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EDITOR-IN-CHIEF
MEYER R. ROSEN

HARRY'S COSMETICOLOGY

VOLUME ONE

NINTH EDITION

NINTH EDITION

Harry’s Cosmeticology

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encourage and support the IFSCC in its mission to bring beauty and health to the many through his clear thinking, close, objective, ever-questioning and challenging examination of the “science” associated with cosmetics and personal care. Once upon a time, he told me that while travelling the world, he always stayed in the same type of room of his favorite hotel chain because “it always made him feel like he was at home.”

A manager I once had told me that if “I had lemons, I should make lemonade”. And so it was with Johann, who turned the enormous amount of time he spent in travelling to producing an incredible volume of questioning, challenging and probing scientific papers for us to read and think about for years to come.

Wherever you are, Johann, I want you to know that your work and critical thinking approach to cosmetic science has impacted us all-and we miss you greatly.

Dedication

This book is dedicated to my wife Selma, my Soulmate, Committed Listener and Partner in the Journey-of-Life.

She who knows, and reminds me, to put the past in the past in order to open the doorway to the creation of new possibilities and generate new directions for growth in areas we do not know that we do not know.

How remarkable it is When mere words on paper Grow together

Beyond themselves.

Such words as these Are tracks in time.

Memories of a mind Focused

A heart and soul

Ensconced.

—Meyer R. Rosen July 4, 2014

VOLUME 1
IN THE BEGINNING REGULATORY REQUIREMENTS THE SUBSTRATES

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IN THE BEGINNING

PART 1.1

MARKETING CONCEPTS TO EMPOWER TECHNICAL PEOPLE

By

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ABSTRACT

Marketing is everywhere. Sometimes it's a good thing, since marketing is what drives revenue for the organization. But sometimes it's not so good. Skepticism aside, it's important to understand that marketing is not merely advertising or selling, but the execution of a carefully planned strategy. Marketing is much more than most people think, and much more than just a great story. In today's hypercompetitive, globalized marketplace, product development is largely a function of assessing market needs and delivering goods and services to meet those needs.

Innovation and R&D are very important, especially in the area of cosmetics and personal products. Ultimately, the marketer is the individual responsible for what will and what will not generate revenue for the organization. Thus, underlying consumer needs and the creativity of ingredient innovators, along with pricing, distribution, regulatory issues, and the promotion of products, is a phenomenon known as the *marketing concept*. The vast majority of new products fail, and so the marketer, at the very least, must ensure that the product looks, feels, smells, and works optimally while also generating the right combination of additional marketing-mix decisions. This chapter explores the methods to the "perceived madness" that often vexes even the most experienced product-development professional. It is intended as a guide to the reasoning behind why marketers do what they do.

We note, in passing, that it may seem odd for a technical book like this to begin with a chapter on the "marketing concept." However, as we open the

panorama of a very detailed description of the "ingredients" that lead to successful cosmetic and personal care products, we must first look at the real world where, inevitably, there's both a marketing and technology push competing with the ever-expanding knowledgeable consumer pull for products that work.

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1.1.1 THE MAGIC AND MYTHOLOGY OF MARKETING

Marketing is indeed everywhere, and depending on one's role within a particular organization, marketing can represent different things to different people. This can become particularly problematic with regard to product development. What exactly is marketing's role in the development of cosmetic products? The purpose of this chapter is to cut through the confusion and to clarify marketing's role in personal care/cosmetics. First, let's mention a few common myths.

1. "Marketing is selling and advertising."—The first thing a student of marketing should learn is just how untrue this assertion is. Marketing is much, much more than selling and advertising, as it involves product, pricing, distribution, and promotional strategies.
2. "The marketer's job is to sell whatever R&D develops."—The second thing a student of marketing learns is that goods and services, in this age of hypercompetition, must be driven by assessing and meeting consumer needs. Goods and services that are not need-based seldom succeed in the marketplace.
3. "My new product has a great chance of succeeding."—Actually, the vast majority of new products (about 90%) fail to meet expectations and are deleted from the company's product mix within a few years.

4. "Word of mouth is a marketing activity."—It is not a marketing activity, but rather a result of our communications efforts. When someone says that most of their marketing is word-of-mouth-based, especially in the age of Internet, be very concerned.
5. "Marketing is about telling consumers what they want to hear."—A marketer must never mislead the consumer. If we overpromise and underdeliver we ultimately lose customers, who are very expensive to acquire. These unhappy people then tweet, blog, Yelp!, and otherwise report on the company's malfeasance. This is not to underestimate the power that media, NGOs, government, competitors, supply-chain members, and other stakeholders have in keeping companies honest.

Indeed these are only a few of the most common misperceptions that both newbies and experienced product developers have when they think of marketing. So what is the true function of marketing?

All organizations have multiple functions within the company's hierarchy. General management, HR, finance, accounting, production, operations, R&D, and IT are just some of these functions. Each has its own primary purpose. Finance manages cash flow and investments, accounting keeps records, HR manages personnel, R&D develops what might be the next big thing, *etc.* The primary function of marketing then, if we must boil it all down to one thing, is Revenue. Marketers must drive revenue or else the organization cannot exist, and to do so effectively, we must first discover what it is that consumers want and need. In some cases, a concept may be so new that consumers may not even be aware they have a need for it.

1.1.2 THE MARKETING CONCEPT

Academic professionals, in their never-ending quest for professional credibility, will often argue over things that seem silly to the rest of us. One of these arguments concerns the difference, if one actually exists, between a "want" and a "need." For our purposes this argument is spurious. Different groups of people have different priorities, and so the idea of what someone wants versus what someone really needs can be debated until the cows come home. The point of the "Marketing Concept" is that we develop no new products unless we can prove that a large enough group of people will buy them. It's as simple as that, but this means that product development is most often driven by marketing analysis and not by innovation, an assertion that does not traditionally sit very well with the

science crowd.

The reality is that the world of marketing is not at all black and white, and that product development is really a carefully planned and effectively implemented process that involves interplay between the folks whose job it is to study consumers (marketing) and the technical folks whose job it is to make a highly effective hair or skin care product. So let us now delve into the new product-development process with a particular focus on developing a strategic marketing plan.

1.1.3 ASSESSING THE MARKETING ENVIRONMENT

New product development begins with an idea. Ideas can come from many places—customers, competitors, employees, supply chain members, universities, and of course the research and development efforts of our cosmetic chemists. In many cases, ideas come from industries that have nothing to do with cosmetics or personal care. An interesting example is the use of information from the carpet industry by hair care product developers (fibers are fibers, after all). The concept of Open Innovation, described elsewhere in this book under Sustainability and EcoResponsibility, describes this process of ideation.

Many ideas are derived from formal brainstorming sessions, usually led by a high-level marketer (i.e., a Technical Marketing Expert) and involves members of all of these groups. Ideas that are initially thought to be feasible undergo “proof of concept” testing wherein relevant parties are interviewed, answer surveys, or participate in focus groups. Here we must prove that the concept *might* work *before* we conduct further analysis; and assessing consumer needs are a huge part of this process.

The next step is to conduct an analysis of the current business environment. This consists of both internal factors, which are controllable by the organization, as well as external factors, which are out of the marketer’s control. The resulting situation analysis (sometimes called business analysis), gives us all of the information that we need to develop a SWOT summary use in decision-making and risk reduction. The analysis itself is a very objective exercise, and as such, opinions are not needed until these can all be summarized as a series of Strengths, Weaknesses, Opportunities, and Threats. The first two areas are derived from the internal factors and the latter two from the external factors.

Internal factors include a full analysis of all the functional areas within an organization that could affect marketing strategy. This includes pretty much

everyone, and a good marketing plan is a cross-functional effort. Involving all relevant aspects of the organization makes for a better-quality plan, and more buy-in among key players as to how the organization is going to achieve its objectives. It all begins with the organizational mission, a statement of scope and purpose, and includes everything that is under the organization's internal control. Finance, IT, current marketing efforts, and R&D are among these, and strengths and weaknesses are what emerges from such analysis.

External factors include the areas that might present opportunities and/or threats to the organization and its attempts to develop and market products that people actually need. An analysis of the industries, consumers, and other factors like the economy and competition is an absolutely crucial part of making the right stuff for the right people. Market segmentation analysis is used—a process whereby a market is broken down into individual consumer segments, each of whose members have many important characteristics in common. Demographics such as age, gender, and income are combined with psychographics (behaviors and attitudes derived from surveys and focus groups) and geographics to form well-defined market segments. Once marketers can understand the people who comprise a particular segment, they are better able to meet their needs through tailoring the right combination of what we call the Four P's of Marketing or the Marketing Mix. This area may require exhaustive marketing research in the form of surveys, focus groups, and other methodologies.

TABLE 1 (*The Green Marketing Model for Strategic Marketing Planning*)

Internal Analysis

- *Mission Statement*
- *Organizational Overview/Demographics*
- *Marketing (Product, Price, Place, and Promotion Strategies)*
- *Information Technology*
- *Finance*
- *Operations/Production*
- *R&D*

External Analysis

- *Industry Analysis*

Competitive Analysis
Market Segment Data
Social Trends
Technological Trends
Economic Trends
Legal and Regulatory Environment
Political Environment
Natural Environment

SWOT

Objectives and Budget
Marketing Strategy and Program
Control and Evaluation

1.1.4 THE FOUR P'S

Although a few P's have been added by overzealous academics over the years, it is really only necessary to understand the "Original Four." Once the marketer has conducted a situation analysis and its companion SWOT summary, it is time to develop the marketing program, the foundation of which is Product, Price, Place, and Promotion strategies.

Product strategy is where the real work between marketing and R&D comes into play. The decision to use certified organic or natural ingredients instead of synthetic ingredients, for example, might be driven by marketing, but the reality of working with these ingredients and still being able to manufacture an excellent personal care product is another story. Product-development chemists and the folks working on the manufacturing end of things might have constraints that the marketer may not fully understand. However, it is the marketer whose job it is to monitor the marketplace and assess market needs. Teamwork and cooperation here are of paramount importance, and this is often where new products fail when cooperation falls below the bar-of-success. The wrong product is made for the wrong target market oftentimes because marketing and other organizational entities won't cooperate, and thus function too independently. Product strategy is the foundation of marketing. The team must decide on the ingredients, the positioning (different from competitors), packaging, branding, and other strategic considerations before pricing.

Pricing the product depends on several factors. First, the cost must be considered, including variable costs (cost of goods sold), as well as fixed costs such as general/administrative and sales/marketing expenses. These costs must be covered, but that's not all. On the demand side of the equation, the marketer must look at the prices of competitive and substitute offerings as well as what prices consumers might be willing to pay. A competitive product and market analysis can run in the several hundreds of pages and such reports may costs tens of thousands of dollars. Unless the marketer decides to sell the product directly to the consumer through the Internet, TV, direct mail, or some other channel, any pricing strategy must also consider both wholesalers (distributors) and retailers. The former intermediary often takes 10% or more of the retail price and the retailer can take as much as 50%. What is left over after cost and channel considerations is profit. This is a lot to consider, and the marketer has dozens of pricing strategies from which to choose.

Placing the product involves distribution decisions and choosing a mix of retail channels to sell it. Should the product be distributed exclusively, selectively, or intensively? Should it be found in higher-end natural products stores, or should it be sold in Walmart and through the grocery channel? Such decisions should not be taken lightly, and they depend fully on both the product and pricing strategies.

Promotion is what most people think of as "marketing communication." Marketers have a myriad of promotional strategies and tactics to choose from and must find an acceptable mix under the marketing budget. Advertising, public relations, direct marketing, sales promotion, and personal selling are all important elements of marketing communication. Much of this communication is moving toward the digital medium, but it is important to realize that the majority of marketing is conducted through what we would call more traditional media such as TV, radio, magazines, newspapers, outdoor and indoor signage, *etc.* The advent of social media and the proliferation of digital advertising have made the marketer's job far more difficult in recent times; but, on the bright side, they have made the control, evaluation, and quantification of marketing activities much easier. Marketers must engage in both "push" marketing to encourage distributors and retailers to carry the product, as well as "pull" marketing to inform consumers what the product is, what it can do (claims), and where it is available. This can be rather expensive as you might imagine, but without it, the product has no chance whatsoever of succeeding.

Before we look at the remaining steps in the product-development process,

something should be said about product claims. Historically the marketing of ingredients with great stories behind them (traditional use, exotic derivation, etc.) has forced marketers to make product claims for what the product is supposed to do, and unfortunately many product developers fail to include an efficacious amount of the ingredient, thus rendering the product ineffective—a phenomenon known as "dusting." The FDA is watching ever more closely, and manufacturers are under increasing pressure to make products that really work. Not only this, but the nature of claims (especially with the FDA's mandate to eliminate false or misleading structure-function claims) is of critical importance to providing proper information to the consumer looking for a solution to a problem. The natural and organic ingredients business has been plagued by this, but mainstream cosmetic and personal care also suffer from this syndrome. When products fail to meet expectations and marketers make promises that product developers cannot keep, the brand and ultimately the entire organization suffer.

1.1.5 DEVELOPMENT, PROTOTYPES, TESTING, AND COMMERCIALIZATION

Once a business analysis has been conducted, preferably in the form of a written strategic marketing plan, it is important for the product-development team to develop a prototype, or in the case of personal care, a final formula for testing purposes. Obviously shelf aging stability, bacterial contamination, and other testing are conducted until the results are satisfactory and any necessary changes are made to the formula to make it robust under foreseeable conditions. Even if the actives have been clinically tested, it is a good idea to test the product itself so that any claims made can be substantiated. Additional market research may also be conducted at this stage if the picture is still incomplete. Once the formula has been "finalized," labels, containers, applicators, and all other considerations should be considered since packaging is such an important part of the consumer experience in this industry. In this regard, Part 1 of this book also includes a detailed description of how attractive packaging is designed. When several mockups have been made with finished product inside, it's time for employees, suppliers, friends, family, and others who are closest to the organization to get an initial feel for the finished product.

In many industries, a product is market tested before it is released to the broader population. This is often done in specific stores or regions of the

country. Such test marketing can tip off the competition, but it may be important to reduce the risk of utter failure by doing a bit of test marketing. The personal care industry admittedly doesn't do much of this, but it is an important step to mention in any interpretation of the new product-development process. Hopefully, the organization's finance function has liberated enough cash flow to produce large volumes of the product and support it with the proper marketing efforts. Since marketing planning is always time-bound, the period of evaluation will culminate in an actual evaluation of what happened. Did the product introduction work? Why or why not? What should we have done differently?

Table 2 ***The Green Marketing New Product-Development Process***

Step 1 Idea Generation
Step 2 Concept Testing
Step 3 Business Analysis/Marketing Planning
Step 4 Product Development
Step 5 Market Testing
Step 6 Commercialization
Step 7 Evaluation of Market Results

1.1.6 THE TRUTH ABOUT INNOVATION

Here is an important question to ponder. Just because an ingredient or a product is "new," does that make it "innovative"? It seems that this word has been thrown around quite a bit and has lost much of its meaning, similar to the way that social media, and Facebook in particular, have somewhat diluted the word "friend." And this begs the question: Are ingredients truly innovative, or is this just a bedtime story that product developers tell themselves so that they have a better reason to rise and shine in the morning? Just because a new botanical or ingredient blend is new or popular doesn't necessarily mean that it is innovative in any real sense. Let's explore this rather controversial assertion further.

The term "innovation" has been used to describe all kinds of underwhelming product developments, and the popular understanding of the term is that it means "new." I think we can all agree that the telegraph, automobile, light bulb, radio, television, computer, and smartphone were all groundbreaking innovations; but what about each iteration of the iPhone? Is a new emollient truly innovative? What about a new natural preservative? Or, a new, naturally derived active ingredient?

Innovation and Product Development

Product Development is one of the author's favorite university courses to teach, and in this important class we learn that there are three forms of innovation.

1. A **continuous innovation** describes a new product that requires almost no change in consumer behavior, and so it represents very minor changes in an existing product type. An example of this would be an existing hand lotion product that features a new nonactive ingredient, or if you prefer a technological example, each and every iteration of the iPhone would be continuous. Many experts do not consider a continuous innovation to be very innovative at all.
2. A **dynamically continuous innovation** involves a major change in a minor behavior or a minor change in a major behavior. An example would be a hand lotion (either an existing product or a new one) that features an entirely new way of dispensing the liquid, such as moving from a jar to a pump. Or technologically speaking, it's the difference between a tablet and a laptop.
3. A **discontinuous innovation** can be very disruptive to the industry. An example in personal care would be a product (or a newly discovered ingredient) that has an entirely new market application, such as hair regrowth. The decade-old advent of skin and hair care products in the form of ingestible nutritional supplements may have been the last true discontinuous innovation in our sector. In tech terms, the inventions mentioned earlier in the article "Which Ones?" would qualify as discontinuous innovations.

In most cases, it's not really the ingredients that are innovative, but the finished product. And in every case, it's always the degree of consumer behavioral change required, and not the ingredient/product itself, that determines the type of innovation. It may sound academic, but what happens is that people in general become desensitized to words they hear all the time such as "innovative," and when they are underwhelmed by what they see, it serves to further numb the target market to the marketing message. I think we can all agree now that the vast majority of new products are continuous innovations. The really groundbreaking product, featuring an entirely new ingredient with an entirely new application, can only be introduced once. After that, successive products will be dynamically continuous or continuous innovations. As such, in almost all cases, "hot" ingredients are merely popular, and not really innovative.

The Patent Process

Now let's look at the patent process. In order to qualify for a patent in the U.S., an invention must be novel, involve an inventive step not obvious to a skilled person, and have an industrial application. If approved, the patent is exclusive for 20 years. Patents are awarded in cosmetic and personal care products all of the time, mostly in the form of process and composition of matter inventions. The vast majority of these do not require changes in consumer behavior, and so they cannot be considered innovations in any respect, and in too many cases the changes are things the consumer doesn't even notice. It's no small wonder then that many observers, including the author, believe that patents are far too easy to obtain and that this is actually very bad for innovation.

Unfortunately, this is often lost on most industry people (who have never taken business classes), but it is very basic science to those of us who have. We learn that new products (which fail 80–90% of the time) should be need-driven in almost all cases (excepting the area of needs that consumers didn't know they had in the first place, which is rare). The firm's job is to address, and in many cases exceed, expectations in meeting the market need while differentiating their offerings from competitive and substitute products. This attempt at differentiation generally results in a whole heck of a lot of noninnovation, some continuous innovation, and an occasional dynamically continuous innovation, but rarely does it result in something that disrupts the industry.

Nevertheless, marketers and media alike will continue to push "the next big thing" as manufacturers look for the next incredible ingredient and retailers tailor their product mix to meet the demands of their customer base. The author will take the road less traveled here and remind everyone that *new market applications* are what spawns innovation in the personal care world, and not necessarily a new ingredient that meets an existing need. So let's dispense with the hype and begin talking about ingredients and products in terms of how consumers view them rather than "innovations" that do not matter to the marketplace. Indeed market applications are crucial to justifying the use of certain ingredients as well as the development and commercialization of the products themselves.

1.1.7 THE MISSING LINKS

Our discussion has obviously been rather academic. The reality is that many product-development teams, not unlike groups of all kinds, are very dysfunctional. Obviously, we know that this perfect world exists only in the

minds of academics, and that the reality of product management is rather messy. Yet, as marketing professionals we must agree on a foundation of theoretical concepts and best practices so that we know how to approach each and every unique situation. Indeed the "science of marketing" must be applied as a basic framework for achieving organizational objectives.

In today's hypercompetitive marketing environment, three areas in particular must be better addressed by corporate leaders if organizations are to increase the chances of their products' success:

1. More cooperation is needed among all functional areas of product development, including marketing, R&D, and manufacturing, for a more seamless product introduction. Today's company needs an interdepartmental understanding that products must be based on needs; that innovations come from R&D and can be incorporated into this need set, or in some rare instances, consumers might discover needs they didn't know they had; and that all of this has to be feasible for manufacturing.
2. More cross-functional employees with multiple degrees would help matters vastly. It is entirely conceivable that members of the staff can have both marketing (business) degrees and chemistry (science) degrees. Dual-degree programs are rather common in today's university environment and a second degree might only require one extra year of education. In addition, a person who has a BS-Chemistry can easily get an MBA or an MS-Marketing with an additional one or two years of education. Why must it always be one or the other? It is our observation that individuals who rise in companies the best are those who have both marketing and technical experience and background.
3. Over the past decades, companies have vastly reduced the amount of money they spend training existing employees, hoping that the universities have done their job. Don't bet on it! Universities are great with theory, but application of theoretical concepts leaves a lot to be desired, and some of this burden must be reappropriated by the organizations themselves. Hire people with great aptitude and basic knowledge, and then train the heck out of them.

Marketing is really everyone's responsibility. Inadequate efforts led by a failure to understand and apply the principles of marketing will almost guarantee product failure in an environment that sees 80–90% of new products languish on the shelves, slated for eventual deletion. In-depth business analysis and market testing, anchored by intensive market research, will greatly reduce a marketer's

risk and will give the scientist more confidence that his/her creation will be a hit with consumers. Flying blind in an age of radar and GPS technology is ill advised even though it may be an exhilarating experience. *A failure to recognize and apply the Marketing Concept at all levels of the organization is akin to flying blind, and there is no glory in product failure.*

PART 1.2

CREATING THE RIGHT FRAGRANCE FOR YOUR PERSONAL CARE PRODUCT

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ABSTRACT

It has been said that no matter how good the technology in the bottle, when the consumer opens it, if it doesn't smell good, look good, feel good, and rub in well, then they won't buy it. As such, fragrances play an incredibly important role in the presentation of a cosmetic or personal care product.

Fragrances are a complex mixture of natural and synthetic materials designed to cover the malodor of a product base, make a cosmetic and personal care product smell appealing, and most importantly, reinforce the product's benefits to the consumer. They are used in nearly every product type and category!

The overall performance of a fragrance in a product is therefore critical to its commercial success. While products are typically developed with technology that works and claims are made as to what to expect, it is common practice for product developers to approach fragrance houses for advice as well as for recommendations as to what to purchase that will enhance the overall presentation of the product and achieve the goals of the brand.

This unique chapter is essentially divided into two parts. The first provides guidance and a general understanding of what, *and how*, to request product and services from a fragrance house. It also describes what can and should be expected of the fragrance house.

The chapter then goes into detail about the composition of fragrances and introduces the reader to the detailed thinking and tools that describe how a fragrance is composed by the Perfumer, and the best way to evaluate the performance of the fragrance in a product. This approach is key to choosing a successful fragrance. The chapter also contains discussion of the impact of EU (European Union) regulations on fragrance formulating and labeling relative to alleged allergens. Finally, it concludes with a focus on three important points: 1) a comprehensive discussion of the many different types of fragrances; 2) insight into making the best choice of asking about what the product formulator and marketer want to achieve; and 3) tips on how to best label these emotive actives.

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1.2.1 THE FRAGRANCE HOUSE

AS the phrase implies, a fragrance house specializes *solely* in the creation of fragrances. Most fragrance houses are structured in a similar way with respect to personnel and project flow. Usually, a Salesperson interacts with the cosmetic chemist to obtain information about the potential project. The Salesperson may ask the cosmetic chemist a variety of questions to help define potential fragrances for their products. For example, questions would be asked about the type of products in which the fragrance will be used; the type of fragrance or odor character desired; and any benchmarks or olfactive targets to consider; information would be required about the brand and target market, the desired price point of the fragrance, or the dosage of fragrance in the product. Other areas of interest will be the price per unit, regulatory considerations, and the expected volume of the fragrance to be sold per year if the project launches. This information is provided to the **Sales Management team** in the fragrance house to determine whether enough information has been given to assign a priority to the project.

Overall decisions are typically made using the financials of the project and the relationship of the fragrance house with the requesting company. If the project passes review, it is passed to the **Evaluation Department** to determine whether the fragrance requested already exists in the “library” (fragrances already created and approved by an Evaluator, but not sold to any company) at the fragrance house, or whether the request needs an investment of a perfumer to create a fragrance to fulfill the request. If suitable types of fragrances exist in the fragrance house's library, these will be chosen by the Evaluator, compounded, evaluated in a product base, and sampled to the cosmetic chemist for review. If no suitable fragrance exists internally, a Perfumer or Perfumers will have to create a fragrance for the request. The new fragrance creations will be reviewed by an Evaluator iteratively in accordance with the protocol described above, until they meet the requirements of the request.

The Perfumer–Evaluator team is a very important part of the fragrance house. An Evaluator is much like a food critic, or a golf caddy, whereas the Perfumer is much like a chef, or a pro golfer. Part of the role of an Evaluator is to understand the cosmetic chemist's request and extrapolate the request into an

olfactive structure that a Perfumer can create against. Evaluators also have a good understanding of current olfactive trends in their specialized product areas, and will use this information to guide the Perfumer to ensure the result is “on-trend” in odor, but is also pleasing overall. Evaluators don't tell Perfumers which specific materials to use, but rather what odor impressions or characters should exist in a creation and whether they are on-trend as per the Evaluator's understanding of their specific area of expertise. Evaluators provide a sounding board for the Perfumer to discuss olfactive directions and act as an impartial critique of a creation, much like a caddy provides a technical view of the shot at hand. Using the feedback and input of the Evaluators, Perfumers are free to use the materials that would be appropriate for the product type but also meet the regulatory restrictions of the particular geographical targets for the product. Due to the broad knowledge of the olfactive market involved, Evaluators generally specialize in fields such as Air Care, Household Care, or Personal Care. In the case of cosmetics and personal care we are primarily concerned with the evaluation points of personal care products that are used on skin and hair.

The Perfumer is a very specialized position. Most Perfumers have been trained by experienced Perfumers in an apprenticeship-type job training that takes from five to ten years. Many start as Fragrance Compounders and then move up to Perfumer's Assistant to Junior Perfumer and then Perfumer. During this time, a potential Perfumer studies and understands the general structures of classic and current fragrances as well as developing their own new creations. A Perfumer has committed to “olfactive-memory” up to 3,000 individual fragrance raw materials during their training. The majority of the required talent and creativity of the perfumer is in understanding how combinations, or “recipes” or “accords” of these materials interact with each other in a product base to form beautiful fragrance compositions that will meet the requirements of a request. The Perfumer is also expected to understand the current regulatory landscape and to know general stability parameters of the individual fragrance components in a variety of product bases such as shampoo, bodywash, conditioners, lotions, and styling gels so that the fragrances will be likely to perform as intended. Additional support is given to the Perfumer–Evaluator teams by the fragrance house's Applications department, which incorporates fragrances into product bases. The bases may be those supplied by the requesting company for a specific project, or may be a “house-base” developed by cosmetic chemists within the fragrance house.

An additional part of the Applications department's responsibility is to handle

any requests for stability testing and to create various product prototypes needed for Sales, Evaluation, and Perfumery. Other key teams that support Perfumery and Evaluation but have different duties include Marketing, which interacts heavily with Evaluation for defining overall trends and providing other marketing support.

The **Analytical Department**, extracts and analyzes the composition of specific fragrances of interest on the market that have not been created by the fragrance house in order to provide current “competitive” information to the Perfumery Department. The **Regulatory Department** provides the final determination as to whether a submitted fragrance meets all the requirements necessary for the request, and their information is invaluable not only to the Perfumer for a creative project, but also to the Evaluation department in the case of a fragrance chosen for a project from the library.

The actual creation of a new fragrance by a Perfumer generally occurs in front of a computer with a specialized software system that has a list of materials available to a Perfumer, and the final formula based on a weight or volume percent is quite like a recipe for baking or cooking. As the formulas are optimized over the Evaluation process, they are streamlined to include only the necessary materials at the proportions required for the performance of the fragrance. The Perfumer can recall the odor of several thousand materials and will first develop the beginnings of the fragrance formula in their “mind's-nose,” or they will start with a part of a formula that they have worked on in the past. The Perfumer may start a new project by putting together a fragrance formula that is about 85–90% complete and use the materials they are sure they want to use, in a ratio that they know, or can reasonably be sure will be appropriate for the request at hand. This methodology is highly useful in order to streamline the required iterative creative process. This “partial fragrance” is compounded by the Fragrance Compounder and returned to the Perfumer. If it is what the Perfumer was expecting, the Perfumer may ask the Applications department to apply the fragrance to a product base.

After evaluation in product base by the Perfumer and/or the Evaluator, the Perfumer may create several trials to complete the last 10–15% of the fragrance composition. Each trial is a specific twist on the fragrance that uses materials whose effects are more difficult to predict, or some untried out-of-the-box ideas that may or may not work well. These trials are also applied to the choice of product base, and these choices are evaluated and compared to each other and to the cosmetic chemist's request by the Perfumer and Evaluator. One or more of

the trials may go forward, or the Evaluator may ask the Perfumer to continue to further develop the concept and create more trials. This process is repeated until there are several fragrances ready to submit to the cosmetic chemist.

Usually from five to 15 trials are needed by a Perfumer to create a new fragrance that is deemed worthy to show to a cosmetic chemist. Depending on the project, several Perfumers may be assigned and then the Evaluator will make a selection of the chosen fragrances across multiple perfumers to give to the Salesperson to show to the cosmetic chemist in order to ensure each submitted fragrance is different enough to stand on its own. The cosmetic chemist will also be informed that the entire chosen range of fragrances is appropriate for his/her request. The fragrances that are completed, technically acceptable, and olfactively sound *but are not chosen by the Evaluator* are placed into the fragrance house's library for possible future use.

As a result of this process, a Fragrance House's library may have tens of thousands of finished formulas available for internal use. This is why most creative fragrance houses do not offer a “catalog” of fragrances . . . there are simply too many to catalog, they are too difficult to describe thoroughly, and since they have to be designed for a specific product type (for both regulatory and technical reasons described elsewhere in this chapter), it would not be a useful exercise.

If a Perfumer is unable to smell due to a cold or other reason, creative work continues since the formulations created by the Perfumer on the computer can be compounded and then smelled by the Evaluators or other Perfumers in a group setting. As the cosmetic chemist's request is always used as the criterion for being chosen, the team members can provide feedback to the Perfumers to help them improve the fragrance until they are able to smell again. When smelling for a living, the Perfumers and Evaluators learn to take very shallow breaths and to clear their airways in between evaluations. Most professional Perfumers and Evaluators simply space their smelling sessions strategically throughout the day rather than pollute their noses with strong-smelling materials such as coffee beans. After the Evaluator and Perfumer decide on the fragrance selection to be offered, the Salesperson shows the chosen fragrances to the cosmetic chemist. After some form of evaluation, the chemist should give feedback to the Salesperson on each of the submissions. This feedback goes to the Evaluator–Perfumer team and helps them for future requests, or helps to optimize a specific fragrance for a current request. The feedback can be about the fragrance's performance in the product base, the overall odor character, or any comment

about how well the fragrance fulfilled the request. All feedback is helpful to the fragrance house to understand their customer (the cosmetic chemist).

Once a fragrance is chosen by a cosmetic chemist and the decision is made to purchase the fragrance from the fragrance house, the Perfumer's formula is activated into the fragrance house's Production system, and is removed from the fragrance house's library so that it will not be used again for other customers. The Perfumery department sends a sample of the fragrance to be purchased to the Production's QC Department to be used as a standard. The flash point, refractive index, general color range, and specific gravity are already known from the trial samples sent to the cosmetic chemist. The QC chemists are trained to compare the standard to their productions via a “triangle test” to determine if the odor is comparable to the standard. (Each panelist is presented with two samples of the control and one sample of the test material, or two samples of the test material and one sample of the control, and they are asked to locate the “odd” sample; the results are statistically analyzed to determine whether the samples are different or not.) In addition, the refractive index, specific gravity, and color specs are compared to those that exist for each formula.

1.2.2 FRAGRANCE MATERIALS

Individual fragrance raw materials may be natural or synthetic. Each of these materials is carefully characterized by RIFM (Research Institute for Fragrance Materials—http://www.RIFM.org) and is regulated by IFRA (International Fragrance Association—http://www.ifranaorg). RIFM conducts safety and environmental studies on the fragrance raw materials and maintains the “master list” of materials that are permitted as fragrance materials. IFRA regulates the amount of each material that can be used in a fragrance and ultimately in a finished cosmetic product, for specific product categories, which is delineated in the QRA (Quantitative Risk Assessment) document. There are currently 11 product categories addressed in the IFRA regulations. They range from lip care to shampoos to wipes to candles. These categories are based on the expected dosage of a fragrance material that the public is expected to be exposed to and depends on the type of product used. It is useful for the cosmetic chemist to know in which of the IFRA categories their product falls, particularly when requesting paperwork from the fragrance house. IFRA actively educates the public as well as the chemist about the safety, history, production, creation, and use of fragrances via video and statements on their website and through social

media.

Natural fragrance materials are most often categorized as *essential oils*, *absolutes*, *concretes*, or *natural isolates*. **Essential oils** are usually obtained by steam distillation of the part of the plant that contains the essential oil. During the distillation process, the essential oil floats on the water. It is then separated from the water and any remaining water is removed. The odor of the essential oil is compared to standards for composition and overall odor. In the case of citrus oils, the essential oil is obtained by squeezing the peels of the fruits. As with other plants, the citrus oil is removed from any water that may be present after squeezing. Essential oils generally are relatively expensive versus a synthetic material (except, depending on the year, citrus oils—since the citrus industry produces a large amount of food products, and the peels are byproducts that can be used for the fragrance industry).

Essential oils are normally used in a standard fragrance (one that is not designed to be all natural) at levels from ~1 to 20%, while the remainder of the fragrance is synthetic. Essential oils add complexity and a certain "naturalness" to a fragrance, but they also add cost, as their pricing is dependent upon supply. These oils can fluctuate in cost dramatically depending on yield, political climate of the producing country, and even time of year. Weather disasters in citrus regions can create serious supply issues.

Concretes are obtained by the extraction of a plant using a hydrophobic solvent. This approach yields a product that is an essential oil mixed with waxes and any other hydrophobic material soluble in the solvent employed. A hydrophobic solvent is removed to yield the **concrete**. Further extraction of the concrete with ethyl alcohol and removal of the alcohol yields an **absolute**, which is the essential oil that contains any other materials soluble in ethyl alcohol. The waxes and other ethyl alcohol-insoluble materials are then removed, yielding a product that is completely soluble in ethyl alcohol. Absolutes of extremely faithful odor quality may also be obtained by supercritical carbon dioxide extractions of the plant as well. Concretes and absolutes are very expensive and are used as "touch" ingredients in fragrances. This means that in a fragrance they may each range in concentration from 0.001 to 0.100%. Even at these low levels they add significant cost, but they also add significant "odor value." If they are removed their removal is quite noticeable, as the fragrance would smell less "rich" and less "extravagant." Most of the time, these materials are only used in expensive, fine fragrances and very high-end creams and lotions.

Natural isolates are obtained by varying physical means from essential oils by

crystallization (such as crystallizing menthol out of peppermint oil), or fractional distillation, where an essential oil is distilled using heat and the various raw materials are isolated by means of separation, employing their differing boiling points. There are no chemical transformations made on natural isolates—they are merely components of essential oils that have been removed from the intact essential oil. These materials are generally also expensive since one must first obtain the essential oil, then put in some additional amount of work to obtain the isolate (usually by distillation). The use of these materials is discussed later in this chapter.

Synthetic fragrance materials are made by chemical reactions on starting materials. Many synthetic materials are derived from crude sulfate turpentine chemistry (as an example, see http://www.renessenz.com/site/production). Some are derived from petroleum products, and yet others are made starting from complex molecules isolated from plants. The majority of the commonly used fragrance materials are synthetic and form the framework of the fragrance. Advantages of synthetic materials are that they are typically very completely characterized by the supplier, are in plentiful supply, are relatively stable in cost, and are not seasonally dependent. There are more varied odor characters available in synthetic ingredients, and they are currently what consumers are used to smelling in their products.

Botanic materials are different than fragrance materials in that they are typically not used to impart an odor to the finished product but are used for marketing or performance reasons. These materials may be derived from any part of the plant, even those parts of individual plants that are not used to provide an essential oil (which vary per plant), with almost any appropriate solvent. Botanics are regulated on the cosmetic product's label by the PCPC (Personal Care Product Council, formerly the CTFA—http://www.personalcarecouncil.org/).

1.2.3 WHAT IS A FRAGRANCE COMPOSITION?

As we have stated earlier, fragrances are complex, well-balanced mixtures of individual natural and synthetic fragrance materials. A fragrance is perceived as an entity with its own aesthetic identity. The unique characteristics of each of the individual fragrance ingredients stay in the background and contribute to the fragrance as a whole without being suppressed.

The identity of the ingredients used in fragrances varies depending on the

desired odor profile, final product category, and form. For example, this would include but not be limited to oil-in-water emulsions or a transparent micellar solution. Fragrance houses and perfumers study which individual fragrance materials perform the best in each product type. They use this experience to combine the ingredients to create complex fragrances that should perform well in that product type. *Since the performance is primarily a qualitative assessment, consultation with an experienced fragrance house is essential if product developers are committed to producing a final acceptable product presentation.* It is also of importance to recognize early on that each product has a number of key points of evaluation in the use-cycle and that these must be determined and evaluated for optimal overall use results.

The creation of a fragrance also differs based on the desired effect upon the consumer. For example, technology is available to provide a refreshing burst of fragrance in use, or long-lasting soothing type or even multiple fragrance notes, delivered over time as the consumer wears it. Perfumers also understand which materials and overall odor characters can create an emotional response such as refreshment or soothing, as well as many other types of emotions and product properties. They are skilled at creating their fragrances to optimize the multiple connotations of these effects. Much work has been done in this area, as the impingement of fragrance molecules on the sensors in the nose is directly transmitted to the brain. The mechanisms of the relationship between pleasant and unpleasant, stimulating or calming, and other effects, have been well studied and will be presented in the Nose section below.

In view of the above discussion, one can see, for example, the actual fragrance materials and the balance of those materials used in a fresh green rosy musky fragrance (intended for use in a soft deluxe facial crème) will probably differ dramatically from a fresh green rosy musky fragrance designed for use in a shampoo, but they actually may smell quite similar.

1.2.4 CREATION / CONSTRUCTION OF FRAGRANCES

a. Top, Middle, and Base Notes

Fragrances are composed of a blend of top notes, middle notes, and base notes (“notes” may be used interchangeably with "character"), a blend that is often represented as a pyramid as seen in Figure 1. A “note” or “accord” is defined as a type of odor character —almost like a mini-fragrance in itself. For example, a

"fresh rose" (perhaps versus a "dark red rose") will likely be composed of two to 20 individual fragrance materials, each of which will contribute a facet to the overall "note." For example, some of the fragrance materials will be grassy green in character and provide freshness, while other materials will be rosy for the overall character. Yet others may add a deep wet or dewy effect to that accord.

Figure 1 Fragrance Pyramid

To make the fragrance, several accords or character notes are combined in different ratios. Fragrance houses will provide on request the “breakdown” of the identity of the top, middle, and base-note characters using an industry standard known as a pyramid—type diagram or something equivalent. It should be emphasized that listed notes or characters are not usually single materials, but are the qualitative perceivable odor characters that the Perfumers and Evaluators have used to provide the overall effect. While artificial “mechanical noses” are being experimented with, at this time the perfumer's process remains a creative one. Such artificial noses are being used as a limited quality-control method in a production setting.

Top Notes are materials and olfactive characters that are immediately perceivable and tend to be the most volatile (volatility is explained in detail in the next section) materials in the composition. Citrus, green, marine, ozone, and some lighter fruity notes are often in the top notes of a fragrance.

The **Middle Notes** are the “Coeur”—the French word for “heart”—of the fragrance. These notes are often the most characteristic and recognizable part of the entire composition. Some florals, sweet, gourmand (edible), and some deeper fruity notes and lighter woody notes can be middle notes.

The formulation of a fragrance is truly a creative process. There is no quality control on fragrance notes during the process. The Evaluators have their own vocabulary, and that is what is taken to be the “standard.” This subjective approach may not be quantitative, but *creativity demands a right-brain approach*.

The **Base Notes** of a fragrance are the longest-lasting part of the fragrance and tend to be the least volatile (i.e., have the lowest vapor pressure) of the notes in a composition. They act to support, round, and extend the richness of a fragrance. The base notes tend to be the most expensive part of a fragrance, so having them is important in order to ensure that a fragrance does not smell “cheap” or “thin.” Musks, woody notes, amber (rich sweet nonedible) notes, and deep heavy floral notes tend to be in the base note.

b. Fragrance Characters

Fragrances are designed by a Perfumer to have a specific odor character overall and to perform well with a defined character at discrete times in the product-use cycle. We use the term “character” as a term of art in this context and distinguish the term “characteristics” as being more consistent with measurable physical properties. By character, we mean the individual odor components involved in making the fragrance. The variation of the intended characters in different product types is what makes perfumery such a high art—extremely difficult and hard to predict. However, understanding the general processes at play can help a cosmetic chemist and marketing manager decide if a fragrance is appropriate for a product or not.

1.2.5 INTERACTION OF FRAGRANCE MATERIALS WITH PRODUCT BASES

A fragrance may smell one way out of the bottle but after being added to the product base it may smell differently in unexpected ways. Product bases in this chapter are generally those that are applied to skin or hair. For example, a facial crème generally is an oil-in-water emulsion, while a shampoo or body wash is a micellar solution and a bath oil is a solution of fragrance in oils with additives.

A review of how fragrances are able to be perceived is useful to help understand changes in fragrance character that may be seen after a fragrance is applied to a product.

There are three major hurdles that have to be surmounted for a fragrance material to be smelled, and therefore for a fragrance to be perceived as intended by the Perfumer. Each hurdle will be discussed in detail and summarized below.

First, Fragrance components need to be *volatilized* into the air in order to enter the nose and interact with the nasal sensors and be perceived by the brain. This is dependent on the intrinsic or inherent volatility of each component, or, how easy

it is to vaporize the component. A good measure of this parameter is the material's boiling point and vapor pressure. A low boiling point (high vapor pressure) means that it is easy to vaporize (out of the “neat” state, without other interactions), and a high boiling point (low vapor pressure) means that it is more difficult to vaporize at the standard conditions of use (i.e., temperature and pressure).

The ability of a fragrance component to be volatilized may change versus its behavior from the neat state due to its solubility in, or interaction with, a product matrix (Raoult's Law). This phenomenon is often due to its hydrophobicity or hydrophilicity. This variation of solubility character is one that can be quantified and indexed for comparison purposes as the ability of a material to partition between water and octanol. This measurement is represented as the log K_{ow} (Octanol-Water Partition Coefficient) of a material.

Each fragrance component has a *threshold concentration* that must be reached in the air in order for it to be perceived by a working nose. This is called the odor detection threshold. In other words, enough of the vapor has to be present at the nasal sensors to be able to perceive it, but that amount differs dramatically between fragrance materials and people.

a. Volatility / Boiling Point

As we mentioned earlier, the relative volatility of a fragrance material can be indicated by its boiling point. The higher the boiling point, the more energy it takes to vaporize a material, which means it is relatively less volatile. Conversely, the lower the boiling point of a material, the higher the volatility. Broadly speaking, materials that give the grassy green odor characters, citrus notes, and some fruity and lighter floral notes are the most volatile, while the heavier florals, sweet, woody, and musky odor characters are the least volatile. The pyramid chart of Figure 1 can be enhanced to show general fragrance character and volatility relationships in Figure 2.

Figure 2 Volatility vs. Character

b. Hydrophobicity / Hydrophilicity (Octanol-Water Partition Coefficient, log K_{ow})

The solubility of a fragrance material in a homogeneous product base may influence the driving force for the material to vaporize in order to be smelled. Absolute water solubility is not an ideal measurement for how a fragrance material will perform in a product matrix. Of more importance is an understanding of the partitioning of a material between a water phase and an oilier phase (such as octanol). This view provides great insight into how a fragrance material will interact with the base.

A useful, simple description one learns early on is the saying "like dissolves like." This means that hydrophilic materials are soluble in water, and hydrophobic materials are soluble in oil. Dissolving the hydrophilic sugar sucrose in water is easy, while dissolving sucrose in oil is difficult if not impossible. It is probable that some very small amount of sucrose is dissolving in the oil, but the ratio of concentration of sucrose dissolved in the oil to the amount of sucrose dissolved in the water is very small. Conversely, mixing kerosene with gasoline is easy, but mixing kerosene with water does not yield a homogeneous mixture. Again, there may be a very small amount of kerosene dissolved in the water, but the ratio of kerosene dissolved in the oil to the kerosene dissolved in the water will be extremely large.

It is easy to see in Figure 3 what this conceptually would look like. For the mathematically minded chemist the equation is shown in Figure 4, but the important idea is to understand that given a choice of oily phase or water phase, for every 1 unit of log K_{ow}, 1,000 times more material prefers to be in the oily phase as opposed to being in the water phase.

Figure 3 Octanol – Water Partition Coefficient

Figure 4 Octanol – Water Partition Coefficient

Abbreviation: K_{ow}, Octanol-water partition coefficient

The same ideas are at play when fragrance materials are introduced into cosmetic products. Overall, fragrance materials are hydrophobic. Only a select few materials are truly hydrophilic and will dissolve to an appreciable amount in a water phase, but there is a range of solubilities and K_{ow}'s across fragrance materials and therefore to some extent across fragrance characters. Once again we must emphasize that fragrance materials are individual components while fragrance or odor characters are how they smell. A single fragrance material can have several odor characters and can be comprised of several fragrance raw materials. This is very generally described in Figure 5.

Figure 5 Volatility & Log K_{ow} vs. Character

c. Odor Detection Thresholds

Each fragrance material has a concentration at which it just becomes perceivable. From there, increasing the concentration results in increasing the perceived intensity of the material up to some maximum level—after which no further intensity increases are detectable. This is described in Figure 6. The maximum perceivable intensity of each material is different, as is its odor detection threshold. It is more difficult to broadly assign fragrance characters to high threshold materials (need a lot to be able to smell them) and lower threshold materials (very low concentrations are able to be smelled). However, it is not surprising that characters related to ripe fruits and other foods have low thresholds to humans so that we are attracted to them. Also, characters related to fecal matter and decay also have very low thresholds, and result in humans being repelled by them. Characters like flowers, woods, and other "survival-neutral" types have higher detection thresholds. This is represented in Figure 7.

Figure 6 Fragrance Ingredient Perception

Figure 7 Volatility & K_{ow} & Threshold vs. Character

When Perfumers design a fragrance, these thresholds and intensities are optimally incorporated into a formula that smells "balanced" such that no single material is immediately identifiable. Figure 8 The whole mix smells harmonious, and the entire mixture performs across many evaluation or product usage points such as out of the bottle. Figure 9 As products are used, the odor profile of the fragrance changes due to the changes in concentration of the fragrance and the product. For example, when using a shampoo, the fragrance is at its highest dosage while it is in the bottle and applied to the hands. Then, when water is added and the product (and fragrance) is diluted, concentrations of each

fragrance material will eventually fall below their detection threshold and disappear from the odor profile. This shift is seen in Figure 10.

Figure 8 Fragrance Ingredient Mixtures

Figure 9 Fragrance Perception – Concentration

Figure 10 Fragrance Perception – Concentration

There are a few other phenomena that are sometimes mentioned with respect to detection threshold. “Anosmia” occurs when a specific person is unable to smell a specific material no matter the concentration. It has been well documented that many people are genetically unable to smell a certain type of musk, sandalwood, rotten fruit, or other very specific types of odorants. Total anosmia to all odorants is rare, but does occur; however, it is more likely to occur from injury, medications, or other trauma than genetic factors.

“Saturation” occurs when one has smelled a strong material at a high concentration that temporarily blocks any receptors from other odors, presumably due to saturation and overload of the receptor. This phenomenon is reversible and after a period of fresh air or overnight exposure the inability to smell will reverse itself. This may occur when smelling material with very low detection thresholds.

“Adaptation” occurs when an odor is smelled so often that the brain doesn't recognize it any longer. For example, when entering the monkey house at the zoo, the stench is often unbearable, but after a few minutes one doesn't really notice the odor. Upon exit and reentry, the process repeats itself—which could be adaptation and saturation presenting at the same time.

It is also useful to mention that some odorants show themselves not only by smell but by triggering a trigeminal nerve response. For example, ammonia has a characteristic odor but this odor may not be able to be covered as it also has a significant trigeminal response, which is an entirely separate neurological pathway from fragrance detection. It is for this reason that the smell of ammonia is extremely difficult or impossible to neutralize by fragrance alone.

1.2.6 EVALUATION OF FRAGRANCES

Fragrances used in personal care products are judged by the consumer at several stages in a product's use cycle. These use cycles are quite different among products and can have a significant impact on how Perfumers will create a fragrance for a specific product. For this reason it is important to explain to the fragrance house the intended nature of the product as well as its use-cycle in order to facilitate selection of the fragrance(s) that will be recommended. Each major event in a product-use profile has the possibility of being an evaluation point for the consumer, and should also be a point of evaluation for the chemist. Usually one evaluation point is more important than all the others, and it is highly useful to identify that evaluation point and ensure that the fragrance and product perform very well at that key point.

During an evaluation, the consumer (or the Evaluator) will consider the fragrance's character as applied to the product base, and the fragrance intensity. At times, the fragrance can be perfect with respect to character, but one must choose an intensity that is consistent with the product's use to make a perfect product. For example, the fragrance intensity of a high-end facial crème is usually much less than the fragrance intensity of a mass-market body crème. An Evaluator will be well aware of this difference and help recommend a level that is typical and appropriate for a product, while a Perfumer may be more likely to want to push the creative envelope.

a. Product-Use Cycle

The product-use cycle may start at the point of purchase. Many packages can be opened and the consumer can smell the fragrance of the product. On this basis alone, consumers can, may, and do make a decision as to whether the product will be purchased. It is also of great importance that the container for the product (and the fragrance) as well as the final packaging can actually be opened. Fragrance packaging design has been covered elsewhere in this book.

The fragrance house will focus strongly on this evaluation point. Normally in the fragrance house, all the potential submissions of the fragranced product will be evaluated against a control or benchmark if applicable. Products are usually compared to each other in 1–2 ounce wide-mouthed bottles with approximately 50% headspace to standardize the comparison conditions. From that selection, the fragrance house's Evaluator will choose a few to submit to the cosmetic chemist and marketing expert. Normally at least two to three times the amount of

final submissions will be compared internally and the best of these will be submitted. The remainder are put into the fragrance house's library for future use as mentioned previously in this chapter.

For packaging that cannot be opened, the consumer product-use cycle starts at the point where the consumer dispenses the product. This generally involves the dispensing and manipulation of a certain amount of product on the hands, and the evaluation point is then on the hands themselves. This is a difficult point to standardize and is considered by the fragrance house in the overall evaluation process—but not as a standalone evaluation point like many of the key evaluation points.

For rinse-off product categories, generally the next step in the product-use cycle is the addition of water to the product/fragrance in the hands or the application of the product to wet skin or hair. At this stage the “bloom” of the fragrance is very important, as usually the consumer is looking for a *burst* of pleasant fragrance to reinforce the product's claims. This is often the key evaluation point for the rinse-off type categories.

Then the product is rinsed out, leaving a certain amount of fragrance on the wet skin or hair. This is an evaluation point as well, but generally is not considered a key point for cosmetic products as opposed to laundry detergents or fabric softeners, where it may be the significant in-use evaluation point.

Finally, the skin or hair is dried, and there are further opportunities for consumers to evaluate the product's smell. Again this is normally not a key point of evaluation for consumers unless there are some type claims at this step, but the fragrance of the product and what is left after the whole use process should be pleasant.

For **lotion-type products or leave-on products** the point of evaluation after application to the hands is the application step where the consumer will rub or apply the product to the hair or skin in a manner consistent with the product category. The product's fragrance character and intensity at this step is very important to help reinforce the attributes of the product, and this can be very much a key evaluation point for products like facial lotion or hair gel.

For **leave-on products**, the evaluation stage continues for the remainder of the intended product effectiveness time frame with varying importance across product categories. For example, in a body lotion, the consumer may wish to be reassured the moisturizing effect has continued for several hours after application, making this perhaps a key evaluation point. However, for a hair gel,

the fragrance intensity and character after several hours of application is fairly irrelevant as long as it is not unpleasant and the product is still holding the hair firmly. All this is in contrast to a fine fragrance where every single moment from the moment of application to the moment in which the fragrance is washed away could be considered a key evaluation point.

b. Fragrance Complexity

Perfumers must create fragrances to be pleasant and appropriate at each of the key evaluation points in the use process. Multiple evaluation points usually indicate the fragrance will need to be more complex and take more time to create and evaluate within the fragrance house. As such, the fragrance created for this type of situation is usually more sophisticated and will be on the higher end of a price point to make a fragrance that will really “wow” a consumer. Certainly less expensive fragrances can be acceptable, but will compromise on certain evaluation points. It may be up to the fragrance decision-maker to determine if the compromises are within the scope of the project and its intended market. Any unusual information about the relative importance of evaluation points should be communicated to the fragrance house; otherwise, the fragrance house will use what seems to be the most appropriate for the product type.

1.2.7 LIMITATIONS OF FRAGRANCES

Fragrances are used to impart a pleasant odor to a product and cover the usual malodor of the product base, in addition to helping reinforce the product's performance benefits. Unfortunately there are some odors that occur in a product base that are unable to be completely masked by fragrance. Highly volatile (gaseous) and strong odors such as ammonia or sulfur dioxide from bisulfite-containing products can be mitigated to some extent, but because fragrances are made from materials with lower vapor pressures than these gases, there is very little hope that a fragrance will vaporize and reach the nose more quickly than these types of odors. Fortunately, concentrations of bisulfites in products tend to be low enough to allow fragrances to have a positive effect on this odor, but ammonia in products such as hair color is at a concentration such that only a little effect may be noticed.

1.2.8 COST STRUCTURE OF FRAGRANCES

Fragrances are constructed of the odiferous natural and synthetic fragrance raw materials described above and, usually, approximately 5 to 20% an odorless carrier that is technically necessary for the fragrance's physical stability, or is introduced as a processing aid for one or more raw materials. The major cost associated with the fragrance is in the actual odiferous fragrance materials. The carrier materials are normally relatively less expensive, but they still add a certain cost to the fragrance formula as demonstrated below.

Fragrances often contribute a proportionally larger cost to a final product than many of the other materials, or are much more expensive per pound or kilo than many of the other materials in the product. However, it is not a good idea to ask the fragrance house for a diluted version of a fragrance just to reduce the purchase price per pound of fragrance. It is best to calculate the contribution of the fragrance to the final product and use that as a guide to whether the fragrance is affordable or not for that product.

a. Carriers

Fragrance houses often ask which type of product the fragrance will be used for. This information is important for the Perfumers to decide which carrier will be used in the fragrance. Carriers such as dipropylene glycol have little solubility in products such as bath oils or some silicones, while very hydrophobic carriers such as isopropyl myristate that may work in oils and silicones may not be appropriate for a sulfate-free shampoo. Using past experience, the fragrance house will match an appropriate carrier with the product type. If an unfragranced product base can be provided to the fragrance house, they will often make tests to ensure that the fragrance is appropriate for the product and will, generally, be able to provide a recommended use range for the fragrance.

b. Concentration-Cost Considerations for Fragrances

Buying a fragrance that is as concentrated as much as possible is the most cost-effective approach to using a fragrance. An easy way to see the advantage of using a concentrated fragrance is to consider a fragrance that costs 40/kg. If the fragrance is formulated into a product at 0.5%, the cost contribution of the fragrance is $0.20 per kilo of finished product. If the fragrance house is told to reduce the purchase price of the fragrance to $20/kg, and it is just diluted with additional carrier costing $7.50/kg, then the concentrated “smelling” part of the fragrance is now not 50% of the formula. However, due to the cost of the carrier, it will be only approximately 38% of the diluted formula. Figure 11

Figure 11 Dilution Reduces Fragrance Activity

To provide an equivalent olfactive effect (such as achieving the same intensity) as the original fragrance, more of the diluted fragrance would have to be used, so in this example the dosage needed of the dilute fragrance would be 1.3% in the final product (versus the 0.5% of the original concentrated fragrance). The 1.3% of fragrance used in the same product contributes $0.26/kg to the finished product. It is $0.06/kg more expensive to use more of a dilute fragrance in a finished product than it would be to use a lower dosage of a more concentrated fragrance, even though the purchase price of that fragrance as a single ingredient is higher. Aside from cost consideration, the additional carrier may affect the physical properties of the finished product or cause other issues. The bottom line is that it is preferable, when possible, to use as concentrated a fragrance as is reasonable.

1.2.9 TROUBLESHOOTING FRAGRANCES

There are times when fragrances can cause a product base to have a shift in color or odor, or cause separation. Color or odor stability issues may be particularly evident in a rapid-aging testing technique or exposure of the fragrance to UV or fluorescent lighting conditions. Phase stability (separation) issues may present themselves as part of a freeze-thaw process—where the product will turn into distinct layers; or if there are pearlizers used in a product, one may see a separation of the pearlizers upon heating that is not reversed upon cooling. Before approaching a fragrance house to solve an issue, it is imperative to ensure that the unfragranced base under the identical conditions did not have the same

issues as was seen in a fragranced sample. If the unfragranced base turned the same color or phase split under the same conditions, then it is probably not the fragrance alone that is causing the issue.

Once it is ascertained that the fragrance is causing or exacerbating such issues, document the condition(s) in which the issue(s) was noted, the dosage of the fragrance, the packaging in which the condition was noticed, how long it took to occur, and any other details that will help the fragrance house reproduce the problem. Normally, the fragrance house will try to reproduce the change in the actual product base, and have a Perfumer rework the fragrance formula to help alleviate the issue. Much of the time the odor profile of the fragrance will be intact, but there are times that a shift must occur. It is best to provide the fragrance house with an unfragranced base to test variations of the fragrance and to allow enough time for a few cycles of variations to occur.

a. Color Changes

Shifts in color of a finished product are sometimes caused by fragrances. A very sweet, gourmand fragrance containing a lot of vanilla or spun-sugar notes will often turn a product yellowish or sometimes light pink. Many times there is not a good way to keep the same fragrance character and solve the issue of color change. Addition of a chelating agent and ensuring the product is slightly acidic, if possible, can help.

Very grapey, fruity, or thick jasmine or tuberose-type fragrances may turn a product pink. Often the only solution in this case is to reduce the amount of that note in the fragrance, which may change the character of the fragrance.

b. Physical Product Stability

If a finished product is not phase-stable when the fragrance is incorporated, it may be that a change in carrier will solve the issue. A change in carrier or solvent will not affect the character of the fragrance to any appreciable extent. For example, in a bath oil the carrier dipropylene glycol will form a second phase in the product, whereas isopropyl myristate will yield a homogeneous product, due to the hydrophobicity of the carriers versus the product solvent. A very good carrier for all product types is diethyl phthalate, but many consumer-product companies no longer allow the use of this carrier. At this time, benzyl benzoate, isopropyl myristate, dipropylene glycol, triethyl citrate, and Polysorbate 20 are the most common carriers.

c. Odor

For odor changes in a stability test, typically either the fragrance just fades away without having any negative odors, or the fragrance "turns" into something that does not have a pleasant odor. Again, especially in the latter case, it is imperative to determine that the fragrance is the cause of the negative odor, and that this odor is not present in an unfragranced base subjected to the same conditions. If the fragrance is the issue, it may be reacting with the product base or active materials in the base and probably will have to be reformulated or exchanged for a different fragrance without the same issues. Overall, one must consider that the fragrance and the product base are basically in a dance with each other. When negative issues result from their combination, one must not jump too fast to the conclusion that the fragrance is the cause.

1.2.10 FRAGRANCE TYPES DEFINED

There are many different categories of "fragrance," and knowing which one to ask for is important to make a successful and cost-effective product.

a. Traditional or Standard Fragrances

Traditional or standard fragrances are mixtures of natural and synthetic materials in some concentration and a carrier. If desired, the natural materials can be "called out" for inclusion on the label, but certainly the rest of the fragrance is listed on the ingredient label as "fragrance." Most traditional fragrances contain from 5 to 20 percent carrier, which may act as a fluidizer/solvent in the event that the raw materials have a low melting point. Use of a fluidizer/solvent will prevent undesirable crystallization. This approach is useful for ease in production of the fragrance, or may be included by the Perfumer to ensure that any crystalline materials stay in solution when shipping during the colder months.

Traditional fragrances are the most common in the marketplace and they can be made in nearly every odor character imaginable, and for nearly any price point. They are also very versatile, and can usually be modified to work well in almost any type of product. If at all possible, this is the best alternative for a fragrance and one that will most likely be optimal regarding its relative cost-stability, versatility, adaptability, and robustness.

If a fragrance house is just asked for a fragrance without any other information, the supplied fragrance will be a traditional fragrance.

b. EU Allergen Label Free or Allergen Free

Based on a consensus of the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFPP), it has been reported that certain fragrances contain up to 26 dermal allergens as defined in http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_07

As a result, for products sold in Europe, the European Union (EU) has mandated that if any of the 26 purportedly regulated dermal allergens are present above certain levels in a finished product, they will need to be listed as an ingredient on the product label in the Ingredients section per the European equivalent of the PCPC.

If a product will be marketed in Europe, these materials *must* be listed on the ingredient label. The fragrance house should be notified up front that a fragrance needs to be "EU Allergen Label Free" if none of the 26 allergens appear on the product label.

The ramification of having an allergen-label-free fragrance is that the Perfumer's palette becomes limited, as no natural essential oils can be used in the creation of a fragrance containing them. As one might imagine, this regulation has had significant effects on many previously formulated, well-known fragrances.

c. Water-Soluble Fragrances

As described above, the vast majority of fragrance materials have no appreciable intrinsic water solubility, which means that fragrance oils, overall, are not water soluble. However, some finished products, especially those without much surfactant or much of an oil phase, require the use of "water-soluble fragrances" in order to yield a transparent product or one that will not separate into distinct phases. An example of this type of product is a water-based hair gel. The designation "Water-Soluble Fragrance" (often abbreviated by the fragrance house as WS appended onto the fragrance name) is in fact a bit of a misnomer. If asked for this type, a fragrance house will supply what is in essence a "presolubilized" fragrance. This is actually a concentrated fragrance oil highly diluted in an affordable surfactant such as one of the Polysorbates. The purpose of the surfactant as a diluent is to provide enough surface-active compatibilizing agent to solubilize the fragrance concentrate—irrespective of the amount of that material in the final product. The amount of surfactant-compatibilizing agent in the fragrance supplied can be appreciable, and is typically 70–80% of the blend,

since the house will likely overdilute to ensure enough surfactant is present to solubilize the fragrance oil. This means that only 20–30% of a “WS” fragrance is actually providing an olfactive quality to the product, and the rest is odorless surfactant of the fragrance house's choice. If the means are available, it may be much more cost effective to buy a concentrated fragrance from a fragrance house and presolubilize it with an appropriate surfactant for the product at only the required level. This amount could be less than the typical ~70% level supplied by the fragrance houses.

A good starting point to accomplish making a presolubilized fragrance is to mix two to three parts surfactant of choice to one part fragrance oil and check it in the final product at the desired dosage (taking into account the dilution). If the final product is not transparent enough, or if the final product forms two phases, then more surfactant is needed. For some types of fragrances, up to four–five parts of surfactant to one part fragrance oil may be necessary to yield a clear product. It is also appropriate to ask the fragrance house to use dipropylene glycol as a carrier if possible since it has the most water solubility of all the commonly used carriers and will result in requiring less surfactant to solubilize the fragrance. It is also important to note that all-natural fragrances are usually the most hydrophobic types and these will need the highest levels of surfactant-compatibilizers to solubilize them if in fact it is possible.

d. Water-Dispersible Fragrances

A “water-dispersible” fragrance (often abbreviated as WD appended onto the fragrance name) is one in which only enough of the surfactant is added (as described above) to form a semi-stable dispersion of the fragrance oil in water. Typically the “WD” fragrance is 50% surfactant and 50% fragrance oil, but can be as low as 30% surfactant and 70% fragrance. This dispersion will likely be hazy, but will not separate into two phases for some period of time so that it can be used in a production or application process. This type of dispersion is normally used in applying fragrances to products that are fragranced at a low level and require some kind of diluent for the ease of application and for accuracy in measurement of the fragrance dosage. For example, if a facial wipe is to be fragranced at 0.010%, the fragrance may need to be diluted in water so that 0.100% of the resulting mixture is used, and the diluting water is added into the water already in product formulation.

e. INCI Blends

INCI Blends are fragrance mixtures in which each individual material has an INCI name as listed by the Personal Care Products Council (PCPC, formerly the CTFA, website listed above), so that the label of a product, as provided to the consumer, does not need to include the word “fragrance,” and instead need only list the blend ingredients separately. The fragrance house should be asked to provide a listing of the ingredients used in these blends once they are approved. Normally in order to do so there is a confidentiality agreement or equivalent in place. It is absolutely necessary to ask the fragrance house for an INCI blend up front, as they are not the norm, and generally require special creation. These INCI blends are typically extremely simple compared to the average fragrance and have very simplistic and singular odor characters since there are so few materials used in their construction.

1. Natural INCI Blends

Not all natural materials or essential oils have an INCI name, so it is necessary to determine the exact purpose of asking for a natural fragrance. If the product needs to be “All Natural” *and* will list the fragrance ingredients on the ingredient label *and* must exclude the word “fragrance,” then asking a fragrance house for an “All Natural INCI Blend” would be appropriate. If the only requirement is a “Natural Fragrance” and the word “Fragrance” or “Natural Fragrance” can appear on the product ingredient label, it would be more appropriate to ask just for a “Natural Fragrance.”

A Natural INCI Blend is an extremely restrictive requirement, and the resulting blends are fairly limited in scope. Natural materials are generally the most expensive. If pricing restrictions are imposed on the fragrance house, typical cost-reducing carriers would be the highly hydrophobic hydrogenated soybean oil or medium-chain vegetable triglycerides or other inexpensive stable oils. Again, it may be best to have as concentrated a blend as can be afforded. The advantage of this is that one can use less of it as opposed to getting a less expensive diluted natural INCI blend. Such blends can cause phase stability or viscosity issues in the product or necessitate the use of more surfactant to incorporate it into the product. It should also be noted that natural fragrances, whether or not they are INCI blends, are subject to wild fluctuations in cost due to seasonal variations, crop yields, and availability of materials, as described previously.

2. Allergen-Label-Free INCI Blend

An “Allergen-Label-Free” INCI Blend is the most restrictive requirement for a

fragrance oil. In this case, no natural essential oils, absolutes, or other complex natural (i.e., must be from an essential oil, or an "absolute") products can be used, but only chemicals that have an INCI name *and* are extracted from essential oils or have another natural source.

Nearly all natural essential oils or absolutes contain one of the currently recognized 26 EU dermal allergens at levels that would preclude their use completely. In the case of asking for an "Allergen-Label-Free" INCI Blend, one would need to list on the ingredient label the actual chemical names of the blend materials. This approach may defeat the overall purpose of having an INCI blend, since the long, unfamiliar names of the "chemicals" may seem onerous to the final consumer and will not differ from the negative impact and consumer perception of their synthetic counterparts. Most of these blends by definition must be fruity in character. They will have little to no base notes, a limited number of middle notes, and therefore will mostly be composed of volatile top notes. Further, they will not be appropriate for the most part to mask any base odors from the product. Such materials would be most appropriate for very clean bases that need a fleeting hint of fragrance.

3. Traditional INCI Blend

A traditional INCI blend will contain only fragrance materials that have INCI names as per the PCPC (Personal Care Products Council). They will likely contain some natural essentials oils and some artificial chemicals. These blends can be quite beautiful fragrance-wise but are typically somewhat limited in fragrance character. They do tend to be more affordable than natural INCI blends (and in many cases will have similar INCI names). It should be communicated to the fragrance house how many materials (or letters) can be supported on the ingredient label. Generally, the more ingredients, the more acceptable the fragrance character or odor.

4. "Unscented" INCI Blend

An "unscented" INCI blend is a very simple blend of neutral-smelling materials with INCI names that help mitigate the base odor of a product but do not add any recognizable fragrance quality to it. These blends typically contain two to three materials. These types of blends are useful for products like eye cream or facial cream or a type of product that has some odors from the actives but does not need a characteristic fragrance odor. Note that the base odor from the product will not be totally covered but will likely be more palatable to the final consumer.

f. Natural Fragrances

If the only labeling requirement is a "Natural Fragrance" and the word "Fragrance" or "Natural Fragrance" can appear on the product ingredient label, it would be appropriate to ask the fragrance house for a "Natural Fragrance." If the product needs to be "all natural" *and* will list the fragrance ingredients on the ingredient label *and* must exclude the word "fragrance," then asking a fragrance house for an "All Natural INCI Blend" would be appropriate (see above).

Natural fragrance materials can include essential oils, absolutes, concretes, and natural fractions of essential oils (which may be a single material, normally called an "isolate"). Natural materials may also use fermentation reactions and other ways to produce materials that are not synthetic.

Natural fragrances can vary dramatically in price from month to month and year to year, and shortages or quality problems in the starting essential oils can occur.

1. Traditional Natural Blends

Traditional natural blends use a combination of essential oils, absolutes, concretes, isolates, and fermentation products. The result can be a fragrance (although fairly limited in odor character) that is pleasant and somewhat complex and can be referred to on the label as a "natural fragrance." If asked for a "natural fragrance," most fragrance houses will send this type of traditional natural blend.

Natural fragrances are generally three to ten times more expensive than traditional fragrances and the odor characters are somewhat limited. Knockoffs of fine fragrances are impossible, but quite pleasant floral mélanges with some woody base notes and fruity top notes would be possible.

2. Essential Oil Natural Blends

Essential Oil Blends are a very limited version of a natural fragrance. These blends incorporate *only* the entire essential oils obtained from a plant or citrus fruit peels. They do not contain absolutes, concretes, or single material isolates. Such materials are very limited in character because they contain only citrus, leafy herbal, lavender, some woody, and a very few floral characters and their resulting blends. It is necessary to specify to a fragrance house that an "essential oil blend" is needed, and the fragrance house may have questions about the allowed types of materials.

Essential oil blends are the most expensive as well as the most limited in

character, and their pricing over the season varies wildly as many essential oils increase dramatically in price when not in season.

g. GRAS Fragrances (Generally Recognized as Safe)

GRAS fragrances are a niche specialty fragrance type necessary for lip balms or other products that may enter the mouth or penetrate the lips, or are for children's toys. GRAS fragrances are those in which each fragrance material has a flavor FEMA-GRAS number and/or is recognized as a flavor material. These fragrances also must meet all of skin contact requirements for the product category in which they are used. GRAS fragrances, nearly by definition, tend to be fruity or food-related in character. They tend not to be long lasting and are usually quite simplistic in character.

CONCLUSION

Fragrance development is very important to the success of a consumer product. Not only will the character and intensity of the fragrance reinforce the product's benefits, but they will also add appeal to the consumer. Understanding the basic technical requirements of a fragrance and knowing how to interact with a Fragrance house will greatly enhance the success of the cosmetic chemist and ultimately the product.

REFERENCES

http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_07

PART 1.3

FRAGRANCE PACKAGING DESIGN:

A Multi-Sensory Experience from Concept to Consumer

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ABSTRACT

The benefits of cosmetics and personal care products are both tactile and functional, providing a temporary benefit by enhancing the appearance of the face, body, or hair.

By contrast, fragrance transforms the moment, awakens the senses, creates indelible memories, and captivates the people in a room. The consumer's experience of fragrance is subjective, abstract, conceptual, and emotional. The combined expertise of the marketer, perfumer, and designer is dedicated to focusing on the development of a product that communicates an emotional link and provides a cohesive, holistic sensorial experience to the consumer. Sensory cues are used throughout the packaging to communicate the olfactive character of the product as well as the emotional story; and these cues are the foundation of a successful fragrance. In this chapter, experts have been interviewed across all components of the development process, and a review of research has been compiled in order to expand the knowledge base of product developers and educate further about the stages of a cohesive fine-fragrance development process.

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With over 1,000 fine fragrances launched globally in 2011, what is the best way for fragrance brands to stand out in the eye of the consumer and generate revenue for the brand? Expectations are set for the consumer through sensory cues such as the bottle, packaging, color story, and scent. If these elements are not aligned, consumers can experience sensory dissonance. The five senses are core to human experience and are essential to our perception of the world. In today's beauty industry, marketers, perfumers, and designers need to work in tandem in order to meet tight timelines and anniversary sales and to be

competitive in the marketplace. But is the final product always optimized? Were there connectivity points between the team to maintain synergy for the final product? Does it communicate the concept to the consumer?

Primary Question for Product and Brand Development:

What can companies do to integrate their internal process in order to produce a unified product the consumer will positively respond to, accept, and ultimately purchase?

1.3.1 INTEGRATED PROCESS: THE BRIEF

Led by Brand Development or Marketing, a document called the “brief” outlines the objective and strategy for the finished product. The brief is shared with Product Development to initiate the project. After the initial review, additional briefs can be developed, narrowing the focus and outlining evolving details of the project. The initial brief puts the project in action, and detailed briefs such as the fragrance brief and creative brief are viewed on a micro level for the designated department. Product Development also creates a project timeline and a component pricing analysis, which outlines the budget for the product.

Initial Brief or Project Profile

- Brand identity
- Target consumer (demographic and psychographic)
- Quantitative and qualitative research results, if applicable
- Product positioning and white-space opportunity
- Competitive analysis
- Pricing and cost targets
- Quantity
- Distribution
- Target ship date

Fragrance Brief

- Brand overview
- Target consumer with focus on psychographic
- Concept: Olfactive story
  - Mood board
  - Color direction
  - Product personality
- Olfactive category direction: Primary and Secondary
  - Primary: citrus, floral, fougere, woody, chypre, oriental
  - Secondary: citrus, marine, aldehydic, aromatic, green, floral, spicy, fruity, musky, gourmand, leather/animalic
- Oil cost target / Fragrance blend percentage
- Olfactive market inspiration or benchmarks
- Fragrance color
- Distribution (in order to understand compliancy restrictions)
- Regulatory and stability requirements
- Timeline for deliverables

Creative Brief

- Brand overview
- Target consumer
- Product positioning
- Concept
- Component direction
- Color direction
- Olfactive direction
- Existing brand assets (logos, fonts, imagery, color)
- Budget
- Timeline for deliverables

While all the departments are working simultaneously, it is important to have connectivity points throughout the process in order to confirm alignment and clear direction towards achieving the ultimate goal.

Throughout the development process of a product, it is essential to think about the left-brain and right-brain functions you want the consumer to experience with the product. One must ask, "How will the consumer react to the product emotionally, creatively? How will the product function? What will the consumer experience through sensory cues? How will the concept or story be successfully translated to the consumer?" In developing sensory cues it is important to consider how each cue will translate the scent to deliver the concept to the consumer.

1.3.2 SMELL THROUGH HEARING: CONSUMER TESTING

Curate and guide the development process based on consumer findings.

Gail Vance Civille, President of Sensory Spectrum Inc., has pioneered advanced sensory evaluation approaches for industry, academia, and government. She shares that ideas are coming directly from the consumer. Preliminary concept work should involve consumers in order to understand what types of messages are being communicated through product design. A hybrid approach using qualitative and quantitative testing can provide insights as to what the consumer wants and needs, and how the concept and marketing messages are being perceived.

Qualitative Testing

- Field Testing
- Shop-alongs
- In-home visits
- Diaries
- Focus groups
- Interviews
- In-store intercepts

Quantitative Testing

- Surveys
  - Multiple choice
  - Numeric

Mark Knitowski, the “Nose” behind all the Victoria’s Secret scents, does not consider himself the “expert” in developing fragrances for his consumers. Rather, he considers himself the “curator of fragrance” and the “protector of the consumer information.”

Further, Knitowski believes the consumer should not be “sold” a fragrance, but they should embrace a journey with a scent and connect to a well-told story. “The right fragrance should be part of an emotional journey, reflecting how a woman or man feels about themselves and how they want to project the image of themselves to the world.”

By using over 1,000 of the Victoria’s Secret stores as direct access to the consumer, Knitowski involves them in the development process. He spends time in the stores engaging consumers with general day-to-day conversations through in-store intercepts. He may also test two fragrances that he’s been developing to gain feedback and perspective. He first has the consumer smell on skin and then on a blotter, the scent strip used to test fragrances. The consumer then smells the fragrance completely blind without any sensory cues. From their response, Knitowski is looking to understand whether they would purchase the scent, and what adjectives they use to describe it. Through the qualitative learning extracted from his consumer studies, Knitowski further evolves the scents based on the comments from the consumer. Each component, including the fragrance, carton, bottle, and name, are tested independently and then together. If one of these elements does not align to create a holistic finished product, then the consumer will not align with the intended concept.

As an example, Victoria’s Secret product “Bombshell” was the perfect blend of fragrance, bottle, and packaging—but it did not start out this way. The fragrance tested so positively that there were no dislikes out of 150 paneled, yet when the scent launched under the name “Signature” in a rounded bottle, the results were less than successful. After analyzing the less-than-favorable response, the team knew the fragrance was not the problem. This observation became a turning point for creating a product success and is an excellent example of the role of the development team realigning and modifying the product based on consumer results.

The next test that was initiated included the same fragrance with a more faceted bottle and a name that aligned with a recent bra launch, Bombshell. Led by Marketing and Design together, these adjustments built the foundation for a successful fragrance that ultimately won a Fifi award for Best Packaging in the Specialty Market and a Consumer Choice Award; it was also voted Fragrance of the Year in 2011. The positioning within the Bombshell lingerie master brand enhanced the fragrance story by giving it depth and an emotional connection. The adjustments to the bottle further enhanced the cohesiveness of the product because it aligned with the overall product positioning in a way that resonated with consumers.

Marketing and Product Development need to consider both qualitative and quantitative testing. Throughout the testing process it is important for the developers to listen closely to what the consumers are saying, while also maintaining the integrity of the concept. By closely "smelling" the direction through listening to the consumer and curating the learning, a successful fragrance, Bombshell, made history.

1.3.3 SMELL THROUGH SEEING: FRAGRANCE NEEDS COLOR

"Used wisely, color is vitally important and is an instant attention grabber that succinctly gets the message across." (Pantone Color Institute)

Color is perhaps the strongest cue in aligning the consumer's expectations with the desired fragrance experience. Through the fragrance, carton, bottle, and

color, olfactive direction can be communicated, as well as the brand's emotional positioning. The relationship between color, scent, and emotion can impact a consumer's emotions. What does yellow smell like? What branding cues does red communicate? It is imperative to have color direction in the initial product profile or brief because disconnect in the color with the other elements of the fragrance can confuse the consumer.

Here are some examples of the message a color in a fragrance can communicate:

Blue—calm, watery, ozonic

Green—revitalizing, fresh, nature, eco-conscious

Red—sexy, powerful, confident, energizing

Purple—luxury, mystery, exotic, royalty

lesight 2012)

Marketers can leverage color to communicate newness within a portfolio and to distinguish flankers, or fragrance variations, from the original, while also aligning with trends. Fashion color trends relate closely to the beauty industry and change based on the season and geographic location. Forecasting services are a key reference in aligning with trendsetting color palettes, Marc Jacobs's Daisy, a fragrance product, is an example of how color has extended the brand lifecycle by communicating a prestige eau de parfum version through the use of richer colors such as black and gold, silver and gold, and red and black. Also, seasonal color stories stimulated demand and further differentiated a limited-edition bottle. Once consumers see the color and packaging of a fragrance, they already form an opinion and their expectations are set.

Coty's Senior Vice President, Catherine Walsh, is known as the "scent whisperer." She is the generating force behind brands and celebrities such as Jennifer Lopez, Calvin Klein, Marc Jacobs, and Sarah Jessica Parker. Walsh has stressed that it is critical for all teams involved in the development of a fragrance to be aligned all the way through the process. Marc Jacobs's fragrance Dot was launched in 2012, but the initial concept and color direction the Coty team was working towards was very different than the polka dot red-and-black fragrance that hit the market. Marc Jacobs changed the direction because he was inspired by a stronger story that was more meaningful to him and tied to his fashion line. Polka dots have been a recurring theme for the designer, and were a major focus for his Fall 2012 ready-to-wear line. "One design that I always go back to in some way is the dot. I felt like this was something I'd never get tired of, and I'll always find a new way to interpret," Marc Jacobs said. He liked the idea of a hybrid butterfly/lady bug, which communicated the Dot story of free spirit, femininity, playfulness, and luck (Naughton, 2012).

With this significant direction change, Coty communicated all the details of the new concept, the polka dot design and the red-and-black color story through all departments so that Marc Jacobs's strong vision and story could be communicated to the consumer.

As a trend leader in the fragrance industry, Coty utilizes consumer testing and research to better educate the marketers to learn how to lead consumers to the next trend. Trend-leading brands need to maintain the integrity of the brand identity and the character of the concept to offer consumers a product they did not know they wanted until they saw it—and of course smelled it.

1.3.4 SMELL THROUGH TOUCHING: HOW WILL THE CONSUMER FEEL?

The consumer will first see the packaging and the bottle. If the packaging and bottle appeal to the consumer, they will then pick it up: this is the first goal of the marketer. Bottle engineers need to think of the following when developing the bottle: how do you want the consumer to feel emotionally when they pick up the bottle? Does the bottle have soft edges and a lightweight feel or is it rigid and weighted? In one example, Tru Fragrance and Beauty worked with a bottle engineer to create a fragrance bottle for a men's apparel sport line. The Product Development and Creative teams needed to think about this product from an active man's perspective. The bottle was built so the male consumer could grab the bottle and go. Ergonomically tapering through the bottle gives it an aspect of comfort in the man's hand and the built-on spray-through cap allows for easy spraying any time without leaking. The weight was also considered, since the product was designed to be thrown in a gym bag and be a portable fragrance. While the glass is lightweight, the black base and overcap are weighted to give it a feeling of a dumbbell. The development team consciously thought about how the man would use the fragrance and how it would feel in his hand.

1.3.5 SMELL THROUGH SMELLING: TELL A STORY, PAINT A PICTURE

Fragrance is an art. Marketers provide a fragrance brief to the fragrance house. The brief includes as much information as possible about the brand DNA, packaging direction, color direction, target consumer, and store environment. Most importantly, the brief should contain a sensory story and paint a picture in the mind's eye of the consumer. The perfumer is no different than a poet or composer, so a marketer needs to convey the story of the product visually and verbally to stimulate the perfumer. To be successful, consideration should be given to an overall view of the use of the product anticipated. The marketer must set the stage in a variety of ways. Set an environment; describe the dream. What is the temperature? Is it hot like the sun or hot like a fire? How does it feel on skin? Is it humid or comforting? What is the mood of the environment? Is there music on the beach during the day in a tropical place with laughter and dancing, or are you warm and cozy next to a fire in a cabin in the mountains?

A fragrance evaluator, whose purpose is to work closely with the perfumers and optimize their ideas, reviews the brief. The evaluators represent the marketer, and the objective is to really put themselves not only in the shoes of the marketer but really to encompass their identity. They need to take on the role of the marketer, to learn the marketer's tastes and how they think, and to fully

understand them in order to achieve the best fragrance submissions.

CONCLUSION

Falling in love with fragrance. The perfumer begins to blend the "story" based on the brief and visual mood board and works closely with the evaluator throughout the process. Once the fragrance is chosen, it is the marketer's responsibility to take the "story" that is bottled up and make sure that the story is released to the consumer through all the sensory cues so the consumer can experience the story and fall in love with the fragrance.

By means of the use of sensory cues and integrating the development process the concept is successfully shared with the consumer. The story becomes the consumer's dream and each time the fragrance is used, the mood is impacted by how they desire to feel. Sophisticated, romantic, casual, masculine, sexual, playful…the consumer's dream comes true—and this is why people love fragrance.

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UNDERSTANDING THE VALUE OF MOLECULAR CELL BIOLOGY AND GENE ANALYSIS FOR THE NEXT GENERATION OF COSMETIC PRODUCTS

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**** A note from Meyer R. Rosen***

Before we get started on perhaps one of the most complex chapters in this book, we call attention to the fact that many readers will not be familiar with numerous terms used in the text. To this end, a glossary has been created at the end of the chapter. To facilitate understanding, note that in the text all "big" words, unfamiliar to many, have been bolded, and a concise definition is presented in the glossary.

As Editor-in-Chief of this book—I take it as my duty to urge you, the reader (no disrespect intended) to grow in the 50-year-old tradition of Harry's Cosmeticology. The contents of this chapter are deemed so important that,

taking editorial prerogative, it has been put in the first section of the book, "In the Beginning," along with other key areas that provide the foundation for new ingredients and products that are essential for understanding and leading innovation in our industry.

This chapter introduces the basic fundamentals of the principles of molecular biology and why cosmetic industry professionals must understand them. It is our opinion, based on the developmental path being taken by ingredient suppliers and formulators, that, like learning to effectively use social media in the Internet age, if you do not at least have a basic understanding of these concepts, you will be left behind.

Consider this analogy, which is quite prevalent at the time of this writing. When young children age three or so and older, and teenagers, are walking around with their iPads and iPhones, etc., and you—the adult, the parent, the seeker of "how do I text, or use Linkedin," etc.—are your resource for how to do IT. If you do not use that brilliant mind of yours to lead the knowledge pack rather than be a follower, then, my friends, your foreseeable contribution to the development of new cosmetic and personal care products will wither into dust and be blown away by the wind.

And Now, to the Heart of the Matter…

1.4.1 INTRODUCTION

The Human Genome Project revolutionized scientific research in the field of molecular biology. Many of the techniques and gene interaction knowledge base currently in use are the result of the project. The cost and time to conduct such studies continues to decrease, resulting in the generation of information relevant to the formulations chemist. Molecular cell biology investigates how cells develop, operate, communicate, and control their activities. Cells communicate with body tissue, which is composed of cells. Tissues communicate with other tissues and organs throughout the body. Generally the transfer of information between cells, tissues, and organs is through proteins. We now know that genes consisting of deoxyribonucleic acid (DNA) are responsible for biological structure, maintenance, and integration of cellular function. DNA indirectly controls the synthesis of many small molecules including proteins. DNA may be considered a storage form of genetic information. Gene expression is how cells "know" to make the right proteins at the right time in the right quantities.

1.4.2 PRINCIPLES OF MOLECULAR BIOLOGY

Amino Acids and Proteins: Their Role in Cell and Tissue Communication

Proteins are the working molecules of the cell; they carry out the program of activities encoded by genes. To do this a coordinated effort of many different types of proteins is necessary (1). The three-dimensional structure of a protein enables it to regulate concentrations of metabolites, cause cell motion, and provide structural rigidity to the cell. The spatial organization of proteins is critical in understanding how they work (1).

Proteins consist of amino acids, and there are only twenty amino acids from which proteins derive their structure. The three-dimensional structure of the protein is determined by the amino acid sequence. Amino acids can be classified primarily on their solubility in water, which is influenced by the polarity of their side chains. Amino acids with polar side groups tend to be found on the cell surface and make proteins soluble in aqueous solutions. Amino acids with nonpolar side groups avoid water and aggregate to form the water-insoluble core of the protein. Water-soluble amino acids have ionized or polar side chains. At neutral pH, arginine and lysine are positively charged; aspartic acid and glutamic acid are negatively charged and exist as aspartate and glutamate. These four amino acids are the major contributors to the overall charge of a protein (1). Histidine has a pKa of 6.8, the pH of cytoplasm in the cell.

Small shifts in the pH of the cell will change the charge of histidine side chains. The activity of numerous proteins is modulated by pH through protonation of histidine side chains. Asparagine and glutamine are uncharged and have polar amide groups with extensive hydrogen-bonding capacities. Serine and threonine are uncharged and have polar hydroxyl groups facilitating hydrogen bonding with other polar molecules. Hydrophobic amino acids have aliphatic side chains that are insoluble or slightly soluble in water. These amino acids tend to pack in the interior of proteins away from the aqueous environment. Cysteine, glycine, and proline have side chains with unique properties. The cysteine side chain contains a reactive sulfhydryl that can oxidize to form a disulfide bond to a second cysteine. Disulfide bonds are commonly found in extracellular proteins where they facilitate maintaining a folded structure. Glycine, the smallest amino acid, has a single hydrogen atom as its R group. Its small size allows it to fit into tight spaces. Proline and glycine may be found at locations on a protein’s surface where the chain loops back into the protein.

The Cell

All organisms are composed of cells. A cell is a membrane-bound unit that contains DNA and cytoplasm. A plasma membrane encloses each cell, separating its contents from its surroundings. The plasma membrane contains numerous proteins that are responsible for the ability of the cell to interact with its environment. For example, receptor proteins in the membrane interact with hormones and induce changes in the cell in response to the interaction. Transport proteins help molecules and ions move across the plasma membrane either from the membrane at the cell surface to inside the cell, or from inside the cell to the cell surface. Every cell contains DNA, which is the hereditary molecule containing the genes that code for the proteins being synthesized by the cell.

Cells found in animals referred to as eukaryotic cells contain numerous compartments termed organelles and smaller sac-like structures known as vesicles. The largest organelle in a eukaryotic cell is the nucleus, which is the repository of the genetic information that directs all the activities of a living eukaryotic cell. The nucleolus is an area found in the nucleus where intensive synthesis of ribosomal RNA is occurring. A nuclear envelope is located on the surface of the nucleus. The outer membrane of the nuclear envelope is continuous with the cytoplasm's interior membrane system called the endoplasmic reticulum. Scattered over the surface of the nuclear envelope are nuclear pores filled with proteins that act as molecular channels permitting certain molecules to pass into and out of the nucleus. Proteins moving into the nucleus may be incorporated into nuclear structures or catalyze nuclear activities; RNA and protein-RNA complexes are formed in the nucleus and exported to the cytoplasm. DNA of the eukaryotic cell is divided into linear chromosomes, which are fully extended into thread-like strands called chromatin. This arrangement allows proteins to attach to specific nucleotide sequences along the DNA. Without this protein interaction DNA could not regulate the day-to-day activities of the cell. Chromosomes are associated with packaging proteins called histones.

When a cell is ready to divide, the DNA coils up around the histones into a tight condensed structure; this initial aggregation is termed a nucleosome, and resembles a bead on a string (2). After cell division the chromosomes uncoil, permitting RNA polymerase—an enzyme that makes RNA copies of the DNA—to gain access to the DNA. RNA copies of DNA are the only way hereditary information in the DNA can be used to direct the synthesis of proteins. The eukaryotic cell is distinguished from plant cells with the presence of internal

membranes in the cell. The largest internal membrane is the endoplasmic reticulum (ER). The ER is composed of a lipid bi-layer embedded with proteins. The surface is embedded with ribosomes involved in protein synthesis. Proteins synthesize on the surface of the ER (termed rough ER because the surface appears pebbly, like the surface of sandpaper). These proteins contain special amino acid sequences called signal sequences. These protein sequences travel through the ER membrane to the interior of the ER and then move to a vesicle-forming system known as the Golgi apparatus, and is eventually released to the outside of the cell.

Eukaryotic cells contain a variety of vesicles. Lysosomes are membrane-bounded digestive vesicles that contain enzymes catalyzing the breakdown of proteins. Other vesicles, known as peroxisomes, contain the enzyme catalayse, which breaks down hydrogen peroxide into water and oxygen. The breakdown of hydrogen peroxide is important because hydrogen peroxide buildup in the cell is toxic. Mitochrondria are organelles containing proteins that carry out oxidative metabolism of the cell. They have their own DNA-containing genes that produce proteins essential to the mitochrondria's role as centers of oxidative metabolism. Most of the genes that produce the enzymes used in oxidative metabolism are located in the nucleus of the cell. The nucleus is the control center of the cell for direction of protein synthesis and cell reproduction. The nucleolus is the manufacturing site for ribosomal subunits, which are sites for rRNA synthesis, a process necessary for DNA replication. Ribosomes provide a framework for protein synthesis.

1.4.3 PROTEOMICS, GENOMICS AND EPIGENETICS

Protein Conformations and Cell Communication

Mammalian cells contain up to 10,000 different kinds of proteins (1). Proteins must be localized to the appropriate cellular membrane or aqueous compartment to function properly (Figure 1). Proteins are the working molecules of the cell; they carry out the program of activities encoded by genes. The structure of the protein gives rise to its function. The function of virtually all proteins depends on their ability to bind to other molecules, or ligands with specificity. There are numerous types of receptors on the cell surface, and each is essential for signaling between cells. Affinity refers to the strength of binding and specificity refers to the ability of a protein to bind one molecule in preference to another

molecule. In some cases a particular protein on one cell binds to a receptor protein on the surface of an adjacent cell, triggering differentiation.

Figure 1: Overview of cell receptors and cell signaling

Cell signaling can be divided into three stages.

1. Reception: A cell detects a signaling molecule from the outside of the cell. A signal is detected when the chemical signal (also known as a ligand) binds to a receptor protein on the surface of the cell or inside the cell.

2. Transduction: When the signaling molecule binds the receptor, it changes the receptor protein in some way. This change initiates the process of transduction. Signal transduction is usually a pathway of several steps. Each relay molecule in the signal transduction pathway changes the next molecule in the pathway.

3. Response: Finally, the signal triggers a specific cellular response.

Source: Overview of Cell Signaling. http://creativecommons.org/ns#" xmlns:dct="http://purl.org/dc/terms/" about="http://www.hartnell.edu/tutorials/biology/signaltransduction.html

Available at: http://www.hartnell.edu/tutorials/biology/signaltransduction.html

Signal molecules are designated, endocrine, autocrine, or paracrine depending upon the distance over which the signal acts. Membrane-bound proteins on a neighboring cell can also signal to each other. In endocrine signaling the signaling molecules, or hormones, act on target cells distant from their site of synthesis. In paracrine signaling the molecules released are in close proximity to each other. In autocrine signaling cells respond to substances that they release themselves. There are compounds that may act by multiple types of signaling. Epidermal growth factor (EGF) is an example of a protein hormone synthesized in the plasma membrane. Membrane-bound EGF can bind to and signal an adjacent cell by direct contact. Cleavage by a protease releases secreted EGF,

which then acts as an endocrine signal on distant cells (1). There are four major classes of cell-surface receptors: 1) receptors with enzymatic regulatory function; 2) G-protein-coupled receptors, which activate or inhibit an enzyme that generates a specific second messenger or modulates an ion channel, causing a change in membrane potential; 3) ion channel receptors, which change the conformation of the receptor to enable specific ions to flow through the receptor resulting in a change of the electric potential across the cell membrane; and 4) tyrosine kinase-linked receptors, in which ligand binding stimulates formation of a dimeric receptor, which interacts with and activates one or more cytosolic protein tyrosine kinases. Receptors for many cytokines, interferons, and human growth factor are of this type. (See Figure 1)

GTPase is another protein category involved in signal transduction. This group of proteins functions as molecular switches that turn cell signaling on or off. GTPase proteins are regulated in different ways. Protein kinases work through phosphorylation of proteins. Animal cells contain two kinds of protein kinases, those directed towards tyrosine and those directed towards either serine or threonine. Protein kinases become active in response to the stimulation of signaling pathways. The catalytic activity of kinases is modulated by phosphorylation, by direct binding to other proteins, and by changes in the levels of various second messengers. Adapter proteins function as docking sites for other proteins and mediate transport steps of structural proteins in the cell.

Epigenetics

Revising the Paradigm in the Age of Genomics

Epigenetics is defined as somatically heritable states of gene expression resulting from changes in chromatin structure without alterations in DNA sequence. Epigenetic changes are involved in inflammation, obesity, insulin resistance, aging, immune disorders, and cancer. The previous paragraphs summarized cell signaling through numerous protein messengers that activate a cascade of molecular events within the cell and between cells. Many cell-signaling mechanisms are not well understood. It is increasingly becoming evident that cell signaling modifies gene expression of critical genes associated with physiologic and pathologic processes. More recent observations in the field of epigenetics indicate that epigenetic modifications can be influenced by external or internal environmental factors.

It is anticipated that the emerging field of **epigenetics** will dominate the field

of genetics for the next decade and beyond. “Epi,” from the Greek language, means “above or over.” Epigenetics, above genetics, is an influence that affects appearance without changing the DNA code. In other words it is an influence that can affect gene expression without causing a mutation in the DNA code. The mechanisms of epigenetic gene expression can be profiled using a variety of techniques. Commonly used techniques measure the regulation of translation or stability of messenger RNA (mRNA), DNA methylation, and histone modification. Virtually all biological pathways and diseases may have an underlying epigenetic component. The epigenome is dynamic; it exhibits changes during the aging process. As an individual ages, gene promoters become hypermethylated, and many of the age-related DNA methylation markers are also associated with disease. Epigenetics is discussed in more depth elsewhere in this text.

A key element of epigenetics is chromatin and its structure. Chromatin is a DNA complex organized into repeating units called nucleosomes. Chromatin is wrapped around the DNA. The degree of winding plays a role in gene regulation. When the winding pattern is altered by a chemical or environmental stimulus, the change in winding pattern is termed histone modification. Modification of histone winding may result in an altered gene expression. Histone modifications regulate DNA transcription, repair, recombination, and replication. They can alter local chromatin architecture. Histones. proteins found in the cell nuclei of animals, package and order the DNA into structural units called nucleosomes. Nucleosomes are the chief protein components of chromatin.

The best-understood and most commonly studied histone modifications are acetylation, phosphorylation, methylation, and ubiquitination. Histone modification regulates DNA transcription, repair, recombination, and replication. It can alter chromatin structure. DNA methylation is a biochemical process involving the incorporation of a methyl group to the cytosine or adenine on the DNA nucleotide. DNA methylation alters the gene expression in cells and plays a role in many forms of cancer. DNA methylation is involved in the regulation of many cellular processes, including chromosome stability, chromatin structure, embryonic development, and transcription.

A study investigating aging and chronic sun exposure related to epigenetic changes in skin found that DNA methylation plays a role in aging skin exposed to the sun (4). DNA phosphorylation is the addition of a phosphate group to a protein, resulting in a conformational change that initiates protein activation or

deactivation of DNA regulatory mechanisms. DNA acetylation involves the removal of the positive charge on the histones, thereby decreasing the interaction of the N termini of histones with the negatively charged phosphate groups of DNA. As a consequence, the condensed chromatin is transformed into a more relaxed structure that is associated with greater levels of gene transcription. Translation is the process of DNA transferring genetic information via RNA that is processed by the ribosome into a chain of amino acids that become functioning proteins termed messenger RNA (mRNA). Transcription is the protein folding of the resulting mRNA proteins. The changes in gene expression and protein expression are the biological markers measured.

1.4.4 APPLICATION FOR SKIN CARE

The Broader Perspective of Molecular Biology

All life depends on three molecules: deoxyribonucleic acid (DNA), which holds information on how the cell functions; ribonucleic acid (RNA), which acts to transfer information to different parts of the cell and provides the template to synthesize proteins; and proteins, which form enzymes that send signals to other cells to regulate gene activity. Proteins also form the body's major components. A common feature of all organisms is the ability to store chemical energy as adenosine triphosphate (ATP). Genetic information is encoded by DNA, and the genetic information is transcribed by RNA. Translation involves ribosomes that use proteins via shared metabolic pathways. Gene expression is regulated through transcriptional controls that are proteins acting on the region of the gene termed a promoter site. These proteins are termed transcription factors, repressors/inhibitors, or enhancers, based on their influence of the gene. This activity results in epigenic changes.

Skin is the primary interface between the body and the environment. It is the first line of defense against microbial pathogens, and against physical and chemical insults. There is a growing body of evidence suggesting that the immune responses in skin are important in the maintenance of homeostasis and occurrence of skin pathologies. Various cells present in the epidermis, including Langerhans, T-cells and macrophages, mast cells, and nerve-related cell types are also found in the dermis. The dermis is drained by lymphatic and vascular conduits through which migrating cells can traffic (3). Keratinocytes may be thought of as sensors of danger from pathogens, irritants, and other toxins. This

is carried out via numerous molecular pathways in skin. Activation of some of the receptors is through the inflammasome, which is a large multiprotein complex in the cell. Ultraviolet radiation activates inflammasomes in keratinocytes, leading to activation of various inflammatory pathways that in turn activate tissue-resident immune cells. Any type of barrier disruption, including UV light, trauma, irritants, or infection, triggers a coordinated immune response to maintain skin homeostasis. Skin-resident immune cells are important sentinels for homeostasis restoration and maintenance of normal cell activity.

Gene expression studies are currently providing insight into the biological mechanisms of skin aging and other aspects of skin health. The implication of these studies is that we have the capability to improve the appearance of aging skin through epigenic therapy. Skin hydration, barrier repair, renewal of the extracellular matrix, pigmentation, and human antioxidant capacity are examples applicable to skin care. The cost and the time to conduct epigenetic studies continues to decrease, resulting in the publication of numerous papers focused on skin hydration, barrier repair, renewal of the extracellular matrix pigmentation, antioxidant capacity, and certain skin cancers. Common conclusions in the papers suggest that “epigenic therapy” will revise skin care treatments, with additional strategies aimed at improving the appearance of skin. Nrf2 and AMPK signaling pathways are associated with epigenetic effects.

The Basics of Cell Signaling and Gene Expression Analysis

Transcription factors are a group of DNA-binding proteins that control which genes are turned on or off in the genome. They do so by binding to DNA and other proteins. Once bound to DNA, these proteins can promote or block the enzyme that controls the reading, or “transcription,” of genes, making genes more or less active. Frequently a specific cellular signal pathway can activate multiple transcription factors. The significance of this is that expression of a specific gene is under control of multiple transcription factors. Transcription factors are essential for the regulation of genes. For example, different genes turned on in liver cells than in skin cells. Different genes are turned on in cancer cells than in healthy cells. Through the action of transcription factors, the various cells of the body, which all have the same genome, can function differently. These proteins are so important to life that they are found in all living organisms. They play important roles in development, the sending of signals within the cell, and the events in a cell that lead to division and duplication, known as the cell cycle.

Several human diseases are linked to mutations in transcription factors, such as diabetes, autoimmune diseases, and cancer. Cytokines (cell motion) are signaling molecules that have key roles in several biological processes that include cellular growth, differentiation, gene expression, migration, immunity, and inflammation. Malfunction of cytokines can lead to a variety of diseases including arthritis, cardiac-related diseases, and cancer. Various cell types can secrete cytokines and other inflammatory mediators as part of an immune or inflammatory response. Gene expression analysis is the process by which information from a gene is used to direct the synthesis of a functional gene product, generally a protein. Gene expression analysis is the process of understanding the function of the gene, their protein products, and the complex regulatory network that affects biological processes of the cells.

Examples of Gene Expression Studies Relevant for the Cosmetic Chemist

Nrf2

Aging is a process that all living organisms experience. An aging human population is interested in products that promote health and maintain a younger-looking appearance. The molecular mechanism(s) of aging are not completely clear and are an area of intense molecular investigation. An area of current focus is on the Nrf2 signaling pathway. The Nrf2 signaling pathway is a mediator of multiple cell signaling cytoprotective pathways. It is expressed in numerous body tissues at different levels. It activates transcription of more than 200 genes crucial in the metabolism of drugs and toxins, signaling for protection against oxidative stress and inflammation. Nrf2 interacts with other key cell regulators including tumor suppressor protein 53 (p53) and nuclear factor kappa beta (NF-Kβ). These interactions are associated with age-related diseases (5). In 1956 Denham Harman postulated that reactive oxygen species (ROS) cause indiscriminate oxidative modification of cellular components leading to damage that cannot always be completely neutralized by antioxidants (6). Recent studies have resulted in producing data that question the validity of the Harman theory (7).

Many long-lived species of animals are found to have high levels of oxidative damage (8, 9). An emerging theory is that cellular stress resistance associated with extended aging tends to exist for numerous other nonoxidative stressors including heat, heavy metals, chemotherapeutic agents, dietary alterations, and xenobiotics (5). Another theory is that oxidative insult is just one factor of a multifactorial cytoprotective pathway that protects against cellular stressors

including synthesis of molecular chaperones, components of cell cycle surveillance, protein degradation, and oxidative stress. Nrf2 is a stress-sensing genetic transcription factor that appears to be a master regulator involved in many of these defense mechanisms (5). Gene expression studies have shown that phytochemicals work through the Nrf2 pathway. Sulforaphane, a component of broccoli, was found to be effective via this pathway (10). Nrf2 was shown to protect keratinocytes from ultraviolet A (UVA) irradiation by increasing the protein levels of Nrf2 in cell culture (11). Quercetin, resveratrol, caffeic acid, epicatechin, epigalocatechin, catechin chemical components found in polyphenolics are associated with Nrf2 activation (11). In summary it is hypothesized that long-lived species express higher levels of Nrf2. Nrf2 appears to be protective against numerous pathological conditions associated with reactive oxygen species (ROS) damage, inflammation immune responses, cell survival, and carcinogenesis (ROS).

Adenosine Monophosphate Activated Protein

Another signaling pathway potentially relevant to skin care is adenosine monophosphate activated protein (AMPK). AMPK is a master metabolic sensor and regulator of the cell, an enzyme that plays a role in cellular energy homeostasis. AMPK consists of three proteins that together make a functional enzyme. It is expressed in a number of tissues, including the liver, brain, skeletal muscle, and skin. AMPK has a key role in the regulation of cellular lipid and protein metabolism in response to stimuli, including physical exercise and changes in diet. The AMPK pathway interacts with other molecular pathways responsible for tumor formation and DNA damage repair (12). AMPK is switched on by metabolic stresses and various chemical compounds that cause a cellular energy imbalance. Energy status of the cell is critical for cell function, and AMPK has several downstream molecular targets that are modulated by changes in cell metabolism, cell growth, and other related functions that restore energy homeostasis to the cell. The AMPK pathway intersects with the Ras/PI3K/mTor and ERK pathways that are involved in tumor formation and cell growth. It also interacts with p53 and ATM pathways, which are tumor suppressor pathways and genomic gatekeepers (12). AMPK can be activated by resveratrol preventing hydrogen peroxide–induced premature senescence of keratinocyte cells when evaluated in human keratinocyte cell cultures (13). Another of its actions is to regulate the transcription of genes that function in the metabolism of glucose, fatty acids, and cholesterol. The AMPK signaling

pathway may also play a role in stimulating mitochondrial biosynthesis. In a keratinocyte cell culture study, resveratrol was found to activate AMPK and prevented the hydrogen peroxide–induced cellular senescence associated with cellular aging. The investigators note that cellular senescence is also linked with cancer in addition to aging (13). A variety of nutraceuticals and traditional medicines derived from plant products found in foods and beverages have been reported to activate AMPK in cells. In addition to resveratrol, epigallochatechin-3-gallate from green tea, curcumin, garlic oil, and berberines are reported in the literature (14).

1.4.5 (FUTURE PERSPECTIVES) CONCLUSION

Gene expression studies are providing a great amount of data that are not completely understood as applied to our health. While current knowledge of the use of gene expression is limited, the knowledge base is rapidly advancing. Published data indicate that bioactive ingredients have a biological effect that can be detected via various molecular biological assays using known markers. *In vitro* cell culture studies indicate that there may be a difference in biological activity depending upon the cell type studied. Further, it is hypothesized that there may be a difference in the efficacy of a biological active depending upon the existing state of the body when consumed or applied topically. An active may help to prevent a malignancy from developing; yet if applied or consumed after the malignancy has developed, it may in some cases have the opposite effect (12, 14).

In reviewing many of the published papers investigating gene expression, a common comment is “limitation of methodologies available to the investigator.” There are continuous variations in cells during the lifetime of an individual, and epigenic changes may be a consequence of changes in the cellular composition as a person ages over time. There are also synergistic effects that may affect the outcome of a study leading to conflicting data, depending on the variation of study protocol, even if minor. As research techniques in gene expression improve, there is no doubt that data interpretation will improve—leading to enhanced skin care products.

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Figure 1 A schematic of an intracellular signaling pathway. Starting at the top, an extracellular signaling molecule binds with a receptor protein that then cascades a signal via intracellular signaling proteins that continue to cascade to target proteins, eventually resulting in modification of various cell activities as shown. Example modifications are altered metabolism of the cell, altered gene expression, and shape or movement of cells. From Molecular Biology of the Cell, 4th edition A.B. Johnson *et al.* (New York, Garland Science, 2002).

GLOSSARY

Biological Markers

A specific landmark that can be identified. It may be a substance, physiological characteristic, gene, *etc.* that indicates, or may indicate, the presence of disease or a physiological abnormality.

Chromatin

The material of which the chromosomes of organisms other than bacteria (i.e., eukaryotes) are composed.

DNA methylation

The modification of a strand of DNA after it is replicated, in which a methyl (CH_3) group is added to any cytosine molecule that stands directly before a guanine molecule in the same chain. Since methylation of cytosines in particular regions of a gene can cause that gene's suppression, DNA methylation is one of the methods used to regulate the expression of genes.

Endoplasmic reticulum

A membrane network within the cytoplasm of cells involved in the synthesis, modification, and transport of cellular materials.

Epigenetics

The study of inherited changes in appearance or gene expression that may be associated with diet and lifestyle.

ERK, MAPK, the MAPK/ERK pathway

A chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell. The signal starts when a signaling molecule binds to the receptor on the cell surface and ends when the DNA in the nucleus expresses a protein and produces some change in the cell, such as cell division. The pathway includes many proteins, including MAPK (Mitogen-activated protein kinases, originally called ERK, Extracellular signal-regulated kinases), which communicate by adding phosphate groups to a neighboring protein, which acts as an "on" or "off" switch.

Gene expression

Conversion of the information encoded in a gene first into messenger RNA and then to a protein.

Histones and histone modification

Low-molecular-weight proteins that form a complex with DNA in the chromatin and function in regulating gene activity. Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes. Chromosomal processes such as transcription are

influenced by a variety of post-translational modifications to histones, including acetylation, phosphorylation, methylation, and ubiquitination. These modifications may act alone or in concert in a context-dependent manner to facilitate or repress chromatin-mediated processes.

Homeostasis

Homeostasis is the equilibrium of the internal environment within an organism. Disturbances in homeostasis cause decreased function of the cell and organism. It is the drive for efficiency of the cell, tissue, and body.

Inflammasomes

Large intracellular multiprotein complexes that play a central role in innate immunity.

Interferon (IFN)

Proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites, or tumor cells. They allow for communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors. IFNs belong to the large class of glycoproteins known as cytokines. Interferons are named after their ability to “interfere” with viral replication within host cells. IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages; they increase recognition of infection or tumor cells by up-regulating antigen presentation to T-lymphocytes; and they increase the ability of uninfected host cells to resist new infection by virus. Certain symptoms, such as aching muscles and fever, are related to the production of IFNs during infection.

Ion channels

Ion channels are crucial components of living cells. Situated in the cell membrane, they allow ions to pass from one side of the membrane to the other.

Ligands (protein binding)

In molecular biology, a ligand (from the Latin ligandum, *binding*) is a substance (usually a small molecule) that forms a complex with a biomolecule to serve a biological purpose. In a narrower sense, it is a signal-triggering molecule, binding to a site on a target protein.

Lysosomes

Cellular organelles that contain acid hydrolase enzymes that break down waste materials and cellular debris. They can be described as the stomach of the cell. They are found in animal cells, while their existence in yeasts and plants is disputed.

mTOR

A protein that helps control several cell functions, including cell division and survival, and binds to rapamycin and other drugs. mTOR may be more active in some types of cancer cells than it is in normal cells. Blocking mTOR may cause the cancer cells to die. It is a type of serine/threonine protein kinase. Also called mammalian target of rapamycin.

Molecular chaperones

A large group of unrelated protein families whose role is to stabilize unfolded proteins, unfold them for translocation across membranes or for degradation, and/or to assist in their correct folding and assembly.

mRNA (messenger ribonucleic acid)

The type of RNA that codes for the chemical blueprint for a protein during protein synthesis.

Oxidative metabolism

Oxidative metabolism is often referred to as cellular respiration, the process during which chemical bonds of molecules such as glucose are converted into energy that life can use on a cellular level. It is referred to as “oxidative” because oxygen is a primary component of respiration. In the oxidative metabolism of glucose, for instance, glucose and oxygen combine to produce carbon dioxide, water, and energy. Oxidative metabolism occurs in gradual steps that convert the energy in bonds into chemical energy stored in adenosine triphosphate (ATP). ATP’s bonds carry energy throughout cells, releasing it when enzymes catalyze reactions.

Peroxisomes

A type of enzyme package similar to lysosomes. They are small vesicles found around the cell. They have a single membrane that contains digestive enzymes for breaking down toxic materials in the cell. They differ from lysosomes in the

type of enzyme they hold. Peroxisomes hold on to enzymes that require oxygen (**oxidative enzymes**). Lysosomes have enzymes that work in oxygen-poor areas and lower pH. Peroxisomes absorb nutrients that the cell has acquired. They are very well known for digesting **fatty acids**. They also play a part in the way organisms digest **alcohol** (ethanol). Because they do that job, you would expect liver cells to have more peroxisomes than most other cells in a human body. They also play a role in cholesterol synthesis and the digestion of amino acids.

P53 (also known as protein 53 or tumor protein 53)

A tumor suppressor protein that in humans is encoded by the TP53 gene. P53 is crucial in multicellular organisms, where it regulates the cell cycle and thus functions as a tumor suppressor that is involved in preventing cancer. As such, p53 has been described as “the guardian of the genome” because of its role in conserving stability by preventing genome mutation.

PI3K (PI3K/AKT/mTOR pathway)

An intracellular signaling pathway important in apoptosis and hence cancer. http://en.wikipedia.org/wiki/PI3K/AKT/mTOR_pathway - cite_note-7. The PI3K/AKT/mTOR pathway is activated by IGF-1 and has a number of downstream effects that either promote protein synthesis or inhibit protein breakdown. PI3K activation activates AKT, which activates mTOR.

RAS

A protein belonging to a large super-family of proteins known as “low-molecular-weight G-proteins.” These proteins are called “G-proteins” because they bind guanine nucleotides (GTP and GDP). They are called “low-molecular-weight” to distinguish them from another, distinct, clan of guanine nucleotide-binding proteins, the heterotrimeric G-proteins.

Somatically heritable

A somatic epitype is a nonheritable epigenetic alteration in a gene. It is similar to conventional epigenetics in that it does not involve changes in the DNA primary sequence. Physically, the somatic epitype corresponds to changes in DNA methylation, oxidative damage (replacement of GTP with oxo-8-dGTP), or changes in DNA-chromatin structure that are not reversed by normal cellular or nuclear repair mechanisms. Somatic epitypes alter gene expression levels without altering the amino acid sequence of the expressed protein. Current

research suggests that somatic epitypes can be altered both before and after birth, and this alteration can be in response to exposure to heavy metals (such as lead), differences in maternal care, or nutritional or behavioral stress. There is no indication that somatic epitypes are heritable in a conventional epigenetic fashion. Some research suggests that methylation levels (and gene expression) can be reversed for some somatic epitypes by alterations in environmental factors such as diet.

Transcription

The first step of gene expression, in which a particular segment of DNA is copied into RNA by the enzyme, RNA polymerase. Both RNA and DNA are nucleic acids, which use base pairs of nucleotides as a complementary language that can be converted back and forth from DNA to RNA by the action of the correct enzymes.

Xenobiotics

A chemical that is found in an organism, but is not normally produced or expected to be present in it. It can also cover substances that are present in much higher concentrations than are usual. Specifically, drugs such as antibiotics are xenobiotics in humans because the human body does not produce them itself, nor are they part of a normal diet. Natural compounds can also become xenobiotics if they are taken up by another organism, such as the uptake of natural human hormones by fish found downstream of sewage treatment plant outfalls, or the chemical defenses produced by some organisms as protection against predators. However, the term xenobiotics is very often used in the context of pollutants such as dioxins and polychlorinated biphenols and their effect on biota, because xenobiotics are understood as substances foreign to an entire biological system, i.e., artificial substances, which did not exist in nature before their synthesis by humans.

REGULATORY REQUIREMENTS, INTELLECTUAL PROPERTY AND ACHIEVING GLOBAL MARKET SUCCESS FOR COSMETIC PRODUCTS

REGULATORY REQUIREMENTS, INTELLECTUAL PROPERTY AND ACHIEVING GLOBAL MARKET SUCCESS FOR COSMETIC PRODUCTS

Co-Editors' Introduction
Co-Editors

Ruud Overbeek and Meyer R. Rosen

Part 2 of this book is unique since it covers the essential "nontechnical" information needed for achieving product success in a global environment. We have stepped out of the "normal" mode of presenting the technological aspects one might first expect, and thoughtfully placed this information here in recognition and emphasis that the technical aspects of ingredients are, of course, not the only areas of importance to achieving the goal of a successful product.

Once you have a stable product that works, looks good, and goes on well (all of which is covered later in this book), it is imperative that cosmetic and personal care professionals be aware of the rapidly changing world of regulations as well as making sure their technology is appropriately covered by patents.

We recognize that the regulatory environment is ever-changing and, after an overview of this area, we first cover thoughts on how to survive and thrive in this turmoil. We then turn to looking at what it currently takes to successfully market one's products in the different geographical areas. Finally, we cover the essentials of global patenting in general and then by geographical area.

GLOBAL REGULATORY DEVELOPMENTS'THE NEED TO KNOW

For centuries, humankind has utilized cosmetics to enhance or alter physical

appearance, negotiate conceptions of femininity or masculinity, combat external manifestations of aging, and assert social status. This pursuit of beauty is a virtually timeless and universally enduring endeavor. the CTFA President's Message in the Cosmetic, Toiletry, and Fragrance Association (CTFA) 2005 Annual Report noted the wide reach of the industry and its potential for expansion. "In an era of globalization," the president stated, "we are truly one of the world's most global industries. Our products and our innovation know no boundaries. Whether it's Bangkok or Beijing, Baton Rouge or Baghdad, the products that we make are the products that women and families use every single day."

In addition to the global expansion regarding cosmetics for women, the men's cosmetic market is also growing exponentially. Beyond product efficacy, the highest priority for personal care products and cosmetics companies is the safety and health of consumers who use their products. To be able to support health, safety, and global expansion goals, the industry has a constant need and duty to inform itself of existing regulations, by geographical area. Further, it must cope with the seemingly endless evolution of newly developed and emerging changes in global regulatory developments.

Separately, in order to have a chance of success in each global market area, as well as each product category, the industry—ranging from ingredient developers, new product manufacturers, and claim writers—has an unavoidable need to understand the drivers for local and global regulation in order to be able not only to address, but also contribute to and impact the development of regulations. This challenge is a key step towards global alignment and regulatory harmonization.

Since regulatory hurdles affect cross-border trade of goods, reduce global competitiveness, and may harm manufacturers of cosmetics and personal care products, a reduction of international regulatory burdens will allow manufacturers improved access to foreign markets. Crossing these hurdles successfully is a key to global economic health. While regulatory harmonization has a positive impact for the protection of public health and safety, it also offers many benefits to both regulatory authorities and the cosmetics industry. The development of harmonized guidelines would streamline the regulatory assessment process and reduce product and ingredient development times and resources needed. Such harmonized guidelines prevent duplication and minimize use of *in vivo* testing without compromising safety and effectiveness.

The movement towards harmonization has recently been addressed among the

International Cooperation on Cosmetics Regulation (ICCR), an international group of regulatory authorities for cosmetics from Canada, the European Union, Japan, and the United States. The group has held multiple meetings to discuss issues related to cosmetics and cosmetic-like drug/quasi-drug products. This group seeks to promote regulatory convergence, while maintaining the highest level of global consumer protection and minimizing barriers to international trade. ICCR has chosen to include members of consumer organizations and industry associations, with an interest in regulatory issues involving cosmetics. Meetings have focused on key regulatory issues affecting the industry in general:

- Alternatives to Animal Testing
- *In silico* (computer) Prediction Models for Safety Assessment
- Nanomaterials
- Trace Impurities
- Endocrine Disruptors
- Allergens

At this time, the highest-priority topic for regulatory professionals in the cosmetics industry is the discussion around animal testing and alternatives to animal testing. This discussion includes the use of predictive models as well as *in vitro* testing methods. Historically, safety testing of chemicals has relied heavily on animal tests. However, in view of consumer pressure, and regulatory recognition of the lack of humaneness in testing on animals, this approach is in the process of being revolutionized. Today, and in the near future, safety assessment of chemicals will rely increasingly on pathway-based toxicity information largely informed by advanced, human-relevant, non-animal tests that are predictive of human health and safety.

The EU (European Union) has recently phased out the use of animals for cosmetics testing, culminating in a ban on the marketing of animal-tested products in 2013. China's Food & Drug Administration (CFDA) has announced that effective June 2014, China plans to remove its mandatory animal-test requirements for domestically manufactured cosmetic products. Initially the new rules will only apply to cosmetics manufactured in China. However, the CFDA has stated that, once the new system has been established, it may be expanded to include imported products and certain "special use" cosmetics as well. Chinese companies producing "non-special-use cosmetics," including shampoos and skin creams but not hair dyes, sunscreens, or other products with biological activity, will be exempt from carrying out animal tests. Manufacturers will now have the

option to substantiate product safety using existing safety data for raw ingredients, or European Union-validated non-animal tests. This would be a major step towards removing trade barriers between China and the EU. Clearly, legislative endeavors are underway to ensure more consistency between European and other markets, but this topic remains a major area of concern for companies wishing to sell products globally. To effectively address concerns about the safety of cosmetic ingredients, new regulations and harmonization should allow for the use of human-relevant, non-animal test methods. The development of such tests will not only provide international market security, but will help drive regulatory science forward.

The rapidly expanding field of Nanotechnology may be considered a second important area. Driven by health and environmental concerns, legislation is being introduced globally in an effort to "control" this new, exciting, efficacious, and emerging technology. As a result, the subject of nanomaterials has formed part of the recast EU cosmetic regulations and is being seriously discussed in various other countries. According to EU Regulation (EC) No. 1223/2009, nanomaterials are defined as insoluble or biopersistent and intentionally manufactured materials with one or more external dimensions, or an internal structure, on the scale from 1 nm to 100 nm. The European Union's Commission sees in particular a need to better understand whether and how insoluble nanoparticles are used in cosmetic applications and has therefore agreed with cosmetic industry associations that it will set up an inventory of current applications of nanotechnology in cosmetic products together with the regulatory authorities from the U.S., Canada, and Japan. Results of this work will be assessed by the four authorities. Such measures are to help strengthen in-market control of products containing nanoparticles.

In the 2012 FDA Draft Guidance for Industry Safety of Nanomaterials in Cosmetic Products, the FDA summarized that inclusion of nanomaterials, or a change in the nanomaterials used, might affect the quality, safety, effectiveness, and/or public health impact of a nano-based product. Therefore, data needs and testing methods should be evaluated accordingly to address the unique properties and function of the nanomaterials used in cosmetic products. This need extends as well to the questions that continue to remain about the applicability of traditional safety testing methods to products that involve nanotechnology. Further, the FDA expects that the science surrounding nanomaterials will continue to evolve and will be used in the development of new testing methods. Although the FDA has not adopted a formal definition of "nanotechnology,"

“nanoscale,” or related terms, it has stated that the term is perhaps most commonly used to refer to the intentional manipulation, manufacture, or selection of materials that have at least one dimension in the size range of approximately 1 to 100 nanometers.

It is expected that the above topics, as well as other “hot” topics listed by the International Cooperation on Cosmetics Regulation (ICCR), will continue to play an important role in regulatory drivers and discussions. Discussion of such hot topics has especially reached a new level of impact with the emergence of social media. Also contributing to the acceleration of the discussion is an ever-growing, globally spread, public, social, regulatory, and political awareness that is expected to push faster regulatory change and possibly global harmonization. Any bad practice, emerging issue, or discussion about (the need for) new regulation will be very promptly published across social media, which can consequently oblige the regulator and industry to control, add, or remove ingredients from products and applications. Therefore, it is a prerequisite for any professional in the cosmetics industry to understand the regulatory framework and the potential social, political, and economic drivers for regulation in key economies around the globe.

This Section of the book has been carefully placed “up front” to insure that our industry *mus*t be aware of the significant impact of changing global regulations, as well as the need for an in-depth understanding of intellectual property matters in an environment that requires rapid evolution—nay, revolution—in products that really work and are safe and acceptable in the changing environmental context.

Thus, our belief is that this chapter is a must read for those industry professionals seeking globalization and desiring harmonization while pursuing the safety and health of consumers who use their products—be they “chemical-based,” “natural,” nano-type, or cosmeceutical—all of which may have biochemical impact on consumers.

ACHIEVING GLOBAL MARKET ACCESS-FOCUS ON RUSSIA AUTHOR

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ABSTRACT

The expansion of the cosmetic and personal care market to all areas of the globe is indicative of the thirst of the peoples of the earth to look good, feel good, and have a healthy aging process. New markets beckon the world's ingredient developers, marketers, and product manufacturers for the need to expand into these "new" markets. Along with the development of new products for new needs, safety issues and regulations have proliferated. The bottom line here is that if a company wants to sell in a far-flung market, it must become knowledgeable about the changing requirements being promulgated by various countries. One such "new" market is Russia. The Russian and Customs Union markets are highly attractive for cosmetic manufacturers and exporters, as the demand for cosmetic goods increases by 5 or more percent each year. Actually, according to experts of the Russian press agency "Credinform," the annual growth rate could exceed 15% until 2015. As stated in the agency's study, import growth is associated not only with a steady growth in demand for foreign-made cosmetics, but also with a reduction of customs duty on imported products after Russia's WTO accession. The Russian market for cosmetics is one of the most dynamic in the world.

In this chapter we provide a detailed description of requirements for companies wishing to gain a part of this lucrative market, and have designed our description in the form of a series of questions and answers relevant to those seeking access to the expanded Russian market.

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2.3.1.1 INTRODUCTION

Much anticipated by the cosmetic world and generating many questions during the two last years, new Technical Regulation No. TR TC 009/2011 on the “Safety of perfumery and cosmetic products” (approved on 23.09.2011 by Customs Union Commission’s Decree no. 799) finally came into force on July 1, 2012. This document became applicable within the whole Customs Union of Russia, Belarus, and Kazakhstan.

We will discuss the general objectives of the Customs Union (hereafter CU) and the differences between the “old” procedure and the new one. We will address whether the new rules simplify access to Russian and CU markets; how

the new rules are different from European requirements; and how they will affect exports of cosmetics to Russia.

2.3.1.2 WHAT IS THE CUSTOMS UNION AND WHAT IS ITS AIM?

The new regulation does not concern only Russia, but the whole territory of the CU.

The Customs Union between Russia, Kazakhstan, and Belarus came into existence on January 1, 2010. These three states became one region by economic integration and removal of customs borders between Belarus, Kazakhstan, and Russia beginning on July 1, 2010. During the first stage of the integration process, the aim of the Customs Union was to create:

- Common customs tariffs without customs duties inside member-states
- Common customs code
- Other joint measures for foreign trade regulations

From January 1, 2012, the three states moved to the next step and established a single economic area through a number of inter-state agreements such as: “On single principles and rules of technical regulation”; “On single principles of regulating intellectual property rights protection”; “On coordinated macroeconomic policy”; “On single principles and rules of competition” and others. These principles were established in order to build a single market with a single macroeconomic policy that would simplify goods, services, capital, and manpower circulation.

The first joint document of the CU was issued in 2010, establishing unified hygienic requirements and unified assessment rules for various goods, including cosmetics: “Unified Sanitary-epidemiological requirements of the Customs Union” (also known as Decree no. 299 dated 28.05.2010). This document set up mandatory State Registration for the Customs Union instead of previous sanitary-epidemiological legislation in Russia.

Development and approval of the Technical Regulations of Customs Union for different categories of products, including cosmetics, have been the continuity of this reform.

2.3.1.3 WHAT WERE THE OLD REQUIREMENTS AND PROCEDURES OF PRODUCT CONFORMITY ASSESSMENT

AND HOW HAVE THEY PROGRESSED TO DATE?

1. Prior to July 1, 2012

Before July 1, 2012, the majority of cosmetic products (with the exception of fragrances) were subject to a two-stage conformity assessment:

1. Customs Union State Registration
2. GOST R Declaration of Conformity of Russia or GOST R Customs Union Certificate of Conformity

with slight difference for fragrances, which used to be subject to:

1. Customs Union Sanitary Control (testing according single CU requirements);
2. GOST R Declaration of Conformity of Russia or GOST R Customs Union Certificate of Conformity.

Both State Registration and Sanitary Control were done according to Unified Sanitary Requirements of the Customs Union (Decree no. 299).

Once the products passed the second step, i.e., GOST R certification, they used to be marked by the GOST R Mark of Conformity to demonstrate product compliance with the applicable Russian standards.

Relevant authorities for both steps of certification were:

The double conformity assessment takes its origin from the requirements for the Sanitary and GOST, provided by the Ministry of Health and Social Development and the Ministry of Industry and Trade respectively. The basic concern of both authorities and of the Russian certification system was to protect the safety, health, and environment of the Russian people and to prevent dangerous and noncompliant imported products from being placed on the Russian market. Consequently many products entering the territory of the

Russian Federation required double conformity assessment in order to:

1. Confirm that the products met Russian safety standards relative to the environment, life, and health of population

And in practice to:

1. Enable goods pass to through Russian customs
2. Enable goods to be used, sold, or marketed on Russian territory

Assessment documents issued according to the old legislation are still valid until July 1, 2014.

2. Since July 1, 2012

With introduction of the new Technical Regulation as of July 1, 2012, the function and aim of assessment documents remain the same. By contrast, the dual compliance system has been abolished. As of July 1, 2012, only one of following assessment documents is required:

- either CU State Registration
- or CU TR Declaration of Conformity

This topic is expounded in the next chapter.

2.3.1.4 OVERVIEW OF THE NEW CUSTOMS UNION TECHNICAL REGULATION "ABOUT SAFETY OF PERFUMERY AND COSMETIC PRODUCTS"

1. Definition of perfumes and cosmetics according to CU Technical Regulation

Perfumes and cosmetics are defined as "a substance or preparation intended to be applied directly on various external parts of the human body (epidermis, hair, nails, lips, and external genital organs), or on the teeth and the mucous membranes of the oral cavity with a single or primary purpose to cleaning them, changing their appearance, perfuming them, and/or correcting body odors and/or protecting them and/or keeping them in good condition and/or caring for them."

2. Conformity assessment documents

As noted previously, the new Technical Regulation came into force on July 1,

2012. It is designed to unify certification requirements for the member states and to remove the double assessment system.

This Technical Regulation suggests two conformity assessment documents: **State Registration** or TR **Declaration of Conformity**.

a) State Registration

According to the text of the Technical Regulation, only a small list of products is subject to State Registration (higher-risk products). They include the following 13 categories:

1. artificial tanning products (autobronzants)
2. skin whitening (lightening) products
3. cosmetic products for tattoos
4. personal hygiene products
5. products for protection of skin from harmful industrial factors
6. cosmetic products for children
7. hair colorants, bleaches, and highlights
8. hair perms and hair straightening products
9. products made using nanomaterials
10. products for hair removal
11. peelings
12. products for dental and oral hygiene containing over 0.15% fluorine (or over 0.05% for liquid products)
13. Dental bleaching agents containing hydrogen peroxide and other ingredients producing hydrogen peroxide, including carbamide peroxide and zinc peroxide in the concentration of hydrogen peroxide (as an ingredient, or produced) 0.1–6.0%

State Registration certificate can be issued in the name of the manufacturer only, without mention of Russian or CU importer/distributor. By this means, the certificate can be used for the whole territory of the Customs Union and for different importers/distributors, regardless of their location. Its period of validity is not limited.

Information on registered products is entered in the unified CU register of State registration certificates before products are imported into the territory of the Customs Union.

b) TR Declaration of Conformity to Customs Union

All other cosmetics are subject to a CU TR Declaration of Conformity.

Declaration of Conformity is issued on the name of a legal entity registered in the Customs Union (buyer, importer, or distributor) and for one specific contract between the exporter and the importer, so that its validity is limited to one client and to the duration of the contract.

For both documents, testing of products at a CU-accredited laboratory is required. Conformity assessment shall be done before entering the Customs Union territory.

Both the Declaration of Conformity and State Registration to Customs Union are issued for a single product or a group of similar products and are valid until the product name, formulation, or manufacturer are changed. Products can be grouped in one conformity document upon condition that they are produced by the same manufacturer; according to the same technical requirements; and have similar ingredients composition, hygienic specification, and usage. However, they may differ by insignificant differences without impact on the hygienic value (for instance: different form or volume of goods, color or aroma resulting from the use of coloring and flavoring agents).

Requirements for obtaining both documents were brought into line with the most demanding conditions (i.e., those applied to previous State Registrations). All documents for conformity assessment must be presented with Russian legalized translations.

3. Requirements for perfumes and cosmetics

Safety of perfumes and cosmetics is ensured by a set of requirements for:

1. composition (*CU TR Appendices 1 to 5: lists of regulated ingredients*)
2. physical and chemical parameters (*Appendix 6*)
3. microbiological parameters (*Appendix 7, Article 5, item 5 of CU TR*)
4. the contents of toxic elements (*Article 5, item 5 of CU TR*)
5. toxicological parameters (*Appendix 7*)
6. clinical parameters (*Appendices 9, 10*)
7. production (*Article 5, item 7 of CU TR*)
8. consumer packaging (*Article 5, item 8 of CU TR*)
9. product marking (*Article 5, item 9 of CU TR*)

4. Labeling requirements

Perfumes and cosmetics labeling (marking) is "consumer information in the

form of inscriptions, digital, color, and pictograms to be applied to the consumer containers, label, sticker."

Marking of perfumes and cosmetics should contain the following information:

- Name, designation (if any) of perfumery and cosmetic products
- Intended use of perfumery and cosmetic products, unless it is clear from the product name
- Cosmetics for children should have the relevant information in the labeling
- Manufacturer's name and location (legal address, including country)
- Country of manufacture (if the country where the manufacturing facilities are located differs from the manufacturer's legal address)
- Name and location (legal address) of an enterprise authorized by the manufacturer to accept claims from the consumer (manufacturer's authorized representative, importer) if the manufacturer does not accept the claim itself in the territory of the member state of the CU
- Nominal amount (volume or mass) of product in a consumer pack (for solid toilet soap—the nominal mass of a piece, at the time of packaging), except for perfumery and cosmetic products with nominal mass less than 5 gr, or less than 5 ml nominal volume, or free samples of perfumery and cosmetic products
- Color and/or tone (for makeup products and coloring agents)
- Mass fraction of fluoride (% or mg/kg, or ppm) for oral hygiene products containing fluorine compounds
- Shelf life: manufacture date (month, year) and shelf life (months, years), or the notice "best before" (month, year) or "use before" (month, year)
- Description of storage conditions if different from the standard
- Special safety application measures (if necessary), including the warning information stated in Appendices 2–5 of the Technical Regulation
- Batch number or special code allowing identification of the perfumes and cosmetics
- Methods of application of the perfumes and cosmetics, which if not provided can lead to misuse by a consumer of the perfumes and cosmetics
- List of ingredients
- Ethyl alcohol by volume (%)

If the perfumes and cosmetics are accompanied with forwarding information, the products are marked with a graphic symbol of a hand on an open book:

All information must be written in the official language of the member state of the Customs Union in which the product will be marketed: Russian for Russia and Belarus, and Russian with Kazakh for Kazakhstan.

5. Mark of conformity

As per **Decision of the Customs Union Committee N 711 dated 15.07.2011 "On the uniform market circulation marking of products within the Customs Union,"** products that are certified according to the requirements of the Technical Regulations should bear the EAC conformity mark of the Customs Union (EAC stands for EurAsian Conformity), and no longer the Russian Gost-R mark.

Mark of conformity can be placed on each product, package, or accompanying documents in any reasonable way to make it indelible and clear.

Mark of conformity can be placed *only* after successful certification and *before* putting the product on the market. Minimum size allowed is 5 mm.

EAC

2.3.1.5 HOW DIFFERENT ARE THE NEW RULES FROM EUROPEAN REQUIREMENTS?

For cosmetic manufacturers and exporters, the new Technical Regulation provides a double advantage: bringing Russian regulation closer to regulation in the European Union, and ending "the dual compliance system." Furthermore, this Technical Regulation also includes Belarus and Kazakhstan, two other members of the Customs Union, thus allowing access to more markets with one set of documents. Actually, this is true for State Registration but not for Declaration of Conformity, which is linked to specific importers or distributors.

In particular, one of the aims of cosmetic Technical Regulation was to harmonize requirements on cosmetic products for member countries of the Customs Union and make them more aligned with European rules, from the

manufacturing process to product labeling. The content and structure of these regulations have been inspired by both the European Directive 76/768/EEC and the European Regulations 1223/2009. The definition of cosmetics and the lists of regulated ingredients are almost identical (lists of substances prohibited in cosmetics, restrictive list of substances that cosmetic products may contain only under certain conditions, and lists of the only substances that can be used in cosmetics as coloring agents, preservatives, or UV filters).

However, the text still contains many requirements specific to Russia and to member countries of the Customs Union.

These include:

- Maintaining the obligation to confirm product compliance by a third party (Russian/CU-accredited laboratories and certification bodies)
- Maintaining animal testing
- Indicating expiry date and date of manufacture of the product
- Affixing Eurasian Conformity Mark (EAC)
- Indication of fluorine contents by weight (% or mg/kg or ppm) for oral hygiene products
- Setting limits for testing parameters in each category of products

In such manner, the Technical Regulation specifies the pH value of products, their microbiology, and various other aspects not detailed in European Directive 76/768 and its amendments, or in Regulation (EC) No. 1223/2009, which have replaced the Directive as of July 2013.

For instance, a **facial or body cream** or **liquid mascara and eyeliner** are regulated by the following:

	Facial or body cream	**Liquid mascara and eyeliner**
Physicochemical requirements		
pH value	5,0–9,0	5,5–8,5
Content of toxic elements:	perfumes and cosmetics containing natural herbal or natural mineral raw materials in amount exceeding 1% shall show the content of toxic elements not exceeding:	
arsenic	5.0 mg/kg	
mercury	1.0 mg/kg	
lead	5.0 mg/kg.	
Microbiology		
Total aerobic mesophilic and optionally anaerobic		

count: *CFU – colony-forming units per 1 g or 1 ml of a product	10^3 and less CFU* per 1 g (ml)	10^2 and less CFU* per 1 g (ml)
Candida albicans:	Not allowed in 0.1 g or 0.1 ml	Not allowed in 0.5 g or 0.5 ml
Escherichia coli:	Not allowed in 0.1 g or 0.1 ml	Not allowed in 0.5 g or 0.5 ml
Staphylococcus aureus:	Not allowed in 0.1 g or 0.1 ml	Not allowed in 0.5 g or 0.5 ml
Pseudomonas aeruginosa:	Not allowed in 0.1 g or 0.1 ml	Not allowed in 0.5 g or 0.5 ml
Toxicology:		
Skin irritant action:	0 points (none)	0 points (none)
Action on mucous membranes:	0 points (none)	0 points (none)
general toxic action measured by alternative methods *in vitro*:	none	none
Clinical testing:		
Irritant action:	0 points (none)	0 points (none)
Sensitizing action:	0 points (none)	0 points (none)

Similarly to these examples, each category of products has its own requirements. Categories vary depending on the type of testing. For example, cosmetics are divided into 33 categories for pH value, into three groups for microbiological requirements, into 21 categories for toxicological requirements, and into 23 categories (including oral hygiene products) for clinical requirements.

As can be seen from the above, the Technical Regulation requires several additional constraints compared to the rules of the European Union.

2.3.1.6 DO THE NEW RULES SIMPLIFY ACCESS TO THE COMBINED RUSSIAN AND CU MARKET? HOW DO NEW RULES AFFECT EXPORTS OF COSMETICS TO RUSSIA?

All cosmetic manufacturers exporting their products into Russia are expecting simplification of exportation rules and regulation requirements. In fact, the new Technical Regulation seems to simplify these procedures. However, experience

has shown that changes don't always mean simplification, as has been the case with the GOST R declaration of conformity in 2010. Presently, it is quite premature to confirm that simplification has resulted.

At this early stage of promulgation the introduction of new rules is accompanied by typical confusion among the main players involved. Thus, many issues continue to be discussed and amended. For instance, the following projects of amendments are currently under study:

- Rules for issuing of the Declaration of Conformity of Customs Union
- Rules for edition (filling out) of State Registration of Customs Union and its annexes
- Rectification of some terminology (e.g., definition of "peeling")
- Elaboration of support standards for testing methods, labeling, *etc.*

Only recently the authority responsible for application and verification of right application of the Technical Regulation in Russia was named. This function was entrusted to RosPotrebnadzor, the Federal Service for supervision in the area of consumer rights and welfare protection (RF Government decree no. 989 dated September 27, 2012), acting under the Ministry of Health. The decision was made three months after the Technical Regulation came into force. In the same manner, controls under right application of Technical Regulations have been recently assigned to the newly created state authority "Rossaccreditatsia" (Federal Accreditation Service).

The end product of these changes says that the rules are not yet completely fixed.

2.3.1.7 BUSINESS CLIMATE IN RUSSIA AND REFORMS—RUSSIA JOINED WTO

On December 16, 2011, after 18 years of negotiations, Russia was accepted as a World Trade Organization (WTO) member after final ratification in August 2012. In acceding to the WTO, Russia embraces a series of rules and commitments and would ensure that all legislation related to sanitary and phytosanitary measures, technical regulations, standards, and conformity assessment procedures comply with the WTO SPS (Sanitary and Phytosanitary Measures) and TBT (Technical Barriers to Trade) agreements. This should be the next step for facilitating international trade and in Russian efforts to make a more favorable entrepreneurial climate. Today this is evidenced by reducing customs duties for imported goods.

REFERENCES:

I. Decree No. 299 of 28.05.2010 of the Customs Union Commission (Single Sanitary Requirements of the Customs Union).

II. Technical Regulation No. TR TC 009/2011, “Safety of cosmetic and perfume products,” adopted by the Decision of the Customs Union Commission No. 799 of September 23, 2011.

III. Regulation (EC) No. 1223/2009 of the European Parliament and the Council of November 30, 2009 related to cosmetic products.

IV. Ubifrance, the agency for international business development in France (December 2012).

V. Russian press agency “Credinform” publication, July 2012.

KINGDOM OF SAUDI ARABIA (KSA): COSMETICS AND PERFUMERY PRODUCTS: MARKET ACCESS AND REGULATIONS BY

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ABSTRACT

Saudi Arabia is a Middle East country, part of the Gulf Cooperation Council (GCC), which includes five other countries (Kuwait, United Arab Emirates, Qatar, Bahrain, and Oman).

In the past few years, the Saudi market has become a key and strategic market for cosmetic and perfumery products, quickly growing and constantly increasing, with particular demand for international luxury products and attracting the world industry. It is however a specific market, where prevailing social customs play a big role, and in which particular precautions need to be observed.

This section proposes an overview of the cosmetics regulations and of the procedures implemented for the control of perfumery and cosmetic products and its enforcement. It also identifies some key issues that are useful in drawing a risk analysis.

Kingdom of Saudi Arabia (KSA) Cosmetic and Perfumery Products: Market Access and Regulations TABLE OF CONTENTS

2.3.2.1 Regulatory Framework

- **a. Cosmetic ruling authority**
- **b. Cosmetic regulations and sanctioned standards**
  - **1. Sanctioned Safety Standard**
  - **2. Guidance for Products Classification**
  - **3. Product-specific standards**

2.3.2.2 Definition and Scope of Application

a. Definition
b. Classification
2.3.2.3 Labeling
a. General rules
b. Specific rules
2.3.2.4 Market Access
a. Certification process
b. Conformity assessment
c. Key issues
References

2.3.2.1 REGULATORY FRAMEWORK

a. Cosmetic ruling authority

In the Kingdom of Saudi Arabia (KSA), the responsibility for regulation of cosmetic products conformity falls under the Saudi Food and Drug Authority (SFDA), which was created in 2003. Their Cosmetic Department was established in 2008 and is today primarily responsible for issuing regulations and controlling cosmetic product safety (for imported and locally manufactured products), but also for the development and update of standards and for cosmetovigilance.

The national standardization body (Saudi Arabian Standard Organization—SASO) transferred all activities linked to food, drug, and cosmetics standards to the SFDA in 2011; but it should be noted that although the responsibility for creating new standards now lies with SFDA, the applicable standards in Saudi Arabia are still the ones issued or adopted by SASO.

SFDA however published a draft version of its first own Cosmetic Safety Standard in December 2012. This standard is not approved yet, but it is aimed to replace all current SASO standards for cosmetics, including the specific product standards.

b. Cosmetic regulations and sanctioned standards

1. Sanctioned Safety Standard

SFDA official sanctioned standard is the Gulf standard: “GSO 1943/2009 –

Cosmetic Products Safety Requirements." This GSO Standard is equivalent to SASO 1953/2009; however, SFDA refers to the GSO reference in all their documents and communications.

This document is based on the EU Directive 76/768/EEC and follows the same structure:

→ Table 1: Substances that must not form part of the composition of cosmetic products
→ Table 2: Substances that must not be contained in cosmetic ingredients except subject to certain restriction and condition
→ Table 3: Coloring agents allowed for use in cosmetic products
→ Table 4: Preservatives that cosmetic products may contain
→ Table 5: Permitted UV filter that cosmetic products may contain

In addition to this standard, SFDA has published other ingredient prohibition/restrictions through its Guidance for Products Classification. This document includes additional restrictions that were previously decided by SFDA through circulars.

2. Guidance for Products Classification

This guideline "SFDA Guidance for Products Classification," published in 2012, determines rules for classifying products (from drugs to cosmetics) relative to definition of products, function, composition, and claims.

Among these rules, there are some significant ingredient restrictions to follow per product category. Cosmetic products are subject to the restrictions listed in the guideline, in addition to the tables under GSO 1943.

These additional restrictions for cosmetic products are:

1. Free of prohibited substances according to GSO 1943/2009 in addition to the following substances:
   a. Tretinoin (Retinoic acid) and its salts not permitted at any concentration)
   b. Hydroquinone (Dihydroxy Benzene)
   c. Triclosan not permitted at any concentration)
2. Compatible with restricted substances according to GSO 1943/2009 in addition to the following restricted substances:
   a. Vitamin A (Retinol) and its esters retinyl acetate, retinyl palmitate (permitted at concentrations equal to or less than 1%)
   b. Sulfur (permitted at concentration equal to or less than 2%)
   c. Urea (permitted at concentration equal to or less than 10%; cosmetics

intended to be diluted in bathwater may contain levels exceeding 10% urea)

d. Salicylic acid (permitted at different concentrations according to its role as shown in the following table):

Role	Salicylic Acid permitted concentration
Active Ingredient	≤ 3% in rinse-off hair products ≤ 2% in other products
Preservative	< 0.5%

e. Zinc oxide permitted at concentration less than 25% (unless it is used for a medical condition, in which case it will be classified as a drug)

f. Alpha-hydroxy acids (AHAs):

   a. Permitted at total concentrations equal to or less than 10%, with a pH equal to or greater than 3.5

   b. The inner and outer labels of all leave-on skin products containing AHAs at concentrations equal to or greater than 3% shall carry cautionary statements in Arabic and English to the effect: "Use only as directed," "Avoid contact with the eyes," "If irritation persists, discontinue use and consult a physician," "It is recommended that prior to exposure to the sun, users cover areas where AHAs have been applied with sunscreen," "Contact of the . . . product with the skin must be of limited frequency and duration."

   c. Preparations intended to be diluted in bathwater may contain levels of citric acid exceeding 10%.

Products falling under the above restrictions will generally be classified as low-risk health products and will be subject to listing at SFDA.

3. Product-specific standards

In addition to the general safety standard, some specific product standards still exist in the KSA. Even if these are not mandatory and not sanctioned officially by SFDA, it should be noted that they may be used for local assessment (for samples drawn by customs and tested in local laboratories). Some of these parameters may not be aligned with the SFDA-sanctioned standards. An acquaintance with the parameters included in these standards is therefore highly desirable in order to achieve a reliable risk analysis.

Example of specific parameters requested by a SASO standard: SASO

1512:2010 (equivalence GSO 1152:2010)

- ▶ pH value shall be in the range of 5–9,5.
- ▶ The total content of fatty substance shall be not less than 15% by mass.
- ▶ The water content shall be not more than 85% by mass.
- ▶ The utility date shall not be more than three years.

Note: All the SASO standards have equivalence at a GCC level with the GSO standards, which may be applicable in other countries within the GCC.

2.3.2.2 DEFINITION AND SCOPE OF APPLICATION

a. Definition

As per GSO 1943/2009, "Cosmetic Products—Cosmetic Products Safety Requirement," the written definition of a cosmetic product is identical to the European definition: *"Any substance or preparation intended to be placed in contact with the various external parts of human body (epidermis, hair system, nails, lips, and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition."*

This standard provides an illustrative list of products considered as cosmetic products, which include but are not limited to the following: – Creams, emulsions, lotions, gels, and oils for the skin (hands, face, feet, etc.) – Face masks (with the exception of peeling products) – Tinted bases (liquids, pastes, powders)

- – Makeup powders, after-bath powders, hygienic powders, *etc.*
- – Toilet soaps, deodorant soaps, *etc.*
- – Perfumes, toilet waters, and eau de cologne
- – Bath and shower preparations (salts, foams, oils, gels, etc.) – Depilatories
- – Deodorants and antiperspirants
- – Hair care products: hair tints and bleaches; products for waving, straightening, and fixing; setting products; cleansing products (lotions, powders, shampoos); conditioning products (lotions, creams, oils); hairdressing products (lotions, lacquers, brilliantines) – Shaving products (creams, foams, lotions, etc.)

– Products for making up and removing makeup from the face and eyes – Products intended for application to lips

– Products for care of teeth and mouth (mouthwash products containing alcohol are considered banned products) – Products for nail care and makeup

– Products for external intimate hygiene that do not contain ingredients that have antimicrobial activity – Sunbathing products

– Products for tanning without sun

– Skin-whitening products (except Hydroquinone-containing products) – Anti-wrinkle products

Note: There is a ban on alcohol in mouthwash; the decision to ban these types of products was taken by SFDA in 2011 due to the existence of permissible and available alcohol-free alternatives.

b. Classification

In 2012, the SFDA Drug sector issued another method to define the cosmetic by issuing the Guidance for Product Classification, which defines cosmetics but also the following product categories:

▶ Drug products

▶ Herbal products

▶ Health products

▶ Cosmetic products

The product category can be generally identified by referring to this Guideline; however, manufacturers can submit a classification request to SFDA to get an official Classification Letter to be sure of the product status.

The other categories are handled directly by SFDA locally and are subject to SFDA registration (for high-risk products: drug, herbal, and certain health products) or to SFDA listing (low-risk health products, cosmetics).

2.3.2.3 LABELING

a. General rules

Product labeling shall comply with the general safety standard GSO 1943/2009. This standard lists the following markings as mandatory:

- Name of the product, Trademark

- Function of the product (in Arabic if the function is not clear from the product's presentation)
- Name and address of manufacturer
- Date of production or batch number
- Durability information (PAO or Best before date)
- Weight/volume
- Country of origin
- Ingredients list (INCI)
- Instructions of use and storage (in Arabic if special instruction that may have an effect on the use of the product for consumer safety)
- Any warning (in Arabic)

Note: A minimum of Arabic translation is always required, such as for the product's function, and safety warnings or special instructions for use.

b. Specific rules

Particular attention needs to be paid to some labeling rules, in order to be in conformity with the Saudi requirements. Among those we can find the following: **Medical claims:**

Medical claims are not allowed, and terms like "treatment" are generally not accepted. Any other claims relative to "regeneration of cells," or "stimulation of collagen production," should be avoided.

Pictures and illustrations: Any pictures or illustrations that are inconsistent with the prevailing social customs and values should not be used.

Country of origin The country of origin marking is mandatory on all products and its immediate packaging; the importance of this marking was reinforced in 2010 by a MoCI decree (MoCI Royal Decree No. M/5) and is applicable to every product entering the KSA, not only cosmetic products. This mandatory requirement is strictly controlled in the country of origin/supply by the inspection agent and by customs in the KSA.

It should be noted that an indication such as "Made in EU" is not accepted; the exact country of origin must be clearly indicated. The rule is also very strict as regards to dual country of origin and labeling such as "Brand of Paris" + made in China; or "designed in France, manufactured in China," which should be avoided.

Expiry date

The marking of the expiration date is not mandatory as per GSO 1943/2009,

which only requests a “best before date” or indication “Period After Opening” (PAO). However, the expiration date is required by some SASO-specific standards, and this contradictory instruction occasionally causes issues during clearance.

Perfumery products Perfumery products standard (SASO 585/2000 – perfumery products based on ethanol) is applied in addition to the general safety standard, due to the presence of alcohol in such products, because of the sensitive issue relative to alcoholic products. This standard imposes the denaturation of ethanol, and some restrictions for products containing a percentage of essential oil/fragrance compounds lower than 8%: volume shall not be more than 250ml, and must be fitted with nonremovable pumps. Furthermore, perfumery products having a percentage of essential oil/fragrance compounds lower than 8% must bear a specific warning in Arabic “for external use only.”

Products packed in ampoules or vials Cosmetic products can be packed in ampoules or vials, even if this may sometimes be considered a medical form, provided that such products bear the following instructions in Arabic and English:

→ For external use only.
→ Avoid contact with eyes.
→ Open with caution.

2.3.2.4 MARKET ACCESS

Any cosmetic product imported into the Saudi market must present a Certificate of Conformity by the manufacturer (hereafter referred to as CoC) during customs clearance. This certificate is requested by law through the MoCI Decree 6386 as well as per the instructions published by SFDA.

a. Certification process

The SFDA regulations that outline the conformity assessment procedures demand that every shipment of cosmetic and perfumery products needs a CoC, issued by an approved inspection company, for each customs entry. The CoC needs to be issued prior to shipment, following a full evaluation of conformity against Saudi Standards and SFDA requirements. Each CoC is valid for one shipment only, and bears all reference specific to this particular shipment (such

as final invoice reference).

In order to perform certification of the country of origin, since 2011 the SFDA has officially subcontracted the evaluation of conformity and the related Certification to approved inspection bodies.

The SFDA requirements, and consequently the inspection bodies' scope of work, are that each shipment should be tested and inspected against the sanctioned standards. The Certificate issued by the inspection bodies should thereafter be presented by the importer to the local SFDA agent and Customs official at the port of entry, in order to ensure that the regulations are fully respected for any cosmetic or perfumery products entering the Kingdom.

It should be noted that the appointed inspection and certification bodies only certify cosmetic products, and not the other products' categories (herbal, health products, and drugs).

b. Conformity assessment

The first step of the certification process in the country of origin/supply, by the inspection agents, is the preclassification of the product. In certain borderline cases, manufacturers can be asked to refer directly to SFDA to obtain a written confirmation of their classification of the relevant product. Once the product is clearly identified as a cosmetic product, it will be assessed as per the sanctioned standards and SFDA requirements. This assessment is done through a documentary review, product testing, and inspection.

A nonmandatory registration system can be implemented by the inspection bodies in order to avoid testing of each shipment.

Once the document review is fulfilled and the products are found compliant, the products are physically inspected prior to shipment, in order to verify the physical integrity of the shipment against the shipping documents as well a verification of the marking of the individual products.

c. Key issues

At the points of entry, a full review of the conformity of the products can happen and samples can be randomly drawn for shipments holding a Certificate of Conformity, to ensure product and certification are correct (if a shipment of cosmetics arrives without a CoC, samples will automatically be drawn for testing).

When this situation occurs, it can happen that the local laboratories do not follow the SFDA-sanctioned safety standard and the local tests are done as per other SASO standards. The risks with such a local test is that a product may fail due to a nonsafety-related test requirement such as fatty matter for a skin cream. It is therefore recommended that a risk analysis be performed as per the specific SASO standards for specifications and labeling requirements, even if these are not officially sanctioned by the ruling authority.

REFERENCES:

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- THE COUNCIL OF THE EUROPEAN COMMUNITIES. COUNCIL DIRECTIVE of 27 July 1976 on the approximation of the laws of the member states relating to cosmetic products 76/768/EEC. Official Journal of the European Union, L 262 of 27 September 1976, p. 169.
- Saudi Arabia Standard Organization. Cosmetic Products – Perfumery products based on ethanol. SASO 585/2000, 6 p.
- GCC Standardization Organization. Skin Cream. GSO 1152/2010, 4 p.
- Saudi Food & Drug Authority. Guidance for Products Classification–version 1. 23 May 2012, 21 p.

Abbreviations

CoC: Certificate of Conformity

GCC: Gulf Cooperation Council

GSO: Gulf Standard Organization

KSA: Kingdom of Saudi Arabia

SASO: Saudi Arabia Standard Organization

SFDA: Saudi Food and Drug Authority

PAO: Period After Opening

ACHIEVING GLOBAL MARKET ACCESS-FOCUS ON CHINA AUTHOR

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Intertek ABSTRACT

The purpose of this chapter is to give a brief overview of the cosmetic legislation and regulatory status for importers who are interested in supplying their cosmetic products to the Chinese market. The difference and similarity between EU and Chinese regulations are summarized. Cosmetic legislation, definitions, the registration process, and new ingredient requirements are also discussed to help the reader understand how to enter the marketplace in Mainland China.

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2.3.3.5 Safety assessment for ingredients and finish products
2.3.3.6 Comparison of EU and China regulation requirements
2.3.3.7 Existing and new cosmetic ingredients in China

2.3.3.1 CATEGORY OF COSMETICS IN CHINA: SPECIAL USE AND NONSPECIAL USE COSMETIC

Cosmetic regulation in China is based on Regulations Concerning the Hygiene Supervision over Cosmetics (1990). “Cosmetic products” are defined as those daily-used chemical products (substance and mixture) applied on the surface of any part of the human body (such as skin, hair, nails, oral, and lips) by way of

smearing, spraying, or other similar methods to keep the body clean; to get rid of undesirable smell; to protect the skin; to make up the face, and to increase appearance and beauty. The definition is similar to that of the EU Cosmetic Directive 76/768/EEC and includes prohibited and restricted substances that are listed in the EU Hygienic Standard for Cosmetics (2007). The main difference between the EU and Chinese regulations is that cosmetic products are divided and managed in two categories: “special use” and “nonspecial use” products. Nonspecial-use cosmetics include hair care, nail care, skin care, perfumes, and makeup. Special-use products include hair growth, hair dye, hair perm, hair removal, breast shaping, fitness, deodorizing, spots removal, and sun block.

2.3.3.2 ORAL PRODUCTS REQUIREMENTS

Oral care products are not listed in either of the two categories, and instead are regulated according to other separate national standards (e.g., GB 8372-2008 gives specified requirements for toothpaste). Furthermore, there are guidelines, national standards, and industry standards on cosmetic products that present specific requirements. These include, but are not limited to, testing methods, labeling, and registration. Consequently, compliance with the legal requirements is made difficult to understand for importers or distributors foreign to China. The authorities have noticed that and now are discussing the matter with industry; it is very likely that oral products will be treated as “cosmetic” and regulated by China FDA.

2.3.3.3 DOCUMENT AND TESTING REQUIREMENTS DURING PRODUCT REGISTRATION

For imported cosmetic products, except oral care products, and unlike the trend in the EU and U.S., registration and animal testing are required by China’s State Food & Drug Administration (CFDA) before marketing. Such testing can take several months or more to perform before registration is possible. The nonspecial-use cosmetics do not require registration, but notice should be provided to provincial authorities if the products were manufactured by domestic companies, including internal brands that have manufactured products in mainland China. The Registration Documents are listed in the Table 1. The application form and all other documents should be written in simplified Chinese and the original language of the technical document should also be submitted to

CFDA together with its translation.

Table 1: China FDA required registration documents for cosmetics

Nonspecial-use cosmetics	Special-use cosmetics
Application form for imported cosmetics (nonspecial use)	Application form of imported cosmetics (special use)
Product formula—INCI name in both English and Chinese	Product formula—INCI name in both English and Chinese
Quality standard of product (specification)	For products with the function of slimming, breast care, and hair growth, documents should provide the effective ingredient based on scientific data and a brief introduction and sketch of production process.
Testing report of inspection institute accredited by Ministry of Health (MOH) and relative documents	Brief description of manufacture technology and procedure chart
Original package (including label) or package (including label) designed for the Chinese Market	Quality standard of product (specification)
Certification documents of sale permission for the registered product in country of origin (region) or manufacturing country (region)-free sale certification	If products contain ingredients that endanger health, documents should provide the related safety assessment data (test report for concerned impurities).
If products contain ingredients that endanger health, documents should provide the related safety assessment data (test report for concerned impurities)	Testing report of inspection institute accredited by Ministry of Health (MOH) and relative documents
“BSE” certificate (prepared by responsible unit of China)	Original package (including labeling) or package (including labeling) designed for the Chinese market and instruction for use in Chinese
Business license copy of responsible unit in China	Certification documents of sale permission for the registered product in country of origin (region) or manufacturing country (region)-free sale certification
Authorization letter—it will be needed if the unit responsible is to apply for registration	“BSE” certificate (prepared by responsible unit of China)
Consigned manufacturing agreement, GMP, or ISO certificate	Business license copy of responsible unit in China

Certificate of an agency issued by the unit responsible if needed	Authorization letter—it will be needed if the unit responsible is to apply for registration
Other documents that may be helpful for the record	Consigned manufacturing agreement, GMP or ISO certificate
Unopened samples	Certificate of an agency issued by the unit responsible if needed
	Other documents that may be helpful for the record
	Unopened samples

2.3.3.4 ANIMAL TESTING REQUIREMENTS IN CHINA

Animal testing for finished cosmetic products is a mandatory requirement and should be conducted by Ministry of Health (MOH) accredited laboratories in China. Rats, rabbits, and guinea pigs are normally used under finished product registration. Alternative methods are normally not accepted by CFDA even if they have been validated by OECD or other international bodies; a good example is the Guinea Pig Maximum Test (GPMT), which has been prohibited in EU. Instead, the Local Lymph Node Assay (LLNA) is recommended in order to comply with the 3R principle (Replace, Refine, and Reduce) for investigating skin sensitization potential, but at the moment China FDA only accepts the GPMT method. As a consequence, all cosmetic products that have not been tested on animals are excluded from the Chinese cosmetic market. Under pressure of international animal rights organizations and the cosmetic industry, the CFDA finally changed its attitude and approved the first *in vitro* method of 3T3 Neutral Red Uptake Phototoxicity Test in February 2012. However, a CFDA prohibition of animal testing is currently thought to be a long way off.

In the European Union, all animal testing has been banned for finished products, as well as ingredients, as of March 2013, but it is clearly mentioned that the ban is only applicable to those animal tests designed for *this regulation* in article 18 of EU cosmetic regulation EC 1223/2009. Therefore, exemption conditions exist for those tests designed to fulfill other countries' legal requirements (such as China's); the relevant data should be documented in a Product Information File (PIF) and made as transparent as possible. The authorities may not consider these animal tests to be in violation of EU cosmetic regulation.

2.3.3.5 SAFETY ASSESSMENT FOR INGREDIENTS AND FINISH PRODUCTS

A toxicological risk assessment (cosmetic safety assessment) is required in China, but this report mainly focuses on identifying prohibited substances or toxic impurities in the ingredients. For example, phenol is a common impurity in phenoxyethanol, and a testing report of phenol would normally be provided either for phenoxyethanol itself or a finished cosmetic product. Other routine test substances such as Dioxane, Methanol, Acrylamide, and N-nitrosodiethanolamine (NDELA) and their safety levels (Margin of Safety) should be calculated to demonstrate their safety if trace levels are present in the finished formulation. This is different from the EU cosmetic Regulation EC 1223/2009, which emphasizes the toxicity of intentionally added ingredients rather than the impurities. Comparable EU and China regulation requirements are summarized in Table 2.

2.3.3.6 COMPARISON OF EU AND CHINA REGULATION REQUIREMENTS

Table 2 comparison of EU and China regulation requirements

Requirement	**EU cosmetic regulation/directive**	**China legislation**
Category	Leave-on and rinse-off products	Special and nonspecial-use products
Oral products	Cosmetics	National standards
Animal testing	Banned	Mandatory
In vitro test methods	Accepted	3T3 Neutral Red Uptake Phototoxicity Test only
Notification	Yes	Yes, but for domestic manufacturers' nonspecial-use products
Registration	No	Yes, all imported cosmetics and domestic manufacturers' special-use products
Duration of notification or registration	Minimum	Notification is minimum but take several months, possibly years, for registration.
Claim of effects	Yes, document evidences in the Product Information File	Yes, reviewed by committee during registration

Testing report(s) for high-risk impurities	Not mandatory	Yes
Hygienic testing report(s)	Not mandatory	Yes
Testing samples	Not mandatory	Yes, for animal and clinical testing
Language	English and member-state official language	Simple Chinese
Organic cosmetic	Not regulated	Prohibited
Labeling	**26 allergen**	Ingredient INCI Chinese name in the label
Infant or child products	No specific requirements	Yes
Existing cosmetic ingredient	No	Yes, 3667 in three batches
New cosmetic ingredient registration	No	Must be approved before use
Nanomaterials	Notify authorities and provide safety data	Not specified, not recommended in infant or child products

Any claims of performance effect(s) are reviewed by the CFDA committee, and substantiation of these claims, such as clinical research reports for the ingredients or products themselves, shall accompany the submitted dossier. Cosmetic products intended for pregnant women and mothers during lactation are not accepted by CFDA as they cannot be justified or assessed for potential risk.

The principle of “organic cosmetics” is not recognized in China according to GB/T 19630 Organic Products, the authority AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People’s Republic of China). Products containing 95% or above “organic ingredient” may use the “organic” mark after the official certification process. Third party “organic” certifications are not accepted and cosmetic brands are basically prohibited from advertising their products as “organic” in mainland China.

This approach is similar to that used for “cosmeceuticals,” which is also not a recognized term. If a product has drug-like properties, it must meet the requirements for drugs as defined in the Drug Control Law of the Peoples Republic of China.

Cosmetic labeling should comply with GB5296.3-2008, and normally the approach that industry takes is to design a “patch” in Chinese and then paste it onto the original label and package. However, warning statements will not be covered by this patch. The CFDA committee obligation is to check the claim(s) on the patch but not to check the original language or the company’s official websites. Importers or suppliers to the Chinese market therefore simply need to design this “patch” rather than modify their products’ claim(s) in the original market.

To protect consumers against allergic contact dermatitis in the EU, the seventh amendment to the cosmetic directive required the listing of any one of 26 allergen names on the packaging should the concentration exceed 0.01% for rinse-off products and 0.001% for leave-on products. No such labeling is required in China. The unique requirement for labeling is that ingredients be listed by their Chinese INCI names, and if a Chinese INCI name does not exist for an ingredient, importers must apply for one. The Chinese INCI name can be identified in the Catalogue of International Cosmetic Ingredients published by the Ministry of Health (MOH).

“Nanomaterial” in cosmetics has been a hot topic in recent years, understandably so as they often provide unique and fascinating properties for products. Unique or not, the safety of these materials requires further investigation. Elsewhere in this text we cover the growing and complex field of the characterization of nanomaterials. While notification is required for products containing nanomaterials according to EU Cosmetic Regulation EC 1223/2009, the Chinese CFDA has not yet given any official opinion on use or labeling—although use of nanomaterials is not encouraged in products intended for infant or child cosmetics.

A new guide for products used on infants and children was published on October 12, 2012. One important requirement is that these types of cosmetic products should minimize the total number of ingredients relative to classes of colors, fragrances, and preservatives. It also requires proof of need for their inclusion.

2.3.3.7 EXISTING AND NEW COSMETIC INGREDIENTS IN CHINA

Existing and new cosmetic ingredients are defined in the Guidelines for the Registration and Evaluation of New Cosmetic Ingredients (2011). A new

ingredient is defined as any ingredient that is used in cosmetics for the first time in China. Basically, a “new ingredient” can be defined as any ingredient that is currently not included in the current CFDA cosmetic ingredient list—even if it is already being used in EU or U.S. markets. China FDA has published an Inventory of Existing Cosmetic Ingredients in China (IECIC 2003 & 2011), and three additional batches of existing ingredients were released in May, July, and September 2012 containing respectively 1674, 637, and 1356 substances. The lists are not exhaustive, and CFDA experts have suggested that more lists will be released over time.

Maximum approved concentration (MAC) and/or maximum used concentration in approved cosmetic products (MUCAP) are available for each ingredient. In these, if MUCAP limits are exceeded then evidence will be required to prove the safety of the finished cosmetic product during use. New ingredient registration requiring exhausted toxicity and safety data and process is extremely time consuming and expensive. CFDA took over the regulation of cosmetics from the Ministry of Health (MoH) in 2008, and since then only three new cosmetic ingredients have been approved for use. Therefore, industry has no choice but to design formulations based on the currently existing list; otherwise companies may have to wait for prolonged periods of time—sometimes years—to gain authorization to market cosmetic products containing “new” ingredient(s).

For all EU and U.S. cosmetic companies, tolerance and patience with the complex registration process are, regrettably, key factors needed to enter this huge and important market.

NANOMATERIALS IN COSMETICS: REGULATORY AND SAFETY CONSIDERATIONS

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ABSTRACT

Nanomaterials are present as ingredients in numerous types of cosmetic products that are marketed worldwide. Regulatory and safety evaluations of nanomaterials and nanomaterial-containing cosmetic products continue to evolve with the accumulation of knowledge on the physicochemical and toxicological properties of particles at the nanoscale. This chapter provides an overview of the current state of regulation of nanomaterial-containing cosmetic products in Europe, the United States (U.S.), and Canada. It also outlines some considerations for the design and conduct of evaluations of nanomaterial toxicity that can support cosmetic product safety.

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2.3.4.1 REGULATION OF COSMETICS CONTAINING NANOMATERIALS

a. Definition of Nanomaterial

The regulation of cosmetics containing nanomaterial ingredients depends, in part, on the definition of a nanomaterial, which in and of itself varies among jurisdictions. For reference, Table 1 presents a summary of the current definition of a nanomaterial for cosmetic regulation purposes in Europe, the U.S., and Canada.

Table 1 Nanomaterial Definition in Different Jurisdictions

Jurisdiction	Definition of Nanomaterial	Reference
Europe	● "Nanomaterial" means an insoluble or biopersistant and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.	**1**
United States	● FDA has not adopted a formal definition of "nanotechnology," "nanoscale," or related terms.	**2**
	● Although there are numerous definitions of "nanotechnology," the term is perhaps most commonly used to refer to the intentional manipulation, manufacture, or selection of materials that have at least one dimension in the size range of approximately 1 to 100 nanometers.	
	● While one definition for "nanotechnology," "nanoscale material," or a related term or concept may offer meaningful guidance in one context, that definition may be too narrow or broad to be of use in another.	
	Any manufactured substance is a nanomaterial if:	

Canada	● It is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale; or ● It is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena. For the purposes of this definition: ● The term “nanoscale” means 1 to 100 nanometers, inclusive; ● The term “nanoscale properties/phenomena” means properties that are attributable to size and their effects; these properties are distinguishable from the chemical or physical properties of individual atoms, individual molecules, and bulk material; and, ● The term “manufactured” includes engineering processes and the control of matter.	**3**

b. Regulation in Europe

The European Commission was the first regulatory body to formally regulate the use of nanomaterials in cosmetics. As of July 11, 2013, cosmetic products placed on the European market must conform to the procedures and data requirements contained in Regulation (EC) No. 1223/2009 of the European Parliament and of the Council 1. As of this date, a Product Information File (PIF) for each cosmetic product marketed in Europe must be maintained by a “Responsible Person” within the Community (defined as "a legal or natural person established within the Community"). Each PIF contains a cosmetic product safety report and other pertinent information for a given cosmetic product and must be made available to regulatory authorities if requested. Moreover, the Responsible Person must notify the Commission prior to the introduction of a cosmetic product to the European market. While notification for cosmetic products that do not contain nanomaterials can be made immediately prior to placement on the market, notification for cosmetic products containing nanomaterials must be made six months prior to placement on the market 1. In the latter case, the notified information must contain the following:

1. The identification of the nanomaterial including its chemical name (IUPAC) and other descriptors
2. The specification of the nanomaterial including size of particles and

physical and chemical properties

3. An estimate of the quantity of nanomaterial contained in cosmetic products intended to be placed on the market per year
4. The toxicological profile of the nanomaterial
5. The safety data of the nanomaterial relating to the category of cosmetic product, as used in such products
6. The reasonably foreseeable exposure conditions

The Regulation does not provide specific guidance on how to address some of the items listed above (e.g., the specific types of toxicological data required), but such guidance is available from the European Commission's Scientific Committee on Consumer Safety (SCCS). Specifically, guidance is available that assists with understanding what needs to be considered when evaluating the safety of cosmetic ingredients in general 4 and when conducting physicochemical characterization, exposure assessment, hazard identification and dose response, and overall risk assessment evaluations for nanomaterials in cosmetics 5. Based on SCCS guidance, the following parameters are considered important for identification and characterization of nanomaterials in cosmetic products 5:

1. Chemical identity
2. Chemical composition and purity
3. Size and size distribution
4. Morphology
5. Surface characteristics (charge, modifications, etc.)
6. Solubility
7. Surface area
8. Catalytic activity
9. Concentration
10. Dustiness (for dry powder products)
11. Density and pore density
12. Redox potential
13. pH
14. Viscosity
15. Stability
16. Other aspects (e.g., ultraviolet absorption, light reflection)

In the event that information supplied for a given nanomaterial raises concerns about its safety, the Commission may request the SCCS to issue a formal opinion. These opinions focus on the safety of the nanomaterial for use in the

relevant categories of cosmetic products and on the reasonably foreseeable exposure conditions. Timelines have been established for the SCCS to request additional information to assist with its evaluation of a given nanomaterial, for such information to be provided, and for a final opinion to be issued, all of which is made publicly available.

Unlike in other regions, special labeling requirements have been established for nanomaterial-containing cosmetics in Europe. Specifically, all nanomaterial ingredients present in a cosmetic product marketed in Europe must be clearly indicated in the list of ingredients and be followed by the word “nano” in brackets. Moreover, the Regulation calls for the creation and maintenance of a publically available catalogue of all nanomaterials that are used in cosmetic products placed on the market. This catalogue will be made available by January 11, 2014 and will indicate categories of cosmetic products that contain nanomaterials and the reasonably foreseeable exposure conditions. In recognizing that understanding nanomaterial safety is a pragmatic process, the Commission has set a deadline of July 11, 2018 for undertaking the first review of the provisions of the Regulation that concern nanomaterials, and will propose suitable amendments to the provisions where it is deemed necessary.

c. Regulation in the United States

As outlined in a recent draft guidance document on the safety of nanomaterials in cosmetics 2, cosmetic ingredients (with the exception of color additives) and cosmetic products are not required to be notified and do not require premarket approval from the U.S. Food and Drug Administration (FDA) in order to be placed on the U.S. market. With some exceptions (e.g., color additives and ingredients that are prohibited or restricted from use by regulation), a manufacturer may use any ingredient in a cosmetic product formulation as long as the use of the ingredient does not otherwise cause the product to be adulterated or misbranded. The manufacturer is responsible for ensuring that the cosmetic product is not misbranded or adulterated, and manufacturers or distributors should have obtained all data and information needed to substantiate the safety of the product before marketing.

The safety of any cosmetic product to be marketed in the U.S. should be evaluated by assessing the physicochemical properties and relevant toxicological endpoints for each ingredient in relation to the expected exposure levels that will result from the intended use of the finished product. For cosmetic products

containing nanomaterials, the FDA recommends that safety assessments address several important factors, including the following 2:

1. Physicochemical characteristics (including size and size distribution, aggregation/agglomeration, surface chemistry, morphology, solubility, density, stability, and porosity)
2. Agglomeration and size distribution of nanomaterials under the toxicity-testing conditions that should correspond to those of a final product
3. Impurities
4. Potential product exposure levels, and the potential for agglomeration of nanoparticles in the final product
5. Dosimetry for in vitro and in vivo toxicology studies
6. In vitro and in vivo toxicological data on ingredients and their impurities, dermal penetration, irritation (skin and eye) and sensitization studies, and mutagenicity/genotoxicity studies
7. Clinical studies to test the ingredient, or finished product, in human volunteers under controlled conditions

With regard to toxicity testing for nanomaterials, FDA has indicated that manufacturers should consider modifying traditional methods by giving consideration to appropriate solvents and dosing formulations, methods to prevent agglomeration of nanoparticles, purity and stability conditions, and other variables. FDA recommends that, at a minimum, evaluations be conducted for acute toxicity, skin irritation, dermal photo-irritation, skin sensitization, mutagenicity/genotoxicity, repeat-dose toxicity (21 to 28 days), and subchronic toxicity (90 days), and take into consideration toxicokinetics and toxicodynamics with regard to different exposure routes 2.

Manufacturers of cosmetic products are encouraged to contact the FDA if they are considering using a new nanomaterial or an altered (i.e., nanoscale) version of an ingredient already on the market so that discussions can be held regarding test methods and data needed to substantiate a given product’s safety.

d. Regulation in Canada

Notification is a mandatory requirement for the sale of cosmetics in Canada. A Cosmetic Notification Form must be submitted to Health Canada within ten days of making a cosmetic product available for sale in Canada. This form provides information specific to each cosmetic product, including the address and contact information of associated companies, the purpose of the cosmetic (shampoo,

lipstick, etc.), the form of the cosmetic (gel, liquid, etc.), and a list of the ingredients and their concentrations.

Ultimately, manufacturers are responsible for ensuring that their cosmetic products meet the requirements for cosmetic products under the Canadian Food and Drugs Act and the Cosmetic Regulations. To assist manufacturers in determining whether ingredients are safe for use in cosmetics, Health Canada maintains a list of prohibited and restricted cosmetic ingredients (the “hotlist”). While there were no nanomaterials listed in the cosmetic ingredient hotlist as of late 2012, this list is not exhaustive, and Section 16 of Canada’s Food and Drugs Act permits Health Canada to restrict or prohibit any ingredient it deems unsafe.

Specific guidance is not readily available regarding the regulation of nanomaterials in cosmetics in Canada. In late 2011, Health Canada released a policy statement on its working definition of nanomaterial 3. At that time, Health Canada indicated that existing risk assessment methodologies are applicable to nanomaterials, and that guidance documents would be developed over time for nanomaterials specific to products, substances, or commodity groups. Given its authority within existing legislative and regulatory frameworks, Health Canada may request the submission of information on specific nanomaterials in cosmetics and other regulated products to assist with assessing potential risks due to exposure to nanomaterials via the use of such products. Such information may include the following (when relevant) 3:

1. Intended use, function, and purpose of the nanomaterial, and information regarding any end product in which it will be used
2. Manufacturing methods
3. Characteristics and physicochemical properties of the nanomaterial such as:
   a. composition
   b. identity
   c. purity
   d. morphology
   e. structural integrity
   f. catalytic or photo-catalytic activity
   g. particle size/size distribution
   h. electrical/mechanical/optical properties
   i. surface-to-volume ratio
   j. chemical reactivity
   k. surface area/chemistry/charge/structure/shape
   l. water solubility/dispersibility

m. agglomeration/aggregation (or other properties)
n. descriptions of the methods used to assign these determinations

4. Toxicological, eco-toxicological, metabolism, and environmental fate data that may be both generic and specific to the nanomaterial if applicable
5. Risk assessment and risk management strategies, if considered or implemented

Manufacturers and other stakeholders are encouraged to establish communication with Health Canada early in the development process for products (including cosmetics) that are, contain, or make use of nanomaterials.

On September 13, 2001, the Minister of Health confirmed the government's intention to proceed with the development of new environmental legislation for all products regulated under the Food and Drugs Act, including cosmetics, as their ingredients would no longer be exempt from the requirements of the Canadian Environmental Protection Act, 1999 (CEPA). Health Canada is in the process of developing Environmental Assessment Regulations to address the unique properties of ingredients in cosmetics and other products. However, until the new Regulations are developed, these substances are subject to notification and assessment requirements under the New Substances Notification Regulations (NSNR) of CEPA. The NSNR represent a volume-based, premanufacture/preimport notification program that requires the submission and government assessment of physical and toxicological information for nonexempt chemicals and polymers that are not listed on the Domestic Substances List (DSL). The amount of information and the studies that must be submitted to the government are described in a series of NSNR "Schedules." The specific Schedule that applies is dependent on a variety of factors including, but not limited to: the anticipated manufacture or import quantity of the notifiable substance (i.e., in kg/year); the inherent nature of the substance (e.g., chemical, polymer, or low-concern polymer); the intended use of the substance (e.g., research & development, site-limited, export-only); and whether the substance is listed on the Non-Domestic Substances List (NDSL).

Within the NSNR program, nanomaterials that are manufactured in or imported into Canada that are not listed on the DSL are considered new. Although there is no internationally recognized definition of this type of substance, for purposes of the NSNR, nanomaterials can be described generally as substances having one or more dimensions in a nanoscale range, typically between 1 and 100 nanometers. The nanoscale form of a substance on the DSL is considered a "new" substance if it has unique structures or molecular

arrangements. Substances listed on the DSL whose nanoscale forms do not have unique structures or molecular arrangements are considered existing and are not subject to the NSNR. In addition, incidentally produced or naturally occurring nanomaterials are not subject to notification.

2.3.4.2 SAFETY ASSESSMENT CONSIDERATIONS FOR NANOMATERIALS

a. Study Design Aspects

The safety of nanomaterials contained in cosmetics and other products must be supported by data generated in studies that are of high quality in terms of their design and reporting 6. While not always feasible, such studies ideally adhere to established testing guidelines, such as those published by the Organisation for Economic Cooperation and Development (OECD), and to the principles of Good Laboratory Practice. Tiered strategies have been proposed to evaluate the safety of nanomaterials wherein *in vitro* and *in vivo* screening studies are followed by pivotal *in vivo* studies and supportive mechanistic studies 7–9. While this may be an ideal scenario, the evaluation of nanomaterial toxicity is particularly challenging in Europe given the restrictions that are in place on animal testing in Europe and the fact that validated alternative methods currently available for conventional chemical substances (e.g., skin corrosion, skin irritation, mutagenicity, photomutagenicity, phototoxicity, and dermal absorption) have not been validated specifically for nanomaterials 5.

At the present time there are insufficient data on nanomaterial properties, behavior, and effects to allow for nanomaterial safety to be evaluated using a read-across approach (i.e., based on structural surrogates or categories of substances). Thus, substance-specific data (i.e., those generated with the substance of interest) are generally required to support the safety of a given nanomaterial. In cases where specific endpoints have not been evaluated with the substance of interest, information from the published literature may be useful if it is deemed to be relevant (i.e., based on a substance that is sufficiently similar in terms of physicochemical properties) and of suitable quality. In this respect, it is worth noting that published scientific articles do not always provide data that can be used for safety assessment purposes. Physicochemical characterization is a key element to nanomaterial study design and its importance is discussed further below. In addition, as for any study, those that are conducted with

nanomaterials need to consider the importance of general study design and adequate documentation of methods, materials, and results. Unfortunately, as summarized in a review of the published literature on the safety of food-related nanomaterials 10, there are many examples of peer-reviewed published articles in which it is difficult or impossible to discern simple yet critical experimental details, such as the number of animals per treatment group, the method/route of exposure, or the doses or concentrations of the substance that was tested. Thus, caution must be exercised when attempting to use publicly available information to support the safety of a particular nanomaterial.

b. Nanomaterial Characterization

Characterization of the physicochemical properties of nanomaterials is paramount to understanding their behavior in biological systems and conducting risk assessments related to potential exposure. Lack of adequate nanomaterial characterization limits the value and significance of a given study and renders it impossible to compare studies and recognize parameters that might influence toxicity. The physicochemical characteristics of a given nanomaterial may differ considerably in the “raw” versus the “tested” state and may be influenced by the solvent, test media, or biological environment that is utilized in a particular evaluation. As such, the SCCS has recommended that, unless otherwise justified, characterization of nanomaterials for use in cosmetics be undertaken under at least the following three conditions: i) in the raw form as manufactured; ii) as present in the final cosmetic formulation; and iii) as present during toxicological evaluations (in the dose formulation, cell culture, etc.) 5.

The importance of nanomaterial characterization was highlighted in a review of the published literature on the safety of food-related nanomaterials 10. Among other findings, this review identified that information on nanomaterial purity was lacking in more than half of the published articles that reported on studies of the oral toxicity of food-related nanomaterials. This seemingly simple oversight in many published articles renders the results of such nanomaterial toxicology studies difficult to interpret, and emphasizes the need for sufficient physicochemical characterization in nanomaterial toxicology studies to allow for their inclusion in risk assessments.

Toxicological properties of food-related nanomaterials is of particular importance as the cosmetic and personal care industries move increasingly towards the concept of “beauty from within” and the growing use of

nutraceuticals. Concerns of nanoparticle incorporation in organs of the body and elsewhere would appear to be very important since the size of a nanoparticle is at a level where cell penetration may be of significance. Thus, there remains much to do regarding the reliability of measuring and reporting toxicological impacts of ingested nutraceuticals.

c. Dose Metrics

Due to their large surface area per particle mass or particle volume, traditional means of expressing dose levels as mass or volume units (e.g., mg/kg body weight or mg/L) may not be appropriate for nanomaterials, particularly those that are insoluble in aqueous systems. Other metrics, such as particle number or relative surface area, may be more relevant to dose-response relationships and need to be considered when designing and evaluating the results of toxicology studies 2, 5, 7, 11. Thus, characterization of the physicochemical properties of nanomaterials should be conducted in a manner that allows for the derivation of such alternate dose metrics.

The results of a study reported by Oberdorster *et al.* 12 provide an example of the influence of dose metrics on data interpretation. When expressed in terms of equivalent mass, lung inflammation in rats was observed to be more severe (i.e., the dose-response curve was steeper) following installation of 25 nm TiO_2 nanoparticles than following installation of 250 nm TiO_2 nanoparticles. But when the dose was expressed in terms of relative surface area of the instilled nanoparticles, the dose-response curves for the two nanoparticle sizes overlapped. Thus, for particles with the same chemistry, but of different sizes, particle surface area may be a more relevant dose metric and should be considered when evaluating the results of toxicological studies. Such studies are of particular importance for nano-size TiO_2 particles in view of their wide use in sunscreen agents and other cosmetics.

d. Assay Interference

Various types of nanomaterials may interfere with assays commonly used to determine cellular or toxic effects. Numerous examples have been cited in the published literature and include interference with optical or other detector measurements, interference with colorimetric or fluorometric dyes used in cytotoxicity assays, interference with measurement of reactive oxygen species, and adsorbance to essential growth factors and nutrients in cell culture media

that leads to indirect growth inhibition and apparent cytotoxicity 13–15. Such effects may lead to false positive or false negative readouts, examples of which are presented in Table 2.

Table 2 Examples of Assay Interference by Nanomaterials

Assay	Interference	Altered Readout	Nanomaterial
MTT (cell viability)	Adsorption of substrate	Decreased indication of cell viability (false positive)	Carbon nanoparticles
LDH (cell viability)	Inhibit LDH activity	Decreased indication of cytotoxicity (false negative)	Trace metal-containing nanoparticles
ELISAs (inflammatory response)	Adsorption of cytokines	Decreased indication of inflammation (false negative)	Metal oxide nanoparticles

Abbreviation: ELISA, enzyme-linked immunosorbent assay; LDH, lactate dehydrogenase, MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide).

It is of particular interest to note that the MTT cell viability assay has been reported to be affected by some nanomaterials. This is one of the recommended assays for assessing cell viability in OECD test guideline number 431 (*In Vitro* Skin Corrosion: Human Skin Model Test) 16. As such, the possibility of false positives from this assay, and others, needs to be carefully considered when evaluating skin corrosion and other toxicological endpoints for nanomaterials.

CONCLUSION

Regulatory and safety evaluations of nanomaterials and cosmetic products that contain nanomaterials are evolving processes. Considering that nanomaterials can, and have the potential, to possess properties quite unlike their macroscopic counterparts, the unique physicochemical properties of nanomaterials present many difficulties with respect to characterization and toxicity testing. These differences contribute to the challenges associated with conducting risk assessments for cosmetic products containing nanomaterial ingredients. The continued accumulation of knowledge on nanomaterial properties and safety and the development of additional validated methods for evaluating these aspects will assist regulatory communities around the world in ensuring that cosmetic

products containing nanomaterial ingredients are safe for use by consumers worldwide.

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PART 3

SUBSTRATE:

THE SKIN:
STRUCTURE, BIOCHEMISTRY
AND FUNCTION

PART 3.1

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PART 3.1

THE SKIN: STRUCTURE, BIOCHEMISTRY, AND FUNCTION

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ABSTRACT

The skin is the organ that forms the border between the organism and the environment. Skin prevents dehydration, stops the penetration of noxious foreign materials and microorganisms, cushions against mechanical shock, helps to maintain a constant body temperature, and transduces incoming stimuli. In order to perform these functions, skin must be maintained in good condition, an important objective for cosmetic formulators. For cosmetic and personal care scientists, whether they are concerned with the improvement of the skin by pharmacology or cosmeceuticals, with the prevention or repair of damage, an understanding of skin structure and function is essential. As such, this section has a primary focus upon these issues.

Further, the impact of light on skin and on skin aging has become so important in cosmetics that it too requires a complete discussion. This chapter is carefully and thoroughly designed to provide a basic knowledge of skin structure and function, thereby forming a sound foundation for the reader to understand key issues and provide stimulation towards the generation of new ideas and novel approaches for new ingredients, addressing the global quest for advanced cosmetic and personal care products.

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PART 3.1.1 SKIN MORPHOLOGY

The skin is divided into three layers: the epidermis, the dermis, and the subcutaneous tissue. The epidermis is the outermost layer of the skin and consists of a stratified squamous epithelium. Its thickness varies, depending on location, and varies from 0.05 mm to 1.5 mm. The epidermis is made up primarily of keratinocytes, whose basic function is to produce a filamentous protein, keratin, to serve as a protective barrier in combination with various lipid components. These cells also produce several other proteins, for example, cytokines, which play a role in the cutaneous inflammatory response. Separated from the epidermis by the basement membrane, the dermis is composed primarily of the so-called ground substance, which includes glycosaminoglycans (GAGs) and the structural protein collagen. Its thickness also varies with location from 0.3 mm to 3.0 mm. The dermis is divided into two layers: the papillary layer, which interdigitates with the epidermal rete ridges, and the reticular layer, which extends to the subcutaneous tissue. This deepest layer of the skin, also known as the subcutis or hypodermis, is composed primarily of lipocytes with fibrous septae providing structure and support (Fig. 1.1).

Figure 1.1 Diagram of normal human skin
(Reprinted from Harry's Cosmeticology 8th Ed.2000)

PART 3.1.2 EPIDERMIS AND THE KERATINIZING SYSTEM

The epidermis measures approximately 100 micrometers in thickness and consists of a number of layers: the innermost basal layer (*stratum basale* or *stratum germinativum*) which attaches to the basement membrane at the dermoepidermal junction, the prickle layer (*stratum spinosum*), the granular layer (*stratum granulosum*), the clear layer (*stratum lucidum*) and the horny layer (*stratum corneum*). Together the basal layer and the prickle layer comprise the Malpighian layer. The viable epidermis (the basal, prickle, and granular layers) consists of about 10 biologically active keratinocytes. The stratum corneum is located on top of the viable epidermis. The stratification into the layers is the result of changes in the keratinocytes as they mature and move outward from the basal layer, in which they are continuously formed by the mitosis of self-renewing progenitor cells, which lose their nuclei and are shed from the skin surface (Fig. 1.2).

Figure 1.2. Conversion of individual basal keratinocytes into the flat cells of the stratum corneum (*Reprinted from Harry's Cosmeticology 8th Ed.2000*)

The replacement time for the whole epidermis is about 42 days and it is about 14 days for the *stratum corneum*. While the process of keratinization remains incompletely understood, in normal skin the desquamation of keratinocytes is in equilibrium with the generation of keratinocytes by mitosis of the proliferating cells. The importance of this equilibrium is best understood by studying examples of those skin diseases with abnormal keratinization. Abnormally rapid transformation of basal cells into horny cells of the *stratum corneum* occurs in psoriasis. *Ichthyosis vulgaris*, on the other hand, is a genetic disorder that results from abnormal retention of keratinocytes.

Three other cell types are present in the epidermis: melanocytes, the dendritic pigment-synthesizing cells; Langerhans cells, which are colorless and dendritic in form and perform functions related to immunology; and Merkel cells, which are concerned with sensation. The melanocytes and the Merkel cells are confined mainly to the basal layer, while the Langerhans cells are distributed in the basal, spinous, and granular layers.

a. DermoEpidermal Junction

The basement membrane zone forms the junction between the epidermis and the dermis. Under the electron microscope, it is seen to be composed of four components, listed from the outermost layer: the plasma membrane of the basal keratinocytes, the clear lamina lucida, the electron-dense basal lamina, and the dermal fibrils and bundles of fine filaments [1]. Some details of the components of this junction are provided in a later segment of this section.

b. Stratum Basale

The basal layer is a continuous layer of cells that gives rise to all the keratinocytes through mitotic activity. It is usually described as being one cell thick, but in thick normal or in pathological epidermis it appears that mitosis may not only be confined to cells in contact with the basement membrane. A portion of the basal cells is proliferative; these are the cells that differentiate and move up through the epidermis, eventually to become the components of the *stratum corneum* (essentially “dead” cells that are anuclear) and later desquamate at the top of the skin.

Cells of the *stratum basale* are cuboidal and have a basophilic cytoplasm and dark-staining, elongated nuclei; under the electron microscope their cytoplasm reveals many ribosomes, mitochondria, and in some cases smooth membranes. In addition, they contain numerous fine tonofilaments, about five nm in diameter, which form the developing cytoskeleton. The basal cells also often contain melanin, transferred from adjacent melanocytes. Intercellular bridges, or desmosomes, connect basal cells with one another and with the overlying squamous cells. Modified desmosomes, or hemidesmosomes, connect the basal cells to the underlying basement membrane zone.

c. Stratum Spinosum

The *stratum spinosum* is so called because the cells are given a spiny appearance by the numerous desmosomes. These desmosomes, or specialized attachment

plates for the cellular tonofilaments, correlate with the intercellular bridges between keratinocytes. The glycocalyx is the intercellular cement between keratinocytes and is composed of glycoproteins. In the upper region of the stratum spinosum, lamellar granules, also known as keratinosomes or Ödland bodies, make their appearance. These are ovoid bodies about 100–500 nm long. In the *stratum granulosum* they ultimately migrate toward the periphery of the cell and are discharged into the intercellular spaces. Their appearance there correlates with the degradation of keratinocytes. Their lipid contents act to establish a barrier to water loss and may participate in *stratum corneum* cellular cohesion. Water loss, as is well known, is a critical phenomenon that is measured in the development of new skin care products and relates to some aspects of skin aging.

d. Stratum Granulosum

The thickness of the granular cell layer is usually proportional to the thickness of the *stratum corneum*. It may be only one cell layer thick in thin skin and up to ten layers in the thicker skin found on the palms and soles of the feet. The cells contain basophilic granules of a material called keratohyalin, a material thought to be responsible for keratin filament aggregation. The “hard” keratins of hair and nail lack these keratohyalin granules.

e. Stratum Lucidum

The *stratum lucidum*, not seen in most formalin-fixed sections, is located at the deepest portion of the *stratum corneum*. It can be recognized only in palmar and plantar skin.

f. Stratum Corneum

In the *stratum corneum* the keratinocytes have lost their nuclei and virtually all of their cytoplasmic organelles and contents, including the keratohyalin granules. This layer of cells is about 10–15 cells thick (approximately 10 micrometers) and is located on top of the viable epidermis. The corneal cell layer stains eosinophilic with the hematoxylin and eosin stain due to the absence of basophilic nuclei. The cells are flattened and completely filled with keratin. The keratin is in the form of bundles of filaments embedded in an opaque interfilamentous material. They align into disulfide crosslinked macrofibers under the influence of filaggrin, the protein component of the keratohyalin granule responsible for keratin filament aggregation [2].

The structure of the *stratum corneum* has been compared to that of a brick wall, with the corneocytes as bricks and the intercellular lipids as mortar [3]. Horny cells are continuously shed from the skin surface. *Stratum corneum* lipids have been characterized and consist of phospholipids, glucosylceramides, cholesterol, cholesterol sulfate, cholesterol esters, ceramides, and fatty acids [4]. The makeup of lipids suggests that hydrophilic lipids are excluded from the *stratum corneum* to provide a hydrophobic surface on the skin.

PART 3.1.3 TERMINAL DIFFERENTIATION

Terminal differentiation describes the change of the cuboidal keratinocytes (on the basement membrane) to the flat cellular remnants that are shed from the skin surface. The progressive changes of keratinocytes as illustrated in Figure 1.2 are accompanied by biochemical changes, formation of keratins, formation and hydrolytic changes in lipids, loss of water, and crosslinking of cell envelopes. The formation of keratins proceeds from the intermediate filaments present in keratinocytes. Intermediate filaments of more than 50 types are synthesized in human tissues. In skin, two types (I and II) are specifically expressed in epithelial cells. In this classification, acidic keratins (cytokeratins K9–K20) are identified as type I, while the basic keratins (cytokeratins K1–K8) are classified as type II. In skin, the keratins are customarily dimers of one type I and one type II. The fundamental structure of the intermediate filaments includes coil-coil alpha-helical segments bonded to each other by nonhelical linker segments. Both ends of the rod-shaped filament are terminated by peptides. The exact modus of attachment of these filaments to each other to form the keratins within the keratinocytes is still under investigation. As noted, epithelial cytoskeletal filaments generally belong to one of two keratin (acidic or neutral-basic) groups ranging in molecular weight from about 40 to 70 kDa. Fillaggrin has been identified as one of the keratohyalin proteins forms in differentiating keratinocytes. Filaggrin is involved in the aggregation of keratin filaments to form the keratins found in mature keratinocytes or corneal cells. After filaggrin has served its function as a matrix between intermediate filaments, it is hydrolyzed enzymatically to create various free amino acids that form part of the natural moisturizing factor (NMF).

The hydrolytic changes of the epidermal lipids are also controlled by keratinocytes, which discharge lipids into the intercellular space after forming the lamellar granules. These lipids are distinctly different from the sebaceous

lipids secreted by the sebaceous glands. In the process of terminal differentiation, which requires about three to four weeks, the basal keratinocyte generates a remarkable set of complex lipids (e.g., ceramides). During the cells' passage outward, these lipids are modified (become more hydrophobic) to contribute to the biphasic structure commonly called *stratum corneum*.

During their ascent to the skin surface, the keratinocytes shrink, primarily through loss of water. The fate of this water is not known, but one may safely assume that it becomes part of the evaporating water generally described as trans-epidermal water loss. The loss of water during the maturation of keratinocytes is an important phenomenon that must be considered in studies of skin moisture levels (Fig. 1.3) and the impact of ingredients used in cosmetic and personal care products to improve the skin's ability to retain its moisture. The level of water in the basal layer is about that found in internal tissues, that is, about 80–85%. The water level drops stepwise to about 35% at the border between the *stratum granulosum* and the *stratum corneum*. The water level in the topmost layers of the skin is variable and is under the control of the environment and the evaporative flux from lower skin layers.

Figure 1.3. Water concentration profile in epidermal layers [SC—stratum corneum; GR—stratum granulosum; SP—stratum spinosum; B—basal layer (stratum germinativum. (*Reprinted from Harry's Cosmeticology 8th Ed.2000*)

Finally, the proteins in the cell membranes of the maturing keratinocytes undergo drastic changes due to crosslinking. This provides the terminally differentiated corneal cell with a rigid cell envelope that is chemically resistant and acts as a protective coating. The most important enzymes that play a role in this process are transglutaminases that catalyze epsilon- (gamma-glutamyl) lysine crosslinking. Involucrin is the primary cytoplasmic precursor to the protein making up the cell envelope. Other crosslinked proteins are present and have been identified—for example, loricrin.

Biologists have further identified some layers within the *stratum corneum*.

The desquamating layer at the surface is frequently called the *stratum corneum* disjunction, while the layer below it is known as *stratum corneum* conjunction. To complete this highly simplified discussion of the skin, it is important to note that the latter is frequently identified as viable epidermis. Cells from this layer can be cultured and are commonly used to study the release of cytokines and the like as well as the impact of drugs and/or cosmetic ingredients. In contrast, the nonviable epidermis includes only the dead cells of the *stratum corneum*.

Before leaving the life history of the epidermis, the process of differentiation should be considered as proceeding from the inside to the exterior. The outward movement of biological debris, water, and lipids directly opposes human efforts to drive ingredients/drugs into and through the skin unless special efforts are made to create molecules that are shaped and manipulated to permeate.

PART 3.1.4 PIGMENTARY SYSTEM OF THE SKIN

Melanocytes are dendritic cells that produce and secrete melanosomes, which contain melanin. Melanin is the major determinant of skin color. The number of melanocytes in the epidermis is the same—regardless of skin color; it is rather the number and size of melanosomes produced that determine the color of one's skin. Melanosomes in dark skin are nonaggregated, whereas they are smaller and form membrane-bound complexes in light skin. Melanocytes are derived from the neural crest in the embryo and are seen in the basal layer of the epidermis by the eighth week of gestation. They differ from the other cells of the *stratum basale* by the possession of dendritic processes, by which they transfer pigment to a group of keratinocytes; the whole forming the "epidermal melanin unit" [5]. Typically each melanocyte is associated with about 36–40 keratinocytes in the human epidermis. Melanocytes have no desmosomes and thus, when stained with hematoxylin and eosin, appear to have a halo due to the separation from adjacent keratinocytes. The concentration of melanocytes, though, does vary in different areas of the skin, with the highest concentration on the face and the male genitalia and the lowest concentration on the trunk.

The characteristic feature of melanocytes is a special cytoplasmic organelle known as a melanosome in which the melanin is formed by the action of the enzyme tyrosinase. The melanosomes arise as spherical, membrane-bound vesicles in the zone of the Golgi apparatus and eventually appear as densely pigmented granules [6].

Melanins consist of two kinds of quinoid polymers: pheomelanins and

eumelanins. Pheomelanins are yellow or red in color, and eumelanins produce the brown or black color. Both are formed by the same initial steps, which involve oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and its dehydrogenation to DOPAquinone. The formation of eumelanins then involves several further steps to produce indole-5,6-quinone, which polymerizes and becomes linked to protein. It is now believed that eumelanin is not a homopolymer composed solely of indole-5,6-quinone units but rather a poikilopolymer that includes several intermediates. Pheomelanins are formed by a different route. The DOPAquinone interacts with cysteine to form 5-S- and 2-S-cysteinyldopa, and these isomers are further oxidized to a series of intermediates that then polymerize [7].

The formation of melanin depends on the generation of free radical species. The biochemical pathways leading to the formation of melanin pigments *in vivo* were described by Raper [8] and more recently by Prota [9, 10]. This information can be found in most textbooks of biochemistry and is not repeated here. Once formed, melanin has been identified as a (stable?) free radical that can react with superoxide.

The significance of melanin as a purported photoprotectant and the response of skin to ultraviolet (UV) irradiation are critically important to skin appearance and health, especially with regard to sun exposure.

PART 3.1.5 LANGERHANS CELLS AND DENDRITIC CELLS

The skin provides the first line of immune surveillance against biologic pathogens and chemical irritants through a network of sophisticated and specialized antigen-presenting cells. These include Langerhans cells (LCs) in the epidermis and several distinct types of interstitial dendritic cells (DCs) in the dermis. LCs and DCs are the sentinels that recognize certain antigens as well as recognizing tumors. For example, they recognize foreign environmental antigens such as irritating chemicals (thereby inducing contact hypersensitivity), microorganisms (thus protecting through antimicrobial immunity). They also recognize tumors (providing antitumor immunity) and allogeneic markers (causing skin graft rejection).

The extended dendrites of these unique cells form a network within the epidermis and in the dermis they create an immunologic barrier primed to recognize, capture, and process pathogens and foreign antigens for presentation to naïve and memory T-lymphocytes, thereby initiating and then modulating the

subsequent immune response. Furthermore, the LCs and DCs play a significant role in inducing peripheral immunologic tolerance to innocuous environmental proteins and to self-antigens, thus helping to prevent autoimmune disease.

LCs are the epidermal resident dendritic cell population. They make up about 3–5% of the suprabasal, *stratum spinosum* layer of the epidermis [11] including the mucosal epithelia lining the ocular oral and vaginal surfaces [12]. LCs are scattered without desmosomal connections between keratinocytes. In light microscopic sections, LCs cannot be detected by routine histologic staining, but only by staining with gold chloride (for human LCs).

LCs are characteristically stellate with extensive dendritic fibers stretching in all directions, forming a network within the *stratum spinosum* layer. When viewed by phase contrast microscopy, the DCs extend delicate flower-like projections that bend, retract, and reextend. This shape and motility enable them to accomplish their function of capturing antigens and of selecting and processing the antigens for specific T-cells [13]. By means of high-powered electron microscopy, LCs have been shown to be a folded nucleus and are identified by their distinctive, intracytoplasmic *Birbeck granules* that (when mature) are rod shaped with a vacuole at one end, resembling a tennis racquet. Antigen capture seems to be a consequence of binding to langerin, a calcium-dependent lectin that specifically binds mannose as well as other sugars and is found only in LCs and some dermal DCs [14]. This binding actually induces formation of Birbeck granules and then routes the antigen into the Birbeck granules for further processing.

Langerin is a recognition receptor for carbohydrates on the surfaces of pathogens that routes antigens to different cellular compartments involved in antigen presentation in order to promote recognition by different specific T-cells [15].

In addition to LCs, the skin has at least four other subclasses of DCs in the dermis. The characterization of these DCs is quite complex because DCs can arise from several types of progenitor cells, and different functional phenotypes of DCs can be generated from the same precursor cell (as determined by function required, generating a sequential encounter with specific cytokines). Furthermore, as the LCs and DCs encounter a foreign antigen and migrate, they mature, and their surface markers as well as their functional interactions change. Thus, the steady-state population is different from the inflammatory response populations.

Langerhans cell function is impaired by UVB radiation, resulting in a decrease in the antigen-presenting capacity and in the production of cytokines. This UV suppression leads not only to adverse consequences by impairing endogenous antimicrobial activity and tumor recognition and suppression, but also to possible advantageous therapeutic treatments for skin allergy and inflammatory disease.

PART 3.1.6 DERMIS

The dermis is a tough and resilient tissue that cushions the body against mechanical injury and provides nutrients to the epidermis and cutaneous appendages [16]. It consists of an association of protein fibers within an amorphous ground substance containing glycosaminoglycans (GAGs), previously known as mucopolysaccharides. There are few cells in this matrix; most of them are fibroblasts, which secrete the dermal constituents. Fibroblasts are derived from the mesenchyme. The mast cell, also of mesenchymal origin, houses granules that contain heparin, histamine, and other active substances. The mast cell is an active participant in skin inflammation and irritation, as well as in several other skin disorders. The dermis also houses blood, contains lymphatic and nervous systems, and surrounds the invaginated epidermal appendages. The GAGs in the dermis can hold copious amounts of water and tend to surround the other constitutents of the matrix. Together with the fibrous portion of the matrix, these substances account for the skin's flexibility and resistance to deformation.

a. Collagen

The major fibrous constituent of the dermis, accounting for 75% of the dry weight and 18–30% of the volume, is collagen [17]. Under the light microscope, collagen fibers appear as eosinophilic branching wavy bands. Collagen fibers are loosely arranged in the papillary dermis and are tightly bundled in the reticular dermis. Pilosebaceous units, eccrine glands, and apocrine and dermal blood vessels are surrounded by a thin meshwork of collagen. Collagen fibers display characteristic cross-striations with a periodicity of 60–70 nm. Collagen is rich in the amino acids hydroxyproline, hydroxylysine, and glycine. The fibroblasts produce a precursor known as procollagen, which includes 300–400 additional amino acids in each of its chains; these extensions are removed after secretion, which results in the conversion to the collagen molecule. Collagen fibrils form by the association of collagen molecules. Vitamin C and copper are two of

several cofactors required in the biosynthesis of collagen. Collagen production is a dynamic process that involves continual synthesis by fibroblasts and degradation by collagenases.

b. Elastin and Reticulin

Elastic fibers make up only 4% of the dry weight and 1% of the volume of the dermis. They are delicate, straight, freely branching fibers that prove very resilient. These fibers are thicker in the lower portion of the dermis and become thinner as they approach the epidermis. Elastin differs from collagen not only structurally but also chemically. Desmosine is an amino acid unique to elastin. About 0.4% of the dry weight of the dermis is made up of fine branching fibers that, unlike collagen, stain black with silver stains and are known as reticulin. Their axial periodicity is identical to that of collagen. Reticulin fibers in the papillary dermis serve to anchor the basal lamina [17].

c. Ground Substance

The amorphous ground substance in which the fibers and cells lie contains acidic GAGs. In dermis the major forms are hyaluronic acid, chondroitin sulfate, and dermatan sulfate.

d. Nerves

The skin is supplied with both sensory and autonomic nerves. It is innervated with about one million afferent nerve fibers; most terminate in the face and extremities, and relatively few supply the back. The sensory nerves, unlike autonomic nerves, possess a myelin sheath up to their terminal ramifications.

The papillary dermis is heavily innervated with unmyelinated nerve fibers that transmit the sensations of temperature, pain, and pruritus. Three types of special nerve end organs also exist in the dermis. Vater-Pacini corpuscles are large end-organs that are located in the deeper portions of the dermis and subcutis and mediate a sense of pressure. They measure up to 1 mm in diameter and have their greatest concentration at the tips of the fingers and toes. A few are present in the nipple and anogenital regions. Meissner corpuscles are located in the dermal papillae and mediate the sense of touch. They occur only on the ventral aspects of the hands and feet and are most concentrated in the fingertips. The mucocutaneous end-organs are found in the papillary dermis of the modified hairless skin of the glans, the prepuce, the clitoris, the labia minora, the perianal region, and the vermilion border. The autonomic nervous system supplies fibers

to the arrector pili muscles, the blood vessels, and the eccrine and apocrine glands. The sebaceous glands are not innervated, and their functioning depends on endocrine stimuli. The autonomic nervous system controls vasoconstriction, contraction of the arrector pili muscles, and glandular secretion.

e. Vasculature

The dermal vasculature consists of intercommunicating plexuses. The subpapillary plexus lies within the papillary dermis and runs parallel to the epidermis to furnish a supply of capillaries, arterioles, and venules to the dermal papillae. The deeper plexuses are composed of larger vessels and surround hair follicles and eccrine glands. The dermal lymphatics are associated with the vascular plexuses.

f. Muscles

Smooth muscle occurs in the skin as the arrector pili muscles of the hair to pull the follicle upward with contraction. There are also smooth muscles fibers in the scrotum and the areolas. Striated muscle occurs in the skin within the neck as the platysmas and in the muscles of expression of the face. Special aggregates of smooth muscles are found between the arterioles and the venules in the skin. These serve to shunt blood from the arterial to the venous system directly and thus bypass the capillary system.

PART 3.1.7 APPENDAGEAL STRUCTURES

a. Eccrine Sweat Glands

Humans have several million eccrine sweat glands distributed over most skin sites, but they are more concentrated in the axilla, forehead, palms, and soles. In some areas they number as many as 600 glands per square cm. Eccrine sweat glands are the most numerous skin appendages and are responsible for the production of sweat. They have a cylindrical spiral duct lined with epidermal cells extending from their visible opening in the epidermis down into the deep dermis, where the duct becomes coiled and convoluted into a ball [18]. This secretory coil manufactures the odorless sweat, which rises up the duct to be released on the skin surface. It is thought that the duct of the gland has the ability to modify the sweat as it flows upward by removing salts or water.

The sweat glands control both body temperature and excretion, and they are

under the control of the cholinergic nervous system. The evaporation of sweat has a cooling effect. The glands respond to environmental temperature but also to other stimuli, such as UV light, emotional stress, and increases in body temperature. On the palms and soles, the secretion from the glands serves to increase surface friction.

Sweating appears to involve activation of myoepithelial cells, which line the ducts of the glands. Although sweating is considered to be a continuous process, it seems that sweat is ejected in small bursts, suggesting a peristaltic action by the ducts. The composition of eccrine sweat is similar to that of plasma, although more dilute, and was documented about 40 years ago. It includes, in decreasing relative concentration (mg %): Cl^- (320), Na^+ (200), lactic acid (35), K^+ (20), urea (15), ammonia (5), Ca^{++} (2), glucose (2), Mg^{++} (1), amino acid (1), and creatinine (0.3).

b. Apocrine Glands

The apocrine glands are tubular glands attached to the hair follicle and, like the sebaceous glands, develop in association with it [19]. Although rudiments are found covering the entire surface of the fetus, the glands become canalized and functional almost exclusively in selective areas including: the axillae, the anogenital regions, the areola, the external auditory canal, and the eyelids. In humans, apocrine gland secretions are milky and viscous but without odor. Odor production is related to bacterial action on apocrine secretions at the skin surface.

After puberty, apocrine gland secretion is in response to emotive stimuli. Adrenergic nerves control secretory activity, in contrast to the cholinergic control of eccrine function. The function of the glands in the human species has been much debated; studies have demonstrated that the apocrine skin glands of mammals produce various glycoconjugates and antimicrobial substances, which may serve as a nonspecific defense on the skin surface [20]. In other mammals the glands serve a sexual function.

c. Sebaceous Glands

Sebaceous glands [18] secrete sebum, which forms the majority of the lipid that covers the skin and hair. They are found in all areas of human skin except the palms, soles, and dorsum of the feet. Sebaceous glands are usually associated with hair follicles, except for those on the nipples, areola, and labia minora. The greatest concentrations (reportedly as high as 400–900/cm) are found on the

scalp, face, upper chest, and shoulders.

The glands are holocrine and thus form their secretion by decomposition of their cells. New cells are formed continually from the lining of the gland by cell division to replace those lost. No motor innervation has been demonstrated in humans. During the generation of sebum, cells at the periphery of the lobule undergo division. As the daughter cell moves toward the center of the lobule, it synthesizes lipids. As the sebum accumulates, the cell increases in volume as much as 150-fold. When synthesis is complete cell rupture occurs. This process from cell division to rupture requires approximately 14 days. The relatively long delay must be taken into consideration when designing drugs, cosmeceuticals, and therapies aimed at altering sebum.

Sebaceous gland activity is under hormonal control. It is stimulated by androgens of both gonadal and adrenal origin. In human males the glands are minute during the prepubertal period but undergo vast enlargement at puberty, when their output increases more than fivefold. Eunuchs secrete about half as much sebum as normal males but substantially more than boys; it seems that the secretion is dependent on adrenal androgens. Adult women secrete only a little less than men; their sebaceous activity appears to be maintained by androgens from the ovary and the adrenal cortex. Estrogens and anti-androgens, such as cyproterone acetate, inhibit sebaceous secretion in humans. On the other hand, relatively small doses of potent androgens can cause enlargement of the glands and an increase in sebum production.

Human sebum is composed of triglycerides (57.5%), wax esters (26.0%), squalene (12.0%), free fatty acids (10%), and, to a minor extent, cholesterol and cholesterol esters. Epidermally derived lipids differ in lacking wax esters and squalene and having much higher proportions of cholesterol esters and cholesterol. There are marked differences in sebum composition among species. The origins of sebaceous lipids and their composition are different from those of the epidermal lipids [21]. The purpose of sebum is not entirely understood, but sebum has been shown to have thermoregulatory functions, and plays a role in the regulation of immunological functions and inflammatory processes. In addition, sebum contains lipids with antimicrobial activity [22]. While excessive sebum production has been associated with the development of acne vulgaris, lack of sebum production in prepubertal children is not associated with any skin abnormalities.

PART 3.1.8 HAIR

The hair follicle is quite variable, depending on its location. In adults, deep terminal hairs are found on the scalp and male beard area. Hair on the extremities and trunk is located more superficially in the skin. Vellus hairs are present on the female face and on the nonbearded areas of the male face. Lanugo is soft fine hair that covers the fetus and is shed prior to birth.

In general, the cross-sectional shape of terminal Caucasian scalp hair is round and somewhat curly; African American scalp hair is oval, sometimes flattened, and usually kinky; hair in Asians is round in cross-section and straight. These relationships do not apply to pubic hair, beard hair, and eyelashes, which have similar features in all races and are typically oval.

Hair color is due to the distribution of melanosomes within the hair shaft produced by melanocytes in the hair bulb. These are transferred to cells of the hair matrix similar to the transfer from melanocytes to keratinocyte in the epidermis. Three types of melanosomes are present in hair. Eumelanins are seen in dark hair, and pheomelanins predominate in blond hair. The intensity of color is related to the number of fully melanized melanosomes produced. Gray hair and white hair are due to a decreased number of melanocytes that produce fewer melanosomes.

Despite the vast body of knowledge regarding the anatomy, biology, and function of hair, humans are still unable to induce hair regrowth in the many disorders that result in hair loss, or to induce permanent hair removal in states of excessive hair. The cost of hair care in terms of time and money spent is huge in many cultures, and the psychological impact of hair disorders should not be underestimated.

PART 3.1.9 NAILS

The nail plate is composed of keratinized cells that originate in the nail matrix. As discussed previously, there are no keratohyalin granules. The proximal nail fold forms the cuticle. The nailbed does not contribute to the development of the nail plate but serves as a base for the adherent keratinous nail plate.

PART 3.1.10 BARRIER FUNCTION AND PERMEABILITY

For the cosmetic industry, by and large, the skin has been synonymous with the epidermal permeability barrier, located in the *stratum corneum*. This is a

somewhat restricted view, but nevertheless remains in place due to the fact that skin moisturization—a function that is of prime interest to the industry—is dependent on a functional permeability barrier. As the saying goes, a good moisturizer is not necessarily a barrier product, but all barrier products are good moisturizers. The two-compartment, "brick and mortar" organization of the healthy *stratum corneum*, with its organized lipid membranes, do "lock in" moisture and prevent dry, chapped skin. However, the significance of other types of barriers in the skin, i.e., UV barrier, antioxidant, and antimicrobial defenses have all begun to be appreciated and emphasized, as evidenced by the proliferation of active ingredients targeted to them. This growing recognition, in concert with an improved understanding of the other areas of the skin, is critically important to novel thinking leading to new functional ingredients for this important cosmetic and personal care category.

a. The Barrier Property of Skin: A Historical Perspective

The barrier property of skin against influx and efflux of water was acknowledged as early as the 1800s, but the conclusive evidence was provided by the work of Irvin Blank and Scheuplein, who convincingly demonstrated its location in the *stratum corneum* (horny layer) [23]. The barrier properties are of interest to different scientific disciplines and industry; especially the pharmaceutical and personal care sectors that are interested in delivering drugs transdermally or active ingredients for topical delivery to improve the skin conditions (anti-aging, moisturizers, skin lighteners, etc.) respectively. For the dermatologists, inherited and acquired skin diseases such as various types of ichthyosis, atopic dermatitis, and psoriasis all have a compelling barrier dysfunction associated with it [24]. Extensive damage to the barrier, as in third-degree burns, can be lethal; attesting to the significance of a functional permeability barrier to prevent life-threatening dehydration of the body.

Details of barrier (and *stratum corneum*) formation, its structural complexity, and chemical characterization

Early investigators interpreted what appeared as a "basket weave" structure of the SC in routine histological studies as an indication that it is a passively occluding structure on the skin surface—a more or less uniform membrane functioning like "Saran wrap." Investigators who followed them have dispelled the notion of the SC as a "basket weave" structure passively occluding the surface. A battery of investigative techniques (electron microscopy, laser confocal microscopy, two-photon microscopy, as well as biochemical

characterization of lipids and proteins) have led to the understanding first of a "brick and mortar" organization of SC, and a later concept of the SC as being a composite material with several characteristics of a "smart" material [24]. In this latest model, the bulk of the SC is made up of corneocytes, which are the "bricks."

Before discussing the secretion of the lamellar lipids (pro-barrier lipids) and the further processing of the lipids in the SC extracellular domains (the "mortar"), a brief description of a multiplicity of the phenomena that occur in the SC is warranted.

These phenomena include: a description of the keratinocyte differentiation, synthesis of proteins (keratins, filaggrin, involucrin, loricrin, desmosomal components, enzymes involved in cornification) and lipids (which are packaged with lipid metabolizing enzymes and antimicrobial peptides within secretory organelles) in the form of lamellar bodies (Odland bodies, keratinosomes).

Keratinocytes (keratinizing epithelia) account for about 95% of the stratified epidermis (other cell types are melanocytes, Langerhans cells, Merkel cells), and based on its position within the strata as well as its progressive differentiation, is designated as belonging to several layers including: the basal layer (*stratum basale*), the spinous layer (*stratum spinosum*), the granular layer (*stratum granulosum*), and the cornified layer (*stratum corneum*). Epidermal stem cells that usually contribute to the maintenance of the interfollicular epidermis are located within the stratum basale. Their division (either symmetric or asymmetric) replenishes the keratinocyte population as cells from the basal layer move to the suprabasal layers and progressively undergo differentiation.

Stratum basale is made up of one layer of columnar basal cells attached to the basement membrane via hemidesmosomes. These cells display a high nucleo-cytoplasmic ratio, cell organelles like mitochondria and RER, as well as keratin filaments inserting into the hemidesmosomes. The cells also connect to the adjacent cells as well as those of the suprabasal cells via desmosomes. Biomarker proteins for the basal cells are keratins K14 and K5.

The *spinous* or prickly layer (*stratum spinosum*) above the *stratum basale* is named for its characteristic histological appearance due to the large numbers of desmosomes. In addition to the typical cell organelles, these cells often display a few lipid-enriched epidermal lamellar bodies (Odland bodies, keratinosomes, membrane coating granules). These will be described later in this section.

The biomarkers that distinguish this layer from the basal layer are keratins K1

and K10. *S.spinosum* also shows an increase in keratin filament content compared to *S.basale*. In the upper layers of *S.spinosum*, the cells begin to elongate and become slightly flattened. Above this layer is the *stratum granulosum*, a few layers of cells that are characterized by the presence of distinct, darkly staining (histological preparations) keratohyalin granules (KHK), composed of profilaggrin, loricrin, and a cystine-rich protein as well as keratins 1 and 10. The filaggrin subunits of profilaggrin play the role of matrix molecules to aggregate and align the keratin filaments.

In the upper granular layers of *stratum spinosum*, the keratin filaments are highly phosphorylated and have an extensive population of disulfide bonds compared to the cell layers below. As the cells of *stratum granulosum* stratify and differentiate, both the protein and lipid synthesis is ramped up, as evidenced from the boost in involucrin, KHKs, and LBs. In fact, the uppermost SG cells show an abundance of LBs (about 20% of the cell volume), which are apically polarized and poised to be secreted out. These details are not seen in histological preparations, but electron microscopy reveals both the internal structure of LBs, as well as tortuous and complex invaginations of the apical cell membrane at the *stratum granulosum–stratum corneum* interface. Secreted contents of LBs fill the interstices between the SG, and the overlying layer of *stratum corneum*, which still remain connected by means of desmosomes. The transition between the granular cell and the corneocyte presumably occurs within the span of a few hours, in response to several signals that involve but are not limited to an influx of calcium ions. The calcium signaling activates Ca++ dependant transglutaminase, which catalyzes the crosslinking of involucrin and loricrin. This process then forms a toughened “cornified envelope” immediately subjacent to the cell membrane. The phenomenon is also accompanied by dissolution of the nucleus, as well as cytosolic organelles, mediated by caspases, especially caspase 14. In fortuitous sections, electron microscopy captures the images of the “transitional cell,” while the images primarily display an abrupt transition between the nucleated SG and the non-nucleated, flattened, organelle-depleted and keratin-filled corneocytes (Figure 1).

Figure 1 (Conventional TEM shows terminal differentiation of nucleated keratinocytes to keratin filled, non-nucleated corneocytes)

Conventional electron microscopy has illustrated the nature of the SC organization (Reference Fig 1), which consists of the secretion of epidermal lamellar bodies (enriched in cholesterol, sphingolipids, fatty acids, and a battery of enzymes and antimicrobial peptides) at the *stratum granulosum–stratum corneum* interface. Hydrophilic tracers (such as colloidal lanthanum) injected subcutaneously have been used to visualize its efflux (and hence of water) through the epidermis. This efflux is blocked at the SG–SC interface where the LB contents occlude the extracellular space [25].

The lamellar bodies are organelles, ranging from 0.2 to 0.5 microns in diameter, which originate from the Golgi apparatus, and begin to appear from the *stratum spinosum* and increase in numbers towards the upper *stratum granulosum*. In the uppermost SG, they occupy nearly 20% of the cell volume, and are polarized towards the apical surface. A recent proteomic investigation [26] identified up to 985 different proteins within the LBs. Freeze fracture studies [27] as well as ruthenium tetroxide post-fixation techniques [28, 29] have unraveled the sequence of events that follow the secretion of epidermal lamellar bodies (Figure 2) (i.e., the fusion of disk-like contents of the LBs into multiple lamellae that occlude the intercellular spaces of the SC). Cytochemical localization, as well as experimental studies with various enzyme inhibitors, delineated the role of enzymes (lipases, phospholipase, sphingomyelinase, B glucocerebrosidase, cholesterol sulfatase) in conversion of these disks into compact lamellar structures that surround the corneocytes akin to the mortar in a brick (corneocytes) wall [4, 30-35]. Investigations on animal models with specific gene defects or knockouts further extended the knowledge on enzymes such as B glucocerebrosidase [36], stearoyl CoA desaturase (SCD) [37], and

Elov 14, which are involved in elongation of long-chain fatty acids crucial for ceramides [38, 39] as critical to barrier competency. In addition, proteolytic enzymes like kallikreins that lead to progressive dissolution of the corneodesmosomes for orderly desquamation of corneocytes [40, 41] and antimicrobial peptides such as beta defensins and cathelicidins [42] are co-localized to the lamellar bodies and within the extracellular matrix of SC, linking the formation of permeability barrier with an antimicrobial barrier [43] and innate immunity.

Figure 2 (Arrow indicates transformation of secreted LB contents to tight lamellae in upper SC)

The epidermal lamellar bodies initially considered to be discrete organelles (0.2 to 0.5 micrometers in diameter) were later found to form an interconnected network within the cells of the *stratum granulosum* [44], enabling a well-coordinated secretion in response to acute barrier disruption, as in tape stripping. Conventional TEM of a lamellar body in perfect cross-section shows the disc structures well, but not all LBs show such crisp morphology, a fact thought to reflect the plane of sectioning (oblique sections or grazing sections along the outer surface). LBs deficient in internal contents ("motheaten appearance") characterize a variety of skin dysfunctions [25], which correlate with permeability barrier defects and increased TEWL. Lamellar bodies are also crucial to the formation of "acid mantle" of the SC, which is of considerable interest to the cosmetic and personal care industry. These fascinating organelles consist of something resembling a cross between a lysosome and a secretory vesicle. They transport and secrete a diverse variety of molecules including: lipids, lipases, proteases, protease inhibitors, beta-glucocerebrosidase, antimicrobial peptides, etc.). An exhaustive proteomic and immunocytochemical analysis of lamellar-body enriched fractions and skin [26] identified about 985

proteins from the LB-enriched fraction: some secreted proteins, those with potential roles in desquamation, lysosomal proteins, related to transport and secretion of LBs, as well as proteins with putative roles in sorting of the LB cargo. It is possible that several subpopulations of LBs exist, and that the morphological diversity seen in electron micrographs may indeed reflect the pleomorphism of LBs, relating to either their contents or functional status. For example, hBD-2, which is virtually absent in normal skin keratinocytes, is induced when stimulated by cytokines or bacteria, and are localized to LB [45]. Most probably, some LBs synthesized in response to the bacterial challenge (LBS designed for microbial barrier?) must have a disproportionate content of the antimicrobial peptide (AMP), and it was speculated that pro-barrier lipids may not be the predominant components of this subpopulation [46]. They also may look different (deficient in lipids) or imperfect under the electron microscope. Could this be the basis of some of the abnormal, deficient LBs seen in various skin diseases such as psoriasis where keratinocytes show a high expression of AMPs? The study by Raymond *et al.* [26] showing 31 LB proteins corresponded to lysosomal components suggest that LBs are a new class of secretory lysosomes. Hence on a broad biological basis, they could be considered as “exolysosomes” involved in the matrix remodeling of the SC, or its exfoliation (the same way that lysosomes function in the molt of crustacean integument).

b. Stratum corneum

As the major barrier to flux of chemicals, water and other molecules into the skin, SC penetration pathways have attracted considerable attention. Since the SC is a two-compartment, composite system, three different routes of penetration have been proposed: transcellular, intercellular, and trans-appendageal (follicular) pathways (see for more details in the next section of this chapter). Much of the experimental results support the follicular and the intercellular pathway models. The intercellular pathway through the lipid domains is a tortuous path, and possibly both nonpolar and polar pathways are localized to this region. A true transcellular pathway (envisioned as a network of microor nano-channels) may only be opened up with the aid of microneedles or power-jet (gene gun) types of devices. The polar pathway may be located intercellularly and comprises aqueous regions surrounded by polar lipids, which create the walls of such microchannels as surmised by results of experiments designed to elucidate the contribution of extracellular lipids and intracellular

keratin to the structure of this pathway. This has been demonstrated by means of using baclofen, a model zwitterion, *in vitro* using human cadaver skin [47]. This contention is in conformity with the morphological characterization of a "pore pathway" through the mortar lipids, as proposed by Menon and Elias [48].

c. Other Barriers:

Other effective barriers in skin are antimicrobial barriers (AMP-mediated), immune barriers (innate and adaptive) mediated by Langerhans cells, oxidant barriers (keratinocyte and sebocyte derived antioxidants), and barrier to UV radiation (melanin-mediated). These diverse barriers are interdependent components of the skin barrier, as exemplified by facts that the permeability barrier recovery is faster/more efficient in darkly pigmented skin; the Langerhans cells are affected by a breach in the permeability barrier; and antimicrobial peptide synthesis is also up-regulated by challenges to permeability barrier.

As the tight junctions are also crucial for epidermal permeability barrier (the knockout models develop defective epidermal lipid synthesis and secretion), it is interesting to note that the Langerhans cells have been reported to extend their dendrites through the TJs, during the "search" mode when they scan the sub-SC domains for any sensitizers that have crossed the primary barrier. Such phenomena have only recently gained the attention of scientists and the appreciation of the cosmetic and personal care industry.

d. Penetration of Cosmetic Actives Through the Stratum Corneum

One of the most recurring debates in cosmetics has to do with the penetration of ingredients. How do manufacturers ensure optimal and targeted penetration of ingredients for efficacy at specific locations within the structure? What is the optimal penetration? How do water, lipophilic molecules, liposomes, or nanoparticles penetrate (if at all) through the *stratum corneum*, deep in the epidermis and below? From a scientific point of view, these questions are imperative towards the industry's goals of achieving improvements in compromised skin or anti-aging effects. We point out that the (global) regulatory definitions and distinctions drawn between "drugs" and "cosmetics" are carefully excluded in this scientific discussion and merely note that if one is to actually impact the multiplicity of biological phenomena occurring in the skin, then, inevitably, the schism between the legal definitions of "drug" and "cosmetic" must merge into a single "zone," and the difficulties in defining the

zone are indeed a challenge for all concerned.

In this section, we will take a somewhat different approach to exploring the question of penetration. We will focus on the *stratum corneum*, the outermost layer of human skin, which optimizes the delivery and efficacy of topical products in the skin. We will investigate now only how molecules are indeed able to penetrate this outermost layer, but also focus on the fact that even if they penetrate no deeper, they play an essential role in skin health and youthfulness.

As described above, the *stratum corneum* is the outermost layer of the skin. It consists of two distinct structural components: corneocytes and the intercellular lipid matrix. The corneocytes are differentiated keratinocytes (i.e., anucleated cells without intracellular organelles such as nuclei or mitochondria). They provide the structural support for the *stratum corneum* and act as a barrier against environmental aggression. Corneodesmosomes interconnect corneocytes as desmosomes interconnect keratinocytes in the epidermis. Essential corneodesmosomal proteins include desmocollin and desmoglein. The intercellular lipid matrix is located between corneocytes. The lipid species are composed of ceramides, fatty acids, cholesterol, and glucosylceramide derivatives. These lipids are synthesized by the keratinocytes in the upper layer of *stratum granulosum*. The covalent bonds between the ceramides of the intercellular lipid matrix and the structural proteins of corneocytes membrane create, through ester bonds, a dense and impermeable network [49].

Having identified what the *stratum corneum* is, let us now turn to the question at hand, namely: How do molecules of different sizes and forms, which have different physicochemical properties, penetrate into the *stratum corneum*?

Penetration

In general:

As discussed above, there are various ways for molecules (using passive diffusion) to penetrate the *stratum corneum*: intercellular, transcellular, intrafollicular, and polar. The intercellular pathway allows for a sinuous penetration of molecules between the corneocytes, through the intercellular lipids. The gaps between the intercellular lipids, which enable for penetration of molecules, measure 0.013 μm. This intercellular pathway is how most hydrophilic and lipophilic molecules penetrate through the *stratum corneum*.

The **transcellular pathway** involves corneodesmosomes creating bridges between cells and becoming sufficiently amphiphilic to enable transportation of molecules. The transcellular pathway is how amphiphilic molecules, as well as small other molecules, penetrate through the *stratum corneum*. These substances are able to integrate at the double layer of phospholipids constituting the cell membrane and can penetrate into the corneocytes. The speed of penetration by this pathway is very slow, but it is offset by the fact that the surface area is significant.

The **intrafollicular pathway** enables penetration through hair follicles (which account for 1 to 2% of the total skin surface). As the pilosebaceous follicles are located in deep invaginations of the epidermis, in the dermis, they lead molecules to the reticular dermis. Furthermore, hair follicles do not have a highly developed *stratum corneum*, which further facilitates penetration at this level. This intrafollicular pathway is how large molecules, which are typically highly insoluble, penetrate through the *stratum corneum*.

Finally, the **polar pathway** enables penetration of molecules through the polar pores. These pores are present between cells and are surrounded by polar lipids, which generate discontinuities in the organization of the lipids. This pathway is hydrophilic by nature. For example, sucrose penetrates into the epidermis this way.

Figure 1: Various pathways of penetration through the *stratum corneum*. There are various pathways for molecules to penetrate the *stratum corneum*: intercellular (penetration between the corneocytes through the intercellular lipids), transcellular (penetration through the corneocytes), intrafollicular (penetration through hair follicles), and polar (penetration through the polar pores).

Penetration of a substance through the skin depends on many parameters that can interact with each other. These parameters are biological factors such as skin condition and age, the site of application, and skin hydration. Physicochemical factors, in particular those related to the substances themselves, also play a role, for example the size of the molecules or their attraction with the surface of the skin. These different pathways are passive diffusions through the *stratum corneum*. Only small molecules (molecular weight below 500 Da) penetrate through the pathways. It should be noted that the vehicle that carries the active ingredient can facilitate the penetration through the *stratum corneum*.

Penetration of water into the *stratum corneum*

The *stratum corneum* is virtually impermeable except for a small quantity of water that is delivered to it by the epidermis. This transferrable water hydrates the SC's external layers, helps maintain its flexibility, and enables adequate enzymatic reactions and corneodesmolysis. The passage of water in the other direction, from the body through the skin into the outside environment, is called trans-epidermal water loss or TEWL. Transepidermal water loss is influenced by the concentration of water in the epidermis, by cellular integrity, by relative humidity, by diffusivity of water in the *stratum corneum*, and by the thickness of the *stratum corneum*.

The ability to maintain water in the skin depends on the arrangement of corneocytes, the composition and structure of intercellular lipids, and the

presence of naturally moisturizing factors. Naturally moisturizing factors are made of water-soluble compounds and absorb water from atmosphere; they are typically amino acids or other chemical substances that hold water and rehydrate the *stratum corneum*. Naturally moisturizing factors are formed during the epidermis differentiation and represent 10% of corneocyte mass.

Penetration of lipophilic molecules into the *stratum corneum*

Lipophilic molecules of low molecular mass enter the *stratum corneum* essentially by the intercellular pathway (a sinuous penetration between the corneocytes through the intercellular lipids). These intercellular spaces are composed of lipids providing the last step of epidermic differentiation (such as free or esterified sterols, free fatty acids, triglycerides, sphingolipides), arranged in oriented bilayers bounding hydrophilic and lipophilic areas. The higher the concentration gradient of cutaneous lipids in lipid bilayers, the lower the penetration speed of hydrophilic molecules will be. Hence, by increasing the fluidity of cutaneous lipids, penetration can be facilitated.

Penetration of liposomes into the *stratum corneum*

Liposomes are lipid vesicles that can be used as carriers for hydrophilic substances solubilized into aqueous domains, or lipophilic substances solubilized into lipidic membranes. These colloidal carriers are formed as concentric bi-molecular layers of phospholipids. The presence of cholesterol may improve the bilayer characteristics of liposomes by increasing the microviscosity/interfacial rheological behavior of the bilayer, thereby reducing the permeability of the membrane to water-soluble molecules, or stabilizing the membrane and increasing the rigidity of the vesicle.

Liposomes represent an alternative method to enhance the permeation of substances through the skin. There are five possible mechanisms for liposomes to penetrate into the *stratum corneum* [50]:

1. Liposomes release the active substances that then penetrate independently into the skin;
2. Liposomes are first absorbed and then fused with the skin's surface and constituents, then change the ultrastructure of the intercellular regions in the deeper layer of the *stratum corneum*. This decreases the impermeability of the *stratum corneum* and enhances the penetration of the encapsulated molecule [51];
3. Liposomes gravitate to the surface of the *stratum corneum*, resulting in a direct transfer of the molecule encapsulated from the liposome to the skin.

Vesicles could also fuse and mix with the lipids matrix of the *stratum corneum*, increasing the distribution of molecule into the skin [52];

4. Liposomes penetrate into the skin as an intact form. Although it is now generally accepted that conventional liposomes do not penetrate directly into the skin, this penetration mechanism has been described by Cevc *et al.* for deformable liposomes, also known as transfersomes [53].

In view of the presence of a surfactant in their bilayer, these vesicles exhibit viscoelastic properties that allow them to deform and pass between the cells of the *stratum corneum*, thus arriving intact into the epidermis. This mechanism is similar to the deformation of red blood cells as they change shape in order to pass through narrow blood vessels whose diameters are smaller than the undeformed blood cells. This penetration is made possible by the presence of a natural trans-epidermal water gradient, which is created by the hydrophilicity of phospholipids. Furthermore, these liposomes avoid dry environments and will follow the gradient of moisture, moving into the deeper layers and more hydrated skin.

5. Liposomes penetrate into the skin via cutaneous annexes such as sweat glands and hair follicles. An excellent review of these studies is provided in a review by Knorr et al [54].

The work with liposomes suggested that carrier/vesicle size may have an influence on skin penetration as well as deposition into hair follicles. The nanosizing approach has the potential to offer increased skin deposition of compounds and also the more efficient delivery of agents to hair follicles. Recent work by the group of Michniak-Kohn *et al.* suggests that some nanoparticulate carriers are able to deliver various lipophilic compounds to skin fairly efficiently while making sure that no compounds traverse the skin layers (no *trans*dermal penetration occurs). Additional benefits of these carriers included high solubilization (in one case 5000-fold) and improved stability of the encapsulated compounds. There are many other such examples in the literature, and nanosized particles have been used for a long time in sunscreen formulations and facial foundations.

Penetration of nanoparticles into the *stratum corneum*

The penetration of nanoparticles occurs through two possible pathways: intercellular and intrafollicular pathways. While the transfollicular pathway plays a central role in the study of the penetration of nanoparticles [55], it is not yet clear if nanoparticles penetrate into the hair follicles, or if they are stored in the outer layer of these hair follicles.

Intelligent Targeting Carriers

Intelligent Targeting Carriers have the ability to be recognized by fibroblasts, melanocytes, or adipocytes, thus delivering anti-aging, whitening, or slimming active ingredients, directly to the cells where they have to act.

Capsules are formed by a matrix of poly (lactic-co-glycolic acid) PLGA with an outer polyvinyl alcohol (PVA) shell with the addition of N-pantothenic-peptide [56]. A proper ligand is covalently bonded to the surface of the capsule. These ligands (mostly peptides) are recognized by receptors in the surface of the target cells. The cells are thus able to uptake the particle by endocytosis, while the particles are able to escape from the endosomal compartment, then degraded and therefore able to deliver the active to the cytoplasm.

Cruz *et al.* suggested that the PLGA capsules used as intelligent targeting devices increase the activity of the encapsulated active ingredients by concentrating them solely in the place where they can be biologically active. This is thus of interest to personal care products (anti-aging, whitening, tanning, slimming) [57].

Figure 2: Examples of penetration pathways by various molecules. The lipophilic molecules of low molecular mass penetrate through the *stratum corneum* by the intracellular pathway while the hydrophilic molecules prefer the polar pathway. The liposomes may penetrate via the transfollicular pathway and the transferosomes (i.e., deformable liposomes), pass between cells, and prioritize the intercellular pathway.

e. Penetration Versus Protection

It is not clear yet whether there truly is real *in vivo* penetration of the ingredients incorporated in cosmetic and personal care formulations. While many will propose that this means cosmetic formulations lack clear efficacy, we propose rather that the lack of penetration of a product's active ingredients need not be a

negative characteristic. Indeed, these ingredients still possess the ability to protect the *stratum corneum* against environmental stressors and damage. This concept is significant since the *stratum corneum* constitutes only 10% of the entire skin, yet it contributes to over 80% of the cutaneous barrier function. For example, the use of antioxidant molecules in cosmetic products can help the *stratum corneum* regenerate and protect itself and thus also regenerating and protecting the underlying epidermis and dermis from the harmful effects of UV and other environmental toxins.

In summary, while the individual penetration of water, lipophilic molecules, liposomes, and nanoparticles through the *stratum corneum* is becoming better understood when viewed through their intercellular, transcellular, intrafollicular, and polar pathways, the penetration mechanisms of cosmetic formulations remain poorly understood and are an area in need of further exploration.

In this review, we presented not only the known and various pathways of penetration through the *stratum corneum*, but also the fact that even if cosmetic formulations penetration is no deeper than the SC, their efficacy is demonstrable. Indeed, the *stratum corneum* represents the barrier function of skin, and thus needs to be protected from environmental toxins. By regenerating and protecting the *stratum corneum*, active cosmetic ingredients protect the epidermis and dermis.

PART 3.1.11 IMMUNOLOGICAL FUNCTION

The skin is the largest organ of the human body and possesses the unique function of protecting the body from allergens, chemicals, and microbial infection [58]. It is an immunoprotective organ that comprises of specialized cells called Langerhans cells in the epidermis. The dermal collagen network comprises of dendritic cells, T-cells and T-cell subtypes, B cells and mast cells. Langerhans cells are activated in response to allergen-mediated contact sensitization that elicits an immune response [59, 60]. Resident dermal dendritic cells in addition are induced in this phenomenon. As a result, the Langerhans cells migrate into the lymph nodes in response to chemokines such as CCL19. The migrated cells activate T-cells that undergo differentiation and proliferation, which results in the selective expansion of clonal populations of allergen-specific T-cells. The activation of Th1 and/or Th2 clonal T-cell populations ultimately results in allergen-specific sensitivity [61].

After first phase of infection with a pathogen, dendritic cells resident in the

dermis present antigens to naïve T-cells in the skin-draining lymph node, initiating an adaptive immune response that generates central memory T-cells and effector memory T-cells. At second round of infection, dendritic cells present antigen to central memory T-cells in skin-draining lymph nodes, giving rise to another population of effector memory T-cells that migrate into skin and attempt to destroy the infection.

There is a marked decrease in number of Langerhans cells with age. This affects the ability of TNF-alpha to induce Langerhans cell migration in response to an infection. There is an overall reduced cutaneous immune function with aging, thus rendering aged skin more prone to infections. There is a reduction in dendrite formation, reduction in Birbeck granules, and reduced antigen trapping capacity. Thus, skin immune function is associated with and decreases with aging.

UV radiation results in changes in the immune response. It suppresses the immune response and results in skin cancer development [62]. In harsh circumstances, it also destroys the Langerhans cells on skin.

Psoriasis is a common skin condition characterized by immune and genetic factors. The symptoms of the disease include skin lesions, dryness, redness, and irritation associated with inflammation. The process is an inflammatory skin disorder that results in dermal infiltration of T-cells, dendritic cells, macrophages, and neutrophils [63]. Anti-TNF alpha as well as anti-IL-12 and anti-IL-23 therapy and methods to exfoliate the skin lesions including reduction of inflammation are suggested interventions to treat psoriasis. A systems biology approach to characterize the transcriptome of the diseased state versus normal skin can be indicative of suggested therapeutic options to combat the disease.

PART 3.1.12 CYTOKINES

Cytokines are small proteins or glycoproteins produced by epidermal keratinocytes, Langerhans cells, monocytes/macrophages, fibroblasts, and lymphocytes in the skin. The cytokines bind to receptors on target cells and initiate an active immune response. The cytokines produced by keratinocytes have been most extensively studied. Keratinocytes produce interleukins (IL-1, IL-3, IL-6, IL-7, IL-9, IL-10), interferons (IFN-α, IFN-β), cytotoxins (TNF-α, TNF-β), colony-stimulating factors (G-CSF, MCSF, MG-CSF), and growth factors (bFGF, EGF, KGF, MGSA, NGF, TGF-α, TGF-β, PDGF) [64]. There is a relatively low basal level of cytokine production in normal healthy skin;

however, in the presence of a challenge (e.g., viral, bacterial, fungal), cytokine production increases. This is regulated by membrane proteins, known as toll-like receptors, that bind certain molecular components of pathogens [65]. Different toll-like receptors recognize different molecular components of the pathogen. The ligands include bacterial DNA, lipopolysaccharide, flagellin, β-glucan, *etc.* Cytokine production is altered in a variety of skin diseases.

PART 3.1.13 ENZYMES

Enzymes within the skin layers play an important role in maintaining the biochemistry of the skin, supporting the synthesis of its components and hence cell replication. This results in skin lipid production and the formation of the *stratum corneum* barrier properties that play such a crucial role in the penetration of cosmetic actives into the skin layers. In addition, the presence of enzymes influences how cosmetic actives and formulation excipients are metabolized. Also, enzymes function as skin protectors by scavenging free radicals and maintaining a pH gradient that influences the number and type of bacterial flora on the skin surface. They are one of the most essential components of the skin.

Enzymes in the dermis are responsible for continuous remodeling of the extracellular matrix [66]. In the basal layer of the epidermis, the metabolic activity of most cells is low. The exceptions are the few basal cells undergoing replication, where enzyme activity is high. As cells move up from the basal layer and enter into differentiation, there is a general increase in the enzymatic activities necessary to synthesize the components of desmosomes, keratohyalin granules, keratin proteins, and lipids [67]. In the spinous layer most energy is produced by mitochondrial activity, but as cells reach the upper granular layer, the mitochondria are degraded and energy production is dependent on anaerobic glycolysis with lactic acid as a by-product. Most of the protein synthesized during differentiation is keratin, which makes up the keratinocyte cytoskeleton [68]. Most of the lipid that accumulates with differentiation is packaged into lamellar granules [69, 70]. In the late stages of differentiation, the lamellar granules migrate to the apical end of the cell, and the bounding membrane of the lamellar granule fuses into the cell plasma membrane [71, 72]. The lipids and a battery of hydrolytic enzymes are extruded into the intercellular space. At about this time, some proteins from the keratohyalin granules are deposited on the inner surface of the plasma membrane [73]. A high-molecular-weight, histidine-rich and highly phosphorylated protein, profilaggrin, is dephosphorylated and

proteolytically clipped to small filaggrin units [74]. On passage into the *stratum corneum*, the filaggrin units induce aggregation of the keratin filaments into bundles of keratin that lie parallel to the plane of the skin. The filaggrin proteins are subsequently acted upon by proteases until they are degraded to their component amino acids [75]. This results in a very high osmotic strength in the corneocytes. At about the time of the transition from the granular layer to the *stratum corneum*, a calcium-dependant transglutaminase crosslinks the proteins on the inner surface of the plasma membrane through formation of isopeptide linkages [73]. There is further crosslinking of these proteins through the spontaneous formation of disulfide linkages. ω-Hydroxyceramides derived from the bounding membrane of the lamellar granules becomes ester-linked to the outer surface of this crosslinked protein layer [76]. Thus the original plasma membrane of the keratinocyte is replaced by a compound envelope consisting of a thick crosslinked band of protein with a covalently bound lipid layer on the outer surface.

The main source of carbon for epidermal lipid biosynthesis appears to be acetate from the circulation [77]. The rate-limiting enzyme for synthesis of fatty acids is acetyl-CoA carboxylase [78]. This is a biotin-dependent enzyme that converts acetyl-CoA to malonyl-CoA at the expense of one ATP. The fatty acids are incorporated into phosphoglycerides, sphingomyelin, and glucosylceramides in the viable portion of the epidermis. The rate-limiting enzyme in synthesis of sphingolipids is serine palmitoyl transferase [79], and the rate-limiting enzyme in cholesterol biosynthesis is β-hydroxymethylglutaryl-CoA reductase [80]. HMG-CoA reductase uses two molecules of NADPH to reduce HMG to mevalonate-CoA.

The lipids found in isolated lamellar granules include phosphoglycerides, sphingomyelin, glucosylceramides, and cholesterol [69]. One unusual glucosylceramide contains 30-through 34-carbon long ω-hydroxyacids amide-linked to a mixture of sphingosines and dihydrosphingosines with linoleate ester-linked to the ω-hydroxyl group and glucose β-glycosidically linked to the primary hydroxyl group of the base [81]. This unusual lipid is thought to be a major component of the bounding membrane of the lamellar granule, and is the precursor of the aforementioned covalently bound lipid of the envelope. Enzymes associated with the lamellar granules include ceramide glucosyl transferase indicating that lamellar granules arise from the Golgi apparatus, since this enzyme is considered a biochemical marker for the Golgi apparatus [82]. Hydrolytic enzymes associated with the lamellar granules include β-glucosidase,

acid phosphatase, amino-glycosidases, galactosidase, aryl sulfatase, phospholipase A, sphingomyelinase, and several proteases [69, 70, 83]. The combined action of some of these enzymes converts the cholesterol-phospholipid-glucosylceramide mixture extruded into the intercellular space into the cholesterol-ceramide-fatty acid mixture of the *stratum corneum*.

A minor lipid in the *stratum corneum* is cholesterol sulfate. This lipid has been shown to be a serine protease inhibitor [84]. It is thought that this protease inhibitor protects the corneodesmosomes, until in the outer layers of the *stratum corneum*, sterol sulfatase degrades the cholesterol sulfate, and this permits the serine proteases to break down the corneodesmosomes leading to desquamation.

3.1.14 ULTRAVIOLET RADIATION-INDUCED PHOTODAMAGE AND SKIN CANCER

Photocarcinogenesis is a cosmetic concern because the UV light-absorbing agents used in cosmetic (over-the-counter) sunscreens help to lower the incidence of skin cancer.

Exposure to UV radiation causes not only photodamage with the appearance of premature aging, but also precancers and cancers of the skin. Actinic keratoses are premalignant lesions in which the epidermis proliferates, producing hyperkeratotic lesions characteristic of photodamaged skin, especially in individuals with skin types I, II, and III. These lesions have a high percentage of DNA mutations, particularly at sites p53 and p16 which are characteristic of nonmelanoma skin cancers (NMSC). Although actinic keratoses occasionally spontaneously disappear, they should be medically treated to prevent their becoming squameous cell carcinomas.

Skin cancer has been classified as nonmelanoma skin cancer, and as cutaneous malignant melanoma. NMSC has the highest incidence of any cancer, approximately one million new cases each year in the United States. One in five Americans will get a skin cancer in the course of a lifetime. Mortality from NMSC is low, but morbidity can be considerable with possible disfigurement, especially on the face and neck.

Basal cell carcinoma (BCC) is the most common NMSC (equal to about 75% of the incidence of all skin cancers). Although BCCs do not metastasize, they are locally invasive and disfiguring if not treated early. Squameous cell carcinomas (SCC) account for about 20% of all skin cancers; approximately 2% of SCCs (particularly those originating on the head and neck) metastasize with possible

mortality. Melanomas make up about 5% of all skin cancers. If excised before they become invasive, melanomas can be definitively treated; however, if allowed to invade the dermis, melanomas metastasize with high mortality.

Sun exposure (and the use of tanning beds) is linked to the skin cancer epidemic as the incidence of NMSC increases in proportion to the cumulative sunlight exposure and is highest in people with skin types I and II. In the case of melanoma, the effects of UV are not clear except in lentigo maligna melanoma (a subclass of cutaneous melanoma), for which sun exposure has been shown to increase the incidence.

UV radiation causes skin cancer by inducing mutations in DNA which lead to damaging the ability of the skin cells to control proliferation. UVB harms mainly the epidermis and UVA, the dermis. The primary damage by UVB is *via* direct absorption by DNA to generate mutations through the formation of dimers between adjacent pyrimidine residues, leading to specific UVB "signature" mutations that can accumulate over time. UVB can also indirectly induce oxidative damage by the formation of reactive oxygen species (ROS) that indirectly damage DNA. If not repaired, both the direct and indirect DNA mutations persist through subsequent subdivisions causing precancers (actinic keratoses) and skin cancers. Reactive protective mechanisms by the epidermis cause thickening of the outer layers of the skin to create a UV-protective "outer shell" that gives an unattractive leathery texture and increased pigmentation that results in mottled discoloration, both of which are characteristic of the appearance of photoaging. UVA penetrates deeper to harm the dermis. UVA damages DNA indirectly through absorption by chromophones that generate ROS which mutate DNA *via* mutagenic oxidative intermediates. UVA also activates metaloproteinases which directly break down collagen and elastic tissue and act on other transcription factors to inhibit repair, thereby causing the sagging and wrinkles of photoaged skin.

Furthermore, recent research shows that UVA in combination with common environmental pollutants is synergistic in generating oxidative damage, thereby significantly increasing visible photodamage and the risk of skin cancer [84.5]. Polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BaP) are ubiquitous in the air, food, and water as a result of incomplete combustion of fossil fuels (from power plants, car exhaust, heaters) and from cigarette smoke (the most prevalent indoor pollutant). These chemical pollutants exist at levels below damaging or tumorigenic levels. However, when combined with UVA exposure, there is a synergistic increase in oxidative damage with the

consequence of even more severe photoaging and increased incidence of skin cancer.

Thus UV radiation is a complete carcinogen; it can act alone as an initiator and a promoter in formation of cancer. UV can also act as a promoter with initiating events inside the cell, such as DNA mutations arising from DNA polymerase incorporation errors, depurination, deamination of 5-methylcytosine, or oxidative damage from free radicals. The cell can respond to the damage by repairing the DNA to avoid the effects of the mutations or, if the damage is too great, by inducing cell death through apoptosis to remove potential cancer cells from the population. Immunosurveillance aids recognition of potentially damaging mutated cells so that apoptotic mechanisms can be initiated. UVA decreases this cellular immune recognition and response.

PART 3.1.15 GENE EXPRESSION IN SKIN CARE

The Human Genome Project (HGP) was a 13-year effort that was coordinated by the U.S. Department of Energy and the National Institutes of Health. The project, which was completed in 2003, produced sequence information for the entire 3 billion base pairs in the human genome. The work cost more than one U.S. dollar per base pair, but generated over 265 times that in economic impact and job creation [85]. More importantly, the HGP gave rise to new, high-throughput technologies used to analyze genes and ultimately, a new field of science—*genomics*. From genomics, other high-throughput technologies were developed, giving rise to a suite of "omics" disciplines (e.g., proteomics and metabolomics). In combination, "omics" data provide a comprehensive view of how cells and biological systems work together in health and in disease. This information has enabled advances in dermatology and in the cosmetic and personal care industry [86]. In this section, the application of genomics-based methods to cosmetics and personal care is reviewed.

a. Genomics Technologies

Researchers have been studying the function of genes and proteins in the skin for decades using technologies such as Northern and Western blotting, and traditional polymerase chain reaction (PCR). These methods are labor intensive and only allow for analysis of a few genes or proteins at a time [87-92].

More recent technologies, including microarrays and quantitative real time PCR (qPCR), have enabled simultaneous analysis of thousands of genes and

more accurate quantification. Microarrays are now the "go-to" method for screening cells and tissues to identify novel biomarkers and understanding mechanisms of action. Both microarrays and qPCR technologies are used to detect changes in the sequence of DNA (i.e., mutations), as well as gene expression (an indicator of which genes are "turned on or off") in a given cell type. When a gene is "turned on," or activated, it is transcribed from DNA into a complementary strand of RNA (ribonucleic acid)—the RNA strand serves as a template for protein synthesis, directing the ribosome as to which amino acids to put together to form specific proteins. On the other hand, when a gene is "turned off," the cell stops transcribing the DNA strand and protein synthesis is terminated. Gene expression analysis is used to determine how cells and tissues are affected by specific conditions. The methods provide information at the molecular level as to how specific cellular functions are altered from normal homeostasis. Gene expression profiles can be used as a guide for creating products or targeted treatments that will convert an aberrant profile back to homeostasis.

b. Genomics and the Skin

To date, thousands of peer-reviewed research studies have been published using high-throughput genomics methods to elucidate molecular mechanisms associated with skin biology [93, 94]. Studies have been conducted across multiple disciplines, from embryonic development to aging, as well as all areas of skin ailments [95-97]. Many studies have identified unique gene expression profiles in skin disorders such as atopic dermatitis, acne, rosacea, and psoriasis [98-102]. A large number of whole-genome sequencing studies have been conducted to elucidate genetic mutations and predisposition related to rare skin conditions [103, 104].

Molecular studies of the epidermal skin barrier provide a good example of how genomics has improved our understanding of skin biology [105, 106]. The skin barrier is our body's first line of defense against environmental insults such as chemicals, radiation, pathogens, and other stimuli. The formation and maintenance of a functionally intact epidermal barrier involves the coordination of complex regulatory networks that involve the expression and interaction of hundreds of molecules. Some of these major processes include keratinocyte differentiation, apoptosis and maturation into corneocytes, cell migration and desquamation [107]. Gene expression studies have been used to identify the expression of key molecules involved in each one of these processes [108, 109].

In other studies, microarrays have been used to identify large sets of genes [110]. For example, Taylor *et al.* [111] used microarrays to identify genes expressed by differentiating keratinocytes at different time intervals. The study identified 1282 genes differentially expressed; specific genes include those that code for structural molecules and innate immune molecules. Other studies have identified microRNAs that regulate genes, such as p63, in proliferating and differentiating keratinocytes [112, 113]. Again, a better understanding of how specific molecules and pathways interact will facilitate the development of high-performing topical products for cosmetics and for treating specific skin conditions.

Additional studies have identified genetic mutations associated with compromised barrier function [114, 115]. Identifying mutations in genes, such as filaggrin, may aid in creating future diagnostic tools and advanced therapies. Filaggrin is a filament-associated protein that binds keratin fibers in skin epithelial cells. In the *stratum corneum*, filaggrin monomers are incorporated into the lipid envelope that forms the skin barrier. Filaggrin undergoes further processing in the upper *stratum corneum* to release free amino acids that assist in barrier formation and water retention. These proteins can also interact with keratin intermediate filaments. Other research has shown that CYP26b1 is expressed in keratinocytes in the epidermis. CYP26b1 is a member of the cytochrome enzyme superfamily, a group of enzymes that are essential for performing metabolic functions in the cell. CYP26b1 is essential for metabolism of retinoic acid [116]. Okana *et al.* [115] has recently demonstrated that genetic ablation of CYP26b1 disrupts epidermal barrier formation by inhibiting retinoic acid metabolism.

c. Gene Expression Analysis: A Breakthrough for Cosmetic Science

Gene expression technologies in cosmetics and personal care are now routinely used in product development pipelines. Drawing on information produced in basic research studies that identify molecular biomarkers associated with phenotypic endpoints, cosmetics and personal care companies are performing studies to define the skin's response to topical materials and formulations [117-120]. Skin care scientists are currently utilizing gene expression methods to screen raw materials for biological effects, identify dose-response relationships, and understand how combinations of ingredients may work together—this may be additive, synergistic, or hormetic.

To date, gene expression studies have been used to:

- Demonstrate efficacy and understand mechanisms of action
- Streamline product formulations during development
- Create performance products that ultimately improve marketability, consumer confidence and brand loyalty

While most work has been conducted to improve product formulations and validate efficacy, genomics-based screening methods may also provide new and improved tools for assessing safety and toxicity [121]. This will require carefully planned studies to develop and validate improved tissue models that contain immune cells as well as sophisticated informatics and algorithms to easily analyze and interpret the datasets.

d. Challenges

Understanding how specific patterns of gene expression translate into a biological response is one of the greatest challenges in interpreting high throughput genomics data. For now, genomics data are best used as a roadmap to generate hypotheses and guide follow-on studies that utilize additional cell biology methods better suited for interrogating functional relationships.

Another challenge in conducting genomics-based studies for cosmetic science is the lack of fully representative experimental tissue models. Studies conducted for the cosmetics industry are typically carried out using *in vitro* cell cultures or artificial skin. While these models provide useful information regarding the keratinocyte and fibroblast response, they lack melanocytes, Langerhans cells, and an epidermal barrier consistent with *in vivo* human skin. *In vitro* models are an ideal tool for conducting early screening studies that should be followed up with *in vivo* clinical work.

e. Looking Ahead

Since the inception of genomics, newer, state-of-the-art technologies such as NextGen sequencing have been developed. These methods can rapidly sequence the entire genome in several days, perform RNA sequencing, and analyze epigenetic mechanisms (i.e., DNA methylation). While epigenetics is now a popular buzzword in cosmetic science, a greater understanding of these mechanisms in skin biology will help elucidate phenomena such as aging, photoaging, and skin disease. Groundbreaking epigenetic research in skin biology is beginning to emerge and will soon be translated into product development.

Another emerging concept is the promise of “personalized skin care” [122].

This approach uses an individual's genetic footprint to develop skin care products to address the specific needs of an individual. Companies are beginning to explore this opportunity, and there will most likely be an increased amount of data and ultimately, product development using the "personalized medicine" approach in the next few years.

f. Recommended Reading

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GLOSSARY

1. Epidermis - composed of the outermost layers of cells in the skin—"epi" in

Greek meaning “over” or “upon,” which together with the dermis forms the entire skin layer termed the cutis.

2. Dermis - a layer of skin between the epidermis and subcutaneous tissues that consists of connective tissue and cushions the body from mechanical stresses. It is divided into two layers, the area adjacent to the epidermis called the papillary region and a deeper and thicker area known as the reticular dermis.
3. *Stratum corneum* (Latin for “horned” or “horny” layer) - the outermost layer of the skin, consisting of dead cells (corneocytes) that lack nuclei and organelles. This layer is responsible for the barrier function of skin.
4. Glycoaminoglycan - any of a group of high-molecular-weight linear polysaccharides with various disaccharide repeating units and usually occurring in proteoglycans, including the chondroitin sulfates, dermatan sulfates, and hyaluronic acid. Abbreviated as GAG.
5. Collagen - protein made up of amino acids, which are in turn built of carbon, oxygen, and hydrogen. Collagen contains specific amino acids—glycine, proline, hydroxyproline, and arginine. There are many types of collagen that have been identified. Collagen is synthesized most commonly by fibroblasts.
6. Keratin - a family of fibrous structural proteins. Keratin is the key structural material making up the outer layer of human skin. It is also the key structural component of hair and nails.
7. Keratinocyte - the main cell type in the epidermis, the outermost layer of the skin. Those keratinocytes found in the basal layer (*stratum germinativum*) of the skin are sometimes referred to as “basal cells” or “basal keratinocytes.”
8. Corneocyte - a terminally differentiated keratinocyte that forms part of the stratum corneum, the outermost part of the skin. The cell is regularly replaced through desquamation and renewal from the lower epidermal layers, making it an essential part of the barrier property of the skin.
9. Fibroblast - a type of cell that synthesizes extracellular matrix and collagen, the structural framework for the skin, and plays a critical role in wound healing. Fibroblasts are the most common cells of connective tissue and in particular the dermal layer of the skin.
10. Cytokine - (Greek “cyto,” cell; and “kinos,” movement) are any of a variety of compounds secreted by specific cells of the immune system. They carry signals locally between cells and thus have an effect on other cells. They are a category of signaling molecules that are used extensively in cellular communication. They can be proteins, peptides, or glycoproteins.

11. Merkel cells - clear cells found in the *stratum basale* (at the bottom of sweat duct ridges) of the epidermis, approximately 10 µm in diameter. They also occur in epidermal invaginations of the plantar foot surface called rete ridges. Most often they are associated with sensory nerve endings, when they are known as Merkel nerve endings.
12. Langerhans cells - dendritic cells (antigen-presenting immune cells) of the skin, containing large granules called Birbeck granules. They are present in all layers of the epidermis, but are most prominent in the *stratum spinosum*. They also occur in the papillary dermis, particularly around blood vessels.
13. Dendritic cell - immune cell forming part of the immune system. Main function is to process antigen material and present it on the surface to other cells of the immune system. Dendritic cells are present in tissues in contact with the external environment, such as the skin (where there is a specialized dendritic cell type called Langerhans cells).
14. Birbeck granule (also known as Birbeck bodies) - rod-shaped cytoplasmic organelles with a central linear density and a striated appearance.
15. Fillagrin - a filament-associated protein that binds to keratin fibers in epithelial cells.
16. Involucrin - a protein component of human skin. In humans it is encoded by the *IVL* gene. In binding the protein termed loricrin, involucrin is involved in the formation of the cell envelope that protects corneocytes in the skin.
17. Melanin (from the Greek “melas” meaning “black”) - a natural pigment in skin derived from the amino acid tyrosine.
18. Melanosome - an organelle containing melanin, the most common light-absorbing pigment found in the mammalian kingdom.
19. Pheomelanin - yellow to red-brown pigment produced by melanocytes.
20. Eumelanin - black to brown pigment produced by melanin.
21. Golgi apparatus (also known as the Golgi complex, Golgi body, or simply the Golgi) - an organelle found in most eukaryotic cells. Part of the cellular endomembrane system, the Golgi apparatus packages proteins inside the cell before they are sent to their destination; it is particularly important in the processing of proteins for secretion.
22. Macrophage - any of the large, mononuclear, highly phagocytic cells derived from monocytes that occur in the walls of blood vessels and in loose connective tissue. Macrophages are usually immobile but become actively mobile when stimulated by inflammation; they also interact with lymphocytes

to facilitate antibody production.

23. Elastin - a key extracellular matrix protein that is critical to the elasticity and resilience of many tissues including skin.
24. Reticulin - name given to the chemical substance of reticular fibers, which were formerly thought to be distinct from collagen by reason of their distinctive structure and staining properties but are now regarded as type III collagen.
25. Apocrine gland - a sweat gland composed of a coiled secretory portion located at the junction of the dermis and subcutaneous fat, from which a portion inserts and secretes into the infundibular portion of the hair follicle.
26. Eccrine gland - a sweat gland that is not connected to hair follicles. It functions by responding to elevated body temperature due to heat or physical exercise.
27. Sebaceous gland - small oil-producing gland usually attached to hair follicles and releasing a fatty substance, sebum, into the follicular duct and thence to the surface of the skin.
28. Sebum - consists of a mixture of fats (triglycerides, wax esters, squalene, and cholesterol) and cellular debris, which is discharged through the sebaceous duct.
29. Kallikreins - these belong to a class of serine proteases with a high degree of substrate specificity. The enzymes are structurally related to trypsin. Kallikrein (kininogen, kininogenase = EC3.4.21.8) cleaves proteins between arginine and lysine residues.
30. Keratinosomes (otherwise known as membrane-coating granules [MCGs], lamellar bodies, lamellar granules or Odland bodies) - secretory organelles found in keratinocytes. Keratinosomes fuse with the cell membrane and release their contents into the extracellular space.
31. Androgen - a male sex hormone such as testosterone.
32. Ichthyosis - a family of genetic skin disorders characterized by dry, scaling skin that may be thickened or very thin.
33. Atopic dermatitis (eczema) - an itchy inflammation of the skin. It is usually a chronic condition that may be accompanied by other conditions such as asthma.
34. Psoriasis - a chronic skin disease that affects the life cycle of skin cells. It causes cells to build up rapidly on the surface of the skin, forming thick silvery scales and itchy, dry, red patches that are sometimes painful.

35. Transepidermal Water Loss = TEWL - This is defined as a measurement of the water amount that diffuses or evaporates from inside the body through the epidermis to the surrounding atmosphere. TEWL can increase due to disruption of the skin barrier through wounds, scratches, burns, exposure to solvents or surfactants, extreme dryness) and is affected by humidity, temperature, season, and moisture content of the skin.
36. Liposomes - nano/micro-size spherical drug carriers that can be produced from natural or synthetic phospholipids and cholesterol. They form spontaneously when phospholipids are combined with water and bilayered spheres form, since the phospholipid molecules orient themselves according to their polarity (one end of each molecule is water soluble, while the opposite end is water insoluble).
37. Rheology - a science dealing with the deformation and flow of matter and including the measurement of viscosity.
38. Colloids - a solution that has particles ranging between 1 and 1000 nanometers in diameter and evenly distributed throughout the solution.

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Abbreviation: GAGs, glycosaminoglycans; NMF, natural moisturizing factor; DOPA, 3,4-dihydroxyphenylalanine; UV, ultraviolet; LCs, Langerhans cells; DCs, dendritic cells; SC, stratum corneum; RER, rough endoplasmic reticulum;

KHK, keratohyalin granules; LBs, lipid bodies; SG, stratum granulosum; SCD, stearoyl CoA desaturase; TEM, transmission electron microscopy; AMP, antimicrobial peptide; TJs, tight junctions; TEWL, TransEpidermal Water Loss; PLGA, poly(lactic-co-glycolic acid); PVA, polyvinyl alcohol; ATP, adenosine triphosphate; NADPH, nicotinamide adenine dinucleotide phosphate; HMG, Hydroxymethylglutaryl-CoA; HGP, Human Genome Project; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

SUBSTRATE: SKIN II

CLASSIFICATION SCALE FOR SKIN COMPLEXIONS AROUND THE WORLD

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ABSTRACT

Cosmetologists and aestheticians are daily faced with a wide variety of clients. In order to provide their skin with optimal health and beauty services these practitioners must accurately look at skin of different races and determine appropriate treatment protocols. Early projections of the changing global skin color landscape have now been proven to be accurate, and the main categories of skin color are no longer just Caucasian-white, but have evolved towards a dramatic escalation of brown, black, yellow, and the many shades in between.

The current scale available to the industry is the Fitzpatrick scale (Figure 4), which was developed in 1975 by Harvard dermatologist Dr. Thomas B. Fitzpatrick. The Fitzpatrick scale is described in this chapter as a custom and practice foundation for current practitioners. It is simple and easy to use; however, the method leaves a gap in the ability to classify skin in light of the evolving epidemiological landscape, and the system fails to accurately predict skin type and precautions for race, complexions, and skin characteristics.

Extensive research over the last decade reveals that beyond color alone, other key factors such as race, country of origin, and ethnicity play an important role in how the skin will respond to cosmetic and personal care products and procedures. Further, when the world is viewed as a whole, and countries are thoughtfully divided into sections, there appears to be an order that helps define how skin from similar regions acts in similar ways. This observation provides the basis for a new, more detailed skin classification scale. A proposal for this

new scale is at the heart of the current chapter. The scale has been called the EP Global Skin Classification Scale (Figure 1) and is focused on an all-encompassing global approach that is comprehensive and easy to use for the skin care professional. (Figures 2 & 3 show additions to EP Global Skin Classification Scale.) **TABLE OF CONTENTS**

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3.2.1 WHY WE NEED AN UPDATED SKIN CLASSIFICATION SCALE

The changing of global skin color landscape is now a reality; the majority is no longer just Caucasian but Asian, with the many shades of yellow undertones and shades ranging from light to dark olive complexions. The common skin conditions among the different races also differ; therefore skin has to be diagnosed and treated according to color and race and a one-size-fits-all method of the past no longer works.

The world is divided geographically into continents:

- Asia is the most populous continent, with its 4.2 billion inhabitants accounting for over 60% of the world population. (The world's two most-populated countries alone, China and India, together constitute about 37% of the world's population).
- Africa is the second-most-populated continent, with around 1 billion people, or 15% of the world's population.
- Europe's 733 million people make up 11% of the world's population.
- Latin American and Caribbean regions are around 600 million (9%).
- Northern America, primarily consisting of the United States, and Canada, has a population of around 352 million (5%).
- Oceania, the least-populated region, has about 35 million inhabitants (0.5%).

Worlds Population by Race

East Asian 24% (Korea, Mongolia, China, Japan)
South Asian 21% (India, Sri Lanka, Pakistan, Bangladesh, Nepal)
Southeast Asian 9% (Cambodia, Burma, Philippines, Malaysia)
Black 15%
White 15%
Hispanic 8%
Middle Eastern 8%

USA

The USA is a diverse country of many races; the chart below shows the growth and census population over the past decade.

Race	**Census 2010, population**	**Percent of population**	**Census 2000, population**	**Percent of population**
Total Population	**308,745,538**	**100.0%**	**281,421,906**	**100.0%**
Single race				
White	196,817,552	**63.7**	211,460,626	**75.1**
Black or African American	37,685,848	**12.2**	34,658,190	**12.3**
American Indian and Alaska Native	2,247,098	.7	2,475,956	**0.9**
Asian	14,465,124	**4.7**	10,242,998	**3.6**
Native Hawaiian and other Pacific Islander	481,576	**0.15**	398,835	**0.1**
Two or more races	5,966,481	**1.9**	6,826,228	**2.4**
Some other race	604,265	.2	15,359,073	**5.5**
Hispanic or Latino	50,477,594	**16.3**	35,305,818	**12.5**

NOTE: Percentages do not add up to 100% due to rounding and because Hispanics may be of any race and are therefore counted under more than one category.

Source: U.S. Census Bureau: National Population Estimates; Decennial Census.

The current scale that has been industry standard for the past 38 years is the Fitzpatrick scale.

The Fitzpatrick Classification Scale was developed in 1975 by Harvard Medical School dermatologist Thomas Fitzpatrick, MD, PhD. This scale classifies a person’s complexion and their tolerance of sunlight. It is used by many practitioners to determine how someone will respond or react to facial treatments, and how likely they are to get skin cancer.

Skin Type	Skin Color	Characteristics
I	White; very fair; red or blond hair; blue eyes; freckles	Always burns, never tans
II	White; fair; red or blond hair; blue, hazel, or green eyes	Usually burns, tans with difficulty
III	Cream white; fair with any eye or hair color; very common	Sometimes mild burn, gradually tans
IV	Brown; typical Mediterranean caucasian skin	Rarely burns, tans with ease
V	Dark Brown; mid-eastern skin types	very rarely burns, tans very easily
VI	Black	Never burns, tans very easily

Fitzpatrick Classification Scale (Table 1)

The method leaves a gap in the ability to classify skin in light of the evolving epidemiological landscape, and the system fails to accurately predict skin type and precautions for race, complexions, and skin characteristics.

The EP Global Skin Classification tool was designed to fill the gaps by expanding the categories and taking into consideration global skin. The following categories were implemented:

- Race
- Country of Origin
- Complexion
- Skin Characteristics
- Skin Type
- Precautions

(See Figures 1-3)

EP Global Skin Classification Scale

EP #	Race	Country of Origin	Complexion	Skin Characteristics	Skin Type
I	Caucasian I	Belgium, Denmark, Estonia, Finland, Germany, Iceland, Ireland, Latvia, Liechtenstein,	Pale, Very Fair. Ruddy	Sensitive, thin fragile skin. Burns easily in sun, never tans, Age's rapidly. Fine wrinkling occurs early.	Hyper-Sensitiv Environmental Damage. Acne

		Lithuania, The Netherlands, Norway, Sweden, Switzerland, United Kingdom, Canada,Greenland	Freckled complexion.	Orange peel skin as they age. Scars usually thin. Skin tends to be dry.	Prone. Skin Ca most common this type.
II	Caucasian II	Andorra, Austria, Belarus, Bosnia & Herzegovina, Croatia, Czech Rep. France, Greece, Hungary, Italy, Kosovo, Luxembourg, Moldova, Monaco, Montenegro, Poland, Portugal, Romania, Russia, San Marino, Serbia, Slovakia, Slovenia, Spain, Ukraine, Vatican City, USA.	Fair. Light Olive undertones.	Sensitive, fairly thin skin. When exposed to sun will first burn then tan. Signs of aging appear early. Nominal Scarring. Dry to normal skin.	Sensitive Phot Damage Prema Aging Acne Pr Greater chance skin cancer.
III	Caucasian III	Australia, Bulgaria, Cyprus, Georgia, Greece, Macedonia, Malta, New Zealand, Turkey, South Africa, Zimbabwe	Fair Medium Olive undertones.	Seldom burns when exposed to sun, will tan, excessive exposure to sun will cause the skin to look leathery. Signs of aging appear later. Fine wrinkling less common. Scarring maybe thicker and darker. Normal to oily skin.	Environmental Damage Exces Photo damage Prone Skin Ca common
		Argentina, Belize,			

IV	Hispanic/Latino	Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, Falkland Islands (Malvinas), French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Paraguay, Panama, Peru, Suriname, Uruguay, Venezuela.	Olive undertones. Medium to Dark Olive; wide range of Olive tones.	Depending on skin tone will rarely burn; will always tan, aging process is moderate. Darker, thicker scarring. Normal to oily skin.	Photo Damage Environmental Damage Hyperpigment Acne Prone Sk Cancer less common
V	Mediterranean/Asian I	Afghanistan, Albania, Algeria, Armenia, Azerbaijan, Bahrain, Iraq, Iran, Israel, Jordon, Kazakhstan, Kuwait, Kyrgyzstan, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, Tajikistan, Turkmenistan, UAE, Uzbekistan, Yemen	Medium-Dark Olive undertones	Seldom burns, will tan. Aging process is moderate. More advanced if over exposed to sun. Wrinkles appear later in more localized areas. Darker, thicker scarring. Normal to oily skin.	Photo Damage Environmental Damage Hyperpigment Melasma Acne Prone Post Inflammatory Hyperpigment (PIH) Skin Ca rare
VI	Asian II	Brunei Darussalam, Cambodia, China, Hong Kong, Indonesia, Japan, Korea, Laos, Macao, Malaysia,	Medium – Dark Yellow-	Seldom burns, tans about average. Ages well, signs of aging appear late. Prone to	Hyperpigment Melasma Acne Prone Post Inflammatory

		Mongolia, Philippines, Singapore, Taiwan, Thailand, Timor Leste, Vietnam.	Olive undertones.	Pigmentation changes. Scarring can be thicker and darker. Normal to oily skin	Hyperpigment (PIH) Skin Ca very rare
VII	Asian III	Bangladesh, Bhutan, India, Maldives, Myanmar (Ex-Burma), Nepal, Pakistan, Sri-Lanka, Tibet.	Light to dark Olive tones. Yellow undertones	Rarely burns, always tans. Pigmentation changes may occur with over exposure to sun. Ages well, signs of aging appear late. Fine wrinkles do not occur. Darker thicker scars more common	Hyperpigment Melasma Activ Acne/ Acne Pr Post Inflamma Hyperpigment (PIH) Skin Ca very rare.
VIII	Native Hawaiian and other Pacific Islanders	Fiji, French Polynesia, Guam, Kiribati, Marshall Islands, Micronesia, New Caledonia, New Zealand, Papua New Guinea, Samoa, Solomon Islands, Tonga, Vanuatu.	Medium-dark Olive tones.	Seldom burns, tans well, thicker skin allows better sun tolerance. Ages moderately. Thicker and darker scars. Thick Oily skin.	Photo Damage Environmental Damage Hyperpigment Acne Prone Sk Cancer rare
IX	Native American Indian and Native Alaskan	There are over 70 Native American tribes and 8 Alaskan races *See attached list.	Medium Olive tones. Red undertones.	Rarely burns, tans well, high sun tolerance. Skin has a tendency to turn leathery with age and excessive sun exposure. Pigmentation	Environmental Damaged Excessive phot damage Hyperpigment / Melasma Ski

				changes may occur. Thick dark scarring. Normal oily skin.	Cancer rare
X	Black or African American	Eastern Africa, Middle Africa, Northem Africa, Southern Africa, Westem Africa, The Carribbean. *See attached list.	Medium-Dark Brown Tones.	Never burns with sun exposure, tans well. Pigmentation issues common. Very little fine wrinking. Formation fo Keloids possible. Oily skin.	Hyperpigment Melasma Acne Prone Post inflammatory Hyperpigment Pseudo follicul Skin Cancer ve rare
XI	Mix of two or more races	Any combination	Varies depending on race combination.	Take the predominant traits into consideration.	Treat with cau Use milder programs to st graduating to stronger produ absence of irritation.

Figure 1 (EP Global Skin Classification Scale)
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EP Global Skin Classification Scale					
Race Section X (10) - Country of Origin					
Africa					
Eastern Africa	**Middle Africa**	**Northern Africa**	**Southern Africa**	**Western Africa**	**The Caribbean**
Burundi	Angola	Algeria	Botswana	Benin	Anguilla
Comoros	Cameroon	Egypt	Lesotho	Burkina Faso	Antigua and Barbuda
Djibouti	Central African Republic	Libyan Arab Jamahiriya	Namibia	Cape Verde	Aruba
Eritrea	Chad	Morocco	South Africa	Cote d'Ivoire (Ivory Coast)	Bahamas
Ethiopia	Congo (Brazzville)	South Sudan	Swaziland	Gambia	Barbados
Kenya	Congo, Democratic Republic of the	Sudan		Ghana	Bonaire, Saint Eustatius and Saba

Madagascar	Equatorial Guinea	Tunisia		Guinea	British Virgin Islands
Malawi	Gabon	Western Sahara		Guinea-Bissau	Cayman Islands
Mauritius	Sao Tome and Principe			Liberia	Cuba
Mayotte				Mali	Curaçao
Mozambique				Mauritania	Dominica
Reunion				Niger	Dominican Republic
Rwanda				Nigeria	Grenada
Seychelles				Saint Helena	Guadeloupe
Somalia				Senegal	Haiti
Tanzania, United Republic of				Sierra Leone	Jamaica
Uganda				Togo	Martinique
Zambia					Monserrat
Zimbabwe					Puerto Rico
					Saint-Barthélemy
					St. Kitts and Nevis
					Saint Lucia
					Saint Martin
					Saint Vincent and the Grenadines
					Sint Maarten
					Trinidad and Tobago
					Turks and Caicos Islands
					Virgin Islands (US)

TABLE 2 (Race Section X (10) - Country of Origin)

EP Global Skin Classification Scale				
Race Section IX (9) - Country of Origin				
***American Indian and Alaska Native tribes**				
Alaskan Athabascan	Crow	Menominee	Puget Sound Salish	Yaqui
Aleut	Delaware	Navajo	Seminole	Yuman
Apache	Eskimo	Osage	Shoshone	
Blackfeet	Houma	Ottawa	Sioux	
Cherokee	Iroquois	Paiute	Tlingit-Haida	
Chippewa	Kiowa	Pima	Tohono O'Odham	
Comanche	Latin American	Potawatomi	Ute	
Creek	Lumbee	Pueblo	Yakama	

TABLE 3 (Race Section IX (9) - Country of Origin) **3.2.2 THE**

METHODOLOGY IN WHICH THE STUDY WAS CONDUCTED:

The research was carried out at Skin Rejuvenation Clinic in San Francisco during 1997–2005. All subjects were residents of USA; a total of 588 subjects, both female and male (majority female) were chosen; they were of different races.

Charts and records were followed during this period to gather the information for the study. All data were manually collected through these charts and records collected from each individual client visit. The initial consultation is where the data were collected from the client. The minimum treatment process was eight visits over a four-month period, two visits a month. Thereafter monthly maintenance visits.

During the initial visit a client filled out a three-page Skin Evaluation questionnaire that asked several questions pertaining to several different factors, which included:

Skin Evaluation	
Skin Type	
[ ] Very Dry	Dry all over—all the time, tight, easily irritated, sensitive
[ ] Dry/Mature	Mostly dry, tight, maturing/aging, sun/photo-damaged, loss of tone
[ ] Normal to Dry	Normal in the T-zone, dry on the sides of face
[ ] Normal	Slightly oily on the T-zone, normal on the sides of face
[ ] Normal to Oily	Oily in the T-Zone, slightly oily on sides of the face
[ ] Oily	Very oily in the T-Zone, slightly oily on the sides of the face
[ ] Very Oily	Very oily all over, all the time, prone to breakouts and acne
[ ] Blemished	Oily with tendency to active acne or acne-prone skin

History/Pigmentation Country of origin and ancestral race

What is your ancestry? Parents:_____ Grandparents

Pigmentation (How do you tan?)

How do you tan? [ ] *Burn [ ] Usually Burn [ ] Burn then Tan [ ] Usually Tan [ ] Always Tan* Pigmentation tendencies: [ ] Even [ ] Uneven [ ] Hyperpigmented [ ] Melasma **Vascularity** *Broken capillaries: [ ] Nose area [ ] Cheek area [ ] Chin area [ ] Forehead*

[ ] Entire face

Acne *Do you have a history of any of the following?*

[ ] Acne [ ] Whiteheads [ ] Blackheads [ ] Pimples [ ] Enlarges Pores
[ ] Flakiness [ ] Scars [ ] Erythema [ ] Post-Inflammatory Hyperpigmentation
[ ] Pits [ ] Cysts

Sun Exposure/Environment Do you spend time in the sun?

[ ] Seldom [ ] Occasionally [ ] Frequently Approximately how many hours do you spend in the sun a week?

Do you use a sunscreen on a daily basis? _____ What SPF? [ ] 15 [ ] 30 [ ] 30+

At what age did you start to use a sun block on a regular basis?

What percentage of time do you spend in the sun?

Summer _____ Winter

Where did you reside the first 20 years of your life?

Country _____ Region/City

Please note that most damage is accumulated during the first 18 years of your life.

Have you actively sunbathed in the past? [ ] Yes [ ] No Do you currently sunbathe? [ ] Yes [ ] No Do you use tanning beds? [ ] Yes [ ] No Have you ever had skin cancer? [ ] Yes [ ] No Do you have a family history of skin cancer? [ ] Yes [ ] No Relationship _____

Free Radical Exposure Do you smoke? [ ] Yes [ ] No How much? _____

Do you consume alcohol? [ ] Yes [ ] No How much? _____

Do you have a healthy diet? [ ] Yes [ ] No Do you exercise on a regular basis? [ ] Yes [ ] No Do you take vitamins? [ ] Yes [ ] No Which ones?_____

Lifestyle Sleeping habits [ ] Poor [ ] Good [ ] Minimal [ ] / 6 hours [ ] 7 hours [ ] 8 hours Stress level [ ] Minimal [ ] Moderate [ ] Excessive Do you meditate? [ ] Yes [ ] No How often? _____

Healing Ability Does your skin appear fragile and thin? [ ] Yes [ ] No Do you bruise easily? [ ] Yes [ ] No Do you scar from cuts or burns? [ ] Yes [ ] No Do you have a tendency to keloid? [ ] Yes [ ] No Have you had a deep

peel? [ ] Yes [ ] No Did you heal well? _____

Do you have facial laser treatments? [ ] Yes [ ] No When the skin evaluation was completed, the skin was further categorized and documented by complexion and skin characteristics of various skin types from different genetic backgrounds.

Complexion/ Skin Characteristics ***Skin Characteristics of people with Anglo-Saxon origins*** **Fair, dry thin-skinned**

Scars heal well

Signs of aging appear earlier

Burn easily in the sun

Bruising more obvious

Greater chance of skin cancer

Skin Characteristics of people with Southern Mediterranean origins Oily, olive-dark complexion

Signs of aging appear later

Cartilage tends to droop

Darker, thicker scars more common

Wrinkles appear later and in more localized areas Skin cancer rare

Skin Characteristics of people with Northern European origins / German and Scandinavian Fair, blue-eyed, blonde

Thin skin

Scars heal well

Signs of aging appear early

Bruising more obvious

Greater chance of skin cancer

Skin Characteristics of people with Southern European origins Dark, oily brunette complexion

Signs of aging appear later

Fine wrinkling less common

Bruising lasts longer

Scars may be thicker and darker

Skin cancers less common

Skin Characteristics of people with Northern European/Irish and Northern England Ruddy freckled complexion

Red hair
Scars usually thin
Signs of aging appear later
Bruises easily
Pigmentation problems
Skin cancers most common in this type

Skin Characteristics of people with African origins Signs of aging appear very late
Very little fine wrinkling
Formation of keloids is possible
Pigmentation changes may occur
Thicker cartilage hard to change
Skin cancers very rare

Genetically, this skin type is less susceptible to damage from UV radiation, although the skin can still get burned.

Skin Characteristics of people with Asian origins Signs of aging appear late
Fine wrinkling does not usually occur
Pigmentation changes may occur
Eyelid surgery more difficult
Skin cancers very rare

The completed questionnaire was then evaluated and categorized according to primary skin condition to be treated and the secondary skin condition to be treated. A woods lamp was used during the initial consultation to further check for photo-damaged, congested skin, hyperpigmentation/melasma This data collected gave further clarity to the client's skin type.

A UV photo was taken through the canfield polarized camera; this visually showed the UV photo damage and was documented.

3.2.3 SUMMARY, ANALYSIS

During the years of 2005 and 2011 the collected data were reviewed and the conclusions were as follows:

- Subjects from a similar geographical region or ancestral region (country of origin) had similar skin types and characteristics.
- Common skin conditions were similar.
- Complexions had similar undertones according to region (lighter complexions in northern cooler climates and darker around the equator where it's the hottest). Undertones of Asian countries were yellow/olive and undertones of Europe more ruddy, red/pink.
- Migration to another country did not change the original skin type or characteristic.
- There were many mixed races that had combined skin types.

3.2.4 WHERE METHODOLOGY MEETS RESULTS

The method in which the data were collected and notes taken during office visits showed that skin from similar races or country of origin had similar skin types and skin characteristics.

Upon completion of the review of skin evaluation data, the skin type section of the EP Global Skin Classification was based on the collective findings of skin type, with sun exposure, environment, and lifestyle data combined.

The compiled data were then assembled into an organized chart, hence creating the **EP Global Skin Classification Scale**. (Refer to Figure 1.)

DERMATOLOGIC DISORDERS IN SKIN OF COLOR

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3.2.2.1 INTRODUCTION

Skin of color is sometimes referred to as pigmented or ethnic skin. It simply means having enough pigment in the skin to impart a color other than white. In general, people of African, Asian, Latin, Native American, Pacific Islander, or mixed racial origins are considered to have skin of color. The demography of the United States is predicted to shift dramatically to the point where the majority of the population will fall under this category by 2050.[1]

There are several dermatologic diseases that are more commonly encountered in this population. In particular, disorders of pigmentation such as melasma and

post-inflammatory hyperpigmentation are among the top skin concerns to prompt a visit to the dermatologist.[2]

3.2.2.2 SKIN PHOTOTYPES

The Fitzpatrick classification of skin types (Table 1) is based on an individual's ability to tan and propensity to sunburn. The skin photoype is generally correlated with degree of pigmentation. For example, Type I skin is typically seen in people of Celtic origin whereas Type VI skin is mainly seen in people of Sub-Saharan African ancestry.

Table 1: Fitzpatrick Skin Types

Skin type	Skin color	Sunburn and tanning history
I	White	Always burns, never tans
II	White	Always burns, tans minimally
III	White	Burns moderately, tans gradually
IV	Olive	Minimal burning, tans well
V	Brown	Rarely burns, tans darkly
VI	Dark brown	Never burns, tans darkly black

a. Melasma

Melasma is an acquired patchy hyperpigmentation predominantly occurring in individuals with Fitzpatrick skin phototypes III and IV. It is a common disorder affecting nearly 5 million people in the U.S. alone. Although most frequently seen in women, 10% of cases occur in men. The reported prevalence ranges from 8.8% of Hispanic women in the Southern U.S. to 40% of Southeast Asian females.[3]

Melasma occurs primarily in 3 clinical patterns: 1.) centrofacial, 2.) malar, and

3.) mandibular, although many patients have a mixture of these presentations (Figure 1). The most common is centrofacial and consists of light to dark brown patches occurring on the forehead, cheeks, nose, upper lip, or chin. The malar pattern affects the cheeks and nose, while the mandibular pattern occurs along the jawline.

Figure 1 Melasma The clinical manifestations of melasma are thought to result from the presence of biologically hyperactive melanocytes in the affected skin. The exact etiology of melasma remains unknown, however, several risk factors have been identified. Affected individuals frequently have a family history suggesting the influence of genetics.

In addition, sun exposure is a common exacerbating factor most likely through upregulation of melanocyte-stimulating factors. The high incidence of melasma in women, especially those who are pregnant or taking oral contraceptives, suggests an important role for estrogen.

The location of excess pigment, which can be assessed using a Wood's lamp, tends to correlate with the response to therapy. In general, involvement of the more superficial epidermis is more amenable to treatment than that of the deeper dermis.

A critical component in the treatment of disorders of hyperpigmentation is sun protection. This is most commonly achieved by wearing sun-protective clothing and a broad-spectrum sunblock. Without these measures, any treatment regimen is bound to fail. Hydroquinone 4% cream is FDA-approved for the treatment of hyperpigmentation and considered by many to be the gold standard. This medication works by inhibiting the melanin-producing enzyme known as tyrosinase. Sometimes higher concentrations are needed which must be compounded by a pharmacy. The addition of tretinoin cream may enhance

efficacy. Non-FDA approved natural alternatives to hydroquinone include kojic acid, arbutin, soy, licorice extract, rucinol, mulberry, niacinamide, ellagic acid, resveratrol, and dioic acid. Other methods of treating hyperpigmentation include laser therapy and chemical peels.[4]

b. Post-inflammatory Hyperpigmentation

An increase in pigment occurring after an inflammatory event is known as post-inflammatory hyperpigmentation (PIH). This cosmetically distressful phenomenon is extremely common in people with skin of color. Frequent causes of PIH include acne, eczema, psoriasis, burns, and certain cosmetic procedures. It is of utmost importance that the underlying cause of PIH be addressed if still present. The treatment of PIH is otherwise similar to that of melasma.

c. Vitiligo

Vitiligo is an acquired disorder of depigmentation estimated to affect 0.5% to 1% of the population.[5] Although vitiligo can occur at any age, almost half present before age 20. Both sexes are affected equally and there is no difference in rates of occurrence according to skin type or race. It can be a psychologically devastating, particularly in people with darker skin, because it is more noticeable.

Vitiligo is divided into three types: localized, generalized, and universal. The generalized type is most common, resulting in widespread symmetric white macules and patches. Areas of predilection include the fingers and wrists, axillae and groin, and body orifices, such as the mouth, eyes, and genitals. There is a propensity for lesions to occur in sites of trauma, which is a process known as koebnerization. The universal type is defined as having at least 80% skin involvement (Figure 2).[5] Lesions begin insidiously and are generally slowly progressive either by centrifugal expansion of current lesions and/or the appearance of new lesions. Spontaneous repigmentation occurs in no more than 15% to 25% of cases.

Figure 2 Extensive facial depigmentation occurring in a patient with vitiligo

Under the microscope, vitiligo is characterized by the absence of melanocytes. The exact reason behind their disappearance is unclear but several theories regarding autoimmune, genetic, oxidative stress, and neural mechanisms have been proposed. Currently, the evidence is most strongly suggestive of an autoimmune process occurring in genetically predisposed persons. Indeed, nearly 20% of people with vitiligo have at least one first-degree relative also affected by the disease.[5] Vitiligo may also be associated with other autoimmune diseases such as Addison's disease, pernicious anemia, diabetes mellitus, thyroid disease, and alopecia areata.

The treatment of vitiligo can be a frustrating process. Numerous treatment options exist but a cure remains elusive. As such, goals for treatment include halting disease progression, inducing repigmentation, and achieving an acceptable cosmetic result. It is also important to address the psychosocial impact of the disease. In general, the response to treatment is often slow and may be modest at best.

Current treatment options include topical and systemic steroids, topical calcineurin inhibitors, topical Vitamin D3 analogs, narrow-band UVB phototherapy, and excimer laser. Among these, the gold standard and most frequently used treatment is narrow-band UVB phototherapy.

Surgical therapy may be an option for people with focal non-progressive vitiligo who have failed phototherapy. Total depigmentation with monobenzone is a last resort reserved for individuals with extensive recalcitrant vitiligo who desire a uniform skin color. Cosmetic camouflage may be helpful in concealing areas of vitiligo occurring in particularly noticeable areas such as the face and neck.

d. Idiopathic Guttate Hypomelanosis

Idiopathic guttate hypomelanosis is a common disorder affecting nearly 80% of people over age 70. Although it occurs in all races and skin types, it is more noticeable in darker skinned individuals. It presents as several, well-defined, hypopigmented 1-6 mm flat spots with a smooth surface on the forearms and shins. The etiology is unknown but sun exposure is thought to play a role. Treatment with liquid nitrogen may be beneficial but must be weighed against the risk of causing post-inflammatory hyper-or hypopigmentation in darkly pigmented skin.

e. Pseudofolliculitis Barbae

Pseudofolliculitis barbae (PFB) is otherwise known as razor bumps or ingrown hairs. The disorder is associated with shaving and is particularly common in the beard area of black men or groin area in women. It is caused by newly shaved tightly curled hairs curving back into and piercing the skin, which results in an inflammatory reaction. PFB presents clinically as inflamed bumps and pustules, which may result in post-inflammatory hyperpigmentation and keloids.

The ideal treatment is to completely stop shaving and instead use a trimmer to leave 1-2mm of stubble. If this is not feasible for personal or professional reasons, a low potency topical steroid and topical antibiotic may be used to reduce inflammation. Other options for treatment include chemical depilatories, laser hair removal, and eflornithine cream.

CONCLUSION

The study of ethnic skin is absolutely necessary to meet the demands of our shifting demographics. Tremendous progress has been made in terms of understanding the biological mechanisms behind disorders of pigmentation. This, in turn, should spur the innovation of more effective medications and cosmeceuticals.

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ASIAN ETHNIC SKIN: SPECIALTY CORRECTIVE COSMECEUTICALS FOR ASIAN ETHNIC SKIN CARE

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ABSTRACT

The darkening of skin color in larger segments of the world's population is an emerging trend according to numerous projections. Concomitant with this change is that new, specific skin care needs are an emerging opportunity for product development in these growing markets. To serve these needs, professionals have to be abreast of the needs of this new and growing sector and they must understand this diverse global consumer. This chapter focuses on Asian ethnic skin, which is a new category as defined elsewhere in this book and known as EP V-VII in the EP Global Skin Classification Scale.

By 2014, skin care will increase its already-sizeable lead in the beauty market

to reach $91 billion (*Euromonitor International*). This increase is driven primarily by expansion of opportunities in the Asian market. Even in USA the Asian population grew faster than any other major race group between 2000 and 2010, increasing by 43% (U.S. Census Bureau).

By 2050, the U.S. population projections are that 51% of Americans will have skin of olive tones and dark-skinned racial backgrounds (Hispanics, Black, Asian, others) according to the PEW Research Center.

The most apparent difference in the skin of those from different ethnicities is, of course, the color, although there are also differences in skin thickness, vascularity, and predispositions to certain skin conditions and diseases. Pigmentation disorders are a primary concern for Asian ethnic skin as well as a tendency for acne for those with normal to oily skin.

The ingredients and methodology applied in creating effective corrective cosmeceuticals for this segment of the population must take these ethnic skin differences into account. Multiple symptoms and conditions, such as hyperpigmentation with oily skin, can then be treated with today's sophisticated formulations.

Market research shows that consumers are streamlining the number of products they buy and focusing on products that are targeted to correct more than one condition. It is therefore important for industry professionals to focus on development of "necessity products" in these skin care ranges.

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INTRODUCTION:

In modern-day India, a woman's worth is all too often determined by the color of her skin. In India, beauty lies not only in the eyes of the beholder but in societal pressure to be fair-skinned. Arranged marriages are still popular and mothers seek fair-skinned brides for their sons; in fact everyone seeks such brides/grooms. Being dark is undesirable and affects the marriage process; a

darker-complexionrf girl, even with beautiful features, is not considered beautiful. Billboards all over India promote fairness, and ads on TV promote fairness with success and beauty.

Culturally this applies to Indians living in foreign countries as well: fair is considered beautiful. My personal experience in the west has been that Asians want lighter complexions and Caucasians want to be darker and tanned. It's like wanting what is not real. I feel we should all be happy with the complexion we are born with and not let societal or cultural pressure tell you otherwise. Taking care of your skin and bringing it back to optimum health by using good products is a necessity and no longer a luxury.

3.2.3.1 DEFINING THE ASIAN ETHNIC MARKET:

Pale skin has always been the ideal for women in Asian cultures for a long time. It has nothing at all to do with wanting to be more "white" in the racial or cultural sense. Up until the 1920s pale, unblemished skin for women was the ideal in western cultures as well. Browned skin was undesirable, since it was considered a trait of only the poor who endured hard labor outdoors. Well-off women used to apply all kinds of products to their skin to whiten them, and both Chinese and Japanese cultures have proverbs that imply "a white skin hides other flaws." Those sayings have existed for ages.

All across Asia, fair skin is highly prized. In dark-skinned South Asia, a fair complexion is considered the epitome of beauty while in already pale-skinned North and East Asia, pearly translucent white skin is a sign of affluence and glamour. In Asia, many people associate dark and tanned skin with menial work in the fields under the hot sun, and a pale complexion with a higher social standing and cultural refinement. Since the 1970s, Asia has been the fastest-growing sector in the global skin-lightening market. Asia is a lucrative market with high-growth potential because of a rising middle-class with increasing disposable income and centuries-old entrenched cultural impressions of beauty.

India's domestic cosmetics industry is expected to grow to US$3.6 billion by 2014, according to the Associated Chambers of Commerce and Industry of India. The skin-lightening cream market alone was worth US$432 million in 2010 and growing at 18% annually.

In Japan the cosmetics industry is growing at 17% a year and is expected to continue that rapid pace for a few more years at the very least, largely because of the hundreds of millions of emerging middle-class women who are spending

their money on cosmetics. (1)

In China, where the skin care market is worth more than 35 billion yuan (US$5.5 billion), whitening products comprise a whopping 71% of the market. Elsewhere in Asia, a survey by the London-based market research firm Synovate found that four out of ten women in Hong Kong, Malaysia, the Philippines, South Korea, and Taiwan use a skin-whitening cream.

The cosmetics industry has also started to target men, selling them the same idea that a fairer complexion would make them more attractive. While skin-lightening products for women have been available for decades, products for men have only appeared in recent years, a trend that is rapidly growing. (2)

Japan dominates the global skin lighteners market with the lion's share, as stated in the new market research report from Global Industry Analysts, Inc. (GIA). The market for skin lighteners in Asia-Pacific is projected to cross the $2 billion mark by 2012, driven by the fast-growing markets of China and India. Apart from Asia, Western countries such as the United States and the United Kingdom are emerging as potential markets for skin-whitening products. The growing proportion of ethnic groups—Asians, Hispanics, and African Americans—in these regions is a major contributor to the enhanced demand for skin lighteners.

In Asia, skin-whitening products are very popular. The West seems to believe that these products are made because Asians want to become more Western, which isn't true. Fair skin has been a staple in beauty for Asian cultures for centuries; even old paintings in Asian cultures portray women as fair with flawless complexions.

Western society's obsession with skin tanning is well recognized, but we're often less aware of the aggressive pursuit of fair skin by those with darker skin. In communities where light skin is associated with success, prestige, and envy, women commonly turn to skin-lightening products to achieve and maintain their desired complexion.

In India, the appeal of fair skin is deeply rooted in the nation's culture and the caste system. Higher caste members traditionally had lighter skin and were less likely to be involved in manual work. Many years later, the ruling colonials had fair skin. And in the last century, film, TV, and advertisements have been saturated with images of attractive western actresses with fair skin. Skin-lightening creams are reportedly the most popular product on the Indian skin care market, with around 60% of Indian women using the products daily.

Data as at May 11, 2011. Source: United Nations World Population Prospects, Haver. Size of bubble indicates 2012 population.

Global diversity continues to grow throughout the world as Asian ethnicity remains a constant that makes up the majority of the world's population. The need for an assortment of products that are relevant to the specific multicultural market continues to drive research and technology in the skin care industry. Although Asian ethnic skin care products exist, the market struggles to find skin care products that are optimal in addressing their particular needs. It's important for brands to connect with ethnic consumers and address specific concerns to a wide array of skin types. Women and men of color around the world experience very distinctive issues related to their skin types, and the amount of pigment varies significantly between many ethnic groups. As noted in the EP Global Skin Classification Scale, each region/race has its unique skin color, characteristics, and related skin conditions.

A. Mediterranean/Asian I (EP Global Skin Classification V)

Afghanistan, Albania, Algeria, Armenia, Azerbaijan, Bahrain, Iraq, Iran, Israel, Jordan, Kazakhstan, Kuwait, Kyrgyzstan, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, Tajikistan, Turkmenistan, UAE, Uzbekistan, Yemen

B. Asian II (EP Global Skin Classification VI)

Brunei Darussalam, Cambodia, China, Hong Kong, Indonesia, Japan, Korea, Laos, Macao, Malaysia, Mongolia, Philippines, Singapore, Taiwan, Thailand, East Timor, Vietnam

In Asia, a lighter skin color is associated with beauty and aristocracy. Asians have skin properties that set them apart from Caucasians. They have more yellow undertones, and skin color ranges from fair to dark. Sometimes Asian skin types can be deceiving and not appear to have a high content of melanin, which is why taking each person's ethnicity into account is crucial. Asian skin

does in fact contain more pheomelanin pigments, which determines the yellow undertone skin color. Despite how Asians of different regions seem to sport different skin colors (Japanese are the fairest, with little redness in their skin; Indians are the darkest, with more skin redness), all actually have yellow undertones. Some might be stronger than others, like Asians of Malay origins (e.g., many Malaysians and Filipinos) have the most yellow "tones." Why yellow? It has to do with pheomelanin: high level of this type of melanin in the skin results in yellow undertones.

C. Asian III (EP Global Skin Classification VII)

Bangladesh, Bhutan, India, Maldives, Myanmar (Ex-Burma), Nepal, Pakistan, Sri-Lanka, Tibet

South Asians have a different skin type when compared to the Caucasians and require a different skin care schedule. Cosmetics used by Caucasians do not suit all Asian skin types. Asian skin is usually brownish, and skin tone varies according to the region of origin. Excessive melanin contributes to the brown color and also excessive melanin in the skin weaves a protection against skin cancer. In spite of excessive melanin content, South Asians are prone to pigmentation, age spots, freckles, *etc.* due to severe climatic conditions. Aging does not show up as wrinkles in South-Asians but rather as hyper pigmentation, dark spots, uneven skin tones, freckles, *etc.*

3.2.3.2 DIFFERENCES BETWEEN CONTENT LEVELS OF MELANIN IN THE SKIN

Differences between skin colors are determined by the amount of melanin produced by the melanocytes. Melanin comes in two different forms: pheomelanin, which gives the skin more red to yellow pigments, and eumelanin, which gives the skin dark brown to black pigment. The relative proportions of

these two types of melanin determine the skin color. For example, lighter skin types produce a lesser amount of melanin whereas darker skin types produce more melanin. Also, some Asian skin types might appear to be fairer with strong yellow undertones; therefore their skin contains higher levels of the pheomelanin pigment. No matter the amount of melanin produced, we all have the same number of melanocytes in our skin, which are the cells that produce melanin (pigment). In darker skin types the melanocytes are more sensitive to irritation and react more aggressively to injuries. An example of injury can be as simple as extracting a blackhead and applying too much pressure on the skin, which results in trauma to the skin where a dark spot is formed, also known as post-inflammatory hyperpigmentation. This is a common problem associated with those who contain more melanin in their skin. Hyperpigmentation occurs more often in those with darker skin, which can be a result from burns, inflammation, scraps, cuts, or even a pimple. Those who suffer with acne, eczema, and ingrown hairs will also result in uneven skin tone and hyperpigmented spots.

Another key point of difference in those with darker skin is the sebaceous glands that produce oil/sebum. These glands tend to produce higher levels of oil, which results in excessive oil production and ultimately results in overdrying the skin. A huge benefit for those with higher melanin content or more color in their skin is that they will tend to see signs of aging later on in life. Melanin acts as a protector for our skin and helps to ward off the UV rays of the sun by absorbing them, and keeping them from penetrating the skin. Whenever the skin detects excess solar radiation it produces more melanin and more melanin means darker skin, hence, the suntan. This not to say that people of color do not get wrinkles; it just means that aging takes place differently and the propensity for irritation and hyperpigmentation is magnified for their skin type. Therefore a greater knowledge is required for product choice and professional treatment options. Most commonly Caucasian women tend to seek out skin care products for anti-aging properties and moisturizing, whereas women with more color look for products to correct and prevent uneven skin tone and blemishes.

3.2.3.3 COMMON SKIN CONDITIONS FOR ETHNIC SKIN AND TREATMENT METHODS

With social media and companies paying attention to wanting to help the consumer solve a skin care problem, I feel that the time has come in the industry where companies can collaborate to help educate consumers on how important

prevention is and making lifestyle changes to support this. For example, if someone in their 30s wants to lighten their skin, it is mandatory that daily use of a SFP30 is also part of the regime. Direct sun avoidance during peak times is essential, and if not, a precaution such as a hat or additional protection measure should be taken.

With a collaborated campaign that is more educational, the message will reach out to consumers and change will happen. Social media plays a big role in that it can assist with getting the right message out to consumers, making it more fun and easier to grasp. In most Asian countries TV and media still play a big role in the lives of the masses, so some form of education via this method may also be very effective. Along with more education will come better preventative steps which will essential be the most successful approach to reduce dark spots.

Prevention is a key to successfully reducing the risk of hyperpigmentation in the future. A good skin care regimen followed a.m. and p.m. will help provide preventative results. A preventative regimen must include some type of mechanical or chemical exfoliation three times a week, a melanin-suppression formula, and sunscreen. The first step is to exfoliate the skin so that the dead skin cells are removed and to reveal brighter skin tone. Exfoliation will help increase the cellular turnover, so results are seen more quickly from the skin-brightening product. The second step is to use a skin-brightening product twice a day after cleansing the skin. There are a variety of skin-lightening products on the market, so the best thing to look for is the product containing the most amount of skin-lightening active ingredients. Some formulas contain hydroquinone and others do not. There are many alternatives in the market to address different skin types and pigment disorders. Refer to the list of skin-lightening actives. The last step will be to protect the skin from the harmful UV rays. Sun is the number one precursor to pigment formation. Protecting the skin with a broad-spectrum SPF of 30 will provide the best protection from UV-A and UV-B rays. If exposed to the sun for a long period of time reapply sunscreen every two hours for adequate protection. Below are common skin conditions and different causes and treatments.

Melasma (also known as chloasma or “pregnancy mask”) is a common skin condition. It causes brown to gray patches of discoloration, very common in women during the reproductive years. Melasma is dark, irregular, well-demarcated hyperpigmented macules/patches commonly found on the upper cheek, nose, upper lip, and forehead. It can also appear on parts of the body that get excessive sun exposure such as forearms and neck and décolleté area.

Causes of Melasma: Hormonal changes in the body, genetics, and overexposure to sun. History of excessive or intermittent sun exposure. Pregnancy, birth control pills, HRT, other medications (diuretics, antibiotics, heart disease, diabetic, hypertension meds). Products or treatments that irritate the skin may cause an increase in melanin production and accelerate melasma symptoms.

People with olive or darker skin, like Hispanic, Asian, and Middle Eastern individuals, have higher incidences of melasma. People with a genetic predisposition or known family history of melasma are at an increased risk of developing melasma. Important prevention methods for these individuals include sun avoidance and application of extra sunblock to avoid stimulating pigment production.

An estimated 6 million women are living in the U.S. with melasma and 45–50 million women worldwide live with melasma; over 90% of all cases are women. Prevention is primarily aimed at facial sun protection and sun avoidance. Treatment requires regular UVA & UVB sunblock SPF 30 application and skin-lightening, -whitening and -brightening creams. (3)

Treatments for Melasma:

Microdermabrasion: utilizes vacuum suction and an abrasive material like fine diamond chips or aluminum oxide crystals to exfoliate the top layers of the skin. The vacuum pressure is adjusted depending on the sensitivity and tolerance of the skin. Typical microdermabrasion sessions can last anywhere from a few minutes to one hour. Minimal to no recovery time is needed after microdermabrasion. Microdermabrasion techniques can improve melasma, but dramatic results are not generally seen or expected after one or two treatments. Multiple treatments in combination with sunscreen and other creams yield best results.

Chemical peels: Many types and strengths of chemical peels are available for different skin types. The type of peel should be tailored for each individual and selected by a physician.

TCA: TCA peels are excellent and safe in the hands of an experienced dermatologist. This is a good option, particularly if you have severe melasma.

AHA +combinations: In treating melasma, 30–70% glycolic acid peels are very common. Various combination peels, including a mix of glycolic acid, kojic acid, lactic acid, etc., can be used to treat melasma. Usually six sittings at a gap of ten days produce good results.

Ultrasonic: Treatments with whitening jelly can be used in conjunction with chemical peels or MDA to further enhance results.

IPL treatments with pigmentation filter or currently Fractional Laser Treatments are being used to reduce pigmentation. Usually three to four treatments are needed at a gap of 15–20 days.

Although melasma tends to be a chronic disorder with periodic ups and downs, the prognosis for most cases is good. Just as melasma develops slowly, clearance also tends to be slow. The gradual disappearance of dark spots is based on establishing the right treatment combination for each individual skin type. Melasma that does not successfully respond to treatment is because sun exposure is not avoided.

Peri-Orbital Hypermelanosis (Undereye dark circles): This is a very common disorder among the Asian population, especially the Indian population. Undereye circles, which are dark pigments around the peri-orbital area, may be hereditary, or due to stress or eye strain. Also, those suffering from allergies seem to have dark circles. Smoking, drinking a lot of alcohol, coffee, soda, or caffeine beverages will usually make dark circles worse. Diet also plays a role and not having a healthy diet can affect it as well. A lack of sleep is a big culprit, so pulling all-nighters—whether for school or fun—can also contribute to dark circles around the eyes.

Treatments of Peri-Orbital Hypermelanosis: Once the cause has been identified there are several available solutions for treatment of this common disorder. Using a good eye cream that has ingredients specifically targeted to this delicate area, twice a day, will help prevent moisture loss, hydrate the area, and assist the delicate skin in absorbing the moisture and active ingredients, while stimulating the blood supply. Also wearing sunglasses can help prevent further pigmentation when exposed to the sun.

Photomelanosis: This is increased pigmentation due to sun exposure; it is also known as sun damage or photo-damage. The pigmentation occurs on exposed skin, commonly on the face, neck, and back. The pigmentation may be patchy or a diffused darkening of the exposed skin.

Hyperpigmentation: Hyperpigmentation is the production of excess melanin causing dark spots on the skin. Age spots, liver spots, freckles, sun spots, and pregnancy mask are all types of hyperpigmentation.

Causes of Hyperpigmentation: Common causes of skin hyperpigmentation are excessive sun exposure, hormonal changes, heredity, acne, and irritations

from skin treatments and products. Hyperpigmentation may also be caused by inflammation, or by other skin injuries, including those related to acne vulgaris. In situations where hyperpigmentation is caused by acne or some other cause of skin inflammation, it is known as PIH, or post-inflammatory hyperpigmentation. People with darker Asian, Mediterranean, or African skin tones are also more prone to hyperpigmentation, especially if they have excess sun exposure.

Many forms of hyperpigmentation are caused by an excess production of melanin. Hyperpigmentation can be diffuse or focal, affecting such areas as the face and the back of the hands. Melanin is produced by melanocytes at the lower layer of the epidermis. Melanin is a class of pigment responsible for producing color in the body in places such as the eyes, skin, and hair. As the body ages, melanocyte distribution becomes less diffuse and its regulation less controlled by the body. UV light stimulates melanocyte activity, and where concentrations of the cells are denser than surrounding areas, hyperpigmentation is affected. It can also be caused by using skin-lightening lotions. (4)

Post-Inflammatory Hyperpigmentation: a very common condition for those with more color in their skin. Many types of trauma, inflammation, medications, cosmetics containing alcohol or fragrance, and skin conditions including: acne, eczema, contact dermatitis. Systemic disease such as Addison disease, liver disease, pregnancy, and pituitary tumors can also cause hyperpigmentation.

Acne Vulgaris: one of the biggest challenges to women with brown skin who seek clear, glowing complexions. In addition to the acne, women with brown skin must also face hyperpigmentation—skin darkening in spots or patches—which occurs in response to the acne outbreak.

Acne Rosacea: not one of the skin problems typically associated with skin of color. A main feature of rosacea is redness or erythema of the face. But rosacea does occur in people of color, including African Americans, Latinos, and Asians, and is often undiagnosed or misdiagnosed.

Eczema: one of the more common skin problems for individuals with brown skin, including those of Asian, Latino, and African descent. It is felt to be the second-most-common skin disease in African Americans. Although it is unknown if the incidence of eczema is increased in Latinos, one study found a significantly higher percent of Mexican American adolescents with eczema than white and

African American adolescents.

Keloids: Abnormal healing of the skin occurs frequently in individuals with brown skin. When skin is injured, it may heal with one of several types of scars (keloids): normal (level with the surrounding skin), atrophic (depressed), hypertrophic (slightly raised), and keloidal (large and raised).

Seborrheic Dermatitis: often referred to as dandruff, is a common problem for many women with brown skin. Areas of involvement are the hairline and scalp, as well as the eyebrows, the area between the nose and corners of the mouth (nasolabial folds) and the ears.

Skin Cancer: People with brown skin often have a false sense of security when it comes to skin cancer. While individuals with increased skin pigmentation have added protection against the UV rays of the sun, it is dangerous to assume that darker skin exempts one from this serious skin problem.

Hypopigmentation: Areas of the skin that have lost their pigment completely when there are no longer functioning melanocytes in that area to create pigment, leaving white spots or patches on the skin. Hypopigmentation can result from overbleaching, or extensive trauma from UV light, lasers, or heat. Examples of hypopigmentation include:

Vitiligo: Vitiligo causes smooth, white patches on the skin. In some people, these patches can appear all over the body. It is an autoimmune disorder in which the pigment-producing cells are damaged. There is no cure for vitiligo, but there are several treatments, including cosmetic cover-ups, corticosteroid creams, or ultraviolet light treatments.

Albinism: Albinism is a rare inherited disorder caused by the absence of an enzyme that produces melanin. This results in a complete lack of pigmentation in skin, hair, or eyes. Albinos have an abnormal gene that restricts the body from producing melanin. There is no cure for albinism. People with albinism should use a sunscreen at all times because they are much more likely to get sun damage and skin cancer. This disorder can occur in any race, but is most common among whites.

Pigmentation loss as a result of skin damage: If you’ve had a skin infection, blisters, burns, or other trauma to your skin, you may have a loss of pigmentation in the affected area. The good news with this type of pigment loss is that it’s frequently not permanent, but it may take a long time to repigment. Cosmetics can be used to cover the area, while the body regenerates the pigment.

(5)

3.2.3.4 TIPS TO ACHIEVE HEALTHY, BEAUTIFUL SKIN

- Mandatory daily use of broad-spectrum UVA & UVB Sunblock – SPF30+
- Wash skin daily with mild nonsurfactant cleanser, use a clean towel to pat dry, do *not* rub the face harshly, or share towels with others. The face should be washed twice, both morning and evening.
- Drink water—an average adult typically needs 10–12 eight-ounce glasses a day. I prefer to drink room-temperature or hot water with a slice of lemon.
- Use quality skin care products that hydrate and repair the skin, and avoid products that clog your pores.
- Use lightweight facial makeup foundation or powders; this will allow the skin to breathe and prevent further breakouts. Personally I feel the less used the better; if you take care of your skin you will have a natural radiance.
- The skin is a very sensitive organ and given the level of abuse it goes through, is remarkably resilient. However, this resilience decreases as we age, and opens the door for bacteria, viruses, microbes, parasites, *etc.* that invade the skin. In climates such as Asia where it is hot and polluted, especially in the cities, environmental damage also plays a role in the aging process.
- If you have a tendency to break out, seek professional help to treat it and go for the root cause of the blemish, rather than using makeup to cover skin blemishes and trauma. This can save you permanent damage with scars and or ice-pick marks.
- Avoid skin trauma like tattoos, body piercing, *etc*. These abuses can age the skin, and expose it to bacteria and other viruses.
- When you notice skin discomfort, dryness, itchiness, reddening, discoloration, blemishes, lesions, bruising, tags, moles, bumps, warts, corns, calluses, or other skin trauma, seek professional help, as some conditions may get worse over time if not treated.
- Diet plays a crucial part in skin health, so eat wisely. Your diet should contain plenty of green leafy vegetables. Eat seasonal fruits and add nuts to your diet; if you have health issues it is wise to seek a nutritionist, or dietician, to establish a healthy diet to suit your unique skin and bodily needs.
- Make regular monthly visits with a skin care professional to help maintain

healthy skin. Having topical treatments such as Lactic Peels, chemical peels, microdermabrasion, and laser therapy specific for Asian skin.

- Treat yourself to regular spa treatments; having a regular full-body massage is not only relaxing but good for you too. A deep massage is good to maintain healthy skin, since the kneading breaks up old fat deposits and releases toxins. The sauna and steam bath are recommended to help keep skin moist, supple, healthy, and toned. Drink water to hydrate before and after your sessions.
- Minimize exposure to tanning machines, lamps, beds, *etc.* The tanning process is actually a form of skin abuse, and can result in premature aging of the skin and promote hyperpigmentation.
- Always use high-quality and well-made skin care products. Using a daily program that consists of cleanser, exfoliator, moisturizer, and SPF 30 is highly recommended. The reward is to enjoy clear, healthy, blemish-free, radiant, glowing, beautiful skin for many years.
- Avoid cheaply made skin creams with a lot of chemicals. Products that are highly fragranced, with artificial colors, binders, emulsifiers, etc., can cause some people to develop allergies, leading to rashes and pigmentation issues.
- In order to maintain healthy skin and overall health, a total avoidance of alcohol, tobacco, and illegal drugs is advisable. These are all toxic to the body and after many years of such abuse, the skin can become wrinkled, saggy, dry, and itchy, and can lose its suppleness. Reducing or eliminating caffeine, sugar, and soft drinks will also benefit the skin and overall health.

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PART 3.2.4

COMPROMISED SKIN IN THE ELDERLY

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ABSTRACT

This chapter provides a thorough look at the structure and chemistry of the skin. It draws upon, and consolidates, the enormous body of literature on the subject and brings it together to form a solid foundation for the reader's understanding of skin aging as well as describing treatments of aging skin with selected effective "cosmeceuticals." We live in a culture that values a youthful appearance at a time when the population is aging rapidly in developed countries. According to the United States census there were ~57 million Americans age 60 and older in 2010, nearly a 25% increase in the ten years since the 2000 census. As people live longer, populations in Western Europe and Japan are also aging.

It is well known that the appearance of the skin can have dramatic effects on the overall impression of age. Both wrinkles (1, 2) and inhomogeneity of skin color (3–6) contribute significantly to the apparent age of individuals. The combination of the effect of skin appearance on apparent age and an aging population with the desire to look youthful has, not surprisingly, led to enormous interest in products that might make skin appear more youthful.

Skin aging is often broken into two categories referred to as intrinsic aging and extrinsic aging (7–9). *Intrinsic aging is the unavoidable effect of time.* It occurs on all body sites and is considered to be independent of lifestyle. Extrinsic aging results from lifestyle choices. The main factor is photoaging brought about by exposure to ultraviolet (UV) light from the sun (7, 10–12). While sun exposure is considered the main extrinsic factor leading to skin damage, it is becoming increasingly clear that exposure to cigarette smoke can lead to significant skin compromise both in smokers(13–21) and in nonsmoking spouses of smokers (22).

This chapter reviews the consequences of both intrinsic and extrinsic skin aging. Mechanisms underlying photoaging will be discussed, as will attempts to improve aging skin and at least partially reverse the effects of time and extrinsic insults.

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3.2.4.1 STRUCTURE OF THE SKIN

We begin with a brief review of skin structure to facilitate our discussion of skin aging. This subject is discussed in more detail in other chapters to this book. Discussion of the structure of skin will naturally refer to its various layers (23, 24) or strata. The skin's multiple layers work together to perform its multiple functions. Figure 1 is a hematoxyln and eosin (H&E) stained image of a cross-section of human skin.

Figure 1 (H&E stained cross section of human skin labeled to show the various layers. The "basket weave" appearance of the *stratum corneum* is an artifact of processing. http://en.wikipedia.org/wiki/Epidermis_(skin) public domain image)

The epidermis and dermis are fundamentally different types of tissue. The epidermis overlays the papillary dermis and is composed of cells. The main type of cell of the epidermis is the **keratinocyte**. Keratinocytes are found from the basal layer to the granular layer where they are transformed into the corneocytes of the *stratum corneum* (SC). Keratinocytes make many proteins including keratins, the main structural proteins of the SC (25). Two other important cells in the epidermis are melanocytes and Langerhans cells.

The **dermis** has two layers, papillary and reticular. The hypodermis or subcutaneous tissue is sometimes considered part of the dermis but will not be considered in this review. The dermis is a fibrous tissue composed primarily of collagen, elastin, and proteoglycans, which form the dermal matrix. The papillary dermis is named for the dermal papilla protruding into the epidermis (Figure 1). The fibroblasts, which make the proteins and proteoglycans of the dermis, are found in the dermal matrix (24).

The main structural protein of the dermis is collagen. Collagen has a unique amino acid sequence. Every third amino acid is glycine, which does not have a side chain. This allows collagen to form a compact triple-helix with three collagen chains winding around each other as if in a braid. Each triple helix is about 300 nm long and 1.5 nm in diameter (26). Collagen is synthesized in fibroblasts as procollagen and exported from the cell into the dermal matrix where it is processed by proteolytic enzymes, which remove sections from both ends of procollagen to form 300-nm filaments. These filaments then aggregate in the extracellular space to form collagen fibers.

The matrix in the papillary dermis is loosely packed with fibers compared to the tightly packed reticular dermis (24). The papillary dermis contains type III

collagen, which is found at relatively low levels in the reticular dermis where type I collagen predominates (27–29). Oxytalan fibers are thin (10–12nm) elastic fibers found perpendicular to the dermal-epidermal junction in the papillary dermis (30, 31). Elaunin fibers are also found in the papillary dermis parallel to the plane of the skin (30).

Elastin provides elasticity to skin (32). In elastin, four protein chains are bound together through the desmosine cross-link. Elastin has an amino acid residue called allysine, made from lysine by the enzyme lysyl oxidase. The desmosine cross-link is formed between three allylisine chains and one lysine chain. Amorphous elastin fibers surrounded by fibrillin microfibrils are found predominately in the reticular dermis (33, 34) (Figure 2).

Figure 2: Electron micrograph of the reticular dermis showing collagen, elastin, a fibroblast, and a mast cell. (© Raymond Boissy, used with permission)

Glycosaminoglycans or GAGS bind water in the dermal matrix (35) keeping the dermis hydrated. Hyaluronic acid (HA), a major component of GAGS, is a very high molecular weight. GAGs such as chondroitin sulfate and keratan sulfate bind to core proteins to form proteoglycans. Proteins called link proteins bind proteoglycans to HA in a noncovalent proteoglycan complex that is very hygroscopic.

Figure 2 is an electron micrograph of the reticular dermis showing collagen and elastin fibers as well as a fibroblast and a mast cell. Mast cells contain histamine granules and release histamine in response to heat or irritants. The reticular dermis is much more densely packed with fibers than the papillary dermis.

The dermal matrix is significantly affected by both intrinsic and extrinsic aging as will be discussed below.

Skin barrier function resides primarily in the epidermis and barrier may be considered "*la raison d'être*" of the epidermis (36). Layers of the epidermis are shown schematically in Figure 3.

Figure 3: Diagram showing layers of the epidermis (illustration by Robin M. Wickett)

The epidermal barrier defends the body from water loss to the environment, absorption of noxious chemicals from the environment, and microbial infection. These defensive functions depend critically on the top layer, the *stratum corneum* (SC), and are thought to be integrated with SC formation and homeostasis (37, 38).

For 30 years the most common model used to organize thinking about the SC barrier has been the "bricks and mortar" model proposed by Elias (39). This model is based on the fact that corneocytes are surrounded by the extracellular lipids of the SC. Thus the corneocytes are modeled as bricks and the lipids as mortar. The model has proven useful for representing the phenomena associated with describing ingredient skin penetration. Moderately hydrophobic molecules are thought to penetrate the SC by diffusing through the lipid mortar, winding their way around the corneocytes bricks (40). Harding has described an updated version of the bricks-and-mortar model that includes the presence of the desmosomes that hold SC cells together prior to desquamation and the role of natural moisturizing factor in SC function (41). This model is illustrated in Figure 4.

Figure 4: Schematic of the "bricks and mortar" model for human *stratum corneum* illustrating the corneocyte "bricks," the intercellular lipid "mortar," and the desmosomes connecting the corneocytes. (M.O. Visscher, used with permission)

The "bricks and mortar" of the SC barrier are formed in the *stratum granulosum* (SG) (Figure 4) where keratinocytes are transformed to form the corneocytes "bricks" and lipids are released into the intercellular space to form the "mortar." The SG is named for the two types of granules that appear in the cells, keratohyalin granules (24) that are composed of protein and lamellar bodies that primarily contain lipids (42, 43).

A complex process occurs in the SG: the nucleus is digested, the cytoplasm disappears, and lipids are released into the intercellular space to form the mortar (39, 44, 45). The keratin intermediate filaments aggregate to form microfibrils (46, 47) inside the corneocytes (48, 49). aided by filaggrin from the keratohyalin granules.

Filaggrin is an acronym for filament-aggregating protein (50–53). Filaggrin contains a high level of positively charged amino acids, allowing it to interact with the negatively charged epidermal keratins (54). Filaggrin is then modified to come off the microfibrils, through conversion of the positively charged amino acid arginine to uncharged citruline by the enzyme peptidyl arginine deminase (PAD) (55, 56). After release from the microfibrils filaggrin is digested by proteolytic enzymes to produce the amino acid components of the natural moisturizing factor (NMF) of the *stratum corneum* (57–62).

NMF consists of lactate, amino acids from filaggrin breakdown, and pyrollidone carboxylic acid (PCA) formed from the amino acid glutamine (63–

65). These natural moisturizers are important to maintain proper hydration of the SC allowing it to be flexible and to desquamate properly (57, 66–69). Digestion of filaggrin to NMF is thought to happen in the mid-*stratum corneum* (70).

In the *stratum granulosum* (SG) the keratinocyte cell membrane is replaced by the corneocyte cell envelope, a very tough structure made of cross-linked protein with lipids covalently attached to its surface (71–75). The resulting corneocytes or squames (the bricks) are flat cells that tend to be in the shape of either a hexagon or pentagon. Corneocyte size and distribution have been investigated by several authors. A single corneocyte is about 25 μm on a side, with a surface area ranging from about 550 to 1300 μm^2 depending on age and body site (76–79), and a thickness of about 0.5–1.0 μm (80, 81). Rougier *et al.* (79) reported corneocyte surface area on most body sites investigated to range from 900 to 1100 μm^2 but to be as low as 500 μm^2 on the forehead. A negative correlation was found between corneocyte size and the penetration of benzoic acid through skin *in vivo*. The *stratum corneum* is 12–16 cell layers thick on most body sites, but it can vary from as little as nine cell layers on the forehead or eyelids to as much as 25 on the dorsum of the hand and 50 or more on the palms or the soles of the feet (82, 83).

The lipids that are released into the intercellular space as the SC forms are glucosyl ceramides, cholesterol, cholesterol esters, and long-chain fatty acids. In the intercellular space the glucosyl ceramides are converted to ceramides (84, 85). SC ceramides are polar lipids, but much less hydrophilic than the phospholipids of the original cell membrane. Figure 5 shows the structure of some of the main SC ceramides with both the original and new nomenclature that is used for them. Phospholipids from the keratinocytes of the viable layers are broken down by phospholipases (86–88) in the lower *stratum corneum*. After this extracellular processing, SC lipids spontaneously organize into multiple layers between the SC cells (89). This layered structure is critical to the barrier function of the skin.

Figure 5: Key ceramides of the SC barrier.

3.2.4.2 SKIN AGING: CHANGES IN THE EPIDERMIS AND *STRATUM CORNEUM*

A general trend for corneocytes to increase in surface area with age has been reported by several groups (77, 79, 90-93). The reported increase in surface area between young adults and adults over 55 ranges from 8 to 14% depending on body site and method of measurement. Figure 6 shows corneocyte size data from two different age groups reported by Grove *et al.* (91, 93).

Note the extreme variability in the data from the older age group compared to the narrower distribution in the younger group. The average size of corneocytes is increased in the older age group but there is considerable overlap between the two data sets.

Figure 6: Projected corneocyte surface area for two age ranges for men (●) and women (○). Reprinted from Grove *et al.* (93) with permission of the Society of Cosmetic Chemists.

While the “bricks” of the SC may increase in size with age, the “mortar” content is apparently decreasing. Imokawa *et al.* reported a gradual decrease in total SC ceramides with age (94). Rogers *et al.* reported progressive reductions in fatty acids, ceramides and cholesterol with age in subjects ranging in age from 21–60 years on the hands, legs, and face (95). The reduction in ceramide I (EOS) linolate was especially noteworthy.

SC barrier function is affected by both intrinsic and extrinsic aging (96), but it is relatively robust to both and changes are not always in the direction that one might expect. The most common and best-validated method for determining SC barrier function *in vivo* is by measurement of the passive diffusion of water through the skin by measuring the rate water evaporates from the surface of dry nonsweating skin. This parameter is known as transepidermal water loss (TEWL) (97–99).

Several groups have studied the effects of age on SC barrier function by measuring TEWL. Results have varied. Roskos and Guy (100) reported no correlation between baseline TEWL on the ventral forearm and age for subjects ranging in age from 20 to 90 years, and Marks *et al.* (101) did not see a significant correlation to age on the forearm with a similar age range, but a trend to *lower* TEWL was observed for subjects above 55. Rougier *et al.* found no significant change in TEWL on the upper outer arm in subjects ranging in age from 20 to 80 years (79). Kligman (102) reported that TEWL on both the forearm and lower legs averaged slightly *lower* for a group of individuals aged 66–81 compared to a 19–26 year old cohort. Leveque *et al.* (77) reported TEWL from the arm to be approximately constant from 25 to 65 years of age but to decline slightly between ages 65 and 75.

Wilhelm *et al.* (103) compared TEWL data from a young group (26.7±2.8 years) and an older group (70.5±13.8 years) on several body sites. TEWL was significantly *lower* from the older age group on all sites except the palm. Saijo *et al.* (104) measured TEWL from the dorsum of the hand and the dorsum of foot on children from one to five years of age, young adults from 21 to 34 years of age, and older adults ranging from 63 to 78 years of age. Aged individuals had the lowest TEWL values on both body sites. There was no difference in TEWL from the dorsum of the hand between children and young adults. Ghadially *et al.* (105) reported average TEWL of 4.43±0.16 g/(m^2-hr) for a group of six individuals over 80 compared to 6.41±0.93 g/(m^2-hr) for a group of 21 subjects under 30.

Schwindt *et al.* (106) found significantly lower TEWL on the backs of

subjects averaging 69.8 years of age compared to subjects averaging 27.7 years old. In a recent study Luebberding *et al.* reported TEWL data from six different body sites on 150 women divided equally into five age groups, 18–29, 30–39, 40–49, 50–59, and 60–80 (107). No correlation was found between TEWL and age on the neck, forearm, or back of the hand. TEWL was negatively correlated to age on the forehead and cheek. Interestingly, TEWL on the décolleté area was positively correlated to age, ranging from 5.62 gm/m^2-hr on the youngest group to 7.86 gm/m^2-hr on the oldest, with a high level of statistical significance. In our review of the literature these data from the décolleté are the only ones to show a statistically significant increase in TEWL with age.

The SC barrier to penetration of compounds into the skin has also been evaluated as a function of age. Roskos *et al.* compared the penetration of six drugs of differing polarity into the skin of subjects 22–40 years of age and over 65 years old *in vivo* (108). Permeation rates for the two most hydrophobic drugs, testosterone and estradiol, were not significantly different between the two age groups but hydrocortisone, benzoic acid, acetylsalicylic acid, and caffeine all penetrated the skin of the older group at a significantly *lower* rate than into the younger. Rougier *et al.* also reported that benzoic acid penetrated older skin significantly more slowly than younger *in vivo* (79).

Analysis of all these results leads to the somewhat surprising conclusion that basal SC barrier function is relatively unaffected by age from childhood through late middle age and then may actually begin to increase slightly sometime after age 60. It is notable that this apparent improvement in baseline barrier function occurs in spite of the reduction in SC barrier lipids discussed above. Rogers *et al.* point out that while lipids decrease, the ratio of ceramides to other SC structural lipids remain approximately constant (95). It is also possible that skin permeation of some compounds through SC is slower because of the larger corneocytes. Rougier at al. found a negative correlation between corneocyte size and the skin permeation rate of benzoic acid *in vivo* (79).

Most studies indicate that the reactivity of skin to irritant may actually decrease with age, or at least is not more marked with age as one might expect. Bettley and Donoghue reported fewer reactions to patch testing with soap in subjects 50 and older compared to subjects 10–49 years of age (109). Grove *et al.* reported lower skin reactivity to a variety of irritants including dimethyl sulfoxide, ethyl nicotinate, and lactic acid (93, 110) in older subjects. Maibach and coworkers (106, 111) and Robinson (112) observed lower reactivity of older skin to sodium lauryl sulfate (SLS). Robinson also reported that reaction to

another strong irritant, octanoic acid, was significantly lower in subjects more than 55 years old while reactions to the weaker irritants acetic acid and decanol were only directionally reduced with age (112). Bowman *et al.* performed a ten-day cumulative irritation test comparing 26 subjects age 18–45 with 26 subjects age 65–80 using 11 products of widely varying irritancy (113). They found no statistically significant differences in response between the age groups, but the response was directionally less in the older age group for all the compounds that produced significant irritation.

While the static barrier function of the epidermis does not seem to diminish with age, recovery of barrier function after disruption has been found to be significantly slower in older skin. Ghadially *et al.* disrupted SC by either tape stripping or acetone treatment to the point that TEWL was increased to the range of 20–30 g/m^2-hr (105). Recovery of the barrier was quantified by measuring TEWL over the next several days. After acetone treatment young skin (under age 30) recovered its barrier function by 50% at 24 hours, while older skin (over 80) had only recovered 15% at 24 hours. Younger subjects took about four days to recover to 90% of their original SC barrier to TEWL while older skin required seven days to reach this level of recovery regardless of the type of insult (tape or acetone). The slower recovery of barrier function may be related to slower rates of epidermal renewal in older skin.

It is well known that corneocytes are continually shed into the environment and are being continually replaced by cells generated in the SG (see above). The SC "turnover time" is most frequently measured by staining the SC with the fluorescent dye, dansyl chloride, and monitoring the rate of dye disappearance with a UV light. When all of the fluorescence has disappeared, the SC has turned over completely (114). Grove and coworkers reported that SC turnover time is increased in elderly skin (76, 93). The turnover time for SC on the volar forearm increased from 19.8 ± 1.4 days in cohort under 35 years old to 28.1 ± 2.7 days in an over 60 age group. Similar changes were seen on the inner arm. These results confirmed the positive correlation between age and the time to turn over the SC reported by Roberts and Marks (115).

Grove found the number of SC cell layers was approximately constant between age groups, indicating that the increased SC turnover time results from decreased epidermal cell proliferation rates with age. Marks reported a small but statistically significant decline with age in the number of epidermal cells in the DNA synthesis phase (116). Engelke *et al.* investigated differences between young normal skin, young dry skin, aged normal skin, and aged dry skin (117)

using an antibody that stains the nuclei of proliferating cells (Ki-S3) (118). Antibody staining indicated a significant reduction in epidermal proliferation rate in aged “normal skin” compared to young normal skin. A slower rate of epidermal cell proliferation is consistent with the increased time to repair a disrupted SC barrier as reported by Ghadially (105). Grove also reported that small chemically induced blisters healed more slowly in subjects between 65 and 70 years of age than in subjects 18–25 years of age (119).

The greater tendency of older subjects to develop dry skin is well known (120–122). Elderly dry skin seems to be especially prone to itching (120, 123–126), which can sometimes be severe. Dry skin in the elderly tends to develop some distinctive patterns, especially on the legs. Figure 7 below shows dry skin that developed on the legs of three elderly subjects who lived in the desert Southwest. These pictures were taken after one week of washing twice daily with a standard soap and no use of moisturizers. Notice the distinct pattern of lines on some of the subjects.

Figure 7: Dry skin on the lower legs of elderly subjects (70–90 y.o.) after washing twice daily with a standard soap and avoidance of moisturizer use.

This pattern of lines frequently develops in elderly subjects but occurs rarely if at all in younger subjects (R.R. Wickett, personal observations). Treatment with an effective moisturizer reduces or eliminates the pattern in some subjects but not in others. In Figure 8 below we see that moisturizer treatment of the right legs has nearly eliminated the pattern in the subject on the left but had little effect on the subject on the right.

Figure 8: Dry skin resulting from soap use on the legs two elderly subjects. After one week of washing with soap and no moisturizer use the right leg of each subject was treated with an effective moisturizing lotion for two weeks while soap treatment continued on both legs.

It is not entirely clear why the elderly are so much more prone to developing dry skin. One possibility is reduction of levels of NMF (see above) in elderly skin. It has been shown that NMF levels are lower in dry skin (62). Data on the effect of age on NMF levels do not present a consistent picture. One of the key moisturizing components of NMF is the very hydrophilic molecule pyrollidone carboxylic acid (PCA) (56, 57) (see above). Harding *et al.* reported lower levels of PCA in elderly subjects and the difference was more marked deeper in the *stratum corneum* (70). Horii *et al.* reported a general decrease in free amino acids with increasing levels of dry skin in elderly subjects (127).

On the other hand, Jacobson *et al.* found that some SC amino acids decreased with age while others increased, but no difference was seen in total NMF free amino acids (FAA) normalized to protein content between young and old subjects (128). Takahashi and Tezuka (129) reported that NMF FAA actually increased in older subjects. However, both of these groups sampled the *stratum corneum* by scraping skin flakes from the surface rather than tape stripping to sample from lower levels of the SC as Harding *et al.* did. Further, as Tagami (96) has pointed out, Takahashi and Tezuka reported their results as FAA/corneocyte. Thus the higher levels of NMF FAA they report could be due to the presence of larger corneocytes in older skin (77, 78, 93) as discussed above.

Lactate is a component of the NMF that may be derived either from sweat (130, 131) or through anaerobic metabolism in the upper epidermis (132). Nakagawa *et al.* investigated the correlation between NMF components and SC stiffness, pH and hydration in summer and winter with healthy subjects (67). The only components that correlated significantly to SC stiffness and hydration were lactate and potassium. One might expect that the slower metabolism of aging skin reflected by the lower SC turnover rate might lead to lower levels of lactate in the SC. However, Prahl and coworkers reported that keratinocytes from aging skin produce higher levels of lactate *ex vivo* (133). It is well established that sweating rates decrease with age (134), which might lead to lower levels of lactate and thus stiffer, less well-hydrated SC.

Another factor in skin aging may be the reduction of sebum production that

occurs with age (135). Fluhr *et al.* have presented data from studies of asebic mice, indicating that glycerol derived from the hydrolysis of sebum contributes to skin hydration (136). Sebum production declines dramatically in women at menopause but does not really decline much in men before age 80 (135), so this seems unlikely to be the whole story either. It seems likely that the tendency for elderly subjects to develop dry skin has multiple contributing factors including lower PCA levels, the slower rate of SC renewal, lower levels of lactic acid due to reduced sweating, possibly reduced glycerol due to reduced sebum production, and other factors that have yet to be discovered.

There are been relatively few studies specifically comparing the effects of intrinsic and extrinsic aging on the SC. Tagami's group studied the dorsum of the hands of Japanese golfers who only wore a golfing glove on one hand (96, 137). Roughness of the skin surface was measured from silicon replicas. Interestingly, there was a significant negative correlation between the difference in roughness between exposed and covered hands and a golfer's handicap. Better golfers (lower handicap) had larger increases in roughness on their exposed hand. Hydration as measured by higher-frequency conductance was lower for the exposed site indicating a drier skin surface, but there was no difference in barrier function as measured by TEWL.

3.2.4.3 AGING AND THE DERMIS

Both intrinsic aging and photoaging affect the dermis far more dramatically than the epidermis. The papillary dermis is strongly affected by both intrinsic aging and chronic sun exposure. Figure 9 shows the effect of aging on abdominal skin of 18-and 76-year-old females. At the time these micrographs were published (1955) the abdominal skin of women received very little sun exposure, so the changes represent the effect of intrinsic aging.

Figure 9: Abdominal skin from an 18-year-old female (left) and a 76-year-old female (right). Note the flattening of the dermal-epidermal junction as the papillary dermis diminishes with age.- (modified from Andrew [138] with

permission of the Society of Cosmetic Chemists).

This flattening of the papillary dermis makes the skin less resistant to shearing stress, and the skin of elderly people is more easily ulcerated than that of younger people.

The thickness of the skin varies with age and changes depend on body site. In general, skin thickness increases for the first two decades of life. After this initial period, skin thickness may be fairly constant until about age 60. De Rigal *et al.* reported ultrasound results showing that skin thickness on both the ventral and dorsal forearm decreased significantly with age, but only after age 60 (139). Gniadecka and Jemec, however, reported a gradual decline in skin thickness on the forearms and ankles starting at about age 20 (140). Skin on the forehead and buttocks was found to increase in thickness with age. Takema *et al.* also found skin thickness to decrease with age on the forearms and increase on the forehead, corners of the eyes, and the cheeks (141). They interpreted this increased thickness as the result of sun exposure on the face.

3.2.4.4 THE DERMATOGLYPHIC PATTERN OF THE SKIN

Young skin shows a complex pattern of shallow lines on the surface. This dermatoglyphic pattern is altered on aging and a pattern with fewer but deeper lines that become more oriented emerges (93). Natural lines of tension in the skin called Langer's lines may influence the directional orientation. Hassan Zahouani and coworkers have studied these line patterns in detail (142–144). Figure 10 shows typical patterns from subject in various age ranges, pseudo-colored to indicate topography.

Figure 10: Changes with age in the dermatoglyphic pattern of human skin.

Figure courtesy of Hassan Zahouani, used with permission.

A highly aligned dermatogyphic pattern can also be seen on the legs of some of the subjects in Figure 8 above.

In addition to the flattened dermal-epidermal junction (Figure 9) and changes in the dermatoglyphic pattern, intrinsically aged skin has diminished collagen synthesis leading to reduced levels of collagen in the dermis (145–147). El-Domyati *et al.* reported that there is gradual loss of the transverse elaunin fiber network and shortening and eventual reduction of the oxytalin fibers, which were nearly gone by the ninth decade of life in sun-protected skin. Underlying elastic fibers in the mid to deep reticular dermis of sun-protected skin remained relatively unchanged in amount with age (146). Focal loss of elastin and clumping of elastin fiber bundles has been observed in sun-protected elderly skin in some individuals (148).

3.2.4.5 AGING AND MECHANICAL PROPERTIES OF SKIN

Skin is a viscoelastic material. Measurement of mechanical properties of skin *in vivo* is complicated by its complex, layered nature (Part 1). It is difficult to deform the SC without producing significant deformations in the underlying layers, and of course it is impossible to deform the dermis without deforming the SC. The general principle is that smaller deformations (minimum strain) will help to localize the response to the SC, and larger deformation will reflect more on the properties of the underlying layers (149).

The most commonly used instrument for measuring skin elasticity is the Cutometer® (150–152). The Cutometer uses negative pressure to pull the skin vertically into the opening of the probe (151, 152). Negative pressures can be controlled between 20 and 500 mbar. The height the skin raises into the probe is determined by an optical system. Probes with diameters of 2, 4, 6, and 8 mm are available. In the most widely used mode, deformation is measured while vacuum is applied quickly, held constant for a defined period of time, and then released (Figure 11).

Figure 11: Schematic of Response of Skin to Cutometer

Table 1 defines parameters that can be obtained from the Cutometer curve.

TABLE 1 Cutometer Parameters from Figure 11

Ue Elastic deformation of the skin due to the application of stress (vacuum)
Uv Viscoelastic creep occurring after the elastic deformation
Uf Total extensibility of the skin during stress application
Ur Elastic recovery due to stress removal
Ua Total recovery at the end of the stress-off period
R Amount of deformation not recovered by the end of the stress-off period
Ua/Uf Overall elasticity of the skin, including creep and creep recovery
Ur/Ue Pure elasticity ignoring viscoelastic creep
Uv/Ue Ratio of viscoelastic to elastic extension, called the viscoelastic ratio
Ur/Uf Ratio of elastic recovery to total deformation

Cua, *et al.* (153) used the Cutometer® to evaluate age and body site differences in the viscoelastic properties of skin in a study with 33 subjects, 17 of them elderly averaging about 75 years, and 16 young subjects averaging about 28 years. Measurements were performed on 11 body sites. Generally, Uv/Ue increased while Ur/Uf decreased with age, but differences were not significant for all body sites.

Smalls *et al.* (154, 155) investigated the effects of age, body site and skin thickness on mechanical properties of skin with the Cutometer® using the 6-mm probe in a 30-subject study. Uf on the shoulder measured by the Cutometer® was negatively correlated to age with $r = -0.78$ and $p<0.001$. Results are shown in Figure 12.

Figure 12: Uf on the shoulder versus age. Slope = –0.78, Reprinted from (155) with permission.

Ur and Ur/Uf also showed significant negative correlations with age.

Krueger *et al.* (156) investigated the effect of age and body site on Cutometer parameters with a panel of 120 women divided evenly into four age groups as follows.

Group	Age Range
I	20–29
II	30–39
III	40–49
IV	50–65

All of the viscoelastic parameters in Table 1 were measured. The mean correlation of Uf with age was –0.56. The parameter that had the strongest correlation with age was Ur/Uf, the ratio of immediate recovery to total deformation (R^2 = 0.656). Figure 13 shows their Ur/Uf results for each age group and body site.

Figure 13: Ur/Uf as a function of age for five body sites. Data taken from

Krueger *et al.* (156).

Ryu *et al.* (157) also reported a strong negative correlation between Ur/Uf and age in a study of 96 Korean women ranging in age from 20 to 75 years.

The reduction in extensibility and even larger reduction in elastic recovery with age is consistent with studies showing changes in the elastic fiber network of skin with aging and photoaging (146, 158–161). Ezure *et al.* (162) reported that Ur/Uf decreased as "sagging" scores increased on the cheeks of Japanese women. Warrier *et al.* found significantly lower elastic recovery (Ur) on the cheeks of Caucasian women compared to age-matched African American women (163) and attributed the difference to less photodamage on the face of African American women because of sun protection by melanin. Other studies have also shown that Ur/Uf is negatively correlated with age (141, 164). Increases in Ur/Uf by product treatment could possibly be used to substantiate anti-aging effects on skin elasticity.

On the other hand, dynamic indentation experiments performed by Boyer *et al.* indicate that the skin becomes more extensible with age (165). The authors attribute this difference to the fact that the indentation experiments were made without a "guard ring." The Cutometer deforms a small, well-defined area, determining the rheological properties of the skin, while dynamic indentation experiments deform a wider area and are proposed to be more reflective of the natural tension in the skin. Further discussion on the measurement and evaluation of skin elasticity and its relation to wrinkles and skin topology are extensively discussed in this book in the section on skin testing.

3.2.4.6 TELANGIECTASIA

Telangiectasia is the presence of visible blood vessels on the surface of the skin. It can occur both in sun-exposed and -protected areas of the skin with age. On sun-protected areas telangiectasia may develop because of thinning of the dermis.

Figure 14: Visible blood vessels (telangiectasia) on the outer thigh of a 64-year-

old male (nonsmoker).

Chronic sun exposure may lead to the development of telangiectasia (166, 167). Yano and coworkers have shown that acute sun exposure induces blood-vessel growth both by upregulation of vascular endothelial growth factor (VEGF) and downregulation of an important endogenous inhibitor of blood-vessel growth, thrombospondin (TSP-1) (168, 169). Upregulation of VEGF and downregulation of TSP-1 was seen in epidermal keratinocytes, endothelial cells, and dermal fibroblasts after UV-B exposure equivalent to two times the minimal erythema dose. Not surprisingly, new blood-vessel growth was also observed (169). The correlation between sun exposure and telangiectasia seems to be stronger in men than in women (21). Current smoking has also been reported to be strongly associated with the presence of telangiectasia (170).

3.2.4.7 PHOTOAGING MECHANISMS

The major factor in extrinsic aging of the skin is chronic sun exposure. It is well known that the majority of negative changes in skin's appearance, especially on the face, are primarily due to photoaging (171). Ultraviolet radiation affects skin cells through two mechanisms. The first is direct damage to DNA by UV-induced formation of DNA lesions, primarily pyrimidine dimers (7, 12, 172–174). DNA may also be damaged by reactive oxygen species (ROS) generated under the influence of UV exposure (175, 176). The ability to repair DNA damage through the Nucleotide Excision Repair system has been reported to decline with age (177), perhaps contributing to further compromise of sun-exposed elderly skin. Telomeres are especially sensitive to UV damage (178) and telomere shortening may significantly affect photoaging (172, 179, 180).

The second major factor in extrinsic aging is damage mediated by ROS, which in addition to damaging DNA, can cause skin changes either by direct action on cell surface receptors or by inducing the generation of cytokines (12, 173, 181–183). Figure 15 illustrates the effect of ROS-induced cytokine release on keratinocytes and fibroblasts.

Figure 15: UV radiation affects keratinocytes and fibroblasts through similar mechanisms. Reactive Oxygen Species (ROS) generated by UV exposure lead to cytokine production that stimulates the Mitogen-Activated Protein kinase pathway. MAP kinases are generated that cause the production of the transcription factor AP-1 and promotion of the synthesis of Matrix Metalo Proteinases (MMP) that break down collagen in the dermal matrix.

In both cases the Mitogen-Activated Protein kinase pathway is activated (181). This pathway involves a complex cascade of protein kinases (enzymes that catalyze the phosphorylation of other enzymes) that leads to the formation of the transcription factor AP-1 (184, 185). AP-1 has two important effects that contribute to photoaging. It promotes the transcription of messenger RNA for the production of collagenase and other matrix metalloproteinases that break down the dermal matrix, and it reduces the sensitivity of fibroblasts to transforming growth factor beta (TGF-β), reducing collagen production (173).

The hallmarks of photoaging in histology are seen in Figure 16. Both type I and type III collagen are reduced (146, 186, 187) except in the thin band right below the epidermis called the Grenz zone. The SC and epidermis may be slightly thickened, though not all authors agree on this point. The major characteristic difference between photoaging and intrinsic aging is the presence of elastosis (146–148, 188). There is actually a large accumulation of elastic fibers in the dermis of severely photoaged skin, but these fibers are dystrophic, with large accumulations of condensates including lysozyme and amyloid P protein (189), which protect the deranged elastin from digestion by elastase (190). The fibers that result from actinic elastosis provide little or no elasticity to the skin.

Figure 16: Hallmarks of photodamage as seen in histology. From Robert Lavker, MD. Skin aging and photoaging: Presented to Congress of the International Society for Bioengineering and the Skin, Orlando, FL. October 28, 2004, reprinted with permission.

As solar damage progresses, inflammatory cells migrate into the dermis. The vasculature changes that may result in telangiectasia have been discussed above.

3.2.4.8 PHOTOAGING AND APPEARANCE

The visual signs of photodamage are well known and include wrinkles around the eyes and mouth, hyperpigmentation (age spots), telangiectasia, and deep furrows in the skin in exposed body sites (158, 191–193). Figure 17 shows classic signs of photoaging on some well-known faces.

Figure 17: Classic signs of photoaging (public domain publicity stills taken from www.skinema.com)

Both subjects have extensive wrinkling in the around the eyes in the peri-orbital or so-called crow's-foot area and around the mouth. The subject on the right spent much time filming in the sun while wearing a cowboy hat. Notice that the skin on the forehead looks better than the skin lower on the face.

Corcuff *et al.* analyzed wrinkling in the peri-orbital area on 81 subjects ranging in age from six to 76 years (194) using image analysis of silicone replicas. Results are reprinted in Figure 18.

Figure 18: Evolution of "crow's-foot" wrinkles with age. Number of wrinkles per cm^2 ± S.E. b. means depth of wrinkles (µm ± S.E.). Reprinted from (194) with permission of the Society of Cosmetic Chemists.

Interestingly, the number of wrinkles peaked at about age 35, but mean wrinkle depth increased by eightfold between age 35 and 65.

Sun-induced changes can occur at an early age if sun exposure is sufficiently extreme. Corcuff, de Rigal, and Leveque did a very interesting study on riders in the 1985 Tour de France (195). Professional cyclists spend many hours in the sun and wear tight-fitting short-sleeved shirts while training, providing a sharp divide between exposed and sun-protected areas of skin. Even though the average age of the riders was 25 years, there were dramatic differences between protected and exposed skin. The line pattern on the exposed skin sites of these young cyclists resembled that seen in the >71-year age group in Figure 10. The average furrow depth in the exposed sites was 42 ± 1.5 mm on the exposed sites compared to 29.5 ± 1.0 mm on protected sites.

3.2.4.9 COMPROMISED ELDERLY SKIN TREATMENTS

a. Sunscreens—an ounce of prevention

Since sun exposure plays such an important role in compromising skin, it seems obvious that sunscreen use is a good strategy to prevent damage. However, this is difficult to prove in humans. Long-term studies are required, and it is not ethical to give people a "placebo" sunscreen.

The most convincing study on sunscreen use and photoaging that has been published to date was done as part of the Nambour Skin Cancer Prevention Trial, a randomized, community-based trial in Nambour, Australia (196, 197). In this study, conducted from 1992 to 1996, some subjects were required to apply an SPF 15+ sunscreen daily while others only used sunscreens at their discretion. The photoaging trial was conducted on a subset comprised of 903 subjects who were under 55 years of age to emphasize the effect of photoaging compared to intrinsic aging (198). Dietary supplementation with β-carotene was also

investigated. Photoaging was measured by using a validated method based on grading silicone replicas from the backs of the hands on a scale ranging from 1 (no damage) to 6 (deep horizontal lines and loss of vertical lines) (199, 200). Reported sun exposure was similar between the daily and discretionary sunscreen groups. Participants with initial grades of three or four showed reduced skin aging in the daily sunscreen group compared to discretionary use and significantly reduced skin aging after 4.5 years in the study, compared to those who only used sunscreens at their discretion. Results with the group given oral supplementation with β-carotene were not different from placebo.

The study described above showed that regular sunscreen use can have a significant effect on the rate of photoaging. When we consider that modern sunscreens tend to be higher SPF and broader spectrum compared to those used in the early 1990s, it seems logical that they could provide an even better protective effect, but the data indicate that very regular use is likely to be required, rather than just casual application when one goes out for prolonged exposure.

b. All-Trans Retinoic Acid (Tretinoin): The Gold Standard

Tretinoin is the only drug approved by the United States Food and Drug Administration for the treatment of wrinkles. It was first reported to have positive effects on photoaged skin by Albert Kligman and colleagues (201) in 1986. During the 1990s, tretinoin was investigated in double-blind, vehicle-controlled clinical studies (202–204). Olsen *et al.* performed a 320-subject multicenter study comparing 0.05%, 0.01%, and 0.001% tretinoin to vehicle on the face (205). After 24 weeks of treatment, 0.05% tretinoin produced statistically significant improvements in grades for overall severity of photodamage, mottled hyperpigmentation, fine wrinkling, and skin roughness compared to vehicle. Other studies showed that tretinoin was very effective for treating “liver spots” associated with photodamage (206) and hyperpigmented spots in Asian subjects (207). Grove and coworkers used image analysis of silicone replicas to demonstrate that 0.05% tretinoin consistently improved skin topography more than vehicle (208).

c. Cosmeceutical Treatments for Aging Skin

Tretinoin is a pharmaceutical product and an approved drug. Cosmetic treatments designed to improve aged skin are often referred to as “Cosmeceuticals.” While Albert Kligman, MD, is often said to have coined this term in the 1980s, it actually predates that time. The first published use of the

term we are aware of is by Raymond Reed, published in the *Journal of the Society of Cosmetic Chemists* in January 1962 (209). However, Reed's definition is so broad as to include simple moisturizers and other ingredients that probably wouldn't be considered "cosmeceuticals" today. Certainly Kligman popularized the term and played a significant role in the development of its current usage to refer to cosmetic ingredients that have activities that provide skin effects such as anti-aging (210). It is also important to keep in mind what the FDA says about "cosmeceuticals (211)"

The FD&C Act does not recognize any such category as "cosmeceuticals." A product can be a drug, a cosmetic, or a combination of both, but the term "cosmeceutical" has no meaning under the law.

Perhaps this is why, even today, the exact definition of cosmeceutical is not totally clear (212, 213). A significant literature exists on cosmeceuticals, even though the FDA says the term has no legal meaning and there are many reviews on the subject (212, 214–227). We will now briefly review a few of the many "cosmeceutical" actives.

d. Vitamin A and "Cosmeceutical" Derivatives

Retinol and retinol esters are popular ingredients in skin care products designed to treat the signs of skin aging. Retinol produces some changes in skin that are similar to tretinoin (228). It is much less effective but also less irritating (228, 229). Pierard-Franchimont *et al.* showed that retinol treatment can produce improvements in the mechanical properties and topography of skin (230). Retinol still has the potential for skin irritation and can be unstable. This has led to investigation of retinol derivatives such as acetate, proprionate, and palmitate esters (216, 231). These esters are presumably metabolized to retinol and the free acid in the skin as has been demonstrated with retinyl palmitate (232). Bissett presented data showing that 0.15% retinol and 0.3% retinyl propionate can have positive effects on both wrinkling and hyperpigmentation after 12 weeks of treatment (216). Retinol has also been reported to improve intrinsically aged skin (233).

e. Niacinamide

Niacinamide, also known as nicotinamide, is the amide of nicotinic acid (vitamin B_3). In the cell it is incorporated in nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, which are vital to energy metabolism. Topical application of niacinamide has been shown to have many positive effects on the skin, including improving barrier function (234–237),

reducing the appearance of fine lines and wrinkles (234, 238), and improving hyperpigmentation (239, 240). The improvement in skin pigmentation is apparently through a reversible effect on the transfer of melanosomes from melanocytes to keratinocytes (240, 241).

f. Alpha and Beta Hydroxy Acids

As pointed out above, the turnover rate of the SC decreases with age. Lactic acid had been well known as a moisturizer for many years (242–244), but the observation that lactic and glycolic acid increase the rate of SC turnover (245, 246) led to great interest in their application as potential anti-aging treatments. Stiller *et al.* published a double-blind placebo-controlled study comparing 8% lactic and 8% glycol acids to vehicle on signs of photoaging on both the face and forearm (247). Both lactic acid and glycol acid were significantly better than vehicle in improving global photoaging scores, and the two hydroxy acids were not different from each other. The so-called beta hydroxy acid products are based on salicylic acid, usually at about 2% concentration. This topic is described in great depth elsewhere in this book under the Ingredient section.

g. Antioxidants

Since reactive oxygen species play such an important role in photoaging, it is not surprising that antioxidants have been a major focus of cosmetic research. Vitamins E and C are probably the most studied for their potential to protect the skin from photoaging and treat its symptoms (216, 248–259). Raschke reported that 3% ascorbate had a significant effect on reduction of facial wrinkles compared to vehicle, but only when delivered from an “optimized formulation” (256). Antioxidants from plant sources are also under study. Green tea polyphenols have been the most studied ingredients in this regard. While studies in murine skin have shown promise (260, 261), human skin clinical trials of both topical (262) and oral (263) green tea treatment have failed to show visible benefits on skin aging. Histological evaluation after topical treatment for eight weeks did show significantly improved elastic tissue compared to placebo (262).

h. Other actives

A wide variety of other ingredients that can potentially improve the condition of compromised elderly skin are under investigation or have been investigated. They include peptides (221, 264, 265), topical application of estrogen cream (266) (a pharmaceutical not a cosmeceutical), anti-glycation compounds (267), and a variety of botanical ingredients (268).

CONCLUSIONS

As we age, our skin undergoes obvious changes. Some changes occur even on body sites not exposed to the sun or other insults. This intrinsic aging results from the unavoidable effects of the passage of time. Some of our daily activities not only accelerate the aging of skin but also alter its consequences, thereby leading to extrinsic aging. The most important contributor to extrinsic aging is exposure to the sun, though exposure to cigarette smoke may also contribute. Sun-exposed skin ages more rapidly than unexposed skin and differences in histology appear. For example, in chronically sun-exposed skin collagen decreases but elastin increases, though it is often highly dystrophic, while in intrinsic aging both collagen and elastin decrease.

This chapter has reviewed the effects of both intrinsic and extrinsic aging on the skin. Perhaps because of its vital function, the *stratum corneum* barrier is relatively unaffected by age, though barrier repair and wound healing may be slowed. The tendency of the Aged to develop dry skin is clear, and this may contribute to the appearance of fine lines and wrinkles. Changes in the dermis result in changes in the dermatoglyphic pattern of the skin and deeper wrinkles. Chronic sun exposure has profound effects on skin, leading to solar elastosis and the development of deep wrinkles and “age spots.”

We have also reviewed prevention and treatment of skin damage. Skin damage from the sun may be reduced or even prevented by frequent use of sunscreens. Tretinoin is the only FDA-approved drug for the treatment of wrinkles. Studies over the past three decades have shown the effect of tretinoin on photodamage, hyperpigmentation, wrinkles, and skin roughness.

Many cosmetic ingredients and products are alleged to provide effective treatment for the appearance of aged skin. The literature on some of the most common of these treatments was reviewed. Retinol derivatives, antioxidants, and niacinamide are among the treatments reviewed. While there is ample evidence that some “cosmeceutical” treatments may be effective for treatment of the signs and symptoms of skin aging, manufacturers must be careful that claims about product efficacy are restricted to those appropriate for cosmetics based on existing regulatory statutes in the U.S. and elsewhere.

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PART 3.3

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THE HAIR

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THE HAIR

Editors Overview

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Over the last ten years our understanding of hair and its care has developed significantly with the application of new scientific approaches and the resulting breakthroughs that have emerged.

In the area of cosmetic science, a large number of the aesthetic properties of hair have unequivocally been linked to the physical and viscoelastic properties of either the cuticle or the cortex. Various layers of the cuticle sheath have been shown to play an important role in hair lubricity, damage protection, and optical properties. The crystalline and amorphous phases contained in the ortho-and para-cortical cells of the hair shaft have been shown to be crucial for most hair-shape aesthetic modifications induced by consumers and cosmetologists' use of harsh cosmetic treatments such as the application of heat, exposure to alkaline formulations such as those used in hair relaxation, and other chemical-reducing aqueous solutions. Denaturation of crystalline regions, disulfide bond scission, and breakage of other protein bonds are all phenomena that have been carefully analyzed to assess their impact on the aesthetic aspects of hair and its damage.

In the area of hair biology, various important contributions have also been made. For instance, the role of stem cells, hormones, and other cytokines on hair growth; the discovery of additional stages in

the hair growth cycle; the biochemical basis for hair graying and aging, and the impact of new bioactives and other nutritional supplements in the health of hair are all relatively new discoveries just made within the last ten years.

With these rapid advances in the various hair-related areas, the science of hair care is unavoidably moving towards a more holistic approach. Such an approach considers the aesthetic properties of hair not only as a by-product of cosmetic treatments to the mature and keratinized form of the hair shaft, but also as a result of the action of bioactives that can enhance or improve the overall condition of hair while its cells are still alive in the hair follicle. This trend is quite significant when one considers that we used to think of hair as “dead,” and now our thinking and knowledge have evolved to focusing on the distinction that the cells in the mature hair shaft may be s “dead” but its proteins are still biologically active. Furthermore, by penetrating to the roots of the hair shaft, we have “discovered” that it is still *alive* while in the follicles, and this realization has created a paradigm shift in thinking of new approaches to bring beauty to what both men and women treasure as a major part of looking and feeling young.

It is within this context that the material in this section was chosen and organized. It starts with an overview of hair structure and function, followed by an overview of hair follicle biology. It then continues with a chapter on aging hair, hair loss, hair dyes, and bioactives, followed by a discussion on the mechanisms of changes in hair shape. The last chapter deals with eyelashes, including their anatomy and methods for increasing their growth.

The five chapters in this part of the book introduce fundamentals of hair science that range from its structure, mechanistic changes in shape and growth, to the action of bioactives to improve its health. This material provides a solid base in what we would now term “Hair Science” for beginners and fresh insights for seasoned researchers. The various contributors to this hair section were encouraged by the

book’s editor-in-chief to not only give an in-depth overview of their various topics but also to present new insights and new ideas emerging in the area of hair care.

AN OVERVIEW OF THE PHYSICAL AND CHEMICAL PROPERTIES OF HAIR AND THEIR RELATION TO COSMETIC NEEDS, PERFORMANCE AND PROPERTIES

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ABSTRACT

In this chapter we present an unusual approach to the subject wherein we consider the system of hair structure and its relation to hair function. The approach used for this purpose consists in reviewing the most recent and advanced hair structural concepts derived from the fields of chemistry and biology, while providing a strong emphasis on the physical, social, and cosmetic aspects of hair function.

The chapter is divided into six parts:

- The four first sections describe the chemical and biological composition of hair, which forms the foundation for its complex physical architecture. Through these sections a detailed analysis is made of the hair internal structure, its viscoelastic and optical properties, and its response to moisture. Wherever possible an effort is made to always connect physical properties and related processes with the functional aspects of hair.
- In the last two sections, a review is made of the various processes taking place in the follicle to create hair. We point out that the follicle is *alive*, and that the native proteins in mature hair still have biological activity and a

function to play. This provides access to cosmetic approaches to improve its beauty, and of course, it is a relatively new concept since the hair protein architecture has long been considered as a structure with no biological activity. There a description is made of the main biological activities occurring in the various zones and phases of the follicle to assemble cells and produce hair. As this description is made, special attention is given to the follicular activities that create, from its inception, the foundations that correlate hair architectural structure with hair function. We assert that this unique approach and a true understanding of the relationship between hair architecture and functional biology opens an entirely new field for the development of new concepts and products for the cosmetic and personal care industry catering to both men and women.

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3.3.1.1 INTRODUCTION

It is a general principle of nature that any living organism, or biological material created by nature, is made and structured in such a way that its properties are optimized to perform a specific function. In this sense, hair is not different from any other biomaterial found in the organs of our body. Contrary to the common belief that hair is a dead material and of little or no importance, hair is an optimized assembly of proteins, lipids, and polysaccharides organized into units or cells for a specific function. All cells in our body undergo a life cycle and are created to fulfill a function. However, in this respect there is a considerable difference between hair cells and other organ cells. Cells from other parts of the body undergo a programmed form of cell death, known also as “apoptosis,” once they have fulfilled their function. After this event, the cells are discarded and eliminated from the body. In contrast, when hair cells die by “apoptosis” in the follicle, their function is not finished; rather, it has just begun. Thus, when hair cells die, they are not discarded by the body because it is then that they are needed the most for their specialized biological function of scalp/skin protection and aesthetic purposes. The short-lived period of hair cells in the follicle is just a preparatory stage for their final function.

Another common belief is that since hair cells are inanimate or lifeless, their structure has no biological activity. This statement is somewhat incorrect because the protein structure of inanimate hair cells still possesses biological activity that is crucial for our living organism. Even when hair cells are dead and inanimate, if their protein structure is damaged, as in the case of enzyme structures, their biological function is compromised. Let us remember that enzymes are lifeless protein structures whose activity plays a critical role in many of our biological processes. Enzymes are not living organisms, and they lose their biological activity when their natural conformations are lost by denaturation due to harsh stresses, either thermal, chemical, or mechanical (1).

Likewise, the proteins composing our hair cells also lose their biological function/activity when they are subjected to harsh stresses, i.e., heat, chemical

treatments, mechanical fatigue, or excessive mechanical elongations. Hair treatments with hot irons and with other chemical processes such as perm waving, bleaching, and alkali straightening are known to cause denaturation and bond breakage in hair's native proteins (2). As a consequence, many hair properties are compromised, i.e., hair loses the ability to control moisture absorption. Its mechanical/thermal/optical response is compromised, and ultimately it loses its aesthetic purpose and ability to protect the scalp. Thus, the chemical composition and protein architecture of inanimate hair cells still possess biological activity enabling hair to fulfill the biological function for which it was created.

3.3.1.2 STRUCTURE AND CHEMICAL COMPOSITION OF HAIR

Hair is composed of proteins and a very small quantity of lipids and polysaccharides. These biomaterials form the hair cells, which are spatially separated and sequentially organized in a brick-and-mortar fashion in the cylindrical hair body. Consequently, they form membranes, amorphous and crystalline protein regions that extend all along the hair fiber, giving rise to the complex architecture of hair (see Figure 1). As will be seen later, these structures are the foundation of hair's remarkable physical properties. Table 1 displays the overall amino acid chemical composition of hair (3); the data in this table clearly show that hair is rich in half-cystine, serine, glutamic acid, and proline, i.e., the amino acids characteristic of a protein known as keratin. The next most abundant materials in hair, although at a smaller scale compared to the amount of protein, are lipids and polysaccharides, which account for 2–10% of the fiber weight. These materials, as will be discussed later, mainly appear in the cement layer gluing cortical and cuticle cells, as well as on the hair surface.

Another substance that is always present in hair is water. Incidentally, water is never considered part of the hair composition. Surprisingly, many scientists consider water to be an integral part of most biologically active proteins and, therefore, for all practical purposes water is also part of the hair composition. Native proteins contain a substantial amount of water in two forms, namely bound and free water. Free water, also referred to as "bulk water" or "loose water," has only a minor influence in the native structure of proteins. In contrast, "bound water," with its strong binding physical forces, attaches to specific protein groups stabilizing the protein secondary structure (4). Bound water plays

an important role in the conformation of exposed side chains, and because of its role on protein stabilization many scientists consider it to be part of the protein structure. Water is thus also part of the hair keratin composition (5) because it is needed for its stability. The role of water on hair behavior will be discussed in further detail later in this chapter.

Percentages amino acids in hair:

Alanine	4.6
Arginine	5.8
Aspartic acid	4.9
Half-cystine	17.8
Glutamic acid	11.4
Glycine	6.4
Histidine	0.9
Isoleucine	2.6
Leucine	5.8
Methionine	0.6
Phenylalanine	1.6
Proline	8.4
Serine	11.7
Threonine	6.8
Tyrosine	2.0
Valine	5.8

Table 1) Amino acid composition of unaltered human hair (3)

Figure 1: Diagram of a hair fiber showing its various components (60).

3.3.1.3 MAIN TYPES OF CELLS IN HAIR

A hair fiber is mainly composed of three types of cells, namely, cortical, cuticle, and medulla cells (see Figure 2). These three types of cells are made, organized, and assembled to form hair fibers by a tiny organ called the follicle (see Figure 3). This small organ sits on the scalp, and there are as many follicles as hair fibers. While hair cells are still alive in the follicle, they acquire a protein structure, composition, and architecture that prepare them for their final function. Before the hair cells undergo apoptosis the follicle carefully feeds and instructs them so they can arrange their internal protein structures into highly specialized conformations. For instance, a large percentage of the proteins found in the cortical cells of hair are arranged into a type of peptide sequence and conformation that is unique among all proteins. This unique protein is called keratin and its helical structure is such that, later, it allows hair to attain a high degree of mechanical strength (see Figure 4).

Keratin is a protein designed by nature to provide mechanical strength wherever needed in living organisms (6). Keratin proteins are usually coiled into filaments and constitute the micro-skeleton that gives mechanical stability to many cells. Without keratin filaments, many living cells would burst or collapse under the action of fluid pressure; i.e., in the liver they act as scaffolding structures that provide mechanical strength to the hepatocytes (7). Depending on their mechanical characteristics, keratins can be divided into alpha and beta keratins, or soft and hard keratins, respectively. Alpha or soft keratins are mostly found in hair, nails, skin, other body cells, wool, and the fur of other mammals, while the hard beta keratins are typical of reptile claws, the shell of turtles, *etc.* (8). Alpha keratins are also classified as type I (acidic) and type II (basic) keratins, and, in hair they are always coiled in pairs (9–10). Keratins can vary in structure and molecular weight, and therefore, there is a large variety of them; however, regardless of their type, nature always uses them when there is need for mechanical strength.

Figure 2: Pictographic representation of three types of cells in hair (60).

Figure 3: Diagrammatic representation of hair follicle with its main general components and the lipid gland (62).

Figure 4: Helical structure of alpha helix in keratin.

3.3.1.4 CORTICAL CELLS AND THEIR ROLE IN HAIR PROPERTIES

a. Cortical cell structure and composition

The cortical cells are elongated and spindle-like in shape; they constitute the major cellular component of hair fibers and are responsible for most of the mechanical properties of hair (10–11). Their irregular circular shape in a grown hair fiber has an average diameter of approximately 20 µm; their entire body length is approximately 100 µm long (see Figure 2). The cortical cells in grown hair fibers are not alive. However, before the hair fibers emerge from their follicles there is a point at which they are still alive. The main task of these cells during their life period is to synthesize keratin proteins in their bodies by assembling key amino acids into helical structures, strand them into filaments—also called intermediate filaments (IFs), and finally, bind them via disulfide bonds to an amorphous protein matrix to form macrofibrils (11–14). The amorphous protein matrix gluing the microfibrils will then be crosslinked with disulfide bonds and hardened as the cells die. The keratin filaments are crystalline in nature and are known to possess remarkable mechanical strength. To further maximize the mechanical resistance of the nascent hair fiber, the follicle facilitates the creation of hundreds of cortical cells and instructs them to interdigitate and produce an extracellular material, the cell membrane complex (CMC), which glues them all together in a cylindrical shape (Figure 5).

Figure 5: Schematic representation of cell membrane complex in hair fiber.

Because the cortical cells contain high levels of keratin, the resistance of a hair

fiber to mechanical breakage is comparable to that of a copper wire of the same diameter. Yet, in spite of this remarkable mechanical strength, the hair fibers do not behave like rigid metallic wires. We all know that hair fibers on the scalp are rather soft and easy to bend and move. This motion allows them to separate and create inter-fiber pockets of air needed for sweat transfer, thermal insulation, scalp protection, and aesthetic purposes (15, 16). Unlike a bundle of copper wires, hair is known for exchanging moisture with the environment by a physical process of absorption and desorption that involves heat transfer with the environment. This process, which occurs also in wool fibers, allows them not only to act as insulating materials, but also as heat buffers that effectively regulate skin/scalp temperature (17, 18). Furthermore, from a cosmetic point of view, hair fibers do not behave like copper fibers since an assembly of hair fibers can be deformed or styled into any desired shape with the aid of water, and it can later recover its original shape.

This particular behavior of hair, which combines mechanical strength with softness and moisture absorption/desorption with heat exchange, is at the base of its biological and cosmetic function. This combination of properties results from the biological activity of hair proteins with their unique architecture within both cortical and cuticle cells. The biggest contribution to this behavior comes, however, from hundreds of keratin microfibrils embedded in a protein-amorphous matrix in the cortical cells. The keratin microfibrils have a high degree of molecular order. Being crystalline, they do not swell in water, while the amorphous phase has no order, and a part of its structure is highly swellable by water. In terms of strength, the combination of keratin microfibrils embedded in an amorphous phase is akin to that of an epoxy composite material reinforced with glass fibers. In this sense a single hair fiber can be considered as an amorphous protein matrix reinforced with keratin microfibrils.

b. Viscoelasticity in hair and cortical cells

In 1959 a mechanical model was proposed by Max Feughelman (15, 20, 21) to explain the remarkable relationship existing between elasticity, softness, strength, and moisture displayed by wool and hair. This scientist considered that the outstanding mechanical behavior of hair and wool and, in general, of most keratin fibers, results mainly from the mechanical response of keratin microfibrils, or intermediate filaments (Ifs), embedded in an amorphous protein matrix forming macrofibrils in the cortical cells.

In order to explain the time-delayed elasticity associated with hair softness and

its dependence on moisture, Max Feughelman introduced the concept of viscoelasticity to keratin fibers. He proposed a two-phase mechanical model that didn't take into account the discrete nature of cortical cells, cell membrane complex (CMC), and cuticle cells. He thus generated a model of the hair fiber as if it were a continuum material—made only of keratin microfibrils embedded in an amorphous protein matrix (see Figure 6). In this model, now supported by a large amount of experimental evidence, the keratin microfibrils (C phase) are considered to act mechanically like rods in parallel with the amorphous phase (M phase M in which they are embedded) (15).

Figure 6: Representation of Max Feughelman mechanical model of keratin fibers showing keratin rods embedded in an amorphous matrix (20).

Since the introduction of the Max Feughelman model, three other models have been proposed to explain the mechanical behavior of keratin fibers. The main differences among all them seem to center on the way the amorphous phase interacts with the crystalline phase. A more detailed discussion of these differences can be found elsewhere (21). For the purpose of this chapter it suffices to emphasize, however, that all of these models recognize the existence of the amorphous and crystalline phases as two separate entities with different mechanical behavior. In all these models it is agreed that the amorphous protein phase corresponds to the zone of scrambled proteins surrounding the keratin microfibrils. The proteins in this zone constitute the "cement" that supports and interconnects, via disulfide bond crosslinking, hundreds of microfibrils to form the macrofibrils we know as "hair."

Within the amorphous matrix, the two-phase mechanical model recognizes the

existence of two regions with different cystine content, namely, the high-and low-sulfur regions. The low-sulfur amorphous region is considered to be in close proximity to the microfibrils, while the high-sulfur region is further away from them. Furthermore, the amorphous phase contains hydrophilic and hydrophobic protein groups that form extended structures all along the cortical cells. Because of these two structures, the amorphous phase can display two distinct patterns of physical behavior. The first one consists of a softening effect usually observed when the amorphous phase interacts with water. The softening effect is due to the hydrophilic extended structure of the amorphous phase and is also referred to as the “gel structure” by Max Fueghelman (20). This structure absorbs large amounts of water, is susceptible to plasticization by moisture, and its viscoelastic response is moisture dependent. In contrast, the non-gel structure of the amorphous phase is hydrophobic, highly crosslinked, and softens at very high temperatures.

Phase C comprises the crystalline keratin rods forming the macrofibrils. As a result of their crystalline character, these rods do not to absorb water, and therefore, cannot be plasticized by water. The macrofibrils thus act like strong micro-springs that respond only elastically to mechanical stresses. Thus, macrofibrils and amorphous phases combine their mechanical and water absorbing/desorbing properties to make hair a viscoelastic material. Because the content of crystalline keratin macrofibril protein in hair is approximately 95%, a hair fiber has a melting temperature (Tm) of ~ 156°C. Hair has also a glass transition temperature (Tg) of ~ 144°C, and it is primarily related to the noncrystalline amorphous matrix (23). Let us not forget that the Tg in polymers or biopolymers is nothing but the temperature at which a hard amorphous polymer becomes softer (24). Therefore polymers that are very hard and brittle at room temperatures can be softened, by raising their temperature to their respective Tg. They can also be softened by adding a plasticizer. In fact, hair fibers are biopolymers, which under dry and room-temperature conditions are mechanically hard but can become softer, either, by raising their temperature above 174°C, or by adding a plasticizer. We are all familiar with the fact that water is the natural plasticizer for proteins. Thus, hair without moisture is hard and brittle, but when it absorbs moisture it becomes softer because the gel structure of the amorphous phase becomes plasticized by water. This plasticization effect by water is clearly shown in Figure 7, where it can be seen that the amount of force needed to deform a hair fiber is always lower when it has higher levels of moisture.

Figure 7: Typical stress-strain deformation curves of hair fibers obtained at three different moisture conditions (60).

The effects of water plasticization/softening in hair are more significant when hair fibers are deformed below 2% water level. The reason for this is that the main hair component that responds to low deformations is the gel structure of the amorphous phase with its large number of hydrogen bonds. At deformations (d), 2% > d < 40% the mechanical response of the fiber is not influenced by the presence of water. Rather, it is mainly controlled by the unfolding of the alpha-keratin into the beta-keratin configuration. Unfolding of the alpha helical keratin in the microfibrils thus allows for larger deformations in the hair fiber, thereby preventing it from immediately breaking in the yield region. The transformation of keratin from the alpha to beta configuration is a thermodynamic phase transition and can be induced in hair by many processes other than mechanical extension. When it is produced by heat or chemical damage, it is usually irreversible; however, when it is induced mechanically it is fully reversible. The beta configuration of keratin sometimes is also called the “ladder or platelet” keratin structure (22, 23). Deformations larger than 5%, giving rise to keratin unfolding, occur in hair in daily grooming practices. They occur mostly upon repetitive combing, in particular, when the hair is wet or when the hair is still wet and is being detangled.

The stress/strain curve of hair is shown in Figure 8, where it can be seen that when we deform a single hair fiber from 0 to approximately 40% in length, the hair displays three different slope regions of force versus deformation. The first line is comprised between 0 and 2 or 5% deformation depending on the relative humidity of the environment (segment A). It is known as the Hookean region and has the steepest slope. This region represents the stress/strain mechanical response of the amorphous and crystalline (alpha keratin) phases. In particular, it

represents the mechanical response of protein region with hydrogen bonds. Since these levels of deformation are easily attained with everyday combing and grooming practices, we stress the amorphous and macrofibril phases of our hair almost everyday. If we continue to deform the hair fiber between 2 and 35% (segment B), the slope stress/strain changes because it is produced by a different phenomenon, namely, the opening of keratin coils in the macrofibrils. This section of the stress-strain curve of hair is known as the yield region. Hair deformations falling in this range are typical during excessive tangling when the hair is wet. Deformations higher than 35% cause the final change in slope before the fiber breaks; the physical phenomenon associated with these deformations seems to be a process of recrystallization of the amorphous phase (segment C) (11).

Figure 8: Stress strain curve for hair up to 30% deformation.

c. Shape-memory properties of hair

It is known from the field of polymer science that the mechanical behavior of polymers can be characterized as either being of the thermoset or thermoplastic type. Thermoset characteristics are those that do not respond to temperature changes, while thermoplastic characteristics refer to those materials that soften when heat is applied.

When polymers contain a high level of crosslinking bonds, they are usually of the thermoset type. In many cases these materials behave also as “smart” materials displaying shape-memory properties (24, 25). Since approximately 90% of the hair is composed of cortical cells filled with crystalline and amorphous proteins crosslinked by disulfide bonds, hair like any other polymeric crosslinked material behaves also as a thermoset, viscoelastic, shape-memory

biopolymer. Thus, hair has a permanent shape that may be temporarily changed, but can also be reversed later under the appropriate conditions. For instance, whether the hair is limp, wavy, curly, or extremely curly, it will always remember its native shape regardless of what type of styling shape has been imposed on it. Furthermore, the fact that the hair geometry can successfully be changed after styling, and held there for a certain period of time without the hair immediately recovering its shape, is by far one of the most important cosmetically useful examples of hair viscoelasticity and memory-shape properties. If the behavior of hair were purely elastic, hair would not be able to easily deform and accommodate for deformations needed for fiber separation, air pocket formation, thermal insulation, scalp protection, and aesthetic purposes. Also, hair would not be able to transiently change its shape during styling for cosmetic purposes. Thus, hair is a viscoelastic shape-memory material, *and failure to comprehend this will hamper our understanding of one of the most fundamental properties of hair's biological and cosmetic behavior*.

d. Viscoelasticity and the shape-memory properties of hair

Because of the great importance of viscoelasticity in the shape-memory properties of hair, a brief review of this concept will be helpful. We are all familiar with the fact that when a small force is applied to a pure elastic material, for instance, to a rubber band, it will deform a certain short distance. Then, when we release the force, the rubber band will recover almost instantaneously to its original shape. In this process it is obvious that the energy applied to deform the rubber band is stored as mechanical tension energy, and then returned when the force is released. In contrast, in a pure viscous material this does not happen. Rather, when we apply a similar force to a viscous material, for instance, to a string of chewing gum, a small deformation is also produced. However, contrary to the elastic material, the string of chewing gum will not recover its original shape once the force is released and it will stay deformed forever. Furthermore, the energy spent to deform a viscous material like the string of chewing gum is not returned but rather, it is dissipated as heat (26). In summary, an elastic material—when deformed—recovers its shape instantaneously when the force causing the deformation is released, while a viscous material does not.

A viscoelastic material, like hair, displays both behaviors simultaneously, namely, viscous and elastic. In fact, we all have daily encounters with materials that behave viscoelastically. For instance, when we apply a force for a short period of time, i.e., for one second, to a piece of plastic film from a sandwich

bag, it will deform slightly. The shape of the film will be recovered instantaneously again when we release the force. However, if we now apply the same force, but for a longer period of time, let's say five minutes, we will still produce the same deformation, but we will notice that when we release the force, the plastic film does not recover its original shape right away. If we wait for some time and pay careful attention to the plastic film, we will notice that it slowly crawls back and eventually recovers its original shape.

In the above example, with a one-second force application, the piece of film displayed typical elastic behavior; yet in the case of a five-minute force application the same plastic film showed initially a viscous behavior followed by a delayed, gradual, and slow elastic recovery. Thus, depending on the time period of force application, the plastic film displayed either pure elastic behavior or a combination of a viscous and elastic behavior. This is the universal characteristic of viscoelastic materials, namely, they either display elastic behavior in response to rapid deformations, or display a combination of viscous and elastic behavior in response to very slow deformations, or to deformations produced by forces applied for long time (27). Hair displays exactly the same behavior. When we deform it rapidly, as in a rapid head movement; or when we pass our fingers between the hair fibers, it behaves elastically, yet, the hair displays viscoelastic behavior when we deform it for long time. For instance, when we sleep with our hair bent or uncombed in an unwanted geometry, or in the morning when we wake up, our hair seems to stay bent and uncombed for long time, i.e., it does not recover immediately; but if we give it enough time it will remember its shape and recover gradually.

In summary, this remarkable mechanical behavior of hair is essentially due to the viscoelastic and shape-memory properties resulting from the disulfide crosslinked amorphous matrix and the keratin crystalline structure in the cortical cells. This behavior has been put in place by nature to provide an optimized assortment of mechanical properties for scalp protection and social communication—perhaps even as a part of the attractive mechanism between members of the opposite sex to facilitate propagation of the species.

The optimum mechanical and biological function of hair is thus achieved by a combination of an amorphous protein phase containing a gel and a non-gel structure, attached to the strong-coiled elastic keratin macrofibrils. For hair to maintain this optimum combination of softness, elasticity, strength, and shape recovery, it is necessary that we keep the biological activity of its proteins unchanged. We can achieve this, first, by helping hair to keep its amorphous

proteins moisturized so they are plasticized; and second, by keeping as long as possible the primary, secondary, and tertiary structure of the proteins in its native structure. We are all familiar with the fact that hair becomes hard and brittle when it lacks moisture, or with the fact that hair becomes weaker when its proteins oxidize or become brittle under the action of heat or prolonged UV radiation. We also know that hair becomes mechanically weaker after chemical treatments that we blithely apply in an attempt to look more beautiful, yet destroy strong hydrogen and covalent disulfide bonds that stabilize its tertiary structure, i.e., its crystalline structure (28, 29).

e. Water and moisture absorption/desorption by cortical cells

As in any other protein biomaterial, hair most of the time contains water, even at very low relative humidities. Water in general plays a crucial role on protein structure and function. It can be found in two states, namely, strongly bound and free or unbound water (30). In the case of hair, strongly bound water is critical for the conformation and mechanical stability of the helicoidal keratin fibrils that strand and form the macrofibrils in the cortical cells (31). This type of water is therefore critical in maintaining the mechanical properties of hair, and it can be found in hair even after it is subjected to zero relative humidity. This behavior is found even with a few water molecules that are strongly attached to protein functional groups and can only be removed from the hair by applying high temperatures close to its denaturation temperature (32). In fact, in the case of hair, denaturation of its proteins is caused by both losses of strongly bound water *and* breakage of disulfide bonds in the protein-amorphous regions.

In most cases, protein plasticization is ascribed to water molecules whose bond strength is weaker and can be broken by the movement of thermal motions of protein chains. This type of water molecule adsorbs onto the protein chains in multilayers and, at high moisture content, takes the form of bulk water. It is the water that causes swelling in hair and in other natural biopolymers (33–36). A great deal of information on the formation of water molecule multilayers and bulk water absorbed can be obtained by looking at the shape of the water absorption isotherm of a specific material. Figure 9 shows the water absorption isotherm corresponding to hair. One can see it is sigmoidal in shape and presents a hysteresis effect (37, 38). Namely, the amount of water content in hair depends on whether we moisturize it from a dried state or whether we dry it from a wet state. In very dry hair there are a few water monolayer or nucleating sites for multilayers to form by a process of condensation, therefore, the amount of water

absorption is low (line A). However, when hair is fully wet, water multilayers are already formed *inside* the hair. Under these conditions the desorption of water occurs by an evaporative process (line B).

Figure 9: Water absorption isotherm of hair (37).

In total, at 100% relative humidity hair absorbs up to approximately 30% of water, and even when moisture is also absorbed by the cuticle cells and the cell membrane complex, most of the moisture is absorbed by the cortical cells (19). Many other synthetic fibers also have the ability to absorb and desorb moisture.

Surprisingly, the process of water absorption/desorption by human hair has an ulterior natural function and it is not like any other ordinary process of moisture interaction with solid matter. It is part of the physiological phenomena used by nature to provide for hair softness, thermal regulation, and sweat transport. Hair interacts with moisture in ways that no other fiber can emulate. Incidentally, wool is the only other fiber that performs like hair, but this is so because wool is also a keratin fiber with a cellular and protein architecture similar to that found in hair. Hair and wool both have water properties that, in principle, appear antagonistic, i.e., they have a hydrophobic exterior that repels water and a hydrophilic protein interior that allows it to absorb and retain water depending on the environmental moisture conditions. These unique and paradoxical properties are not shared by other synthetic fibers. This analogical behavior is at the root of why so much research in developing cosmetic hair products begins in literature reviews of wool.

Because of their crosslinked structure, hair and wool have the ability to absorb large amounts of moisture and sweat without changing their mechanical integrity. The absorption of large amounts of water by hair occurs without the hair feeling wet and soaked and without excessive weakening of its protein

structure. These properties of keratin in hair contrast with those found in keratin of the skin. If we simultaneously submerge a piece of *stratum corneum* and a hair fiber in water we will observe, after a few hours, that cornoecytes in the *stratum corneum* swell without limit and lose their mechanical integrity. In contrast, the cortical and cuticle cells in the hair do not swell without limit and keep their integrity. Furthermore, as the amorphous protein matrix in hair absorbs and desorbs moisture it is able to release and gain heat depending on temperature differences between scalp and external environment. This particular behavior allows it to act as a heat buffer against rapid environmental temperature changes.

The effects of heat release upon moisture absorption by hair and wool, and their ability to keep warm surfaces is well documented (39). For instance, it is considered that if 1 kg of dry wool absorbs moisture from 40 to 70% RH it will release approximately 164 kJ (18, 32). If the wool is dried and absorbs approximately 35% of water it will release ~960 kJ of heat; this is as much heat as an electric blanket will release during eight hours. For these heat effects to take place, the wool or hair has to be dried and absorb moisture from low to higher moisture conditions. Then, as the wool or hair absorbs moisture the temperature will increase due to heat release. The water absorption isotherm of hair associated to this remarkable behavior was already shown in Figure 9; the points in this curve represent equilibrium states. The proteins in the amorphous matrix of cortical cells have various active sites for water adsorption. They are amino, carboxyl, and other hydrogen-bonding groups (40, 41). These proteins also act as nucleation centers for water multilayer adsorption. It has been reported that most of the heat released by wool or hair occurs when their hydrophilic groups absorb moisture in multilayer form at low relative humidities. At high relative humidities, moisture condensation and bulk water formation take place and there is less heat released.

3.3.1.5 DIFFERENT TYPES OF CORTICAL CELLS AND HAIR SHAPE

There are two types of cortical cells in hair; these are the ortho-and para-cortical cells (see Figure 10). Hair fibers from other mammals contain also a third type of cortical cells, namely, the meso-cortical cell, but this type of cell does not seem to be part of the human hair. There must be a reason for nature to have created two different types of cortical cells and confining them in two different

sections of the cortex within the same hair fiber. Existing data, whose interpretation is still controversial, suggest that the presence of ortho-and para-cortical cells is associated to the creation and stabilization of hair shape (42–44). It appears that by creating these two types of cells, nature can control hair shape by making it straight, curly, or extremely curly as a response to environmental adaptation. For instance, the cortical cells of hair fibers that are straight and limp have been found to be mostly of the para type. In contrast, the cortical cells found in hair that is curly are of two types, namely para and ortho, and their location in the cortex is such that they fill two distinct separate regions within the cortex of the same fiber (see Figure 10). Furthermore, the ratio of para to ortho type cells varies with the degree of curliness, i.e., the higher the content of ortho-cortical cells the curlier the hair is.

In spite of their differences, the ortho-and para-cortical cells are still made mostly of keratin filaments embedded in an amorphous matrix. However, because of their different packing density, the viscoelastic response of the hair section filled with para-cortical cells should be different from the section filled with ortho-cortical cells. Furthermore, the ortho-cortical cells seem to absorb less moisture than the para-cortical cells and this should result in a lower degree of water plasticization for this type of cell. This difference in moisture plasticization and viscoelastic response between the two inner sections of a single fiber has been evoked to explain the curliness enhancement seen in wavy and curly hair when moisturized.

Figure 10: Schematic representation of different ratios of ortho-and para-cortical cells in African, Caucasian, and Asian hair (60).

3.3.1.6 CUTICLE CELLS AND THEIR ROLE IN HAIR PROPERTIES

a. Cuticle cell structure and composition

As the follicle produces the cortical cells from stem cells, it also creates the cuticle cells. Like the cortical cells, the activity of the living cuticle cells is also highly specialized. In the process of differentiation they are instructed to become layered and flat in shape, with a final thickness of approximately 0.5 µm (see Figure 1). Subsequently, the follicle arranges them in stacks of 5 to 11 cuticle cells like shingles on a roof all around the cortical cells to create a strong protective shield. Their flat and layered structure organized in ordered stacks allows them to form the cuticle sheath. This form of cellular envelope acts as an effective shield that protects the cortical cells against friction, mechanical abrasion, impact, and other environmental stresses. It has been put there by nature to extend the life of hair to fulfill its function. Without the cuticle sheath around the hair fibers, the cortical cells will fray out and become rapidly damaged by friction. The cuticle sheath acts also as a gate to control incoming and outgoing light from the cortex to provide protection against radiation and color for aesthetic and social communication.

All of these important property/functions of the cuticle cells would not be possible without the unique architecture of their various layers and their specific chemical composition. For instance, the very top layer of a hair fiber, called the F layer, is made up of a fatty-acid chain, the 18-methyl eicosanioc acid or 18-MEA (see Figure 11). This fatty acid chain is attached covalently to an ultra-high sulfur-rich protein on the epicuticle surface (45–47). The attachment is made in such a way that the waxy part of the 18 MEA protrudes away from the epicuticle surface to provide the highly needed lubricity and hydrophobicity of hair fibers. Beneath the monomolecular layer of 18-MEA is the epicuticle followed by the exocuticle. These two layers, which are 120 and 250 nm in thickness, respectively, have the highest degree of sulfur content in the whole hair fiber (48).

Figure 11: Representation of F layer on cuticle cell surface showing 18-MEA attached covalently to the epicuticle (47).

The high sulfur content in these layers is testimony to the high degree of crosslinking bonds needed by the proteins at the surface so that the cuticle sheath can become hard and strong in order to resist mechanical abrasion. Then, beneath the exocuticle we find the endocuticle, this layer is composed of a non-cross-linked negatively charged protein, which is very susceptible to water swelling (49). The absence of crosslinked and crystalline proteins in this layer makes it highly deformable, very soft, and somewhat weak to mechanical stresses. It has been speculated that the soft nature of this layer allows it to act as a soft viscoelastic cushion to protect the hair against mechanical impact.

Below the endocuticle one can find the cell membrane complex (CMC), which is also the extracellular material used by the cuticle cells to bind and cement together. The structure of this cementing substance is very important because it not only glues cuticle and cortical cells but also plays a crucial role in the diffusion of moisture and other chemicals into the hair cortex.

The cell membrane complex is essentially composed of three layers: the upper beta layer, the delta layer, and the lower beta layer (see Figure 12). The beta layers are made of lipid chains arranged in palisades and attached on one side covalently to their immediate near protein network on the epicuticle. The other side of the lipid palisade is not covalently bound to the proteins in the delta layer; it is rather attached by physical forces also called dispersion forces. For this reason, many researchers consider this junction to be the weakest mechanical link in the whole fiber assembly (49–52). Cuticle and cortical cells fail at this junction when hair fibers are elongated to their breaking point and

when the cuticle cells chip away during dry combing. When wet, the cuticle cell fails rather at the soft protein zones in the endocuticle and not at the cell membrane complex (CMC).

Figure 12: Representation of cell membrane complex between cuticle cells (53).

The delta layer in the CMC appears to be made of proteins that are partially hydrophilic and partially hydrophobic. This layer is considered to contribute, in great part, to the diffusion of many liquids into the cortex. In the past, it was thought that the overall composition of the CMC, with its delta layer forming a sandwich between the two beta layers, was uniform all across the hair fiber. However, recent analyses made by C. Robbins and others appear to suggest that its composition changes depending on whether the CMC is cementing the cuticle cells, the cortical cells, or the cuticle/cortical cells interface (53) (see Figure 13). It has been proposed that the beta layers in the CMC gluing the cuticle cells are made of a monolayer of 18-MEA. As it was previously explained, this fatty acid is attached covalently to the epicuticle and physically attached to the delta layer. In contrast, the beta layers gluing the cortical cells are made of bi-layers of fatty acids, cholesterol, and ceramides, all of which are only physically attached on both sides. This important proposition suggests that the lipid of choice by nature to provide for high levels of hydrophobicity and lubricity to the hair surface is 18-methyl eicosanoic acid (18-MEA).

Figure 13: Representation of cell membrane complex between cortical cells of hair (53).

b. Viscoelasticity in hair and cuticle cells

The mechanical properties of the cuticle cells are just as important, if not more important, than those of the cortical cells for the function of hair. In fact, since hair is made of fibers whose length is comparably larger than their diameter, the fiber surface becomes predominant in controlling the mechanical properties of a hair assembly. Inter-fiber separation and air pocket formation for thermal regulation and aesthetic appearance are, therefore, mainly controlled by the mechanical behavior of the hair surface at the cuticle cells. Further, the integrity and mechanical stability of the cortical cells depends on the cuticle cells. Without the five to ten cuticle cells arranged in overlapping stacks to form the cuticle sheath, the cortical cells would rapidly collapse. Moreover, the surface of the cuticle cells not only has a high degree of hardness but also contains a lubricious fatty layer and is therefore very resistant to friction/abrasion. Hair fibers need this type of surface structure as they are constantly in motion, rubbing against each other.

Physical parameters relevant to these properties are the dynamic and static friction coefficients of the cuticle cells, the strength of the cuticle cell cement (CMC), and the Bending, Extension, and Compression Modulus of the cuticle cells. Unfortunately, data on the various mechanical modulus of the cuticle cells is scarce. Experimental studies carried out by various researchers have revealed, however, that the mechanical behavior of cuticle cells is not elastic but, as in the

case of the cortical cells, their behavior is also viscoelastic (54, 55). This is not surprising since the proteins making the various components in hair are biopolymers and, therefore, the overall behavior of each one of these components is also viscoelastic.

The phenomena of cuticle cell lifting and buckling produced by various environmental, mechanical, and thermal stresses, and the time it takes to recover from these deformations, are proof of cuticle cell viscoelasticity (56). Lifting and buckling of the cuticle cells occurs often in hair when subjected to continual elongation during combing, and also by thermal stresses after blow-drying or hot iron applications. Cuticle cell cement breakage and buckling are two of the most common manifestations of viscoelastic material mismatch between the various layers of the cuticle cells. When hair fibers are stressed, either thermally (by rapid compression due to water evaporation), or mechanically during excessive tensile elongation, the internal components of the single cuticle cells, i.e., the epicuticle, exocuticle, endocuticle, and cell membrane complex, do not respond in the same manner. This causes an excessive accumulation of shear stresses at their junctions and, consequently, the weakest link within the cuticle cell fails. Depending on moisture conditions, the endocuticle or the cell membrane complex fails, causing either cuticle cell buckling or breakage (52).

Cuticle cell damage in the form of cement breakage and buckling is commonplace in most people and it is significantly increased by various damaging hair treatments (54–60). This type of hair damage is almost absent in hair sections close to the fiber root and it is commonplace at its tips. Thus, a strong possibility exists that the “cement” becomes weaker by various environmental oxidative stresses during the life of a hair fiber. Gradual oxidation of the various cuticle cell layer components and their junctions will make them more prone to breakage by mechanical extensions during grooming.

Figure 14a shows a typical image of hair near its tip taken from a female subject with long hair (60). The same type of cuticle cell damage can be produced in virgin hair in the laboratory after subjecting it to large elongations and to thermal stresses (see Figure 14b). A very interesting aspect of cuticle cell buckling is that the deformations causing it can be either viscoelastically reversible or irreversible. For instance, it has been found that most of the buckling deformations produced during a hair-damaging process are reversible, i.e., the buckled cuticle cells can actually recover their original shape if enough time is allowed for recovery. There is, however, depending on the damaging process, a fraction of cuticle cells that cannot recover their shape and remain

permanently and irreversibly deformed (61).

It should be pointed out here that cuticle cell buckling always entails breakage of the “cement” or cell membrane complex (CMC). This is true regardless of whether the deformations in them are reversible or irreversible. In most cases, once the cuticle cells have undergone buckling their mechanical integrity is compromised. For instance, simple hair-strand swelling by water absorption and the resulting stresses are not capable of inducing cuticle cell lifting and buckling in virgin cuticle cells. Yet, after they undergo buckling, and after the cells have recovered from their deformations, they can be easily lifted and buckled again by simple water imbibition and resulting swelling. Studies have shown, however, that in certain cases the cell membrane complex by itself may have the ability to partially recement and re-glue the cuticle cells (61, 62). For instance, experiments have shown that, if deformed and lifted cuticle cells whose cement is broken are allowed to recover closing their gap, they will not lift again when immersed in water. In many cases the previously buckled and recovered cells will only buckle again when the levels of mechanical stresses are similar to the ones that initially produced cell lifting. This observation seems to indicate that, under certain conditions, the “cement” is able to partially recement the cuticle cells.

Figures 14a and 14b: SEM pictographs of cuticle cell buckling in hair as follows: (14a) as it appeared in a long hair of a female subject, and (14b) produced in the laboratory by mechanical stresses in an undamaged hair fiber (60).

c. Water and moisture absorption/desorption by cuticle cells

In the previous paragraphs we have pointed out that moisture absorption by the cortical cells has beneficial effects for the function of hair; among these benefits are scalp/skin temperature buffering, moisture transport, and a large contribution

to softness/plasticization of hair fibers. We also pointed out that water is the main protein plasticizer, and we came to the conclusion that moisture makes hair softer because it plasticizes the amorphous protein in the cortex. An observation that is often ignored by many researchers studying keratin fibers is that the cuticle cells also absorb moisture and, most importantly, that their mechanical behavior is very sensitive to moisture. The strongest evidence of this sensitivity is the effect that moisture has on cuticle cell recovery from deformations that were caused by stress buckling. Figure 15a shows an SEM picture of a hair fiber whose cuticle cells underwent buckling deformations after the fiber was subjected to a mechanical elongation of about 15%. The same hair fiber with its cuticle cells recovered is shown in Figure 15b, where recovery of the buckled cuticle cells took place after the fiber was immersed in water absorption (61, 64). Likewise, Figure 16a shows a micrograph of deformed and buckled cuticle cells produced in hair by blow-drying in the presence of water and low-molecular-weight oils (58); while in Figure 16b the same hair fiber can be viewed with its cuticle cells recovered after they were allowed to absorb moisture. The pictures were obtained by optical microscopy using the technique of patterns of light interference analysis described in more detail in the following paragraphs.

Figures 15a and 15b: SEM micrographs of cuticle cell buckling before (15a) and after water immersion (15b). Both micrographs correspond to the same spot in the hair fiber. As a point of reference, observe the elongated deep crack in the top section of the hair fiber (60).

Experimental observations made on those cuticle cells as depicted in Figures 16a show that their deformations do not recover if they are left alone for a good period of time at normal temperature (25°C) and under moisture conditions (53% RH). The experiments showed, rather, that the deformed and buckled cuticle cells recover only when moisture condenses on the cuticle cells, i.e., when the hair surface is above the dew point (65). These simple observations indicate the following: a) that cuticle cells need to absorb liquid water and not gaseous water molecules to recover by water plasticization and, b) that protein

moisturization in the cuticle cells only occurs when multilayer adsorption occurs at protein chains by moisture condensation.

Figures 16a and 16b: Optical micrographs of buckled cuticle cells observed in a hair fiber blow-dried in the presence of low-molecular-weight oils by using the LIP technique (54). Figure 26a represents the state of the cuticles after the hair fiber was blow-dried, and Figure 16b shows the cuticle cells after the hair was immersed in water for a period of one minute (60).

Moisture absorption by the cuticle cells is thus crucial for the cells to recover from deformations and to comply mechanically with the deformation of the cortex and the whole hair fiber. Just as in the case of cortical cells, cuticle cells are also able to perform their beneficial function in hair if they are plasticized by water, i.e., "moisturized." It is important to mention here that the subjective perception of "hair dryness" is de facto associated to a lack of moisture in the cortical cells. However, studies have shown that this is an incorrect conception since hair swatches that were equilibrated at high relative humidities seemed to obtain higher "dryness perception" scores than those equilibrated at low relative humidities (66). An explanation of these contradictory facts has already been offered by some scientists. They have proposed that roughness on the hair surface, and not lack of moisture in the cortex, is what creates the perception of dryness in hair (66). Moisture content in the cuticle cells should, therefore, have a stronger correlation with the sensation of hair dryness.

d. Optical properties of the cuticle cells

Another important property of the cuticle cells is their optical set of properties. All the various layers composing the cuticle cells are transparent. When white light strikes a hair assembly the first component it encounters is the cuticle sheath. The main physical property that determines how a transparent material interacts with light is its index of refraction (IR). The IR of the cuticle sheath has

been reported to be ~1.53 (67); the IR of each cuticle layer is not known, but because each one has a different composition and architecture, it is expected that its behavior will be somewhat different. The behavior of white light striking the cuticle cell—just as in the case of other transparent materials—thus, will be dependent on the IR of each layer. Its value will thus determine how much light is reflected, refracted, and absorbed by the cuticle cells. Reflected light can be of two types, specular or diffuse. If the reflected light is specular it will give rise to shine; if it is diffuse it will give a matt and dull appearance to hair. Light that is refracted passes through the cuticle cells and reaches the cortex, where it interacts with the pigments of melanin. There, a certain wavelength component of the light will be absorbed and the remainder of the light, which will not be white any more but colored, will be reflected back into the cuticle cells. The light will pass, then, throughout the cuticle cells and will appear to the eye as hair color (see Figure 17). If part of the light diffracts, penetrates into the cortex, and is absorbed by the melanin granules, the hair color will appear shiny and black, brown, or blond depending on the type of melanin. As it will be seen later, brown and blond colors are produced by different amounts of a brown melanin granule called eumelanin.

The condition of the cuticle cells thus determines the optical appearance of the hair assembly. If the cuticle cell cement is broken and the cuticle cells are lifted, light will not be properly transmitted to the cortex, and neither will it be specularly reflected. Rather, the light will be scattered and diffused and the hair will partially lose its shine and color, thereby, appearing dull and lifeless. Recently, it has been found that, either, when the cuticle cells are lifted, or whenever there are micro-pockets of air within the cuticle cell layers, the cuticle cells produce colorful microscopic light-interference patterns (LIPs) (see Figures 18a and 18b) (56). It is important to mention here that the vivid colors found in insects, butterflies, birds, and also in some mammals str created by such light-interference phenomena. These colors are usually very strong and they are not created by subtraction of colors from white light due to absorption by pigments. Rather they are created by the same mechanism used by soap bubbles to create color—a phenomenon based on the constructive and destructive interference of light (see Figure 19). This phenomenon is also known as iridescence, and the colors are called structural colors (68, 69). In the case of hair, if the interference colors produced by lifted cuticle cells could be made coherent at the macroscopic level, they would create strong and vivid colors.

Figure 17: Diagrammatic representation of light interacting with hair showing (a) total reflection of colors, and (b) light reflection plus diffraction and absorption by melanin granules.

Unfortunately, the lifted cuticle cell areas containing air in their layers creating these strong LIPs are very small, and light-scattering edge effects dominate. In addition, because of the small size and discontinuity in the lifted cuticle cells, the strong interference colors are not coherent at the macroscopic level. Consequently, their final effect to hair appearance is rather deleterious. However, the weak patterns of iridescence produced by the layered structure of undamaged cuticle cells certainly will contribute to the final optical appearance of hair. Colored light coming from the melanin in the cortex will thus combine with colored light produced by the weak iridescent patterns of virgin cuticle cells, and both, just as in the case of cuticle cells of some mammals, should produce the final hair optical properties. Thus, the weak iridescence patterns of virgin cuticle cells contribute to the unique color properties of undamaged hair.

Figure 18: Micrographs showing lifted and buckled cuticle cells on the same fiber by SEM (left), and by optical microscopy (right) using the LIP technique. Lines and circles in the pictures identify same features (56).

Figure 19: Diagram of lifted cuticle cells describing formation of light interference patterns by the phenomenon of thin-film light interference (56)

The color and shine enhancement often seen in hair when wet is a typical example of improved hair color perception due to changes in the IR of the cuticle sheath. Color enhancement in wet objects that absorb water can thus be explained by two mechanisms. The first is by the decrease in light scattering due to water filling of the otherwise empty pores containing air. The second is color and shine enhancement in hair by moisture absorption, also due to changes in the IR of the endocuticle, which enhances the phenomenon of iridescence. Polydimethylsiloxane polymers, oils, and some other high-molecular-weight polymers can also produce similar effects—as long as they are able either to penetrate into air gaps at the cuticle sheath or to smooth the cuticle cell surface to eliminate diffuse scattering.

e. The medulla cells

The medulla in hair refers to a region of very low density located at the center of a hair fiber (11). It is considered to be porous, irregular in shape; and, in human hair, in most cases, it is not continuous all along a single hair fiber. The regions of low density can be detected either by scanning electron microscopy or by optical microscopy (see Figure 20). In Figure 21, we can see a photograph showing the discontinuous presence of medulla along the hair fiber by which the hair was analyzed by transmission using optical microscopy (70). Not all hair fibers have medulla, but it is frequently found in thicker hair. Analyses have shown that, even in the case of thick hair, hair fibers from the same head may or may not have medulla cells. The medulla region is made of cells that are very different in structure to the cortical and cuticular cells; they are large and cuboidal in shape, and often, but not always, they are very porous. The structure, composition, and role of the medulla cells are not very well known. Because of

their porous structure, the medulla cells are filled with air and it has been speculated that this cell-air system contributes to increase the hair's ability to provide thermal insulation. The medulla cells have also been reported to have a strong impact on hair optical properties (71).

Figure 20: Micrograph of hair showing medulla region in hair (60).

3.3.1.7 MELANIN PIGMENTS IN HAIR

The melanin pigments in hair are made of very interesting molecules that play a crucial role in the function of hair. They act as free radical scavengers, antioxidants, UV light absorbers, heat radiation absorbers, and also as gamma rays absorbers (72–74). Melanin pigments not only protect the proteins in hair against oxidation and UV damage, but also help to protect the scalp from excessive harmful UV and heat radiation. They also provide color to hair for aesthetic and social communication. The protective action of melanin pigments is so important that nature does not use melanin only in hair, but also in the neurons of the brain as well as in the cells of eyes. Melanin has been found to provide important protection effects to the eyes; among these are the following: photoprotection, vision enhancement, decreases in the risk of cataract formation, and a lower risk of macular degeneration in the retina (73, 74).

Figure 21: Micrograph showing discontinuity of medulla region along a hair fiber (60).

Melanin granules in hair are approximately 0.8 µm in diameter (see Figure 22). They are polymeric in nature and depending on their type they differ chemically. The various chemical moieties forming the melanin polymer are 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid molecules (75, 76). A description of their composition is given in Figure 23. Melanin granules isolated from hair or skin display brown, black, green, and yellow colors.

There are many types of melanin and they can be grouped into two main types, namely, eumelanins and pheomelanins. Eumelanins can be further divided into brown and dark eumelanins. Brown eumelanin is common in people with lighter or brown color while dark melanin is typical of people with dark hair. Gray hair is caused by the absence of melanin in general while blond hair is produced by low amounts of brown eumelanin. Red hair contains mainly pheomelanin as pigment, which is also found in small amounts in skin together with eumelanin. The melanin granules are made in the follicle not by the cortical or cuticular cells, but rather, by specialized cells called melanocytes. Once the pigment granules are made, the melanocytes get instructions to excrete them and pass them into the cortical cells, where they are incorporated by phagocytosis (76, 77).

Figure 22: SEM micrograph showing physical aspect of melanin pigments in hair SEM (72–75).

Figure 23: Schematic representation of melanin composition (75).

3.3.1.8 HAIR FUNCTION

As discussed in the previous paragraphs, the sequences of amino acids constituting the primary, secondary, tertiary, and quaternary structures in the proteins of hair are optimized so the fibers are capable of fulfilling their biological functions. Other biological substances in hair—such as lipids, polysaccharides, and complex polymeric molecules—are found in the cuticular cement and in the melanin pigments. These are also carefully structured in the hair cells to fulfill a specific function. This optimization of composition and structure in the cells occurs so that hair can, finally, serve its biological functions of temperature buffer by absorption/desorption of water, protection against harmful environmental radiation, thermal and mechanical insulation, and aesthetic agent in social communication. Hair with an undamaged structure is apt to perform its biological function, and it is not coincidental that undamaged hair is also aesthetically and cosmetically appealing.

3.3.1.9 THE FOLLICLE

As we have stated earlier, the follicle is the smallest organ in the body, and it is there where hair fibers are produced (see Figure 3). Also acknowledged previously, there is one single follicle for each hair fiber. This minute organ is essentially composed of various biological units, namely: bulb, dermal papilla, bulge, and external and internal root sheath (78). With these key basic units, follicles are able to produce and assemble hair cells to form hair fibers. There are between 100,000 and 150,000 follicles in a normal scalp, distributed on its surface in the form of clusters of three or four hair follicles (see Figure 24). Evidence suggests that the number of hair follicles is constant throughout one's life, so the number of hair fibers on a scalp will, at least, while one is alive,

remain constant. The hair follicle, with all its basic internal units, regenerates itself approximately every four to six years. At each regeneration cycle, the hair fiber that has been produced falls out, and a new one starts to grow.

Figure 24: Clusters of various follicles in scalp (60).

a. Different zones in the follicle

All cells forming a hair fiber are made in the follicle from stem cells (79–81). The manufacture of hair cells involves a very well-organized process of stem cell preparation, shaping, and assembly in a way that is akin to a car assembly line. These various processes occur from bottom to top of the follicle in three main zones; namely: a zone of cell proliferation; a zone of gene expression and differentiation, and, finally, a zone of cell hardening or keratinization (see Figure 3) (11).

Each zone within the follicle has specific tasks crucial for hair production. At the bottom of the follicle is the first zone, and it is constituted by the dermal papilla and basal membrane. It is in this zone where epithelial stem cells that descend from the bulge are gathered to receive instructions from the dermal papilla to proliferate by mitosis. Moving towards the tip, in the next contiguous zone, the cells obtain instructions also from the dermal papilla to become differentiated into cuticle, cortical, and medulla cells. During this process they are packed and glued together, forming a cylindrical shape. Moving further towards the tip in the following third zone, located in the middle portion of the follicle, the hair cells are instructed to undergo apoptosis and become hard through a process of keratinization. In this zone, proteins in the cortical and cuticle cells become crosslinked to attain their final optimized, mechanical, thermal, and physical properties. In the zone of keratinization the hair reaches its final form and emerges from the scalp.

Just as in a car assembly line, where total production is controlled by external demands and internal constraints, there has to be a very well-coordinated process of communication within the follicle itself and with other body glands to

successfully produce hair. For a period of approximately four to six years each hair follicle is instructed to keep its key components active making hair. The new portions of hair created in the follicle push out upper sections of the fibers and the hair grows. At the end of the sixth year of activity, the follicle gets instructions from the body's glands and genetic system to cease and stop the production of hair. The ancillary machinery in the various zones of the follicle is then shut down, and the three zones of the follicle become completely inactive. Subsequently, the key components of the follicle are dismantled and discarded as they are pushed out by new ones being formed underneath. In this way, the old grown hair fibers, together with their inactive follicle units, are pushed out from the scalp and the hair fibers fall out. The new components form a new follicle with new zones of cell proliferation, gene expression/differentiation, and cell keratinization, and a new hair fiber grows.

b. Life cycle of the follicle

From the time of its inception until it recedes, the regenerated follicle has a life cycle characterized by three different phases, namely: anagen, catagen, and telogen (see Figure 25) (82). In the anagen phase, the assembly line activity of the hair follicle is invigorated and has its maximum efficiency preparing and assembling cells into fibers. It is at this stage that the hair fibers in the three zones of cell creation, differentiation, and keratinization are continuously assembling and growing. An intensive production of cuticle and cortical cells via stem cell mitosis characterizes this stage. The produced cells are continuously shaped and assembled into hair fibers by instructions from the dermal papilla in coordination with other body glands. For instance, it is well known that the growth rate of hair is strongly dependent on certain hormones and that it is used by nature as a tool of social communication to assert gender differentiation. During the anagen phase most of the hair fibers thus acquire their main physical, mechanical, thermal, and optical properties. The overall characteristics of hair fibers are therefore dependent on nutritional and hormonal variations affecting this stage of follicle development. Then, in the catagen phase, which lasts only two to four weeks, the follicle lowers its cell production and a large part of its cells undergo apoptosis. In the telogen phase, which lasts only from two to three months, the inactive parts of the follicle are pushed out and the old hair falls out.

New research has actually shown that the activity of the follicle is even more complicated than described in the three stages mentioned above. Some researchers have already proposed the existence of various substages for each

one of the three main phases (83). There are two main reasons behind these proposals:

Figure 25: Life cycle of the follicle: anagen, catagen, and telogen (60).

namely, a) each stage has very well-differentiated activities that can be further divided, and b) it has been found that at the end of each stage there are already preparatory signals taking place for the next stage. In fact, the appearance of new follicle components producing a new hair fiber starts in the follicle even before the older fiber is pushed out; so the anagen phase of a new growing hair fiber in some cases overlaps with the telogen phase of a hair that is going to fall. Thus, some of the processes controlling the formation of new hair fibers and the shedding of old ones seem to overlap between phases. This process can easily be perceived in the picture of human scalp shown in Figure 24, where it can be seen that in certain areas there are two hair fibers emerging from the same follicle.

All processes taking place in the follicle, whether they occur in the anagen, catagen, or telogen phase, or in any of their substages, need to occur in synchrony. Their occurrence involves the timely interaction and participation of a large number of biopeptides, enzymes, and other biological factors that act as regulators, communicators, and controlling agents for the production of stem cells, cells division, cell apoptosis, keratinization, *etc.* Furthermore, the maintenance process of the hair shaft, after it has emerged from the scalp in all its length, is partially executed by the biological activity of the follicle. Sebum and enzymes are released from the sebaceous glands in concert with the follicle to maintain the integrity of the hair shaft.

Hair diseases, hair defects, or mechanical weaknesses in hair fibers are caused by internal or external factors that interfere, block, change, or damage the

various biological processes involved in normal hair growth and hair maintenance. Traction alopecia, androgenetic alopecia, chemical alopecia, trichorrhexis nodosa, *etc*. are all hair ailments caused by some sort of internal or external interference. It has recently been discovered that the follicle is the target of major hormonal pathways, and that the follicle and sebum glands, both together, referred to as the pilosebaceous unit, can also act as a peripheral hormone-producing unit for a wide range of steroid hormones, neuropeptides, and neutrophins (84). Thus, proper growth of new hair fibers and shedding of old ones require no obstruction or interference with the biochemical substances involved within the follicle. Any internal or external factor interfering with the function of these biochemical substances will cause some sort of malfunctioning or disease in hair growth.

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AN OVERVIEW OF HAIR FOLLICLE ANATOMY AND BIOLOGY

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ABSTRACT

Since ancient times, the human hair has been an attribute for health, youth, and attractiveness as it plays a vital role in people's self-perception [(1)]. As a result, changes in the appearance of hair such as hair loss, hair thinning, and premature graying often cause psychological distress to the affected individuals.

Hair is produced by a multicellular entity; the **hair follicle**. The hair follicle is one of the most fascinating and dynamic organs of the human body and, unlike the hair itself, which is composed of dead keratinocytes, hair follicles undergo a process of regeneration that controls the production of the hair fiber. One can distinguish between three main types of hair follicles: the **lanugo**, the **vellus**, and the **terminal hair follicle**:

- **Lanugo hair** is very fine and normally shed *in utero*, or during the first weeks of life.
- **Vellus hairs** are very short, nonpigmented, nonmedullated hairs found all over the body surface.

Terminal hair is the large, and usually pigmented and medullated hair that is predominantly found on the scalp. It is estimated that humans have around 5 million hair follicles, of which 80,000 to 150,000 are located on the scalp. Scalp hairs grow at a rate of 0.3 to 0.4 mm per day [(2)]. **No additional follicles are produced after birth**, although the size of the follicles and hairs can alter with time, primarily under the influence of androgens. In pubic, axillary, and beard regions, androgens stimulate the conversion of vellus to terminal hairs, whereas

in frontal scalp regions of genetically predisposed individuals, androgens trigger miniaturization of hair follicles via conversion of terminal to vellus hair follicles [3].

The most unique feature of hair growth is its cycle. Hair follicles undergo repeated cycles of anagen (growth), catagen (degeneration), and telogen (quiescence). During anagen, hair follicle stem cells sustain the steady production of matrix cells in the hair bulb. These proliferate several times and terminally differentiate into the companion layer, the inner root sheath, and the hair shaft.

Unwanted hair loss is a common problem and is suffered by much of the world's population in both women and men. The condition is characterized by premature hair loss and excessive hair shedding. The process is caused by an interruption in the normal hair growth cycle, which can lead to the development of baldness with insufficient replacement of hairs in human scalp. This can have negative effects on an individual's self-esteem and thus products that claim to be useful for treating hair loss target a steadily growing, multibillion-dollar market worldwide.

The field of human follicle biology is progressing rapidly largely because of technical advances in molecular and cellular biology as well as sophisticated cell culture techniques. This chapter reviews the structure of hair, the biological processes occurring during the hair growth cycle, and the changes that occur with hair aging.

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3.3.2.1 HAIR FOLLICLE STRUCTURE

The hair follicle is a three-dimensional tube, composed mainly of epithelial cells,

which protrude down through the epidermis and dermis of the skin (Figure 1).

Figure 1. Human scalp tissue isolated from the scalp. Hair follicles protrude down through the epidermis and dermis of the skin (photo kindly provided by Dr Claire Higgins)

Hair follicles vary considerably in shape and size, depending on their location, but they all have common architecture. Anatomically, the hair follicle is most commonly described when it is in the mature anagen stage (Figure 2A). The mature anagen follicle can be divided into an upper permanent portion (infundibulum and isthmus), which does not cycle, and a lower part (bulb and suprabulbar), which is remodeled in each hair cycle. The infundibulum is the opening of the hair canal to the skin surface, and the isthmus is the permanent middle section extending from the sebaceous duct opening to the point of arrector pili insertion. The lower isthmus also contains the bulge region (Figure 2B), which is a region of functional importance as it is in this location that a reservoir of stem cells reside. Stem cells are unique, as they have the capacity to generate all the differentiated cell types within tissue and to self-renew in order to replenish their pool. Earlier studies have demonstrated that all epithelial layers within the hair follicle and hair originated from bulge cells [4, 5]. Hair follicle stem cells therefore appear to be responsible for regenerating the hair follicle in each cycle. Up until recently the location of the bulge in human follicles was unclear. By contrast with the bulge in rodent follicles, human follicles are not morphologically distinct. However, identification of unique bulge markers in the human follicle has enabled the human anagen bulge to be defined as a distinct region [6, 7].

Figure 2. Structure of human anagen hair follicle. (A) Longitudinal section of a human scalp hair follicle showing the permanent (infundibulum and isthmus) and cycling (suprabulbar region and bulb) components of the hair follicle. (B) High magnification image of the isthmus. The dashed square indicates the approximate location of the bulge. (C) High magnification image of the bulb showing the outer root sheath (ORS), inner root sheath (IRS), matrix zone (M), dermal papilla (DP) and connective tissue sheath (CTS). (D) Schematic drawing illustrating the concentric layers of the ORS, IRS and shaft in the bulb. Current Biology 19, R132-R142 2009 ©2009 Elsevier

The anagen bulb (Figure 2C) is one of the most rapidly dividing tissues in the body. The bulb represents the hair shaft factory; it contains the proliferating matrix keratinocytes and pigment-producing melanocytes (matrix zone). The hair matrix keratinocytes move upwards and differentiate into the hair shaft, as well as into the inner root sheath; the melanocytes transfer pigment into the developing hair keratinocytes to give hair its color [8]. These cells are supplied by the pluripotent stem cells existing in the bulge. The hair bulb surrounds the dermal papilla, which comprises a group of specialized cells (dermal papilla cells) with important inductive properties. The dermal papilla is believed to be one of the most important drivers that instruct the hair follicle to grow, and dictates hair bulb size, hair shaft diameter, length, and anagen duration.[9] The dermal papilla is known as the “conductor of the orchestra” or “command center,” and is an essential source of growth factors (keratinocyte growth factor, bone morphogenetic protein, hepatocyte growth factor, insulin-like growth factor, stem cell factor), all of which are critical for hair growth and melanogenesis [8]. Surgical removal of the dermal papilla and the lower dermal

sheath prevents hair growth, which indicates the importance of these specialized cells as the key signaling center in hair follicles [10]. Interestingly, dermal papilla cells from balding scalp have both higher levels of androgen receptor (AR) and type II 5-alpha reductase, which converts testosterone to dihydrotestosterone (DHT) [11]. DHT can induce dermal papilla cells to secrete factors including transforming growth factor beta 1 (TGFb1) and DKK-1 that inhibit keratinocyte growth [12, 13].

The hair shaft (Figure 2D) is divided into the hair cuticle on the outside and both the cortex and medulla in the center. The cells that make up the hair shaft are known as trichocytes, which are terminally differentiated hair follicle keratinocytes. The hair shaft cuticle consists of overlapping cells that are arranged like shingles pointing outward and upward. The hair shaft cortex, the bulk of the hair shaft, is composed of hair-specific keratin filaments and keratin-associated proteins with melanin produced by melanocytes in the matrix region [2]. The hair shaft medulla, the central part of the hair shaft, is composed of large, loosely connected keratinized cells with large intercellular air spaces. The hair shaft is surrounded and supported by the inner root sheath (IRS), the companion layer, and the outer root sheath (ORS).

The IRS consists of three distinct layers: the cuticle, Huxley's layer, and the Henle's layer (Figure 2D). The IRS cuticle layer adjoins the cuticle of the hair shaft and anchors the hair shaft to the follicle. IRS keratinocytes produce keratins and trichohyalin that serve as an intracellular "cement" giving strength to the IRS to support and mold the growing hair shaft, as well as guide its upward movement [8].

The ORS surrounds the IRS and is continuous with the basal layer of the epidermis. Distinct from the other epithelial components, the ORS does not generate by upwards growth from the matrix zone, but rather directly from the stem cell bulge. The bulge region of the ORS is located just below the sebaceous gland and at the insertion site of the arrector pili muscle. Stem cells leave the bulge in the ORS and migrate toward the matrix zone, where they begin to proliferate and subsequently differentiate into new hair shafts [14].

Hair follicles grow in an oblique angle towards the epidermal surface. This angle can be varied by the contraction of a bundle of muscle, collectively called the arrector pili muscle (APM). The APM is under adrenergic control and thus, in situations of cold temperature and emotional stressors, it gives rise to the interesting phenomenon of involuntary contraction resulting in the hair standing up!

3.3.2.2 HAIR FOLLICLE CYCLING

Hair growth and loss in humans is controlled by a specific follicular cell cycle (Figure 3) that includes three major stages [9]:

- growth and differentiation (**anagen**)
- regression and apoptosis (**catagen**) and
- rest (**teleogen**)

Additional phases recently discovered include exogen, in which the fiber is actively shed [15], and kenogen, when the follicle is empty [16].

Figure 3. Hair Follicle Cycle. The different stages of the adult hair follicle are divided into three main categories known as anagen, catagen, telogen, which continually cycle throughout the life of the follicle. During anagen the matrix cells of the bulb (red) rapidly proliferate and differentiate to produce the hair fibre and layers of the inner root sheath. The hair follicle enters catagen which is characterized by apoptosis of the epithelial cells, and the formation of the club fiber. The dermal papilla (blue) moves up to the base of the permanent section of the follicle, in close proximity to the bulge (green). A resting phase known as telogen then occurs before the renewal of a subsequent anagen phase. Exogen describes the removal of the remaining club fiber which typically occurs close to the time of renewed anagen onset. Seminars in Cell & Developmental Biology 18 245-254 2007 ©2007 Elsevier

Hair follicle cycling is driven in response to continuing molecular interactions between the dermal papilla and the epithelial components of the follicle [17, 18].

The duration of the different growth phases depends on the type and localization of the hair follicle. Scalp follicles stay in the anagen stage for two to six years and produce long hairs, whereas eyebrow or eyelash hair follicles do so

for only one to three months and produce short hairs. An in-depth description of eyelash hair follicles may be found elsewhere in this book. What determines the clock mechanism and the duration of anagen in individual hair follicles is not known, although one candidate for consideration is the circadian clock, a molecular oscillatory system [19, 20].

Following morphogenesis, the hair follicle enters the regression phase (catagen). The catagen stage lasts approximately two weeks, during which time the hair fiber ceases to grow. During the catagen stage, cells located in the lower portion of the hair follicle undergo programmed cell death (apoptosis). The hair follicle shortens and the hair bulb loses its internal and external sheaths. During this process the hair follicle acquires a club-shaped end and is subsequently referred to as the "club fiber." Additionally the dermal papilla condenses, moves upward beneath the hair follicle bulge, and halts its activity. Follicular melanogenesis also ceases during this stage. Catagen has been suggested to occur as a consequence of decreased expression of anagen maintaining factors, such as insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and increased expression of cytokines promoting apoptosis, such as TGFb 1, interleukin-1alpha (IL-1a), and tumor necrosis factor alpha (TNFa) [21].

Upon catagen completion, hair follicles enter a resting stage (telogen) for an average of three months. In the telogen stage, the hair follicle has decreased to about half of its previous size and the stem cells are thought to be quiescent. However, by the end of the telogen stage, hair follicle stem cells become activated by Wnt and TGFb signals from the dermal papilla and form a new hair follicle [22–24]. Anagen involves the complete regrowth or regeneration of the lower, cycling portion of the follicle, i.e., the hair shaft factory. The cross-talk of dermal papilla with neighboring matrix cells results in the maintenance of hair fiber production (i.e., the hair continues to grow). Two secreted molecules that have important roles in hair follicle cycling are IGF-1 and bFGF. Both are produced by the dermal papilla, and their receptors are found predominantly in the overlying matrix cells [25].

Sometime after new shaft production begins, the club fiber is shed [26]. The term 'exogen' has been introduced to identify the hair fiber shedding event as a separate process during hair follicle cycling [15]. Shedding of 100–150 hairs per day is normal [2]. Previously, hair fiber shedding was believed to be passive, but recent research suggests that shedding is an active and highly controlled process [15, 27, 28]. Empty hair follicles, after shedding of the club fiber, but before the onset

of renewed anagen, are in a stage called “kenogen.” Kenogen can be observed in healthly skin, but the frequency and duration are significantly greater in individuals with androgenetic alopecia [16].

In addition to hair follicle tissue modeling, skin innervations and vascular networks also undergo substantial changes with the progression of the anagen stage [17, 29]. Perifolicular vascularization is significantly increased during anagen. It correlates with the up-regulation of the expression of vascular endothelial growth factor (VEGF) mRNA, a potent angiogenic growth factor, produced by keratinocytes of the outer root sheath [8].

Each follicle can undergo repeated cycles until it eventually dies or miniaturizes to give rise to a vellus hair [30, 31]. **Disturbances of hair follicle cycling lie at the heart of most hair growth disorders, and have dramatic effects on visible hair growth and shedding**. For instance the most common alopecia, *androgenetic alopecia*, produces a patterned baldness involving a reduction in the percentage of scalp hair follicles in anagen, a reduction in the anagen growth phase duration, and a corresponding increase in the percentage of hair follicles in telogen and an increase in the duration of telogen. These alterations (in combination with a shift in the exogen phase from primarily occurring in early anagen to initiation primarily during telogen) result in thinning hair coverage [32, 33]. The most effective therapeutic strategies for hair loss therapy inhibit catagen development so as to prolong anagen, to induce anagen in telogen follicles, and/or to inhibit exogen [34].

3.3.2.3 AGING OF THE HAIR FOLLICLE

While consumers are increasingly concerned with diminishing the signs of aging, especially the aesthetic of hair, addressing the cellular mechanisms behind them represents a decisively new direction in hair care strategies. Like all biological systems, the hair follicle also undergoes an aging process. This process is not only characterized by loss of pigmentation, but changes in growth characteristics that include decreased hair follicle density, increased number of telogen hairs, a decrease in the diameter of hair shafts, and slower hair growth rates [35–38].

The color of hair mainly relies on the presence or absence of melanin pigment. Two types of pigment can be produced by the hair follicle. The first type is eumelanin, which is the pigment in brown-black hairs, while the second type is pheomelanin, the pigment in red-blond hairs. Melanin is produced by the

melanocytes, and is the result of a complex biochemical pathway (melanogenesis) with tyrosinase being the rate-limiting enzyme. Hair melanins are formed in cytoplasmic organelles called melanosomes. The melanosomes travel out to the tips of the melanocyte's dendrites where they are transferred to the hair follicle keratinocytes of the hair cortex and medulla. Each hair cycle can produce a long pigmented terminal hair because the follicle is able to replace these melanocytes from a reserve found in the bulge region of the ORS [39]. As individuals age, the melanin content of the hair follicle decreases, causing graying and eventual whitening of the hair. Although poorly understood, numerous mechanisms have been suggested for this loss of hair follicle pigment, including pigmentary machinery malfunction or loss, melanocyte apoptosis, anagen defects, the loss of melanocyte stem cells or their failure to differentiate, and melanocyte migration defects [40].

Hair graying, sometimes referred to as canities, is one of the most prominent signs of human aging. A recent worldwide survey showed that 74% of people between the ages of 45 and 65 have gray hair, and that it occurs earliest in people of Caucasian descent, followed by lesser occurrence in Asians and Africans [41]. The age of graying onset is genetically controlled and inheritable. Hair is considered to gray prematurely only if it occurs before the age of 20 years in Caucasians, before 25 years in Asians, and before 30 years in Africans [42]. As hair color is socially important, premature graying imposes a psychosocial distress and is often perceived as a sign of stress, old age, ill health, and bodily decline [42, 43].

Both the hair and scalp are constantly exposed to various environmental stressors. These include UV irradiation as well as oxidative and chemical stressors. The effect of external stressors on the hair fiber is quite well documented. In particular, repeated sun exposure impairs the structure of the hair shaft keratin fiber, while UV radiations induce hair protein loss and color changes [44]. Recent *in vitro* and *ex vivo* studies have investigated the damage caused by UV radiation and oxidative stress on the hair follicle [45, 46]. Upon exposure to repetitive UVB irradiation isolated hair follicles in culture demonstrated reduced hair shaft elongation and premature entry into the catagen phase. Moreover, oxidative DNA damage was observed in hair follicle submitted to low UVB doses [45]. Gondran *et al.* examined the effects of UV radiation and oxidative stress generated by H_2O_2 on human scalp skin grafts containing hair follicles [46, 47]. *Oxidative stress impaired hair follicle structure as indicated by increased number of vacuoles and edema mainly observed in the outer root*

sheath. Emerging evidence shows that reactive oxygen species (ROS) accumulate in human gray/white scalp follicles up to millimolar concentrations. The presence of ROS is believed to cause oxidative damage (free radical-induced aging) to the hair follicle melanocytes [48, 49]. In addition catalase (the enzyme involved in cell response to oxidative stress), expression is reduced in unpigmented and older follicles [48, 50, 51]. Altogether, the data strongly suggest that the hair follicle itself is a direct target of oxidative stress and underline the interest of applying a specifically developed cosmetic follicle ingredient to protect the hair follicle from endogenous or environmental stressful processes [46, 47].

CONCLUSION

Hair care formulations have traditionally relied on ingredients that treat the hair fiber to improve appearance. However, vibrant and healthy-looking hair starts at the roots and, therefore, cosmetic biofunctionals that target the hair follicle provide product manufacturers with new innovative ways to bring back beauty to the hair.

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GLOSSARY

Alopecia: Abnormal hair loss.

Androgenetic alopecia: Loss of hair caused by miniaturization of genetically predisposed follicles

Anagen: Growing stage of the hair follicle cycle.

Bulb: Lowermost portion of the anagen follicle containing rapidly proliferating cells that generate the hair fiber

Bulge: Site of stem cells within the follicle outer root sheath, located at the insertion site of the arrector pili muscle just below the sebaceous gland.

Catagen: Regression stage of the hair follicle cycle.

Club hair: Fully keratinized, dead hair formed during catagen.

Dermal papilla: Signaling control center of the hair follicle consisting of dermal papilla cells.

Exogen: Stage of the hair follicle cycle when hair is shed from the follicle.

Hair shaft: The hair *per se*, composed of trichocytes, which are terminally differentiated hair follicle keratinocytes. It is composed of the medulla and the cortex.

Infundibulum: Most proximal part of the hair follicle relative to the epidermis, extending from the sebaceous duct to the epidermal surface.

Inner root sheath: Surrounds and protects the growing hair.

Isthmus: Permanent middle section of hair follicle extending from the sebaceous duct to the point of arrector pili insertion

Kenogen: Stage of the hair follicle characterized by empty hair follicles after the hair fiber is shed before a new anagen hair emerges.

Outer root sheath: The outermost layer of the hair follicle. Merges

proximally with the basal layer of the epidermis and distally with the hair bulb.

Telogen: Resting stage of the hair follicle cycle.

Terminal hair: Large, usually pigmented hairs (e.g., those found on the scalp).

Vellus hair: Very short, nonpigmented hairs (e.g., those found diffusely over nonbeard area of the face and bald scalp as a result of miniaturization of terminal hairs).

HAIR AGING: FUNDAMENTALS, PROTECTION AND REPAIR

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ABSTRACT

Aging skin is a concept easily grasped; aging hair is not. However, changes in hair with aging are consumer perceivable and measurable. This chapter describes the structure of hair, fundamentals of aging hair, signs of aging hair, and causes of the visible signs of aging hair. Further, it also covers remedies for protection of hair fibers, the scalp from which it grows, as well as hair products and ingredients formulated to maintain and restore youthful hair. Beyond these approaches, the author has also provided a unique cosmeticological look at selected products and ingredients on the market with commentary on their performance.

While considerable attention has been paid to the many factors affecting aging skin, it is only recently that a shift in the perception that hair is “dead” has begun to accelerate. Actually, while this is true, in part, it is also true that the core of the hair fibers is alive as well as the skin from which it grows, and much has been done and needs doing to bring it to life again.

The understanding of the hair-scalp system described in this chapter provides important leads to ingredient-seekers and product formulators and presents an opportunity for a myriad of novel products that consumers long for. The chapter is also unique in that it provides thoughtful, practical recommendations for hair care and protection. This wisdom goes beyond the traditional approach of finding new ingredients or formulations to achieve the goals of beautiful hair. It is recommended that these approaches be incorporated in recommendations by

product manufacturers as important information to consumers.

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3.3.3.1 INTRODUCTION

Similar to skin, with aging, the scalp and hair are subject to intrinsic or chronological aging, and extrinsic aging due to environmental factors. The changes associated with aging skin and the mechanism of skin aging has been well researched and published extensively over the past decades. According to Mintel's global product database, nearly half of the skin care products in the market are anti-aging products.

While the aging of hair has not been studied extensively like skin aging, there are numerous studies published on hair aging[1–3], and the number of anti-aging hair care products continues to increase year by year[4]. The most obvious sign of hair aging is the appearance of gray hair that correlates closely with intrinsic aging. Melanin pigment is responsible for the natural color of hair and it is produced by melanocytes in the hair follicles. With aging, melanocytes stop producing melanin pigment, resulting in gray hair. The loss of melanin has been linked to a decrease in the number of melanocytes in the hair follicle as well as a possible decrease in the activity of the enzymes involved in melanogenesis[5]. A recent publication by Wood *et al.*[6] showed for the first time high accumulation of hydrogen peroxide in human gray scalp hair shaft, thus supporting the theory

of oxidative stress in gray hair formation. These researchers also demonstrated that H_2O_2-mediated oxidation of methionine in tyrosinase enzyme limits its functionality, which leads to gradual loss of hair color.

In addition to graying of hair, with aging, your hair will become deficient in essential nutrients and the hair follicles shrink, it loses its elasticity, lipid and keratin production diminish, and many hair follicles stop producing new hairs altogether. These internal changes cause hair to become thin, dry, less manageable, brittle and less shiny, and ultimately lead to hair fall.

Externally, the hair shaft is exposed to sunlight, environmental pollutants, chemical and heat treatments, and subjected to daily grooming and mechanical aggression-in-search-of- "beauty." These environmental insults and daily aggressors alter the chemical and physical properties of the hair fiber. When hair is exposed to sunlight, severe damage occurs due to lipid, protein, and melanin oxidation. This results in loss of mechanical strength, loss of color, an increase in porosity, as well as an increase in surface roughness. Other environmental pollutants such as cigarette smoke, car exhaust fumes, and smog also impart hair damage, resulting in dull and brittle hair. These physicochemical changes in hair structure and damage caused by aging are measurable and also perceivable to the consumer.

Several methods involving microscopic techniques, differential scanning calorimetric, wet and dry combing measurements, protein loss, and assessment of tensile properties of the hair are generally used to evaluate the various physicochemical changes to hair[7]. These approaches are useful in characterizing aging changes as well as the effect of "treatments" that may improve the detrimental effects of the various stressors.

Hair loss is another significant aspect of aging that occurs gradually with a progressive thinning of hair. With aging, less protein is produced in the hair follicle that can cause hair to fall out and not to be replaced as quickly as desirable. This causes baldness, as well as thinning of hair. Unlike the long term, initial focus on the skin of women, the baldness phenomenon is also a men's "condition" and any impact of novel approaches to reduce hair loss and hair thinning represents an area of potential product growth for both men and women.

Experimental evidence supports the hypothesis that oxidative stress also plays a role in hair aging. The damaging effects of these reactive oxygen species are induced internally during normal metabolism and externally through exposure to

various oxidative stresses from the environment. The body possesses endogenous defense mechanisms, such as antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidative molecules (e.g., vitamin E, vitamin C, glutathione, ubiquinone), protecting it from free radicals by reducing and neutralizing them.[9] With age, the production of free radicals increases, while the endogenous defense mechanisms decrease. This imbalance leads to the progressive damage of cellular structures, presumably resulting in the aging phenotype.

Nourishment and proper grooming are very important to maintain the beauty of hair as perceived by: good hair texture, healthy growth, shine, and luster. Eating foods rich in vitamin A, B, minerals, and proteins is necessary for promoting a healthy scalp and hence healthy hair growth. Eating a diet rich in dark green vegetables, orange and yellow fruits, sprouted whole grains, cereals, meat, and soy helps maintain a healthy follicle. It is equally important to use good scalp care and hair care products to protect the hair shaft from damage due to sunlight, pollution, and other daily aggressors. For daily washes, use a shampoo that is mild, gentle, and moisturizing. Anti-aging hair care topical and ingestible products enriched with vitamins, lipids, proteins, vegetable oils, and antioxidant rejuvenate the scalp and follicle, and encourage new, more resilient growth from the root.

This chapter describes the fundamentals of aging hair, signs of aging hair, and the causes of visible signs of aging. Further, it also surveys current remedies for protection of the hair fiber, along with hair products formulated to maintain and restore youthful hair.

3.3.3.2 STRUCTURE, COMPOSITION, AND NATURAL COLOR OF HAIR

Hair is composed of two parts, the hair follicle and the hair shaft. The hair follicle is a tiny cup-shaped point located in the dermis from which the hair grows. The hair follicle consists of the hair bulb, the inner root sheath, and the hair shaft (see Figure 1).

Figure 1. Cross-Sectional Image of Hair Bulb Structure

New hair is made inside the onion-shaped hair bulb that lies within the hair follicle. The hair bulb also contains some special cells called melanocytes that produce melanin, the pigment responsible for the natural color of hair. The part of the hair seen above the scalp is called the hair shaft. It consists of three layers; cuticle, cortex, and medulla (see Figure 2).

Figure 2. Structure of Human Hair Fiber

The outermost layer called cuticle is a thin and colorless layer made of several overlapping scale-type structures. It is responsible for the visual attributes of hair such as shine and smoothness. It is also responsible for the friction effect of hair, which determines manageability (i.e., how easy it is to comb or brush hair). The cuticle layer can be damaged by daily grooming such as combing and brushing as well as by chemical treatments such as bleaching or relaxing. The middle layer of the hair shaft is called the cortex, made of keratin fibers. The strength, color, and texture of a hair fiber are provided by the cortex. The innermost layer of the hair shaft called medulla is only present in large and thick hairs.

Melanocytes are the cells responsible for the natural color of hair, which are

large star-shaped cells with branches called dendrites. They are mainly found at the bottom of the hair follicle, where they produce melanin in the form of small grains of colored pigment. Melanocytes inject these pigments into the keratinocytes of the hair shaft by lengthening their dendrites. Melanocyte produces two types of melanin: eumelanin and phaeomelanin. Eumelanin occurs in the form of a small rice-like granule having a color varying between brown and black. Melanin is formed by the oxidation of an amino acid, tyrosine by the enzyme tyrosinase. The higher the concentration of eumelanin, the darker the color of hair.

The actual appearance of hair, and its overall reflective quality, is determined primarily by the pigment type, but also by the density and distribution of the pigment granules. Phaeomelanin has a less precise shape and can be seen in the form of diffuse spots. Its color varies from yellow to red. Phaeomelanin differs from eumelanin because, in addition to tyrosine, another amino acid, rich in sulfur (cysteine), is involved in its production. There are two different types of eumelanin (brown eumelanin and black eumelanin). A small amount of brown eumelanin in the absence of other pigments apparently causes blond hair.

Although people with dark hair may still produce the yellow-orange pheomelanin, it is largely masked by the dark eumelanin pigment and cannot easily be seen. However, the red-yellow pheomelanin is believed to cause the warm, golden, or auburn tones found in some types of brown hair. The range of colors produced by melanins is limited to shades of yellow, brown, red, and black. Gray hairs contain only a few melanin granules, spread throughout the hair. White hairs contain no melanin at all: their whiteness is an optical effect, due to the way they reflect the light.

3.3.3.3 FUNDAMENTALS AND SIGNS OF AGING HAIR

When considering the phenomenon of hair aging, genetic, hormonal, and environmental factors need to be taken into account. Similar to skin, the scalp and hair are subject to intrinsic aging depending on individual genetics and hormonal factors and extrinsic aging due to environmental factors. Intrinsic aging or chronological aging results in graying of hair due to a decrease in melanin production and loss of hair due to androgenic alopecia. Extrinsic factors include ultraviolet radiation, pollution, smoking, diet, and lifestyle. All these extrinsic factors progressively degrade the hair fiber through oxidation of lipids, cleavage of disulfide bond, degradation of amino acids, and oxidation/bleaching

of melanin pigment.

The effects of age-related changes in hair density, hair diameter, hair lipids, and hair color (graying) produce changes in hair fiber properties. These changes in properties impact important consumer hair assembly properties such as ease of combing, hair body, hair coverage, frizziness, shine, manageability, and style retention. There are many visible signs of aging hair that are measurable and consumer perceivable. *The most critical aging attributes are thinning of hair, loss of color, moisture, shine, and manageability.*

Thinning of Hair

Both men and women tend to lose hair thickness and amount as they age. Baldness is not usually caused by a disease. It has been found that poor diet, stress, aging, and pollution are the most common factors that damage hairs and cause hair loss. Scientifically, health experts have found that the growth of dihydrotestosterone (DHT) hormone on the scalp is responsible for hair loss. With aging, hair loss is experienced more rapidly because the amount of DHT increases with increase in age. This hormone makes hair follicles weaker and weaker by blocking the nutrients and water to the hairs. Over time, the follicle can shrink, causing the hair to become shorter and finer and finally the hairs fall from the scalp. Figure 3 shows the picture of a normal hair follicle compared to a finer follicle and a very small hair follicle, which results in baldness.

Figure 3. Picture of a hair follicle showing shrinkage over time

According to the American Hair Loss Association, around two-thirds of U.S. men will have experienced some degree of hair loss by the age of 35, and about 85% of men will have significantly thinning hair by the age of 50. Although hair loss is typically associated with men, figures state that women make up 40% of

all hair loss sufferers in the U.S., showing that hair loss is a condition that can affect us all. Recent studies claim that 16% of women under age 50 are affected, which increases to 30–40% of women aged 70 years and over[10]. The thinning of hair in women may become rather more pronounced after menopause when there are fewer estrogen hormones to counteract the androgens. Men tend to have baldness while women have thinning of hair and preservation of the frontal area.

Hair loss treatment products are mostly designed to act as DHT blockers. In recent years, two pharmacological agents have become available to treat male pattern baldness. Neither is fully effective in all cases. Both need long-term administration or there will be recurrence. Both must be prescribed on private prescription. Minoxidil is provided in 2% and 5% solutions that are applied to the scalp twice daily. The 5% solution is for men only. One study found that the 5% solution was 45% more effective than the weaker strength after a 48-week trial and was the preferred treatment for men[11]. It may well be months before any improvement is seen and it should be discontinued if there is none after a year. A solution of 5% minoxidil in cutaneous foam is now available, which is developed for ease of use. The product instruction requires application of half a capful to the scalp twice a day but the treatment should be stopped if there is no improvement after four months.

Recently, the natural hair loss treatment, **Provillus™**, has gained significant popularity for its effective dual-step formula to treat the hairs of men and women accordingly as it offers different formulations for both. The treatment, clinically proven to regrow hair, consists of a powerful blend of natural herbs and has been approved as safe and beneficial formula by health experts and FDA. The two-step formula consists of a hair regrowth supplement and a hair regrowth spray with 5% minoxidil for men and 2% minoxidil for women, which performs dual action of preventing hair loss first and then promoting hair regrowth. The consumption of this supplement twice a day makes the hairs strong enough to fight against DHT hormone and to survive. The hair regrowth spray is a liquid to be applied over the scalp. The use of that spray twice a day promotes a thicker, stronger, and healthier hair regrowth. The product nourishes the overall health of hairs and makes them healthy, shiny, and beautiful, without any negative side effects.

Change in hair fiber diameter (fine-coarseness) is another parameter that is influenced by age. Large increases in hair fiber diameter occur during the first year in life and during the teenage years. Diameter tends to peak at about age 20 for men and in the mid-40s for women (see Figure 4). Figure 4 shows a steeper

drop for the scalp hair fiber diameter of Japanese males versus Caucasian males. A decrease in hair fiber diameter results in thin hair.

Figure 4. Hair fiber diameter of Japanese males vs. Caucasian males as a function of age (C.R Robbins pp 120)

Loss of Hair Color (Graying of Hair)

Graying of hair is a natural age-related phenomenon that varies between individuals and between different ethnicities. The biological process of hair aging has been attributed to the loss of pigment-forming melanocytes from the aging hair follicle[12]. There are many theories that explain the gradual loss of pigmentation with aging, including inactivation of tyrosinase enzyme[5] and decrease in stem cell factor[13]. Recently, a free radical theory of graying has been proposed by Arck *et al.*[14]. These authors found evidence of melanocyte apoptosis and increased oxidative stress in the pigmentary unit of graying hair follicles. Recently Wood *et al.*[6] have shown for the first time accumulation of high levels, millimolar concentration, of H_2O_2 *in vivo* in human gray/white scalp hair shaft by FT-Raman Spectroscopy. These authors further demonstrated absence of catalase and methionine sulfoxide reductase A and B enzymes. In younger people, catalase enzyme breaks down hydrogen peroxide into water and oxygen, thereby protecting melanocytes from oxidative damage by hydrogen peroxide. As we age, the decrease in the levels of catalase, combined with lower levels of methionine sulfoxide reductase enzymes, MSR A and B, which repair hydrogen peroxide damage, cause hair to turn gray. A recent study by researchers at University of Bradford demonstrated repigmentation of eyelashes and skin after reduction of epidermal H_2O_2 with topically applied narrow-band UV activated pseudo-catalase PC-KUS.[15] This further supports that accumulation of hydrogen peroxide due to decrease in catalase enzyme causes hair graying and reversal of

pigment formation is possible through introduction of pseudo-catalase type molecules through topically applied products.

Figure 5. Picture of a young hair follicle vs. gray hair follicle showing melanin particles replaced with colorless air bubbles (www.nlm.nih.gov)

Hair Dryness, Shine, and Manageability

Hair dryness also results from both intrinsic and extrinsic aging. The sebaceous gland in the hair follicle produces sebum that normally hydrates the scalp and moisturizes the hair by surrounding it with a protecting microfilm. A deficiency or lack of sebum makes the scalp more fragile, resulting in irritation and itchiness. The loss of sebum also results in loss of hair elasticity and the hair becomes dry, dull, and prone to breakage. The intrinsic production of sebum from sebaceous glands in the follicle decreases with age, particularly in females as shown in Figure 6 below.

Figure 6. Variation of sebum production on foreheads with age. Data are from C.R. Robbins, *Chemical and Physical Behavior of Human Hair, 4th edition, p. 79*

Additionally, extrinsic parameters such as frequent washing of hair, use of alcohol-containing hair products, chemical treatments, exposure to sunlight, and frequent blow-drying also cause hair dryness. Each healthy hair strand has an outer protective layer (cuticle), which is composed of fatty acids, ceramides, and cholesterol. Together, these account for 0.7–1.3% of the total chemical content of hair. Integral hair lipids are located in the cell membrane complex (CMC) of hair cuticles, and are important in the maintenance of hair integrity due to hydrophobicity, moisturization, and stiffness.

Exposure to sunlight results in degradation of cuticle lipids by oxidative damage, and exposure to alcoholic cosmetic products solubilizes lipids and removes them from hair surface. Blow-drying and frequent washing with harsh surfactants also removes cuticle lipids. The appearance of your hair, and its reflective quality, depends heavily on the condition of the cuticle. Light reflection from a smooth and intact cuticle gives shine and glossiness, resulting in youthful appearance. Loss of cuticle also makes combing and brushing difficult. A flat and smooth cuticle structure reflects light, and it is important to keep healthy and shiny hair. It is reported that replacing the essential hair lipid, **18 MEA**, results in healthier-looking and -feeling hair through restoration of the hydrophobicity of hair fiber. Further, lipid replenishment protects the hair from further damage[16].

Chemical treatments such as hair relaxing and permanent hair coloring chemically modify the hair keratin structure by disrupting the disulfide bond, which is responsible for the strength of hair. Curly hair has a higher degree of disulfide bond in the cortex compared to straight hair. The process of straightening or relaxing changes curly hair into straight hair using alkaline or other chemical relaxers. The chemical modification of the disulfide bond in the cortex of the hair shaft affects the physicochemical property of hair fiber. Frizzy hair, hair breakage, hair thinning, scalp irritation, scalp damage, and hair loss are some of the common consumer complaints due to the misuse or chronic use of chemical hair relaxers[17]. Permanent hair coloring also changes hair fiber properties due to oxidative damage of hair proteins and lipids due to the presence of ammonia and hydrogen peroxide. The oxidation of lipids makes the hair dry and dull and the oxidation of the disulfide linkage of the keratin protein makes the hair fiber weaker. However, recent hair color products are formulated with great care to minimize damage as much as possible. Additionally, the hair color kits provide after-color conditioners to be used right after coloring to provide conditioning, shine, and color protection. Regular use of products

formulated for colored hair provides conditioning, shine, and overall youthful appearance.

3.3.3.4 PHOTO-AGING OF HAIR

Photo-damaged hair shows dryness, an increase in fiber porosity, reduced strength and an increase in surface roughness. Long-term exposure to UV radiation causes severe chemical degradation to hair proteins and lipids. Photoaging of hair results in lipid oxidation, disulfide bond cleavage, tryptophan degradation, and cysteic acid formation. Hair exposed to sunlight is claimed to be more brittle, stiffer, and drier than before irradiation and exhibits a reduced water-absorption capacity. Hair pigments provide photochemical protection to hair proteins by absorbing and filtering the impinging radiation and subsequently dissipating this energy as heat. However, in the process of protecting the hair proteins from light, the pigments are degraded or bleached. Dark hair is more resistant to photo-degradation than light hair. This is due to the higher photostability of eumelanin compared to pheomelanin[2].

The most obvious effect of the photo-aging of human hair is hair lightening, an effect that is accelerated by moisture. The extent of this photochemical-induced color change is dependent on the nature of the hair pigment and is understood to involve an oxidative attack on the eumelanin or pheomelanin melanosomes. Black hair is more photostable than blond hair. This color of hair seems well protected against UV light, and light brown hair is obviously damaged by a wide range of natural sunlight. The protective action of melanin granules is limited to the melanin-rich cortex of black hair, which shows only a slight modification of fiber proteins under irradiation[18].

3.3.3.5 PROTECTION OF HAIR FROM AGING

a. Scalp Care

Adequate care of scalp and shaft hair is critical to protect the hair from aging and to have healthy beautiful hair. Beautiful hair comes from a healthy scalp and hence taking care of the scalp is just as important as taking care of hair shaft. Hair problems such as thinning or falling of hair, premature graying or breakage, dandruff, and flaking could be symptoms that indicate an unhealthy scalp. Scalp care should be chosen based on the nature of your scalp, whether it is normal, oily, or dry. Normal scalp has the right balance of sebaceous glands, so it is

generally not prone to problems like dandruff or excessive oil deposition. Shampooing three to four times a week with a normal shampoo and a light conditioner will be sufficient for this type of scalp. A warm oil treatment and massaging the scalp one to two times a month stimulates the oil gland and the massaging action improves blood circulation[i]. Coconut oil is great for all hair types. Avocado oil is one of the most emollient and moisturizing oils and is particularly good for really dry and frizzy hair.

If your scalp is oily, it is the most prone to dandruff and flaking. The excess oil that the sebaceous glands in the scalp produce tends to accumulate around the roots, clogging the pores. Such hair is usually limp and flat, making it almost impossible for volumizing or styling products to hold. Shampooing your hair every day with a deep cleansing shampoo is suitable for oily hair. If you suffer from dandruff, choose an anti-dandruff variety. Avoid using leave-in conditioners. A dry scalp causes constant itching and flaking, leaving the hair rough, dull and frizzy. As the oil secreted by the sebaceous glands is insufficient, apply oil externally and massage the scalp regularly to nourish the scalp. For dry hair, shampoo once or twice a week with a product that contains natural oils and moisturizing agents is sufficient. A thick cream-based conditioner and leave it on for about ten minutes before rinsing off makes hair smooth and soft. Get a warm oil treatment every week to stimulate the sebaceous glands. Massage your scalp thoroughly to stimulate oil secretion.

Unilever has recently launched Scalp and hair beauty therapy line of products formulated with Nutrium 10, an advanced moisturizing complex. A well-nourished scalp is the right foundation for strong, beautiful hair. For years women have been treating the wrong end of their hair. Clear Scalp & Hair Beauty Therapy made with Nutrium 10 nourishes the scalp, creating the right foundation for strong, beautiful hair. The regimen of shampoo and conditioner together is clinically proven to help restore scalp’s natural moisture balance to create the right foundation for strong, beautiful hair from root to tip. There are various regimens of shampoo and conditioner to address the needs of different hair types.

b. Hair Care

Most women will see their hair become frizzier and less manageable due to chemical treatments such as coloring, straightening, perms, and frequent washing with harsh shampoos that strip the hair of natural oils. Everyday caring of hair starting with the right shampoo, conditioner, and styling product could

cosmetically improve the appearance of shaft hair. Nourishment and proper grooming is very important to keep the hair texture, growth, shine, and luster in a good condition. For daily care, choose cleansing products that are gentle and moisturizing, preferably without harsh surfactants like sodium lauryl sulphate. Daily massaging of your hair with your fingertips helps proper blood circulation in the scalp. Apply heat-styling products to your hair before blow-drying and try to keep the setting on medium, not high. Use UV-protection hair products or wear a hat if your hair is brittle or color-treated. Choose a demi-permanent hair color product for covering gray; it doesn't contain ammonia and has less hydrogen peroxide. This type of product may not be an option if you have a lot of gray hair (more than 50%) and want to cover it, in which case you'll need permanent color.

3.3.3.6 ANTI-AGING HAIR CARE PRODUCTS

One of the latest trends in the global beauty industry is the proliferation of anti-aging hair products in the marketplace. Consequently, beauty product developers, ingredient suppliers, and brands large and small have been busy addressing the graying population in hopes of attracting the baby boomer marketplace[4].

Ingredients for hair care include the well-established ones such as: silicones for shine, quaternary ammonium compounds for ease of combing, thickening approaches such as proteins and high-molecular-weight polyquaternium polymers for conditioning[19] and polyvinylpyrrolidone for shaping and styling[20].

The hair shaft lacks biochemical processes, which gave the traditional concept that hair is dead and cannot be repaired and restored. As the root of the hair shaft is alive, hair treatments using anti-aging ingredients such as small-molecular-weight peptides, alpha hydroxyl acids, certain vegetable oils, amino acids, peptides, and ceramides can provide benefits to both the shaft and the hair root. In recent times, the hair care sector is being enlivened by entirely new ingredients as well as proprietary packages that combine the old standbys in novel ways. Anti-aging hair care specifically focuses on ingredients and products (applied both topically and in ingestible form) that can repair hair, providing more manageable and healthy looking hair.

Many ingredients are recently launched to address hair and scalp aging at the cellular level, improve follicle health, reduce hair loss, and offer antioxidant properties. These low-molecular-weight compounds penetrate the cuticle layers

of the hair shaft, enhancing its softness, protection, and manageability. They can also penetrate the scalp, enhance scalp microcirculation, and improve follicle health, thereby promoting healthier new hair growth. Recently, researchers at DuPont have shown that hair care products with the well-known anti-aging ingredient, glycolic acid (marketed as DuPont™ Glypure), penetrates through the hair shaft and delivers enhanced protection and manageability to hair by conditioning, moisturizing, strengthening, and preventing breakage[8]. It also moisturizes and exfoliates the scalp, resulting in less flaking to give the scalp a healthy look and feel.

Natural vegetable oils provide nourishing conditioning and shine to hair. Coconut oil, being a triglyceride of lauric acid (principal fatty acid), has a high affinity for hair proteins and, because of its low molecular weight and straight linear chain, is able to penetrate inside the hair shaft[21]. It is an excellent conditioner and helps in the regrowth of damaged hair. It also provides the essential proteins required for nourishing damaged hair and it softens the hair and conditions the scalp. Avocado and Jojoba oil are other oils for nourishing, conditioning, and shine benefits.

Amino acid-like L-Arginine is a nitric oxide precursor found to stimulate hair regrowth by stimulating the production of hair follicles and also to strengthen hair by nourishing the hair roots. Sunscreens are another category of anti-aging ingredients now found in a variety of hair care products, including shampoos, conditioners, and hairsprays. They prevent breakdown of keratin due to sun exposure and also keep hair dye from fading in ultraviolet light. Ingredients like Crodasorb™ UV-HPP from Croda and PARSOL® SLX provide multiple benefits like prevention of color fading, strengthening hair body, and enhancing gloss when delivered through hair color, or hair care products, especially leave-on sprays. The table below lists some advanced functional hair care ingredients popular in today's hair care market.

Table 1. Advanced hair care ingredients popular in current hair care products[ii]

Hair Care Ingredients	**Benefits**	**Supplier**
Crodafos™ HCE	Increases color intensity & color uptake. Provides better color wash fastness. Reduces hair damage as shown by hydrophobicity and combing studies.	Croda Inc
Incroquat™ Behenyl TMS-50	Hair conditioning agent, suitable for cationic emulsions, provides soft touch.	Croda Inc

ChromAveil ®	Protects the hair from the damaging effects of sunlight. Gives color protection benefits to dyed hair from UVA and protects the mechanical properties of the hair from UVB.	Croda Inc
Crodasorb™ UV-HPP	Polyester polyquaternized ingredient that is substantive to hair and protects against the damaging effects of UV-B radiation.	Croda Inc
Lustreplex®	Creates lustrous, healthy-looking hair from anionic systems. Provides frizz control, shine, detangling, and conditioning benefits.	Croda Inc
Dow Corning® CE-8411 Smooth Plus Emulsion	Repairs colored, gray, and heat-damaged hair by restoring moisturized feel, shine, & alignment. Protects hair from further damage and color loss.	Dow Corning
Dow Corning® 5-7113 Silicone Quat Microemulsion	Restores smoothness, shine, and Alignment. Prolongs hair color. Protects hair from breakage.	Dow Corning
HYDROVANCE®	Improves water retention and helps to strengthen and even repair damaged hair, for benefits the consumer can clearly feel and see.	Akzo Nobel
STRUCTURE® PQ-37P	Cationic acrylic homopolymer multifunctional rheology modifier that provides thickening and conditioning.	Akzo Nobel
Procapil™	Reinforces hair anchoring. Reduces hair loss.	Sederma
Glypure® glycolic acid	Glypure® penetrates the hair shaft to enhance the softness and manageability of hair.	DuPont
L-Arginine	Helps hair regrowth by stimulating the production of new hair follicles. It nourishes the hair root and strengthens hair.	Ajinomoto
PARSOL® SLX	PARSOL® SLX is a silicone-based UV-B absorber. Delivers multiple benefits including prevention of color fading, gloss enhancement, and conditioning.	**DSM**

Table 1. Shows ingredient information obtained from supplier websites

Anti-aging hair care products are designed to address the consumer needs such

as thinning, coloring, breakage, and drying, with emphasis on particular ingredients that target specific hair issues. Such hair care formulations are designed to deliver conditioning, thickening, and color protection, strengthening, and nourishing properties through deposition of essential oils, cationic polymers, proteins, and other nutrients. For gray hair, so far, there are no solutions for reversing the color. Hair colorants are used for covering gray, to impart color and shine and hence youthful appearance. Therefore, hair colorants can also be classified as anti-aging hair color products.

Pantene has launched PRO-V Expert Age Defy shampoo, conditioner, and advanced thickening treatment designed to work together for thicker hair strands. These products are claimed to be gentle enough for daily use on color-treated hair. Formulated with a triple blend complex (mixture of amino acids), the shampoo and conditioner help fight seven signs of aging hair: reduces breakage, prevents split ends, reduces frizz, controls unruly grays, minimizes lackluster color, improves a thin look, and minimizes dryness.

There are several professional hair care products formulated with advanced technologies that restore oils vitamins and other essential nutrients. Nexxus has launched its first anti-aging range of products that combats eight signs of aging hair: volume loss, breakage, roughness, less shine, dryness, brittleness, unruliness, and loss of color vibrancy. It replenishes and revitalizes for more vibrant, youthful-looking hair.

Nexxus Youth Renewal™ Rejuvenating Shampoo gently cleanses hair to help rebuild volume, vibrancy, and vitality. Nexxus Youth Renewal™ Rejuvenating Conditioner provides lightweight nourishment to help preserve volume, vibrancy, and vitality. Youth Renewal™ Rejuvenating Elixir lightweight leave-in treatment helps rebuild hair’s strength by reducing breakage while making hair look more youthful and vibrant in just seven days.

Alterna has launched CAVIAR Anti-Aging® Replenishing line of products comprising moisturizing shampoo and conditioner that transforms dry, brittle hair by sealing in a rich blend of lipids and essential ingredients to continually nourish and hydrate the hair. The shampoo is a luxurious, sulfate-free cleanser that restores moisture while protecting hair from color fade, daily stresses, and future damage. Both shampoo and conditioner are infused with Seasilk®, Age-Control Complex®, Enzymetherapy®, and Color Hold®. It not only helps maintain moisture, but also protects from daily stresses and delivers what your hair needs to become strong, healthy, and younger-looking. The anti-aging rapid repair spray helps add moisture and vibrancy to hair while combating natural,

chemical, and environmental stresses. It may be used as a thermal protectant when applied pre-styling, or as a finishing shine spray.

Keranique® has launched a hair revitalization system developed for women to strengthen, fortify, and thicken each and every hair shaft. There are three key components to the Keranique® System. Step 1 includes a revitalizing shampoo and volumizing conditioner. Step 2 has the hair regrowth treatment with clinically proven FDA approved ingredient, Minoxidil, and Step 3 is an amplifying lift spray to provide fullness, volume, and body.

CONCLUSION

Hair, like skin, undergoes aging both intrinsically due to chronological aging and extrinsically as a result of exposure to environmental factors such as sun, pollution, and daily grooming habits. The aging of hair results in a series of visual and measurable changes both in the scalp and hair shaft. The most obvious sign of hair aging is graying of hair while the consumer also perceives a series of changes such as thinning of hair, dryness, brittleness, loss of shine, and manageability. Prevention of scalp and hair aging is not completely possible, but there are ways to protect scalp and hair fiber from damage due to external aggressors and to minimize damage from daily grooming by choosing the right products. The chapter provides an overview of the cause of hair aging, the factors that contribute to hair aging, the visual and perceptional signs of aging, and finally a compilation of some hair ingredients and anti-aging products currently available in the market.

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GLOSSARY

1. **Gray Hair :** Hair without melanin pigment
2. **Hair Follicle:** An organ and part of the skin below the scalp that produces hair.
3. **Hair Shaft:** The part of the hair that is seen above the skin
4. **Oxidative Stress:** An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, is termed ‘oxidative stress’
5. **Chronological Aging:** Age of a person measured in years, months, and days from the date the person was born
6. **Androgenic Alopecia: A** common form of hair loss in both men and women
7. **DHT:** Dihydrotestosterone (a derivative of the male hormone testosterone)
8. **18**-MEA: 18-methyl eicosanoic acid (essential hair lipid responsible for hair shine and hair integrity)

MECHANISMS OF CHANGES IN HAIR SHAPE

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A note from Meyer R. Rosen, Editor-in-Chief

* This chapter brings to mind a phrase that I hope will stick in your memory for a long, long time. At its heart it is Manuel Gamez-Garcia's unique ability to describe:

The Science of Hair Beauty and the Beauty of Hair Science

ABSTRACT

Changes in hair shape can be categorized as permanent and temporary. They can also be classified as cosmetically desirable and undesirable or by a combination of any of these two categories. For instance, examples of hair shape changes desired permanently are those produced by perm solutions and alkaline relaxers. Examples of desirable temporary changes are those resulting from water-setting, hot irons, and styling polymers. Undesirable permanent shape changes are, on the other hand, those leading to frizzy hair, excessive tangling, and lack of manageability.

This chapter provides an exceptionally detailed look at the development of scientific distinctions based on the integration of polymer physics and chemistry with that of hair behavior and what impacts it in our endless search for beauty.

Whether the changes in hair shape are desirable or undesirable, all of them result from a process consisting of three main steps:

1) the softening of hair, which may involve the breakage of chemical or physical bonds, and/or protein rearrangements in the amorphous and crystalline phases,
2) the mechanical imposition of a new shape, and
3) the fixing or locking of the new shape by the re-formation of broken chemical or physical bonds. In shape changes that are desirable the occurrence of these three steps is induced purposely to create a new shape, whereas in those that are undesirable the three steps occur mostly during hair damage. This chapter will focus primarily on those aspects that are desirable from a cosmetic point of view. Those aspects produced by damage have been reviewed elsewhere (1).

We would point out that technology transfer from the world of polymer physics and chemistry has been of great value in the development and application of “novel” concepts and their use as seminal thinking points for cosmetic hair scientists. We encourage the reader to continue this approach, as much of this type of “new” thinking will open fascinating and productive pathways to novel hair care products.

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3.3.4.1 INTRODUCTION

The key physical properties and mechanistic events related to changes in hair shape can be better understood when we recall that hair is a biopolymer that shares many of the physical and chemical features possessed by synthetic polymers. In fact, we can learn a great deal about hair's mechanical behavior by comparing its properties with those of synthetic polymers having a similar structure. For instance, synthetic semi-crystalline polymers, like hair, possess crystalline melting and amorphous phase glass transition temperatures (2–4).

Furthermore, the composition of hair's architecture is such that it can exhibit two types of physical behavior.

- **One type of behavior is related to the hydrophilic structure of the globular proteins in the amorphous phase.** The protein hydrophilic groups in this structure are capable of forming hydrogen bonds with water. This aspect of the structure makes it highly water swellable and susceptible to plasticization by moisture. It is responsible for the viscoelastic and softening effects produced in hair by water; Max Feughelman refers to this aspect as the "gel structure of the amorphous phase" (6).
- The other type of physical behavior stems from the non-gel structure of the intermediate filaments (IFPs). This structural aspect is comprised of an amorphous network of hydrophobic, and stiffer protein segments. These are covalently cross-linked by disulfide bonds, as well as an ordered arrangement of crystalline proteins regions, which are responsible for both shape stabilization and hair strength.

The entire amorphous phase acts mechanically in parallel with the crystalline phase and gives rise to the unique combination of viscoelasticity and strength typical of hair. This behavior is also found in semi-crystalline polymers and, incidentally, there is a remarkable similarity in the architecture and properties of hair, and a particular type of cross-linked semi-crystalline polymers, namely; those that have been synthesized to behave as shape memory polymers (SMP) (7). These polymers, like hair, also exhibit two and even more types of physical behaviors. These multiple types of behavior result in most cases from the various chemical groups or structures grafted onto main polymer chains introduced with the purpose of storing various temporary shapes (8–11).

As will be discussed later in this chapter, hair, with both its amorphous and crystalline structures, can also act as a shape memory polymer (SMP) and the mechanistic aspects related to changes in its shape can be better understood by using concepts from this field of polymer science. We would point out here that this cross-fertilization of ideas, or technology transfer, is an excellent approach for hair product developers to employ when seeking new ideas.

3.3.4.2 THE SHAPE MEMORY PROPERTIES OF HAIR

a. Definition of shape memory materials

In the scientific literature, C. Liu and C.B. Chun (8) define a shape memory material as follows:

"Shape-memory materials are those materials that have the ability to

‘memorize’ a macroscopic (permanent) shape, be manipulated and ‘fixed’ to a temporary and dormant shape under specific conditions of temperature and stress, and then, later, relax to the original, stress-free condition under thermal, electrical, or environmental command. This relaxation is associated with elastic deformation stored during prior manipulation” (2).

Currently, the most rapidly growing area in the field of shape memory materials are shape memory polymers (SMPs), and they can be either purely amorphous or semi-crystalline (12–14). Figure 1 shows a sequence of captions displaying a shape memory polymer changing its shape from a coiled temporary to a straight permanent shape after this event is triggered by elevating its temperature (15). Shape memory polymers can store temporary shapes and can recover their permanent shape by a suitable stimulus, either electrical, chemical, or temperature.

Figure 1) Transition of shape from temporary (spiral) to permanent (rod) of a polymer synthesized from poly-(ε-caprolactone) dimethylacrylate and butyl acrylate (15).

Various scientists working in this field have already listed the main physical requirements for a semi-crystalline polymer to display shape memory properties. According to C. Liu et al., these are as follows (8):

1) a relatively sharp glass transition and/or a high crystalline phase melting temperature that can be used to promptly fix the secondary shape at low temperatures and trigger shape recovery at high temperatures,
2) superelasticity (low loss modulus, high deformability) above the transition temperature that leads the shape recovery and avoids residual strain (permanent deformation) and,
3) complete and rapid fixing of the temporary shape by immobilizing polymeric chains without creep thereafter.

Synthetic polymers with shape memory properties consist primarily of two main features in their chain architecture. These are:

- A primary polymer network with network points, or cross-links that serve as a memory mechanism for the generation of a permanent shape, and
- A structure of secondary groups attached to the main network that serves as memory mechanism for the formation of temporary shapes (8).

These groups can be pendant functional moieties, grafted chains, polymer blocks, interpenetrating networks, or crystalline domains; they act as switches to release the temporary shape when activated by suitable external stimulus (see Figure 1) (7). Moisture, heat, light, and electric fields are examples of external stimulus that can release temporary shapes stored in SMPs with pendant groups or structures sensitive to them (see Figure 2) (8, 9).

Figure 2) Molecular mechanisms of shape memory polymers according to C. Liu *et al.* (8)

b. Hair is a biopolymer with shape memory properties.

In the scientific literature, biopolymers such as silk and collagen have also been reported to have shape memory properties, and thus, they have been classified as shape memory materials (16, 17). **Hair is a biopolymer that also behaves as a shape memory polymer**. Clear manifestations of this behavior and properties are the following:

1) hair has a permanent shape,
2) its shape can be changed either temporarily or semi-permanently, and
3) it can "remember" or reverse to its permanent shape when treated appropriately.

Beauticians and cosmetic scientists, aware of these properties, have been creating fashionable hairstyles for years that can be reversed when desired. An example of hair's ability to recover its permanent shape can be seen in Figure 3, where it is shown that a curly temporary water-setting in a woman's hair (Figure 3a) can return to its original straight shape after washing the hair (Figure 3b).

Also, Figure 4 displays a sequence of micrographs of a hair fiber recovering its permanent shape from temporary straight to its curly permanent shape.

Figure 3) Captions of hair in a woman before (a) and after (b) it recovers its straight shape from a water temporary setting (18).

Figure 4) Captions showing sequence of a single hair fiber recovering its permanent shape from a straight temporary shape to a curly shape after water immersion (18).

As in the case of synthetic polymers, the permanent and temporary shape memory sites in hair reside in its crystalline and amorphous phases. In the case of hair, these polymeric entities are of natural origin and located in the macro-fibrils of the cortical cells. These can be listed as follows:

a) a protein crystalline phase forming intermediate filaments which serves as permanent shape memory and is also capable of storing a temporary shape when its crystalline structure unfolds or when it denatures above its melting temperature Tm.
b) a disulfide bond cross-linked protein amorphous phase capable of storing temporary shapes when it becomes soft at the hair's glass transition temperature Tg, or when it undergoes disulfide bond interchange. This

structure also contributes to the stability of the crystalline phase, and

c) an extended structure of hydrophilic protein groups located at the surface of globular proteins in the amorphous phase. Its structure forms a gel, is sensitive to moisture, and is also capable of storing a temporary shape.

c. Temporary shape memory in hair

The most common temporary or secondary shapes in hair are stored by physical means. The position of storage is in the protein regions with high levels of hydrogen bonding, i.e., in the soft gel structure of the amorphous phase (6). A typical example of this mechanism is water-setting. The gel structure in hair is ubiquitous in all types of hair and plays a crucial role in most temporary shapes imposed by hair movement and daily grooming practices. It is part of the amorphous phase, and behaves like a material that exhibits variable viscosity depending on the shear rate it is exposed to (i.e., pseudoplastic with a yield stress). This type of behavior can turn hair from very soft and flexible to very hard and brittle and, in doing so, it can virtually lock-in almost any temporary shape in hair. The viscosity of the gel structure depends on the hair's moisture content and, when hard and brittle, it presents a high opposition to the imposition or recovery of any type of shape in the hair. Temporary shapes in hair can also be stored by processes involving vitrification of the disulfide cross-linked amorphous phase and rearrangements of the crystalline phase. A typical example of this is the case of setting hair with hot irons.

The ideal creation of temporary shapes in hair shouldn't involve permanent and irreversible modifications to the protein architecture of hair. This means that after creating a temporary shape, the hair's permanent shape memory should remain intact, i.e., irreversible protein denaturation in crystalline filaments or irreversible breakage of covalent bonds in main protein chains and pendant groups should not be allowed to occur. If these irreversible phenomena occur, the hair may lose part or all of its permanent shape, becoming shapeless, and the imposition of a temporary shape will thus be irrelevant. As will be seen later, when hair is heated above its melting and glass transition temperatures with hot irons, temporary shapes can be created but at the expense of producing some of these unwanted irreversible phenomena.

d. The apparent permanent shape in hair

The hair shape that we normally perceive in our natural hair in everyday life is an apparent shape that results from a number of unavoidable mechanical effects.

These unavoidable effects are primarily caused by hair's own weight and by inter-fiber interactions in the hair assembly. Both effects produce a temporary shape that is stored in the gel structure of hair and is always present in our everyday life. It is a form of quasi-permanent water-setting. Therefore, the hair shape that we have in our natural hair is not the "real" hair's native shape, but rather it is a very close representation of it.

We call this close representation an "apparent permanent shape" or temporary shape in equilibrium with the permanent shape. Other factors that contribute to the formation of the "apparent permanent shape" are hair length, relative humidity, and type of hair; i.e., it also depends on whether the hair is naturally straight, wavy, or very curly.

The "real" permanent shape of hair is only manifested when we immerse a single hair fiber in water so that the temporary shape imposed by its own weight and other interactions is released from the gel structure. For instance, experiments have shown that a high percentage of hair fibers from Caucasian origin that appear wavy in shape, regardless of their degree of waviness or curliness, turn into almost perfect circular coils when immersed in water (18)!

We probably have all observed these effects at one time or another upon seeing our own hair fibers floating in a pool of water. These effects are shown in Figure 5 where six single hair fibers from different origins are displayed as follows: (a) Oriental, (b), (c), (d) Caucasian, (e) Mulatto, and (f) of African origin. The shape of the Caucasian and Mulatto fibers was wavy/curly before equilibration in water. Observe in this Figure that the hair fibers of Caucasian, Mulatto, and African origin formed almost perfect circles after ten minutes of water immersion, except for the one of Asian origin. This means that the real permanent shape of most hair fibers that appear wavy in a hair assembly, is actually coiled and circular, and that the wavy pattern results from deformations mostly imposed by their own weight as the fibers hang from the scalp. Another important implication from these observations is that when hair emerges from the follicle, the shape of a high percentage of hair fibers of Caucasian origin is never wavy but rather circular with a predetermined radius of curvature. Thereafter, as the hair fiber grows longer it actually becomes wavy because it is deformed by both its own weight and the interaction with other hair fibers in the scalp.

Figure 5) Picture showing six single hair fibers after their shape was equilibrated in water as follows: (a) Oriental, (b), (c), and (d) Caucasian, (e) Mulatto, and (f) of African origin (18).

Figure 6) Picture showing four single hair fibers before and after they were hung vertically as follows: (a) Oriental, (b) and (c) Caucasian, and (d) of African origin (12). The shape of hair fibers depicted in (a), (b), (c), and (d) captions had been equilibrated by water immersion for ten minutes (18).

The above-mentioned effects are better visualized in Figure 6 where it can be seen that the circular shapes of two equilibrated Caucasian hair fibers (b) and (c) turn into wavy patterns (f) and (g) as the hair fibers hang. In Figure 4 it can also be observed that the circular coil of an African hair fiber (d) presents a very small tight curly pattern, and does not open its coil upon hanging. The hair fiber of Oriental origin (e) did not change its straight shape very much after being hung.

The observations made on the behavior of African hair are not new and rather confirm what is already known by cosmetologists and beauticians; namely, that hair with a very tight coil is not readily opened by its own weight when hanging from the scalp regardless of its length. The tight curly hair will be opened,

however, when we impose a force, either with the fingers or with other devices. As will be seen later in this chapter, hair with very tight curls, such as that depicted in Figure 6d, has a very small radius of curvature, and its modification requires processes that modify a larger number of memory sites in the hair.

The modifications introduced to real permanent shapes in hair by its own weight also explain well-recognized hair shape variations often observed in people's hair when the environmental humidity changes. Higher levels of moisture result in hydrogen bond breakage in the gel structure and, therefore, moisture affects the "apparent permanent shape" of hair.

If the permanent shape is very strong, the hair will recover its real permanent shape, either curly or limp. For instance, people with wavy hair know very well that when the relative humidity is high their hair may become curlier. In contrast, people that have straight or fine straight hair as the real permanent shape may experience a higher level of hair limpness or straightening at high relative humidities. Furthermore, the formation of this "apparent permanent shape" also gives rise to differences in hair shape depending on its position on the head. For the sake of simplicity in the following paragraphs the term "real permanent shape" will be used when referring to hair shapes without any imposed deformation; and the term "permanent shape" will be used to refer to hair shapes in equilibrium.

e. Permanent shape memory in hair

The real permanent shape of hair is cast in the cortical cells during its life cycle through the anagen, catagen, and telogen phases (19–20). It is predetermined genetically and permanently imprinted in the hair follicle by the process of keratinization when the proteins in the amorphous phase are cross-linked by disulfide bonds. Figure 7 shows typical examples of hair with different types of permanent shape. Whether the hair is straight, wavy, or extremely curly, its permanent memory shape essentially resides in the composition ratio of ortho-to para-cortical cells. At the microscopic level, however, the permanent shape of hair is imprinted in the two-phase architectural structure of hair which results from the crystalline keratin micro-fibrils embedded in a highly cross-linked amorphous network of proteins (see Figure 8). More specifically, it is imprinted in the primary, secondary, and tertiary protein structures of crystalline and amorphous phases, chemically and physically stabilized by disulfide and isopeptide covalent bonds and by hydrogen bonds, salt bridges, and dispersion forces. Both protein phases stabilize hair shape and also strengthen the fibers

mechanically.

Figure 7) Captions showing three types of natural permanent hair shape as follows: Oriental (left), Caucasian (center), and African (left).

Figure 8) Schematic representation showing two hair fibers with different shape. The permanent shape of hair, either curly or straight, is imprinted in the hundreds of micro-fibrils and amorphous protein networks forming the two-phase protein arrangement in the cortical cells (18).

f. Changes to the permanent shape in hair

Permanent changes in hair shape have very different requirements than those already outlined for temporary shape changes. While temporary changes do not require irreversible modifications to the hair structure, permanent changes do require this process. Irreversible modifications are needed, first, because it is necessary to erase or delete the permanent shape from the memory of hair, and second, because it is necessary to build a new permanent shape in the hair structure when faced with consumer demands and needs. The latter step usually requires irreversible modifications to the crystalline phase, the introduction of new chemical bonds, and the rearrangement of proteins in the amorphous phase. **Thus, permanent changes in hair shape require irreversible modifications to**

one or two properties of sites where the permanent memory of hair shape resides.

The irreversible changes may be in the form of chain scission, chemical modification of protein-pendant groups, and breakage of disulfide bonds. They can also involve irreversible protein denaturation, irreversible melting of crystalline regions, or a combination of all these factors (21). Thioglycolate salt solutions and very high pH alkaline creams used in permanent waving and hair relaxers, respectively, involve exactly this type of irreversible protein denaturation. The former is selective to the breakage of disulfide bonds in the protein chains and does not have a strong impact on other chemical bonds. The latter also breaks disulfide bonds but is less specific to them and, it breaks other bonds in the protein chains as well. In addition, the phenomenon also produces irreversible modifications to the crystalline regions and to the whole hair architecture itself (22).

g. Shape reversion

One factor often encountered in treatments geared to produce a permanent change in the shape of hair is that in many instances, they do not produce a real durable permanent change. As a result, there is a partial and gradual reversion to the original fiber shape. Lack of performance of active ingredients, poor formulation design, or poor hair treatment conditions are often the cause of this reversal. Consequently, in the case of permanent waving, various treatments have to be applied periodically to maintain the wavy shape (18). In the case of hair straightening with alkaline relaxers, shape reversion is difficult to correct because after the straightening process, the hair fibers are left weakened due to the high degree of broken covalent and other protein bonds produced by these treatments.

Shape reversion phenomena occurring after permanent waving or alkaline relaxer treatments are usually difficult to explain when using conventional mechanisms of disulfide bond breakage and re-formation. However, the shape reversion processes can be easily explained when it is considered that hair is a shape memory biopolymer with a permanent shape stored in its “memory.” As will be discussed later in this chapter, in order to successfully induce permanent changes in hair shape, not only do disulfide bonds have to be broken, but also other chemical and key structural components of the hair memory need to be modified. Otherwise, the induced shape will be semi-permanent and eventually the fibers will undergo shape reversion.

Understanding the shape memory properties of hair can pave the way to optimize processes and produce successful permanent changes in hair shape. Therefore, in the following paragraphs shape memory polymer concepts will be used to review changes in hair shape, and we encourage the reader to use this information as a foundation for innovative thinking in this field.

3.3.4.3 CHANGES IN HAIR SHAPE BY WATER-SETTING

a. The process

Water-setting, or the process of changing hair's shape with water, is by far, among all the temporary setting processes, the easiest to produce in hair. It only entails the use of water and a relatively low-temperature drying process. In order to understand it mechanistically using concepts from the science of SMPs, let us first review the key generic steps necessary to produce shape changes in semi-crystalline polymers (6), and then apply them to hair. Upon thoughtful comparison, polymers also require the same three key steps described above for changes in hair shape:

1) making fluid or softer at least one of the polymer phases,
2) imposing mechanically a new shape followed by stress relaxation in the soft/fluid phase, and
3) immobilizing the soft/fluid phase to lock-in the new shape. In general, temporary shape changes in SMPs also require keeping the polymer structure intact where the permanent shape memory is located (11).

Thus, when we want to impose a temporary shape on a semi-crystalline polymer, or by analogy, in hair, the first requirement is to keep the main polymer architecture intact. To be more specific, in the case of water-setting this implies that the disulfide bond cross-linked protein matrix and the protein crystalline regions should not be modified. Water-setting usually does not produce these modifications except when high temperatures, or large deformations are involved in the production of the temporary shape. High temperatures above 80°C may already start to produce disulfide bond breakage; while deformations higher than 5% already induce alpha to beta transitions in the crystalline phase which may be partially irreversible (23).

b. Physical processes taking place inside hair during water-setting

As outlined in the previous paragraph the imposition of temporary shapes, either

in polymers or in hair, requires three main steps (see Figure 9). For the water-setting of hair, step 1 consists of softening the gel structure of the amorphous phase by wetting the hair and allowing water to penetrate into the cortex. This first step will ensure that hydrogen bonds in the gel structure are broken by water. It is important to mention here that when water breaks hydrogen bonds in hair proteins, after step 1 the hair still has all of its covalent bonds intact, and therefore, the permanent shape memory is also unchanged (see Figure 10). As will be seen later, this contrasts with other processes where the hair becomes shapeless and completely loses its real permanent shape. In step 2, once the weak hydrogen bonds are broken, the hair is set into a new configuration with plastic rollers or with the fingers to create the desired new shape, which can be either that of light hair waves or tighter curls.

Figure 9) Schematic representation of three steps required to create a temporary shape by water-setting. The three-step process is essentially based on the breakage of hydrogen bonds in the gel structure of hair (step 1), imposition of temporary shape (step 2), and re-formation of hydrogen bonds to lock-in the new shape (step 3) (18).

Figure 10) Schematic representation of hydrogen bond breakage by water dipoles during water-setting. Other covalent bonds are not affected.

Finally, in step 3, the hair has to be rapidly dried to extract water molecules in order to re-form the hydrogen bonds in the gel structure. This process will cause

hardening of the hair, thereby locking-in the temporary shape and imposing it over the primary hair shape. Drying can be achieved by just exposing hair to atmospheric conditions or it can be accelerated with the aid of blow-dryers. If the drying process is too slow and the hair rollers are removed, the crystalline micro-fibrils and amorphous matrix with its combined elastic force will push for hair to recover its permanent shape and the temporary shape will be lost. Fast drying with blow-dryers will ensure that water is rapidly removed from the gel phase before the hair can elastically recover its permanent shape. In this manner the new shape will be locked-in.

c. Temporary shapes induced by long-term deformations

Another form of water-setting is the one that takes place when hair is subjected to involuntary deformations for extended periods of time at room temperature and high moisture conditions. This situation occurs, for instance, when sleeping, using hats, *etc.* In these cases the assembly of hair fibers is held in a configuration for various hours, and after freeing the hair from the confined environment a temporary change in hair shape is produced. The phenomenon is caused by stress relaxation in the gel structure of the amorphous phase.

As was previously discussed, the gel phase of the hair is plasticized when the amorphous phase contains moisture. Water from the environment is absorbed in the gel structure and breaks a limited number of hydrogen bonds at protein neighboring sites. When there are no mechanical stresses applied to hair, the broken and unbroken bonds stay in equilibrium. However, when deformations appear as a consequence of imposed deformations, i.e., during sleep, use of hats, etc., broken and unbroken bonds undergo a bond interchange process and the protein chains move to relax internal stresses. This process of internal stress relaxation in the gel structure locks-in the temporary shape that is produced during sleep (bed hair), or with the use of hats, *etc.* (see Figure 11).

Figure 11) Caption showing typical appearance of bed hair (18)

The imposition of new temporary shapes on hair, either during sleep, use of

hats, etc., or during water-setting, will immediately produce internal elastic stresses in the structures where the real permanent shape resides. This occurs primarily in the crystalline regions and in the disulfide protein matrix. These internal stresses will remain there as long as the temporary shape is in place and are needed for the hair to recover its permanent shape. Temporary shapes imposed by water-setting will maintain their secondary character as long as the two following conditions are met. First, the elasticity of the crystalline and amorphous phases does not change, and second, that the gel structure of the amorphous phase remains unchanged. The first condition was previously reviewed and it was referred to as a requirement for superelasticity of the SMPs. This condition is required to ensure that the polymer will always remember its permanent shape. The second condition was also reviewed previously and it relates to the absence of relaxation in the soft phase; it is required to lock-in the temporary shape as long as it is necessary until it is released by an external stimulus.

Shape changes induced by water-setting can, rapidly or almost instantaneously, be reversed by water-wetting. The process of liquid water absorption by hair causes rapid hydrogen bond breakage, softening, and swelling of the gel structure, thereby releasing the temporary shape and allowing the permanent shape to be quickly recovered. If the hair didn't behave like a shape memory biopolymer, it would not remember and recover its permanent shape and would stay deformed like a viscous resin. Thus, water-wetting acts as an external stimulus to quickly release the temporary shape from hair.

d. Limitations of water-setting

Unfortunately, shape changes induced by water-setting in hair are temporary and cannot be maintained as long as we would like nor can they be dormant forever. *It is known, for instance, that shape changes induced by water are slowly recovered even when no water-wetting processes are applied.* Temporary shapes stored in the gel structure are gradually lost because, as has previously been explained by Max Feughelman (6), the gel structure of phase M in hair is viscoelastic, and even at very low moisture contents it will always relax to the Elastic Modulus of the crystalline phase. Thus, all types of temporary shapes induced by water-setting eventually revert back to the permanent shape by a stress-relaxation process and this occurs even under very dry conditions. The process may take months but the shapes will reverse! The memory shape properties of the gel structure in hair are transient, and temporary shapes induced

by water-setting cannot be maintained for very long periods.

Another important aspect of water-setting is that, depending on the type of hair, there is a limit on the type of initial shape that can be imposed. This fact becomes more clearly understood when we analyze the following questions:

- How tight can we induce a curl in hair that is very straight, or how straight can we render hair that is very kinky?
- Can this induced curl be achieved just by using a water-setting process? In other words, is it possible to put very tight curls in natural straight hair just by breaking and re-forming hydrogen bonds? Or is it possible to render very kinky hair straight just by breaking and re-forming hydrogen bonds?

Obviously, the answer to the above is no. Everyone who has tried to straighten extremely curly hair just by using water-setting will fail.

This failure can be explained by the fact that the gel structure has only limited mechanical stiffness, and therefore, cannot completely counter the strong elastic recovery forces of permanent shape coming from the crystalline and disulfide cross-linked protein phases. In other words, the gel structure is a weak physical memory and cannot store large changes in hair shape. Thus, the strength and degree of hair curliness is very important in temporary shape changes induced by water-setting. The straightening of extremely curly hair may require additional physical modifications of other, stronger protein structures that store temporary shape. Likewise, this type of reasoning can be applied for the imposition of tight curls in straight hair with water-setting.

According to the ongoing discussion, there appears to be a rule that relates strength of shape change, and number of memory sites required to achieve the changes in shape. This rule *always* prevails when imposing temporary changes in hair shape. In the case of straightening curly hair, the rule is: **the tighter the curl, the more the elements in the architectural sites in hair's memory need to be modified to make it straight.** This means that for any particular type of hair, there is a limit in the degree of shape changes that can be imposed by water-setting. In fact, this is the reason for the use of harsher temporary hair-shaping techniques, other than water-setting, for the straightening of curly hair, such as the use of hot irons and other treatments. As will be seen later in the following paragraphs, the rule of changing shape has important consequences not only for the imposition of temporary shapes but also for engendering those of permanent nature.

3.3.4.4 CHANGES IN HAIR SHAPE BY HOT IRON TREATMENTS

a. Introduction

Changing the hair shape with hot irons is a very old practice. Museums of history already report drafts of hot iron designs made in the 18th century (see Figure 12). Hot irons became popular in the early 1900s for the straightening of extremely curly hair of African origin (24). Later, over the years the application gradually extended to other types of hair to create/enhance stronger curls or to create straight patterns in hair. Currently, there are two main types of hot irons, namely, those of cylindrical shape and those with flat plates (see Figure 13). The use of hot irons with flat plates has become very popular for the straightening of wavy hair. The wanted effects from those who use it nowadays range, in terms of achieving long-lasting behavior—from totally irreversible, or permanent types, to those of long duration and reversible behavior. Most hot iron treatments used today leave only a short temporary change in hair shape. As a result, cosmetic formulators are perennially looking for new hot iron technologies that can extend the persistence and long-lastingness of heat treatments.

Figure 12) Caption displaying draft describing hot irons used in 1858 (24)

Most of the progress made in the treatment of hair with hot irons has produced innovations in their design and in the use of new ingredients or formulations to aid in the hot ironing process. Innovations in hot iron design comprise, to mention a few: more efficient temperature control, new ceramic materials to improve surface smoothness, better and effective hot iron/hair contact area, use of steam, and the incorporation of infrared heating elements (25). To this end, progress in the area of ingredients and formulations has led to the development

of two types of products; namely,

1) those based on the use of polymers geared to enhance shape effects and also to reduce hair damage (26), and
2) those using ingredients that modify or introduce new bonds in hair.

The latter type of products in most cases requires the incorporation of heat to modify the permanent shape memory of hair; either by introducing new cross-links, or by changing the disulfide cross-linking structure of the proteins. Examples of these are the formaldehyde (Brazilean) and sodium bisulfite (Japanese) treatments; both will be reviewed later in another paragraph.

Figure 13) Main modern types of hot irons, flat hot iron (top), and rod hot iron (bottom)

b. The process

A typical hair-straightening process with hot irons consists of the following steps: 1) pre-wash and condition hair with the shampoo and conditioner of choice, 2) dry hair until it keeps about 80% of its moisture content, 2) divide hair into thin swatch sections and then apply the hot iron. The temperature of the hot iron, depending on the type of desired effects, is usually selected between 160 and 230°C. The hot iron is passed two or three times over the hair swatches from root to tip at a speed of approximately one inch/sec. During application the hot iron is held firmly but gently with the hand, so the resulting force of friction will allow smooth sliding over the hair surface, and also, will enhance heat transfer at the interface of hot iron/hair. Excessive force application with the hand should be avoided since this will unnecessarily increase friction at the hot iron/surface interface causing the hot iron to slow down or completely to stop. If this happens the hot iron will damage the hair swatch. If properly used, flat hot irons produce immediate straightening effects in hair (see Figure 14).

Figure 14) Changes in hair shape before (a) and after (b) treatment with a flat hot iron. The hair shape has been changed from permanent semi-curly to temporary straight.

Good contact between the hot iron and hair is always necessary for efficient heat transfer. It is important to recognize that heat transfer from the hot iron plates to the hair swatch is always poor because hair, like wool, is a poor heat conductor. Furthermore, because of hair irregularities at the cuticle sheath surface and in view of the formation of air pockets located between hair fibers in the swatch, there is always poor thermal contact between them. This condition causes uneven temperature gradients between hot iron surface and hair swatch. Measurements carried out by placing a thermocouple inside a hair swatch have shown that hair fibers placed in a row 100 micrometers below the hot iron surface always sense at all times an average of 20 to 25°C less than those at the row surface contacting the flat iron plate (27).

c. Mechanical action of hot irons

The mechanical action of hot irons on hair is different depending on whether we try to impose a curl on straight hair or whether we try to straighten curly or wavy hair. For instance, if we place a swatch of straight hair in a hot iron of cylindrical shape at 210°C, and press on the hair statically without motion, the hair will acquire a curled shape. In contrast, if we place a swatch of wavy or curled hair between the plates of a flat hot iron at 210°C and let it be there statically without motion, it will not become straight; the action of flattening alone will not lead to changes in hair shape from wavy/curly to straight. For hair to become straight it needs to be in motion so its length can be stretched at least until the curl opens and the hair becomes straight. Thus, there is a big difference in the way the hot iron acts mechanically. This difference depends on whether the change in shape is from straight to curly or from curly to straight. Very curly hair needs a relatively high friction imposed at the interface between the hot iron and the hair

surface so the iron plates can stretch and open the curl. In contrast, the process of changing straight hair to curly is not dependent on whether the hair is in motion or not.

Other factors that influence the friction process during the straightening of wavy hair with flat irons are the type of products applied to hair before hot ironing. Hair swatches to be ironed may contain polymers, surfactants, and oils on the surface prior to the ironing process. These ingredients, in particular, include polymers that have been placed there to aid in the styling process. The polymers appear to have an effect in preventing hair damage by eliminating uneven harsh temperature gradients that may create hot spots on the hair swatch (28). Polymers and oils can have, however, a negative effect on the hot ironing process as they can excessively increase or lower the interfacial friction between the iron and the hair surface (34). High levels of friction may cause the hot iron to stop or to produce lifting of cuticle cells and tear them away from the hair (see Figures 15 and 16), while very low friction may reduce the uncoiling of the curl compromising the efficiency of a hair-straightening process (27).

Fig 15) Micrograph of a single hair fiber showing cuticle cell deformation and lifting after treatment with a hot iron at 210°C. The colored sections represent lifted cuticle cells showing patterns of light interference (27).

Figure 16) Micrograph showing fragments on cuticle cells left on plate of hot iron after a hair bundle was treated with a hot iron. Cuticle cell removal was produced by excessive friction at hot iron/hair interface (27).

3.3.4.5 PHYSICAL PROCESSES TAKING PLACE INSIDE HAIR DURING HOT IRONING

a. Water evaporation

Anybody who has witnessed for the first time the application of hot irons to hair is struck by the hissing sound of water vapor or steam coming out of the hair as the hot iron is passed over the hair swatches. At the microscopic level this means that a large quantity of “free” and “bound” water molecules are being rapidly extracted from the protein sites in the amorphous phase. Water-absorbing groups such as amino, carboxyl, and other functional groups capable of forming hydrogen bonds lose their water in this process. As a result, the gel structure of the amorphous protein phase changes from a soft and highly plasticized gel to a hardened amorphous mass.

Experimental observations indicate that rapid water evaporation from hair starts to take place when the hot iron reaches temperatures slightly higher than 100°C. Moreover, if the temperature of the hot iron is maintained at 120°C for 15 seconds while it is in contact with the hair swatch, the hair will be fully dehydrated. This is not surprising since bulk water starts to evaporate rapidly at 100°C. Yet, observations made by scientists in the laboratory and by many stylists confirm that when the temperature of the hot iron is set between 100 and 120°C, it is difficult or almost impossible to efficiently impose a temporary shape on hair, either straight or curly (27).

Furthermore, work carried out in this area indicates that the shape of a wavy hair swatch does not change efficiently from wavy to straight even when the temperature of the hot iron is increased to 150°C. These experiments also indicate that it is not until the hot iron reaches temperatures between 150 and 230°C, that a noticeable and efficient change in shape from wavy to straight starts to appear in hair (27). These observations indicate that the physicochemical processes that are responsible for inducing changes in the shape of hair occur at temperatures ranging between 165 and 230°C, and when the hair is almost fully dehydrated. Incidentally, the only protein processes that take place inside hair within this temperature range are the melting of crystalline

proteins and the softening of the amorphous matrix. The relation of these two processes with shape changes in hair will be discussed below.

b. Phase changes and transitions in hair

The thermodynamic transitions taking place in hair when its temperature is increased from 25 to 240°C have been the subject of numerous studies (29–31). Most hair types have been shown to undergo two main thermodynamic transitions, namely a first-order transition associated to the denaturation and melting of its crystalline structure, and a second-order transition associated to the softening or glass transition of its amorphous protein structure. The melting of the crystalline structure in virgin hair occurs at Tm *156°C, and the softening of the amorphous disulfide cross-linked protein phase occurs at its glass transition temperature, Tg* 144°C.

The occurrence of these two transitions in most types of hair can be detected and confirmed by Differential Scanning Calorimetry. For instance, in Figure 17 can be seen a typical DSC thermogram showing the endothermic peak related to the melting of the hair crystalline phase. Thus, since the working temperature of a typical hot iron treatment ranges between 150 and 230°C, it is straightforward that both of these transitions take place in the hot iron straightening process. In fact, as will be seen later, the better efficiency in changing hair shape temporarily with hot irons is due precisely to the occurrence of these transitions, as they are the sites with temporary memory properties.

Figure 17) Caption of a typical thermogram obtained by DSC from hair (32).

c. The mechanisms of water and hot iron setting are different.

With the material presented thus far, it is not difficult to anticipate that the mechanism by which hot irons impose a temporary shape in hair is very different

from that occurring in water-setting. Setting hair with hot irons does not involve the key and crucial typical step of water-setting, namely that consisting of the breakage of a high quantity of hydrogen bonds to soften the gel structure followed by imposition of the new hair shape.

Setting hair with hot irons involves the formation of hydrogen bonds, but they do not contribute to imposing a new shape. In order to better understand the role of hydrogen bonding during hot iron treatments, let us start by analyzing the role of water on the properties of the gel structure in hair. When water molecules penetrate into the hair structure they adsorb and intercalate on protein-functional sites breaking hydrogen bonds between them. The breakage of these hydrogen bonds renders the gel structure of the amorphous phase softer (6). When the water molecules are removed by evaporation, the internal hydrogen bonds between neighboring protein chains re-form and transform the soft gel structure into a hard viscous gel mass.

Hair swatches to be treated with a hot iron are, however, not required to be wet, for they already contain small amounts of moisture in their gel structure. However, this limited amount of water only breaks a limited number of hydrogen bonds, and therefore, it produces a semi-soft gel structure. When hair tresses with such limited amounts of moisture enter into contact with a flat hot iron at high temperatures, there will be a rapid process of heat transfer into the hair cortical regions. For instance, if after heat absorption, the temperature of the amorphous protein and crystalline phases rises rapidly to levels higher than 160°C, there will be violent evaporation of this limited amount of water, thereby producing the hissing sound typical of hot iron treatments.

At temperatures higher than 160°C the hair will have lost most of its moisture, causing all broken hydrogen bonds to re-form with the consequent hardening of the gel structure. Systematic hair-straightening experiments carried out with Mulatto hair using hot iron temperatures ranging between 110°C and 130°C have shown that hair swatches already become fully dehydrated at these temperatures. Even in the face of full dehydration the process leads to very poor or almost no hair straightening. These observations clearly indicate that rapid hardening of the gel structure attained during hot iron treatments does not allow for an efficient change in hair shape by hydrogen bond re-formation. *In other words, hot iron treatments harden the gel structure of hair before the temporary shape is imposed on it* (27).

d. Hot iron setting: partial denaturation of micro-fibrils and vitrification of

the amorphous phase

According to the ongoing discussions, hydrogen bonding does not contribute much to changes in hair shape by hot irons. There are, however, two other important physical phenomena occurring in hair between 130 and 230°C capable of inducing temporary shapes. These are the partial melting or denaturation of the crystalline protein phase at Tm and the softening of the disulfide protein matrix at Tg. Incidentally, both types of phase transitions occur also in cross-linked semi-crystalline polymers with shape memory properties (SMPs) and are commonly used to impose temporary shapes (8). In Figure 18 a schematic diagram explains the mechanism of temporary shape storage by semi-crystalline SMPs. The mechanism involves three key steps:

a) heating the polymer above its crystalline melting and glass transition temperatures to soften the polymer,
b) deforming the polymer to impose the temporary shape, and then,
c) cooling down to lock-in the temporary shape.

Figure 18) Schematic depiction of three steps needed for temporary shape fixing in cross-linked semi-crystalline shape memory polymers according to C.Liu *et al.* (8). Step 1 comprises melting of crystalline regions (a), step 2 requires the imposition of deformations to create the temporary shape (b), and step 3 involves cooling of the polymer for the recrystallization to lock-in the new shape (c).

Since hair is also a cross-linked semi-crystalline polymer, it is straightforward that hot irons must store temporary shapes in the hair structure by a similar process. Namely, when the hot iron is set at temperatures above 160°C and passes over the hair, it melts/denatures the crystalline regions and softens the amorphous phase. Then, as the hair is in motion the hot iron plates open the curl by friction and impose the straight shape by mechanical pressure. Once the hair moves out of the hot plates with its straight shape, it cools down. Cooling at

room temperature induces a vitrification process of the amorphous phase and also a recrystallization process or a reorientation of crystalline regions (see Figure 19). In this process the molten or denatured crystalline regions and the softened disulfide cross-linked proteins vitrify; while the non-denatured crystalline proteins reorient to comply with the new shape. This process locks-in the new straight shape in hair. Because hot irons use vitrification of amorphous and reorientation of crystalline proteins as processes to store temporary shapes, the stability and durability of the shape is longer than that obtained with water-setting.

It should be mentioned here that the denaturation of crystalline regions at high temperatures in hair is always an irreversible process and, therefore upon cooling, recrystallization of denatured proteins does not occur. Experiments have shown, for instance, that as the temperature of the hot iron rises between 160 and 220°C, the level of irreversible protein melting/denaturation also increases (27, 33, 34). Furthermore, it has been observed that as the level of denaturation increased in this temperature range, and while the hair curl was maintained open under the hot iron, the character of hair straightening shifted from temporary to more permanent (27). These observations clearly indicate that hot irons induce temporary shape changes in hair by a softening and vitrification process of its two protein phases. The amorphous phase locks-in the new shape by a pure vitrification process, while the crystalline phase, by either a reorientation process of the non-denatured crystalline regions or by vitrification of the denatured ones. The straightened shape becomes more permanent in character when the level of denatured crystalline regions that vitrify increases.

Figure 19) Diagrammatic representation of three steps required for the creation

of a temporary shape in hair with hot irons. In step 1 the hair is heated to its Tg so the crystalline regions partially melt irreversibly and the amorphous protein matrix softens, in step 2 a straight shape is imposed on hair, and in step 3 the hair is cooled down to vitrify the amorphous protein regions to lock-in straight shape (27).

The hot iron process could also be detailed as follows: hot irons increase the temperature of hair above its Tm and Tg. This causes the disulfide amorphous protein matrix to soften and become fluid-like while the crystalline regions start to undergo a kinetic melting or denaturation process. The degree of crystalline protein melting will depend, however, on the thermal stability of the amorphous matrix (29). If provisions are taken to reduce disulfide bond scission, then, most of the shape change induced by the hot iron will be temporary and due to vitrification of the amorphous phase and to a reorientation of crystalline regions. However, if disulfide bond breakage occurs readily in the amorphous proteins, the changes in shape will be due to a vitrification of amorphous and denatured proteins and the changes in shape will take a more permanent character (27).

For demonstration purposes Figure 20 shows three hair fibers whose semi-elliptical shape underwent a softening and vitrification process with a hot iron at 220°C. Fiber (a) in Figure 20 shows a pronounced flattening effect on its semi-elliptical shape as a consequence of the softening/vitrification process during hot iron treatment, fiber (b) shows imprints of neighboring fibers, and finally, fiber (c) shows shape flattening due to a similar vitrification process. All these effects indicate that at a certain point during the hot ironing process at 220°C the hair proteins became very soft, viscous, and fluid-like and were able to change their geometrical configuration to comply with the hot iron geometry. Then, upon cooling, the proteins vitrified and reoriented into the new shape. The experiments also showed that these effects are only temporary, i.e., when the fibers are immersed in water they recover their original shape (34).

Figure 20) Micrographs of shape distortion produced by 4 cycles of hot irons at 210°C on hair fibers of Caucasian origin as follows: (a) flat shape distortion obtained on a single fiber with a 40 g compression force, (b) imprint left on fiber by neighboring fibers in a hair tress, and (c) deformation left in a fiber selected from a hair tress after treatment with hot iron (34)

Since temporary shape changes induced by hot irons involve only physical mechanisms, the conditions for their recovery also entail physical phenomena. These conditions are met when the protein chains in the vitrified amorphous phase enter into faster motion again. This could be done by increasing again the hair temperature above Tg or when the hair is wet. Thermal motion of main chains in polymers and proteins always occurs at room temperature, and their degree of motion is relative to their Tg. The higher the Tg in a polymer, the slower is the thermal motion of its main chains at room temperature (2). Thus, in theory, temporary shapes induced with hot irons will be very long lasting but unfortunately, the principle of superposition in polymers (2–4) also dictates that the Tg of a polymer can be lowered by a plasticizer, thereby increasing chain mobility.

Let us also remember that water is the main plasticizer of proteins, and it is also the main plasticizer of the gel structure in the hair amorphous phase (5). Even when water cannot penetrate and plasticize the crystalline regions, the effects of water in the amorphous phase have an impact on the orientation and arrangement of the crystalline phase. When the hydrophilic groups of the amorphous phase absorb water the amorphous phase relaxes, and this may result in a reorientation of the crystalline regions storing the temporary shape. The final result is a loss of temporary shape. Thus, even when temporary shapes induced by hot irons are of longer duration than those attained by water-setting, their shape is also lost when the hair absorbs water.

e. Heat transfer from hot iron to hair

It should be mentioned here that because of poor hair thermal conductivity, nonhomogeneity of cuticle cell surface, and packing irregularity of hair fibers, not all hair fibers in a hair swatch exposed to a hot iron surface will experience the same temperature. Those hair fibers in contact with the hot iron will certainly be expected to equilibrate rapidly to the hot iron surface temperature. However, those a few millimeters away from the surface will experience a much lower temperature. Systematic experiments using a thermocouple embedded in a hair

tress have shown that hair fibers in the middle of a 2 mm hair tress experience ~ 25°C less than those in contact with the hot iron (see Figure 21). Furthermore, additional experiments carried out with hair swatches ~ 1 mm thick and placed on a hot plate at 200°C showed that it takes time for heat to be transferred across a hair swatch into a metallic heat sink to equilibrate temperatures. Once equilibrium conditions are established, the temperature at the hair swatch surface not in contact with the hot plate is always ~ 60°C less than that in contact with the hot plate (see Figure 22).

The simple experiment described above does not represent realistic conditions of hot iron treatments because only one surface of the hair tress is exposed to high temperature. However, the experiment helps us to visualize the time delays needed for temperature to equilibrate across the hair tress. Therefore, we will expect that when a hot iron is passed at 160°C over a hair swatch only those fibers in contact will rapidly equilibrate temperatures with the hot iron; and these fibers will experience melting of crystalline regions and softening of the disulfide cross-linked matrix. Those fibers underneath may not experience this phenomena.

Furthermore, occurrences of the above-described transitions in hair are not only dependent on the position of the hair fibers relative to the hot iron surface but also dependent on the heating rate. For instance, it has been shown that the faster the rate of heating, the higher is the melting temperature of the crystalline regions (29). Since the hot iron is passed swiftly along the hair fibers we would expect, therefore, that if the hot iron temperature is set to 160°C, large numbers of hair fibers will not undergo these transitions. Thus, if we want all hair fibers in a swatch to experience the thermodynamic transitions required, two actions must be taken, namely, 1) to increase substantially the hot iron temperature above 160°C, or 2) to improve heat transfer across fibers. From the practical point of view, only the former action can be easily done and this can be accomplished just by setting the hot iron temperature to higher levels. This analysis helps explain why setting the hot iron temperature in the range of 170–210°C improves substantially the efficiency in hair straightening (27).

Figure 21) Schematic representation of hair swatch between hot iron plates. The experiments show that hair fibers in the middle section of the hair stress sense temperatures ~20–25°C lower than those at the periphery (27).

Figure 22) Graph showing variation in temperature as function of time (a) in a thermocouple placed at the top of a hair tress placed on a hot plate (b) an (c). The temperature of the hot plate was preset at 200 C and, then, at t=0 the hair and thermocouple were place on it (27).

f. Unwanted consequences of friction and rising hair temperature above Tm and Tg

Setting temporary shapes in hair with hot irons has advantages over water-setting or any other types of temporary setting. This is true, first, because hot irons introduce stronger changes in shape, and second, because the shapes last longer. In fact, an ideal hot ironing process would occur if the duration of the temporary shape could be controlled at will and the mechanism of shape storage would only involve reversible changes to the amorphous and crystalline phases. Unfortunately, it is very difficult to control water absorption by hair and also to prevent irreversible disulfide bond breakage and other forms of damage that occur in hair at high temperatures. Scientists studying heat damage in hair have reported the occurrence of protein oxidation, cuticle cell lifting/bulging,

formation of pores inside the hair (see Figures 23 and 24), degradation of tryptophan, and disulfide bond scission (35–37). *All these effects make the process of flat ironing far from ideal.*

The first cause of damage in hair treated with hot irons comes from the fact that the thermodynamic transitions responsible for storing the temporary shape in hair lead to processes that are not fully reversible. For instance, upon cooling, the softened disulfide cross-linked protein matrix does not return to its original native vitrified state; nor do the denatured crystalline regions recover their original crystalline structure (29). Extensive research carried out in various laboratories has already shown that when hair is heated above its Tm a process of partial irreversible protein denaturation takes place. Lack of full recovery of both amorphous and crystalline phases has been ascribed to various thermal damaging factors. Among these thermal damaging factors the most important one is irreversible disulfide bond breakage. This process causes covalent cross-linking points in the amorphous phase to decrease, thereby producing a different state of vitrification and a process of irreversible destabilization of the crystalline regions. Both processes contribute to a gradual loss of permanent shape and undesirable reduction in hair strength.

Figure 23) Micrograph of a hair fiber (φ ~ 76 µm) subjected to five treatments of hot ironing showing the presence of pores at a depth of 10 µm inside the cortex (a). By using digital filters in the image, the pores were separated and the remainder of the picture was transformed into a black background (b) (34).

Figure 24) Micrographs of a hair fiber (φ ~ 64 µm) showing image of surface

obtained with no shift in plane of focus (11a). Later the plane of focus (p.o.f.) was shifted in 27 and 32 µm, respectively; the white spots in the image are voids with sizes ranging between 3 and 10 µm. The hair fiber was subjected to five hot iron treatments with the hot iron at 190°C and a speed of 0.2 in/s (34).

Cosmetic scientists and formulators are continually working in this area to alleviate these damaging effects and to improve the widely used hot ironing process. Efforts geared into this direction have already resulted in the use of polymers that enhance straightening and decrease damage. Some groups have introduced the use of formaldehyde in hot iron applications. The use of this ingredient in combination with hot irons is very effective in almost permanently changing the shape of hair. However, because of its toxic properties its use has been banned in the U.S. The mode of action of this ingredient will be reviewed later.

3.3.4.6 HAIR SHAPE CHANGES INDUCED BY PERMANENT WAVING SOLUTIONS

a. The Process

The use of thioglycolate salt solutions to chemically reduce or break disulfide bonds in hair forms the basis for one of the most effective methods for permanently changing the shape of hair from straight into a more wavy and curly configuration. Essentially, the process consists of three basic steps that are similar to those already described for other forms of hair setting:

1) breakage of bonds to soften the hair, i.e., in this case the disulfide bonds,
2) imposition of a new shape, and
3) re-formation of bonds to lock-in the new permanent shape.

Breakage of disulfide bonds in hair is carried out by treating hair in a 7–9% solution of ammonium or sodium thioglycolate at a pH of ~ 9.5. Imposition of the new shape is made by winding hair swatches on plastic rollers. Finally, re-formation of the broken disulfide bonds is accomplished by applying a solution or cream containing 3% hydrogen peroxide to reoxidize broken bonds (see Figure 25).

Figure 25) Diagrammatic representation of three steps required for the creation of a permanent shape in hair by permanent waving treatments. In step 1 disulfide bonds are unzipped to erase memory in hair, in step 2 a curly shape is imposed, and in step 3 the broken disulfide bonds are re-formed to lock-in the curly shape (18).

b. Physical and chemical processes taking place inside hair during permanent waving

The role of step one described above in permanently changing the hair shape with ammonium thioglycolate is not just to soften the hair, but mainly to delete the permanent shape in the hair architecture. This process is carried out by the thiolate ion which unzips disulfide bonds in the amorphous structure of hair and produces a certain degree of destabilization in the crystalline regions. Then, once the old shape is partially removed and when the hair is somewhat shapeless, a new shape is introduced in the protein chains by mechanical deformation of plastic rollers. Finally, the new shape is stored in the amorphous protein phase by re-zipping the same bonds in a different configuration by using hydrogen peroxide. Unfortunately, the disulfide breakage and re-formation process modifies only one part of the memory where the permanent shape of hair is stored. Namely, it only modifies part of the tertiary structure of the amorphous proteins. However, it does not modify the crystalline phase nor it does modify the primary and secondary structure of the proteins in the amorphous phase. This means that after the ammonium thioglycolate treatment, there are still shape recovery internal stresses coming from these structures. These stresses will push gradually for the recovery of the original permanent shape in the hair fibers.

Changes in the hair shape memory with ammonium thioglycolate are consequently partial, and will result in a very slow shape reversion process. At the molecular level this process is driven by the recovery of protein conformations to their native state. The forces driving this phenomenon stem from the primary and secondary structures of hair proteins in the crystalline and

amorphous phases. It should be mentioned here that the phenomenon of shape reversion is common in a host of denatured proteins and enzymes and, in particular, when it is related to disulfide bonds.

In a famous experiment, C. Afinsen showed that disulfide bond breakage/re-formation in ribonuclease leads to a mixture of denatured and scrambled protein conformations with no enzymatic activity (38). He also observed that the process of protein scrambling by disulfide bond breakage/re-formation was not sufficient for the enzyme to completely lose its shape memory or ability to revert to its native shape. For instance, Afinsen found that traces of mercaptoethanol/urea in combination with the scrambled enzymes in solution were sufficient for the enzyme to reverse and recover its native shape (see Figure 26).

Figure 26) Schematic representation of C. Afinsen's experiment with ribonuclease showing that this enzyme is able to recover its shape even after its disulfide bonds were re-formed with the proteins in a scrambled configuration (38).

It would thus appear that the ability of proteins to recover their native shape is not completely lost when their disulfide bonds are arranged in a different configuration. This particular phenomenon exhibited by proteins helps to explain why the application of treatments that produce only disulfide bond breakage/re-formation in hair are not sufficient to produce irreversible changes in its protein architecture, so that they will support the desired permanent shape changes. Disulfide bond interchange, in combination with recovery stresses coming from the residual memory in the protein architecture of the amorphous and crystalline phases, will be able to drive the reversion process.

From the practical point of view, however, thioglycolate systems are

probably the best compromise that can be made between hair damage and persistence in shape changes. Ammonium thioglycolate systems have been shown to produce shape changes that are quasi-permanent and last for months. Certainly, more effective treatments could be designed, but they will probably have to include permanent changes to the primary, secondary, and tertiary protein structures of both the amorphous and crystalline phases. Such a process could be devised but will introduce irreversible damage into other regions of the hair fiber, not to mention the practical loss of recurring business and socialization phenomena that are embedded in the perennial trek to the local cosmetologist. In summary, the process of disulfide bond breakage/re-formation for storing permanent shape changes is not 100% efficient, but it is in a good position as a system because it is very selective to disulfide bonds. It is for these reasons that many scientists working in this field have rather opted for improving the system.

In fact, there is still a great deal of improvement that can be done with these systems. For instance, the disulfide bond breaking/re-formation process is usually assumed to be fully reversible, i.e., it is commonly believed that the amount of disulfide bonds that break can be completely re-formed. Scientists have found that this is not the case and that a small percentage of these bonds always remain permanently broken (39, 40). Much effort has also been dedicated to understanding the effects of temperature, thiolate ions diffusion, and reaction kinetics inside the hair (41, 46). The process of disulfide bond re-formation by means of oxidizing agents such as hydrogen peroxide and other reagents has also been the subject of numerous studies. Effect of fiber diameter on shape changes with these systems has also been the object of concern (43–46). All these studies represent efforts geared to improving the efficiency and effects of the disulfide bond breakage process.

Surprisingly, changes in the crystalline regions after permanent waving have not received much attention and only a few studies have appeared in the literature (47). These studies, however, do not seem to correlate efficiency of shape change with levels of irreversible change in crystalline regions. Certain substances such as urea in combination with ammonium thioglycolate salt solutions appear to enhance destabilization and breakage of hydrogen bonds, apparently improving the efficiency of permanent shape changes, yet their effect on hair’s crystallinity has not been reported (48).

3.3.4.7 HAIR SHAPE CHANGES BY ALKALINE RELAXERS

a. The process

The straightening of hair with alkaline cream relaxers belongs to the category of permanent changes in hair shape. It is the most damaging process of all known hair permanent reshaping cosmetic treatments. This is so because during the alkaline straightening process not only are disulfide bonds broken, but also a series of other processes occur that damage the hair architecture. Among these processes are: permanent denaturation of hair proteins, irreversible breakage of disulfide bonds, protein alkaline hydrolysis, protein loses, and excessive swelling of hair (49–51). All alkaline straightening processes are based on the chemical reactivity of the OH ions towards the groups in hair proteins that store the permanent curly shape. The pH of these creams depends on the tightness of the curl, and it ranges between 11.5 and 14.0. As a result, the concentrations of alkaline ingredients in these creams often vary between 1.5 and 3%. Strong chemical bases are used for this purpose and these include sodium hydroxide, lithium hydroxide, combinations of lithium/sodium hydroxide, and guanidine hydroxide produced *in situ* by the combination of calcium hydroxide and guanidine carbonate.

There are various classifications of cream relaxers in the market. These are known as lye, no-lye, base, no-base, and mix, no-mix, *etc.* Regardless of their classification, the key active chemical in all of them is always the OH ion. Lye relaxers are those based on sodium hydroxide; no-lye relaxers are those containing any other ingredient than sodium hydroxide. A base relaxer refers to one that needs a layer of petrolatum on the scalp prior the straightening process to protect it from irritation; the no-base relaxer does not need petrolatum separately as it is already formulated in the cream; and finally, a mix relaxer refers to one needing the mixture of two ingredients to produce the OH ions, as is the case of the guanidine carbonate/calcium hydroxide relaxer.

Other types of chemicals used in the straightening of curly hair comprise the use of sodium bisulfite and thioglycolate salts; these systems are referred to as the Japanese and Thio relaxers, respectively. The action of these two ingredients is not based on the action of the OH ions but rather on the reactivity of the sulfite and thio groups. They are more selective to the breakage of disulfide bonds, and as will be seen later, they have advantages and disadvantages over alkaline cream relaxers. A typical salon application of a relaxer consists of three main steps: relaxer cream application, neutralization, and hair conditioning. During

the application step the cream is evenly distributed over the selected hair swatches. Almost immediately after application a diffusion of OH ions is triggered from the cream into the hair cortex via the endocuticle and cell membrane complex. As the ions diffuse they start to react with the hair proteins in the amorphous and crystalline phases. Experience shows that 15 or 20 minutes is sufficient for the cream to produce desired hair-relaxing effects.

Relaxer creams in many cases are not designed to reach reaction equilibrium conditions with the proteins in hair. Usually, their OH ion concentrations, which depend on the pKb of the base, are above that required for the hair-straightening process. Consequently, if they are left for longer periods of time on the hair, the effects can be partially depilatory. Thus, as soon as the beautician considers that the hair has been properly relaxed, the cream is rinsed out with water. At this point the OH ions are still reacting with the hair internal proteins; therefore, a neutralization process must be immediately introduced to stop the OH ion/protein reactions. The neutralization process is usually carried out with a shampoo containing citric acid. During this process a strong odor is perceived coming from liberated sulfur. Also, experiments show that the neutralizing solution turns turbid, indicating extraction of protein fragments from the hair structure (52). Because of the high level of damage to the whole hair architecture, a conditioning process is immediately introduced, mainly, to lessen fiber entanglement due to friction effects.

b. Physical and chemical processes taking place inside hair during alkaline straightening

The alkaline reshaping of very curly hair also starts by deleting the hair's permanent shape from the memory located in its architectural structure. In the case of permanent waving this could be done partially by using thio or sulfite compounds to unzip all disulfide cross-linking bonds and allow reorientation of the crystalline regions. The use of these ingredients for the straightening of very tight African-type hair is usually not recommended as it leads to a gradual shape reversion. This happens because disulfide bond breakage alone is not sufficient to delete enough shape memory from the curly hair so the straight shape will resist reversion. The degree of natural curliness in African hair is very tight and strong, and therefore, making it straight requires more changes in its protein chemical structure. This is true, in particular, on sites where the curly shape is imprinted. In other words, African hair was casted from its inception at the hair follicle with a primary, secondary, and tertiary protein architecture complying

and supporting very small radius of curvature and also high degrees of fiber elliptical shape (see Figure 27). This means that at the microscopic level the proteins in the amorphous and crystalline phases in these fibers are oriented and chemically bonded to accommodate for these geometries (see also Figure 8).

Thus, in order to attain a complete straightening effect in these curls a sizable amount of shape change or shape deletion is required. If the change in shape were from very tight curly to open curly, the amount of shape deletion in the hair architecture will be less, and this type of mild shape change would not even require the formation of new bonds to lock-in the new shape. However, complete straightening of very tight curly hair requires a higher degree of structure modification; first, because the tight curly shape has to be erased and, second, because the new straight shape needs to be locked-in. It should be recognized that the complete removal of curly shape from a curly hair fiber implies the breakage of chemical bonds that stay irreversibly broken, thus weakening the overall structure of hair. Therefore, the process of introducing a new permanent shape such as in the case of straightening curly hair may require either full reversibility of bond breakage/re-formation, or the introduction of new bonds to stabilize the new shape and also to strengthen the fiber.

Figure 27) Micrographs of a single fiber of African origin showing tight coil radius of curvature and high degree of ellipticity (18)

As was previously stated, there is a rule relating shape changes and number of sites in memory for curly hair, the rule is: **the tighter the curl, the more the elements in the architectural sites of hair's memory need to be modified to make it straight.** Sizable permanent changes in hair shape always require the annealing of stresses in the internal structure of hair. Otherwise, they will produce strong internal fiber stresses that will drive a shape reversion process. Consequently, large changes in hair shape always need the modification of a higher number of memory sites to eliminate strong reversion forces. The degree of memory deletion process can be outlined in the following manner:

- Weak curls require the breakage of a few disulfide bonds.
- Medium strong curls require a larger amount of disulfide bond breakage.
- Tighter curls require, in addition, denaturation of crystalline regions.
- Very tight curls require all of the above, plus breakage of amide linkages in the proteins as well.

By the same token, the level of chemical and physical stabilization required by the new straight shape increases in the same order.

However, regardless of degree of initial hair curliness, the steps needed to straighten hair with any type of cream relaxer are all the same and almost identical to those required for other changes in hair shape:

1) the softening of hair, which may involve breakage of physical bonds, breakage of covalent bonds, and softening of the crystalline and amorphous regions,
2) imposition of a new shape, and
3) fixing or locking the new shape by re-forming broken chemical or physical bonds, and by rearranging crystalline and amorphous regions.

The role of the first step is not only to chemically soften the hair, but in doing so, also to delete the permanent shape from the hair architecture or memory. In the second step, a new shape is introduced in the shapeless hair, and in the third step, the new shape is stored and imprinted in the memory structure of hair. If we pay close attention to these steps we will immediately realize that the efficiency of changing hair shape, from curly to straight, does not depend only on how much memory is erased but also on how efficient the process of imprinting and storing the new shape is.

In the scientific literature it has already been shown that alkaline creams lead to substantial chemical and physical modifications to the hair structure (53–57). Among the main chemical reactions and physical processes are breakage of weak and strong hydrogen bonds, breakage of salt linkages, and the formation of the following products: lanthionine, cysteic acid, sulfur, and protein fragments. These reactions proceed according to the paths shown in Figures 28 and 29.

Figure 28) Diagram showing reaction of cystine and OH ions. The reaction takes place during the alkaline straightening of African hair at pHs ranging between 13.5 and 14.0, and it leads to the production of sulfur and lanthionine (32).

Figure 29) Diagram showing additional reactions of cystine and OH ions occurring in the alkaline straightening of African hair at pHs ranging between 13.5 and 14.0 (32).

Because of the high level of bond breakage, alkaline relaxers also produce

high levels of protein denaturation in both the amorphous and crystalline regions. Figure 30 shows typical denaturation enthalpy reductions of various hair fibers subjected to a combination of alkaline and permanent waving treatments. The smaller the bar size in the graph, the higher is the degree of denaturation or disarray in the crystalline regions. All treatments in these experiments showed a decrease in the enthalpy of denaturation and some of them were more pronounced than others (32). Those treated with the alkaline relaxers alone, i.e., with NaOH and No-Lye are more real representations of the irreversible denaturation process taking place in real straightening hair practices, and they show reductions that range between 30 and 50% in their denaturation enthalpies.

Figure 30) Denaturation enthalpies of hair fibers after various treatments as follows: Untreated = fiber with no treatment, PW = fiber with permanent waving treatment, No-Lye = fiber treated with No-Lye relaxer, No-Lye+ PW = fiber treated with a combination of No-Lye straightening + permanent waving, NaOH = fiber treated with a relaxer based on NaOH, and finally, NaOH+PW = fiber treated with a combination of a relaxer and permanent waving (32).

This is the reason why alkaline relaxers are very efficient in deleting the permanent shape from the hair architecture—namely, because they modify a large number of hair's physical and chemical features in the protein structures containing the permanent shape memory; *i.e.* they break disulfide bonds, strong and weak hydrogen bonds, salt linkages, and a certain amount of amide bonds. By breaking all these bonds the OH ions render the amorphous phase extremely soft; in this process the crystalline regions also become scrambled and highly plasticized as well. The hair becomes, therefore, extremely soft and shapeless. The memory deletion process obtained by these effects does not guarantee however the imposition of a new shape, nor the process of locking-in the new

shape. For this to happen, new chemical bonds have to be introduced in combination with a process of reconstitution of the hair architecture.

c. Shapeless hair and a new straight shape

As previously discussed, when the alkaline straightening process starts to delete the tight curly shape from the hair memory, the hair fibers gradually become soft and shapeless. The new straight shape is also gradually introduced during the alkaline straightening process by the rubbing of the cream into the hair. The process of new bond formation also appears to occur as the cream is deleting the old curly shape. Experiments have shown, for instance, that if the process of hair straightening is carried out while the hair maintains its coiled shape, the straightening efficiency is less than if the curl is uncoiled and extended (52). Furthermore, if the acid neutralization step is done while the hair is kept in a more straighten configuration the efficiency in straightening also increases (52). These observations indicate, thus, that a certain amount of new bonds, most certainly lanthionine (see Figure 28) (53–59), are gradually introduced while the hair is still reacting with the cream. The straightening efficiency observed during neutralization, on the other hand, is certainly due to a vitrification process of the amorphous phase, and also to a partial reconstitution of the crystalline regions as salt linkages and strong hydrogen bonds re-form upon elimination of the OH ions.

The phenomena of fiber supercontraction and denaturation observed to occur in alkaline treated hair fibers, and reported by many scientists (60–62), cannot explain the straightening process when considered alone. These phenomena are at most manifestations of disulfide and hydrogen bond breakage and also of changes in protein conformation in the crystalline regions. Like the phenomenon of excessive water swelling, they do not impose by themselves the new straight shape or cause the locking-in of the new shape in the hair architecture. Furthermore, in terms of shape changes there are substantial differences between the processes of protein denaturation, supercontraction, and hair swelling. Protein denaturation does contribute to the removal of permanent shape from the hair architecture; while supercontraction and swelling do not, they are physical phenomena associated to destabilization of the protein structure. Protein denaturation per se, however, does not impose a straight shape; nor does it lock-in a new shape.

Currently, there are three main mechanisms that have been proposed to explain the straightening of very curly hair by alkaline creams. The oldest one

centers its arguments on the formation of lanthionine as the new chemical bond that is formed during the reaction of OH ions and hair. This bond is proposed to lock-in the straight shape (59–61).

Another mechanism introduced later bases the explanation on the phenomenon of supercontraction. This mechanism essentially proposes that the high level of contraction observed to occur in hair and wool when treated with high pH solutions is responsible for the imposition of the straight shape (60).

Yet, a more recently proposed mechanism suggests that the occurrence of protein denaturation is crucial and that lanthionine and the formation of any other products are just secondary to the straightening process (62). However, when we use concepts from the field of shape memory materials, we realize that the permanent straightening of hair must include the following steps: 1) removal of protein configurations that support the curly shape, 2) imposition of straight shape, and 3) locking-in of the straight shape, either physically or by forming new bonds.

It is quite possible, thus, that the straightening of curly hair by alkaline relaxers occurs by a combination of all three of the proposed mechanisms. Namely, 1) breakage of disulfide and protein denaturation are needed to delete the curly shape from the memory of hair, 2) a certain amount of fiber supercontraction aids during the cream rubbing process to create the straight shape, and 3) lanthionine formation is needed to stabilize the new straight shape.

Finally, it is obvious that the introduction of a straight shape in curly hair needs to be cosmetically acceptable. This means that both the new straight shape and the hair fibers need to have strength so the shape can be maintained to resist external forces without further unwanted deformation or breakage. The hair with the new straight shape has to be able to resist stresses coming from the grooming of hair, i.e., from its own weight and from hair movement produced by combing or styling, either when wet or dried. Experience tells us, however, that relaxer technology still is far from achieving this objective satisfactorily. Currently, many scientists are working to bridge this gap, and it has already been shown that for certain cases, i.e., for medium curly hair, it is possible to impose a straight shape without causing much damage.

These processes involve the introduction of new strong bonds in the protein chains, bonds that are capable of overcoming internal recovery stresses from the old permanent shape. Unfortunately, most of these new technologies are based on formaldehyde. This chemical is known to cause protein cross-linking in

proteins and therefore when used in hair it introduces new cross-links in the hair structure (63). Formaldehyde is, however, a potential carcinogen and its use has been banned in cosmetic applications.

Other hair-straightening technologies involving sodium sulfite or thiogycolate salts are more specific to disulfide bonds, but by their nature they are not efficient in fully removing the very tight curly shape from the hair memory. Consequently, later on the hair undergoes total or partial reversion to its curly shape. In summary, it can be said that the alkaline straightening of curly hair by OH ions is characterized by an efficient shape memory deletion process. It introduces a new straight shape, but it falls short of forming new bonds to lock-in the new straight shape so the final hair shape and hair fibers stay strong. We would be remiss if we did not point out that one other area needing research and improvement is the undesirable occurrence of chemical burns on the skin from the highly alkaline processes described above.

3.3.4.8 HAIR SHAPE CHANGES OCCURRING DURING GROOMING PRACTICES

a. Static vs. dynamic changes in hair shape

The imposition of temporary changes in hair shape is always carried out by external means, and depending on the way hair is handled they can be classified into two types: 1) shape changes imposed while hair is under static conditions, and 2) shape changes imposed while hair is under movement. In the first category we find water-setting, permanent waving, and alkaline straightening. All these shape changes are imposed with external devices that force the hair to stay statically or quasi-statically with almost no movement while the new shape is being imposed. By contrast, in the second category, shape changes are also imposed externally, but the temporary shape is imposed while the hair fibers are in a dynamic free or forced intensive movement. Thus, the final shape attained by the fibers is an average shape, and depends on fiber motion in the assembly. These are, for instance, shape changes produced by blow-dryers, combing devices, and by drying towels. In shape changes of the first type, the interaction of fiber to fiber is not important, while in those of the second type this interaction is extremely important as inter-fiber motion in the assembly determines the final average shape. In the following paragraphs we will focus our attention on the latter types of shape change.

b. Friction vs. bending forces in dynamic changes in hair shape

Movement of hair fibers in hair assemblies always occurs by currents of air, head motion, grooming devices, and even when wet hair is allowed to air dry. Because of hair flexibility, length, and motion intensity, the hair fibers bend, flex, and rotate inside the hair assembly. This flexion and rotation imposes instantaneous transient deformations on the fibers and ultimately leads to the formation of transient temporary shapes in the hair assembly. The transient deformations are, however, immediately countered by internal stresses coming from the permanent shape of hair. The hair assembly will stay deformed in a transient state or it will recover its shape depending on whether inter-fiber slippage occurs between fibers.

Ultimately, the degree of fiber inter-slippage will depend on the balance between single fiber bending and its frictional forces. For instance, slippage among fibers can be prevented if the inter-fiber frictional forces are larger than the bending forces. Under these conditions the hair fibers will behave as semi-solid ropes and the contact points between fibers will serve as strong welding joints that restrict inter-fiber movement. This condition occurs for low bending curvatures and high surface frictional forces, or when styling polymers are used. Polymer spot welding and high friction sites restrict fibers form moving and prevent the fibers from slippage when the hair assembly is being deformed.

Conversely, if a high degree of slippage occurs between hair fibers, then, the low surface frictional forces will not restrict inter-fiber movement. Consequently, slippage among fibers will occur and the permanent shape of both single fibers and hair assembly will be rapidly recovered while in motion. This occurs at sufficiently large bending curvatures or when there are relatively low frictional forces at the hair surfaces. These conditions correspond to the case when silicones or efficient conditioners are applied to the hair surface. The final average shape attained by an assembly is dependent on the balance between fiber slippage and friction forces. Figure 31 displays two hair tresses (a) and (b) washed with different shampoos, and showing the effects of these two conditions. Both hair tresses were cut from the same hair batch and were allowed to air dry at room temperature conditions after shampooing. Hair tress (a) was washed with a simple cleansing shampoo, while hair tress (b) was washed with a “2 in 1” conditioning shampoo containing silicone. In Figure 31 it can be seen that hair tress (b) dries with its fibers in a wavy fashion or with a shape that resembles its permanent shape, while hair tress (a) dries into a straight lump. The reason for this is that during fiber movement and drying, fiber inter-slippage in

hair tress (b) was dominant and the final hair shape was driven by natural recovery forces.

Figure 31) Effect of silicone on the temporary shape of hair after drying. Hair tress (a) was washed with a cleansing shampoo while hair tress (b) was washed with a shampoo containing silicone (64).

Thus, the effect of fiber-to-fiber interaction via surface effects has a strong influence on the acquisition of a temporary shape when the hair is in motion.

Fiber-to-fiber interaction has, therefore, a strong impact on the final shape of the hair assembly. This means that if water-setting is taking place during hair movement, the final shape of the hair assembly will be determined by the interplay between hair inter-fiber friction forces, hair drying rates, hydrogen bond formation in gel-structure, and ability of hair to recover its permanent shape. The role that these processes play in setting dynamic temporary shapes is so important that in the following sections of this chapter their interplay will be analyzed in relation to various grooming practices.

3.3.4.9 HAIR VOLUME AND CHANGES IN HAIR SHAPE

a. Definition of volume

Hair volume can be defined as the effect obtained when a bundle of hair fibers, aligned in synchrony, occupy as a whole or individually a physical space larger than their real nominal volume. Volume in hair is more about the space occupied by the hair bundle than about hair mass or fiber density. The larger the space between fibers or the larger the total volume occupied by the hair bundle, the

more volume is perceived (see Figure 32). The definition of volume also includes the ability of the hair fibers to maintain their separate space and shape in synchrony when the assembly of hair fibers is in motion. It should also be perceived when the hair bundle is at rest. These conditions imply that temporary hair shape changes, producing volume should have the strength and elasticity to recover rapidly and in synchrony with shape distortions created during movement. As will be seen later, if this does not happen, the undesirable cosmetic effect will be that of frizzy hair.

Figure 32) Caption of hair assemblies showing hair volume. The assembly of hair occupies a larger space than it would if each single fiber were shaped straight. Observe also that hair fibers are aligned in synchrony with a particular bundle.

Temporary shape changes in hair that create volume effects can be introduced dynamically while the hair assembly is in movement or also statically. The former is the focus of this paragraph, since the latter belongs to the types of static shape changes already described in previous paragraphs. There are two ways of creating volume by a dynamic process of hair in motion:

1) by imposing a wavy or curved shape in a collective assembly of hair fibers in a synchronized manner while the fibers are in motion. This process usually involves rapid water-setting; and/or
2) by increasing the distance between individual hair fibers, either by creating inter-fiber friction or by using styling polymer films to produce light wavy shapes on smaller hair bundles.

b. Main challenges in creating volume

The creation of hair volume in hair assemblies by any of the procedures described previously always entails hydrogen bond formation, even when polymers or styling resins are used. This means that for all practical purposes, the process of dynamic volume creation has many similarities to the process of

water-setting. In water-setting the temporary shape is created statically with plastic rollers, while in the production of dynamic volume the temporary shape is created usually when the hair fibers are in movement. The reason for this is that temporary shapes with volumizing or styling characteristics are commonly created in hair by people after taking a shower, and they are rapidly and conveniently created when the hair is wet and being dried. Therefore, volumizing temporary shapes created by motion are better stored in the hair gel structure of the amorphous phase while the hair is dried. In the following paragraphs of this section, attention will mainly be given to the process of creating hair volume without the use of resins or styling polymers, while the use of styling polymers will be reviewed in a later section.

The main challenge in creating temporary shapes that will result in an increase in hair volume is to coordinate the process of hydrogen bond formation with hair movement in order that an average temporary shape can be captured. Implicit in achieving this is the creation of a temporary shape whose instantaneous strength will be able to resist later shape-recovery forces arising from the permanent shape. While daunting, we note that beauticians have been producing volume in hair just by manipulating hair in motion as it dries from its wet state. As was previously discussed, temporary shapes created by hair fiber assemblies in motion are determined by the interplay between hair inter-fiber friction forces, hair drying rates, hydrogen bond formation in gel-structure, and ability of hair to recover its permanent shape.

In order to better understand the role of hydrogen bond formation and hair movement, let us imagine an ideal and imaginary case of hair volume creation, where the volumizing shapes introduced during hair movement are ideal and will be frozen and stored instantaneously by an extremely rapid process of hydrogen bond formation.

In this ideal process, hydrogen bond formation would be so fast and intense that the instantaneous hair shape acquired during movement would be fixed on hair like a photographic process that freezes a hair shape image while it is in motion. This ideal process would produce a very strong and temporary, rigid pattern of hair volume, somewhat similar to that obtained with the use of styling gels. Such a temporary shape would also resist very well the internal recovery stresses from the permanent shape. Unfortunately, this ideal process does not exist. First, because the rate of hydrogen bond formation by evaporation is slower than the instantaneous shape movement and second, because the instantaneous formation of hair shape during movement depends on the balance

between inter-fiber surface friction and shape-recovery forces. Third, and finally, such an ideal process would create a very stiff and static hair pattern that for all practical purposes contradicts one of the main characteristics of hair volume, namely, that of a synchronized hair movement as well. In layman's terms, it is a beautiful thing to watch a woman with long flowing hair shake her head and all of the long tresses move together from side to side. We state this here to bring the reader back, temporarily, to why we have spent such a significant amount of time and scientific research: in the end, all of this technical understanding is intended to bring beauty to hair!

Let's analyze then, a more realistic case; a good example might be that of a head hair assembly with straight shape fibers that are six inches long and belong to a hypothetical subject. After the hair has been shampooed, conditioned, and towel dried, the subject intends to introduce volume by blow-drying. For the sake of simplicity in the analysis, the effect of conditioning ingredients on the hair surface will be omitted. When the straight hair has been wet and towel dried, it still contains about 80% of its internal moisture and, therefore, its gel structure still is very soft because most of its hydrogen bonds are broken.

If at this point the subject introduces motion into the hair fibers, they will be bent and flexed elastically, and the whole hair assembly will be turned into an elastic scrambled irregular pattern. Then, as the subject dries the hair with a blow dryer, he/she forces the hair to move with a brush or a comb to create wavy patterns. As the hair moves and dries, simultaneously hydrogen bonds are formed in the gel structure of each hair fiber, thereby trapping a synchronized wavy temporary shape into their temporary memory. This temporary shape will increase hair volume because the wavy shape of the hair fibers will occupy more space than when the fibers were in a straight shape.

In this simplistic example only two factors affecting the formation of shape were considered. These were the effects of hair drying and hydrogen bond formation. The effects of hair inter-fiber friction, permanent shape strength, and effects of products on rates of drying were not considered. It is easy to foresee, however, that when the fibers enter in motion, the type of shape that will be produced depends on whether the surface is well lubricated or not. It will also depend on the integrity of the hair fibers. It may be that the hair assembly was previously treated with an alkaline relaxer or with other harsh chemical ingredients and thus has a low content of disulfide bonds. In such a case, the instantaneous shape that would form during movement would be very different from that which would have formed had the hair been virgin. Thus, the

instantaneous shape that forms during hair movement is also dependent on how strong the real permanent shape is. If the fiber strength and permanent shape are weak, the hair fibers will behave more like viscous fibers and will not be able to recover from instantaneous deformations created by hair movement.

Another factor that was not considered in the prior simplistic analysis is the rate of drying. If the hair contains ingredients on its surface that will retard water evaporation, or if for some other reasons water evaporation is slow, then the process of hydrogen bond re-formation to lock-in the shape in motion will be slow as well. This means that the amount of hydrogen bonds that form at each instant will be so slow that the gel structure will not have the strength to counter the internal shape-recovery forces coming from the real permanent shape. Consequently, the hair wavy pattern produced by hair movement will not be locked-in. For the temporary shape to be locked-in, the numbers of hydrogen bonds that form per second during drying have to be enough to build a hardened mass in the gel structure, so that its strength would be able to counter at any time the internal recovery forces coming from the permanent shape.

By the same token, if the ingredients favor faster drying, then the gel phase in the hair fibers will harden more rapidly, and the wavy shape in motion will be entrapped more efficiently. This analysis explains what cosmetic formulations and beauticians already know, namely, that the types of ingredients applied to hair and the rate of hair drying both have an effect on hair volume. The design of formulations that increase volume thus contain ingredients that in some way or another modify inter-fiber friction, hair drying rates, hydrogen bond formation, or permanent shape.

c. Back-combing and “static fly away”

Back-combing and “static fly away” are two different processes also often used to increase volume. They do not involve the creation of temporary hair shapes by free fiber movement and are based on a different mechanism. Back-combing essentially consists of increasing inter-fiber friction to very high levels by lifting cuticle cells (see Figure 34). The protruding cuticle cells created in this manner interlock the hair fibers at various points. The idea is to totally restrict inter-fiber slippage and movement so the bending deformations introduced during back-combing are maintained. This technique is effective in creating volume; however, it is very damaging to hair. Another way to produce volume in hair is by creating electrostatic repulsion between each hair fiber. Electrostatic repulsion in hair, also referred to as “static fly away” by the cosmetic

community, is very common in dried hair, and is due to the buildup of an excessive electric charge in hair. This charge is usually of negative polarity and can also be introduced artificially onto the hair fibers.

Figure 33) Caption showing process of back-combing ("teasing") to produce hair volume

Figure 34) Captions of hair swatches showing effects of "static fly away." There is an attraction between hair fibers of each swatch due to the relative amounts of negative charge in each one, i.e., the hair tress on the right contains a higher level of negative charge buildup (64).

The process of creating an excess of electrical charge in hair fibers is not a difficult task since hair is an electrical insulator, having very poor electrical conducting characteristics. Therefore, hair, like other electric insulators, is prone to accumulate charges on its surface. Charges are easily transferred from one electrical insulator to another when their surfaces enter into intimate contact. The direction of electric current, or flow of electrons, from one material to another has been studied and categorized for a host of insulating materials and put into a table usually referred to as the triboelectric series (see Table I). Materials at the top of the chart have an excess of negative charges trapped into their structures, and therefore tend to transfer electrons to those at the bottom of the chart. In

Table I, it can be seen that hair is below rubber, which is the material often used to make combs. When hair is combed, its surface enters into intimate contact with the rubber in the comb. Electrons are transferred from comb to hair, and thus the hair acquires a negative charge.

Table I) List of various insulating materials and their position in the triboloelectric series (65).

Since all combed hair fibers acquire the same type of charge, they repel each other, thereby creating a larger distance between them and, ultimately, increasing the hair volume of the assembly. Unfortunately, negative charge in hair does not last long since it escapes via moisture in the hair. Also, the presence of moisturizing ingredients on the hair surface reduces its ability to build up charge. Thus, when hair is highly moisturized, it cannot be charged no matter how many times it is rubbed against the comb. The deposition of certain polymers on the hair surface, however, increases charge buildup and can thus create an apparent effect of volume. The production of “static fly away” to increase volume in hair assemblies is, however, difficult to control as it depends on the rubbing intensity against the comb, i.e., excessive rubbing gives rise to excessive “static fly away.” Furthermore, “static fly away” also causes unwanted repulsion or attraction of hair assemblies in proximity, leading to frizzy hair and other unwanted effects (see Figure 34).

3.3.4.10 FRIZZ IN HAIR AND CHANGES IN SHAPE

Frizzy hair can be defined as the visual aspect of a hair assembly resulting from hair fibers that are positioned geometrically out of synchrony with the remainder of the bundle. The out-of-synchrony effect can be very small or large and can come from a few hair fibers, or almost the whole hair assembly (see Figure 35). The maximum radius of curvature of a frizzy hair fiber separating it from the

bundle can be of a few mm or larger (i.e., 1 or 2 cm). Out-of-synchrony effects appear in hair usually immediately after hair drying, and they are more pronounced in hair that has been chemically treated or in hair whose surface has been damaged. In non-frizzy hair, either curly or straight, all hair fibers are aligned in synchrony with their respective neighboring hair fibers. However, in hair with frizz, multiple hair fibers in various sections of the hair assembly separate randomly away from it, forming irregular-shaped curls or waves that are out of synchrony with the rest of the fibers in the bundle (see Figure 35).

Figure 35) Caption of frizzy hair produced by excess bleaching (left), and by repetitive hot ironing (right). Observe in both cases the large number of single hair fibers that separate out of synchrony from the bundles.

The causes of frizz arise mainly from the inability of hair fibers to recover their permanent shape during or after grooming deformations; i.e., after combing, drying, either by towel, blow-drying, or by hair movement during washing. The inability of hair to recover its permanent shape is caused by damage to the hair structure where the shape memory resides. When this happens, the hair partially or totally loses its permanent shape. Frizz can also be caused by excessive damage to the hair surface. Virgin and healthy hair has its permanent shape memory and also its surface intact. As a result, when the hair fibers are deformed, all of them respond in the same manner. In other words, when they are deformed by motion during grooming practices, they rapidly recover their shape and form an aligned bundle.

In contrast, damaged hair has its memory structures or surface damaged. This damage can be in the form of a partial destruction of disulfide networks in the amorphous phase, or in the form of irreversible denaturation of the crystalline phase. It could be said that hair fibers that present frizz do not remember their permanent shape, and therefore, are partially shapeless. Thus, when damaged hair fibers are deformed, depending on their degree of damage, they respond with shapes that are random and different. For instance, if the various damaged hair fibers undergo excessive stretching or bending during washing, combing, or

towel drying, their stretched or bent shape may stay like that or transform into a random shape. The result is that after the hair fibers are dried, their shape is out of synchrony with the remainder of the bundle.

Frizz caused by excessive damage in the cuticle cells of hair fibers is due to very high inter-fiber friction. As was previously discussed, the recovery and final shape in hair fibers during movement is determined by the interplay of the following factors: inter-fiber friction forces, hair drying rates, hydrogen bond formation in gel-structure, and ability of hair to recover its permanent shape. Those hair fibers in a bundle that present high levels of cuticle lifting will not be able to recover their permanent shape after the hair bundle is washed and dried. The reason for this is that high degrees of cuticle cell lifting causes high inter-fiber friction during fiber motion and this phenomenon results in a poor recovery of their permanent shape. Thus, in this case, the hair fibers separate from the bundle, making it appear frizzy. Another cause of frizz in hair comes from excessive static fly away. Typical examples of hair that develop frizz are excessively bleached hair, excessively dried hair, hair often treated with hot irons, and hair damaged during combing/tangling because of a lack of proper conditioning.

Frizz in hair can be reduced or alleviated by temporarily or permanently reconstructing the shape of frizzy hair. This can be done with hot irons, water-setting treatments, or even with treatments that introduce a new shape by re-forming disulfide bonds. When frizz is corrected with temporary treatments such as water-setting or hot irons, the problem is not solved, as the hair fibers will become frizzy again after washing. If it is caused by excessive surface damage, then, frizz can be reduced with treatments that smooth cuticle cells or reduce excessive inter-fiber friction. In many cases, frizz is caused by a combination of damage to the disulfide bond network, damage to the hair surface, and by the appearance of static fly away. In this case, frizz reduction can only be accomplished by a combination of various treatments.

3.3.4.11 BODY IN HAIR AND CHANGES IN HAIR SHAPE

The subjective perception of Body is one of abundant hair that is strong and healthy. It manifests itself during hair movement as a combination of hair mass and hair shape. The closest mechanical descriptor for body is the flexural rigidity of a bundle of fibers. Unlike other mechanical properties that do not take into account shape, flexural rigidity combines Bending Modulus and shape of

materials (66). This property recognizes the fact that the same mass of any material is easier or more difficult to bend depending on the way that it is shaped. For instance, it is difficult to make a sheet of paper stand up straight on a surface because it flexes and bends down easily. However, if we fold the same sheet of paper in half it will be able to stand without bending. Thus, just by changing its shape we have changed its flexural rigidity (see Figure 36).

Figure 36) Effect of shape on the flexural rigidity of a sheet of paper. The sheet of paper cannot stand up straight on a surface because it flexes and bends down easily. Observe that after folding it in half the sheet of paper is capable of remaining straight.

Figure 37) Effect of hair curliness on the flexural rigidity of hair. The bundle in the left with curly hair is able to maintain almost a straight inclination in cantilever without flexing, while the bundle in right with straight hair fibers bends more easily (64).

When we apply these concepts to hair we realize that is easier to flex a hair bundle of very fine hair when its fibers are in a straight fashion, than when a bundle of similar mass has its fibers tightly coiled (see Figure 37). Thus, shaping the fine hair fibers into a coiled configuration allows them to present higher flexural rigidity and higher body. The tighter the coil in hair fibers, the more body the hair assembly has. Furthermore, flexural rigidity in a solid body does not change with movement. However, in a hair bundle, since the hair fibers are

not tightly attached to each other, this property may change if the interplay of inter-fiber friction, hydrogen bond breakage with moisture, and drying rates are not properly set for that purpose. For instance, Figure 38 shows two hair tresses with their half-lengths standing up and their tips pointing upwards. The bundle at the left was treated with a solution of cationic particles that increased inter-fiber friction, while the bundle at the right does not contain any product on its surface. The bundle at the left is stiffer than that on the right, and it shows higher flexural rigidity and more body because its pre-formed shape was not allowed to change by the high inter-fiber friction in the bundle.

Figure 38) Effect of inter-fiber hair friction on the flexural rigidity of hair. The hair swatch at the left was surface treated with cationic particles and is able to maintain an upward position vertically without any support, while the hair swatch at the right with no treatment bends more easily (64).

Body in hair is, therefore, dependent on the following three properties:

a) hair fiber density, i.e., number of fibers per unit area,
b) intrinsic protein density,
c) inter-fiber friction, and
d) temporary shapes created in hair fibers.

Since cosmetic treatments cannot change properties (a) and (b), in order to increase body, formulators always try to maintain temporary shapes with high flexural rigidity by changing properties (c) and (d). Products that increase body are, therefore, those that can influence and create the formation of temporary

shapes with high flexural rigidity, i.e., strong wavy patterns, high inter-fiber friction, inter-fiber spot welding, high drying rates, *etc.*

3.3.4.12 HAIR SHAPE CHANGES INDUCED BY STYLING POLYMERS

Styling polymers have been used for many years to produce or enhance temporary changes in hair shape and to create fashionable styles that are difficult to achieve by water-setting. The ways to produce these styling shapes vary from beautician to beautician but they can be divided into static and dynamic processes.

Styling static processes involve the following steps:

1) applying a new shape to hair with the help of rollers or other devices while the hair still contains ~ 60–80% of its moisture,
2) applying the formulation containing the polymer,
3) letting the polymer and hair dry, and finally,
4) removing the rollers or devices.

Styling dynamic processes involve, on the other hand, the following general steps:

1) inducing movement in hair fibers with blow-dryers or fingers while the style is being created,
2) applying the polymer as the hair moves to entrap an average shape, and
3) stopping movement and polymer application and letting the hair dry.

From the mechanistic point of view, both polymer styling processes are similar to the static and dynamic water-setting described in previous sections. The only difference is that when a styling polymer is used, these materials are applied to hair via various formulations to produce temporary shapes and/or to enhance their durability. In most cases the mode of action of styling polymers in changing hair shape is external and limited to the production of seam and spot-welding junctions between hair fibers to restrict inter-fiber movement, thereby giving rigidity to the hair assembly (see Figure 39). The polymer by itself does not affect the memory shape or intrinsic structure of hair. In other words, the polymer molecules do not interact with hydrogen bonds, disulfide bonds, or the crystalline structure of hair.

Figure 39) Micrographs of a bundle of hair fibers showing formation of seam and spot welding by polymers (64)

However, formulations used as polymer vehicles may contain water or solvents that penetrate into the cortical cells and enhance or inhibit plasticization of the hair gel structure by breaking or re-forming hydrogen bonds. As will be seen later, these effects are not due to the polymer per se and have an influence on how rapidly the temporary shape is entrapped and locked-in. In the following paragraphs an analysis is made of the influence that polymers have in assisting changes in hair shape. Special attention will be given to how the polymers modify the interplay of hair inter-fiber friction, hair drying rates, hydrogen bond formation in gel-structure, and hair strength to recover its permanent shape.

The effects that formulations have on these processes will also be reviewed but to a lesser extent. Properties and composition of the various polymers used in hair styling applications will not be reviewed here, but for information purposes a list of the most common is given in Table II.

3.3.4.13 ROLE OF STYLING POLYMERS IN CHANGING HAIR SHAPE

As was previously discussed, the water-setting of hair, or the entrapment of a hair shape in movement without polymers, is very simple and controlled mainly by the interplay of hair inter-fiber friction, hair drying rates, hydrogen bond formation in gel-structure, and hair strength to recover its permanent shape. Hair shape entrapment with styling polymers adds, however, various levels of complexity to these factors not only because the polymers play externally a role on hair shape formation but also because the formulations influence the shape.

The added levels of complexity brought about by polymers and formulations are due to the following parameters:

1) rates of solvent evaporation; this will determine how rapid the polymer welding seams and spots dry and harden,
2) hair plasticization by the solvent; mainly when water is used as the solvent,
3) polymer glass transition temperature (Tg); this parameter determines how hard/brittle or soft/ductile is the spot welding, and
4) strength of adhesive joints at the hair/polymer interface. In the following paragraphs a discussion of all these factors will be made in relation to each one of the main formulations used in the styling of hair.

a. Temporary setting of hair with aerosols

In terms of entrapping a hair shape in motion, aerosol formulations are the simplest to analyze because the polymer is usually dissolved in ethanol or in combinations of this solvent with water. The reasons for the simplicity stem from the following facts: 1) the ethanol/water solution produces a very low level of hair plasticization; 2) the solvent mixtures have high rates of evaporation and, therefore, the polymer "welding spots" harden very rapidly, entrapping transient hair shapes efficiently. Thus, provided that the polymer or resin has the appropriate Tg, and that it is properly formulated and delivered from the aerosol bottle, and that the concentration of cationic charge along the polymer chain is sufficient, it will rapidly form welding seams and spots across hair fibers, restricting their motion and thereby entrapping the transient hair shape produced by movement (see Figures 40 and 41).

Figure 40) Illustration of hair in mannequin with styling polymer applied (left) and caption showing welding seams and spots (right)

Figure 41) Picture showing action of spraying a styling polymer to entrap shape of hair in motion

Aerosol formulations are also used to enhance temporary shapes produced statically, i.e., when the hair is not in motion. These types of applications do not have the complexities of entrapping an average shape produced by hair motion. The phenomena of fiber-to-fiber slippage, fiber bending, and fiber recovery from instantaneous shape deformations are absent in these cases. Temporary shapes produced under these conditions are preset by any of the methods described before for water-setting or hot irons under static conditions. In these cases the polymers are not used to entrap or capture a temporary shape from hair assemblies that are in motion, but rather are used to enhance long-lastingness of the preset temporary shape.

b. Temporary setting of hair with gels and mousses

Styling formulations like gels and mousses are based on water-soluble polymers and contain high amounts of water. Therefore, they have a double influence on shape formation while the hair is in motion. The water in these formulations diffuses into the hair and partially plasticizes the hair's amorphous phase; meanwhile the polymer/water system is able to provide a certain amount of hold to entrap a temporary shape. With polymer gel or mousse formulations the hair is put into motion with the fingers or a comb to create a temporary shape. During the application step, these formulations are administered to the hair surface with a very high shear rate. Thus, such pseudoplastic behavior results in a low enough viscosity at the high rates of shear to apply easily and effectively, but on stopping the application, the rate of shear goes to zero and the apparent viscosity rapidly increases to a very high value, thereby preventing the hair from recovering its permanent shape. Higher viscosities at zero shear rate (i.e.,

polymers that form a high-yield stress) are optimal for this approach.

Even when these formulations still contain high amounts of water, they are able to suppress internal hair recovery forces and inter-fiber slippage, providing a transient hold. Once the desired shape is attained the polymer/water system is allowed to dry, and subsequently the polymer hardens, forming “welding spots” and also seams, i.e., points of attachment across multiple hair strands, both of which restrict inter-fiber motion and give flexural rigidity to the temporary shape.

c. Mechanical properties of welding seams and spots

The complexity of hairstyle and hold ultimately depend on the mechanical properties, size, and distribution of welding seams and spots. Properties such as the glass transition temperature (Tg), susceptibility of the polymer to be plasticized by moisture, and adhesive strength at the polymer/hair interface are crucial in the final retention of temporary shape or style hold. Aerosol or sprays produce an irregular distribution of spot welding while gels and mousses yield more uniform welding patterns (see Figure 42). Furthermore, the welding patterns left by gels are, in particular, thicker and more uniform, and this explains why gel formulations, which create high-yield stresses at zero shear rate, are more suitable to create extreme styles. The distribution and amount of polymer that remains after gel application is such that the hair undergoes a transformation from a pure hair assembly to a polymer/hair composite with very different mechanical properties (68–71). The formed polymer/hair composite, depending on the polymer properties, is able to create shapes with very high flexural rigidity that can withstand high levels of relative humidity (see Figure 43).

Figure 42) Micrograph of a bundle of hair fibers showing seam welding by a gel formulation.

Figure 43) Effect of two gel formulations on the temporary hold of hair after five seconds of water immersion. The hair of the mannequin at the left was treated with a gel containing a polymer with a high Tg and low resistance to moisture, while the hair of the mannequin at the right was treated with a polymer with a high Tg but with high resistance to moisture (67).

d. Effect of polymer glass transition temperature (Tg) on styling

The glass transition temperature of a polymer is a parameter that relates to its viscoelastic behavior. Once the style is set and hair shape has been changed, the polymer welding seams and spots must withstand stress/deformations in the hair assembly arising from head movement and from the hair's own weight. The polymer also has to efficiently counter recovery stresses coming from the memory shape of hair in the amorphous and crystalline phases. These stresses will push the hair fibers for recovery of their permanent shape.

If the Tg of the polymer is too low or if its value decreases because of water plasticization, the spot welding will flow like a viscous "chewing gum" and will not be able to retain the temporary shape (see Figure 44). In contrast, if the Tg is too high the polymer will be very hard and brittle and the seams and spots will break by stress/deformations arising from hair movement. The final effect will be losses in the hairstyle and the production of "flakes" in the hair (see Figure 45).

Figure 44) Micrograph showing effects of low Tg in the seam welding of a styling polymer (65).

Figure 45) Micrograph showing flaking of a polymer due to a very high glass transition and lack of plasticizer (64).

These two effects are better visualized in Figure 46, where three typical graphs depicting changes in hair Elastic Modulus as a function of temperature are shown. The graphs correspond to three hypothetical polymers (A), (B), and (C) with different glass transition temperatures. In theses graphs, the solid line (a) represents the location of room temperature, the dotted line (b) the glass transition temperature (Tg) of the polymer, and the curved line (c) represents the variation of Elastic Modulus with temperature.

Figure 46) Graphs showing the relationship between Elastic Modulus and Temperature for three polymers with different glass transition temperatures (64).

Observe in this graph that when the glass transition temperature (Tg) of the polymer is way above room temperature, i.e., polymer A, the polymer is very stiff, and we will expect that the welding spots and seams will also be very hard and stiff. In the graph at the center with polymer B, the Tg is close to room temperature, and therefore, the seams and spots will be flexible. Finally, in the graph at the right with polymer C, the Tg is below room temperature and the seams and spots will behave extremely soft and will flow viscously. The glass transition of hair therefore has to be properly adjusted so it can provide the necessary hold without having the inconveniences of being very soft or extremely hard.

e. Adhesive strength at the polymer/hair interface

The adhesive strength at the polymer/hair interface of welding seams and spots is another important parameter in maintaining hairstyle (68, 69). The reason for this is that the polymer must be able to counter permanent shape-recovery stresses coming from the disulfide bonds and crystalline regions, and these stresses are transferred from hair into polymer via the adhesive joints. Further, the polymer needs to comply with the strong forces of hair contraction, which are produced during the de-swelling of hair. If the bonding strength of the polymer with the hair surface is weak and breaks, either because of contraction or recovery stresses, then there is no resistance to recovery, and the hairstyle will be lost soon.

In summary, styling polymers help in locking more elaborate hairstyles and temporary shapes, but they also have limitations in terms of the temporary shapes they can induce. For instance, styling polymers, no matter how high their Tg is, or how they are formulated, may not be able to straighten extremely curly

hair, or vice versa, they may not be able to produce tight curls in very straight hair. The reason for this is, again, that the polymer cannot counter the strong recovery forces coming from the permanent shape stored in the hair's memory. *Styling polymers can however contribute substantially to attaining these changes when combined with other treatments, i.e., with hot irons or other chemical treatments.*

The styling polymers used currently also have limitations in terms of complying with increasing demands of performance and with restrictions in the use of volatile organic compounds in aerosol formulations. These issues have put a lot of pressure on polymer designers and formulators to create novel ingredients that can meet performance and regulatory issues. New polymers are required to be completely soluble in water and/or at least to be partially soluble in it. This task represents a major challenge because in terms of performance, styling polymers are required to resist plasticization by moisture. Polymer synthesis scientists have responded to these challenges by creating polymers with particular pendant groups and domains that appear to provide water solubility and yet are able to transform into hydrophobic materials once they cast into dried films. The field of polymer innovations is vast and most certainly in the years to come there will be a solution to all these issues. Examples of some of these styling polymers are shown in Table II below.

Gels	**Styling Polymers – Sprays**

PVP Polyvinylphrrolidone	Octylacrylamide/Acrylates/Butylaminoethyl Methacrylate
VP/Dimethylaminoethylmethacrylate Copolymer	

Copolyme	
PVP/ DMAPA Acrylates Copolymer	VA/Crotonates/Vinyl Neodecanoate Copolymer
Hydrolyzed Wheat Protein/PVP Crosspolymer	

Acrylates Copolymer	
VP/VA Copolymer	Acrylates/Dimethicone Copolymer (Elasteesse)
Vinyl Caprolactam/VP/	Digylcol/CHDM/Isophthalates/SIP Copolymer
Dimethylaminoethylmethacrylate Copolymer	Octylacrylamide/Acrylates Copolymer

Acrylates Copolymer	Ethyl Ester of PCM/MA Copolymer
PVM/MA Copolymer	
Polyquaternium-4	
Polyquaternium-11	

Chitosan

Chitosan PCA	
Polyacrylate-3	

Mousses
Polyquaternium-4
Polyquaternium-11

Hydrophilic Polyester Polyurethane

PVP	
VP/VA Copolymer	
VP/Dimethylaminoethylmethacrylate Copolymer	
PVP/ Acrylates/Lauryl Methacrylate Copolyme	
Hydrolyzed Wheat Protein/PVP Crosspolymer	
Acrylates/ Allyl Methacylate Copolymer?	

Acrylates Copolymer	
Polyquaternium-37	

(Table II) List of main hair styling polymers

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EYELASHES: ANATOMY AND CONDITIONERS FOR INCREASING LENGTH AND FULLNESS/THICKNESS

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ABSTRACT

This chapter describes the anatomy of eyelash hair and conditioners as well as methodology to beautify one of the most beguiling substrates related to beauty.

The history of eyelash beautification is a long one. It was first documented in Egypt with the use of a mixture of donkey feces and kohl pigmentation to paint on lashes. Lash transplant was developed about a decade ago. Commercial lash conditioners first arose in early 2000 and were composed of oil and vitamins. They only produced modest results. A flurry of prostaglandin-containing lash conditioners were developed in 2007 with one published study described below. Subsequent second-generation bioactive peptide lash conditioners and recent third-generation adipose stem-cell secreted peptide are currently on the market. In view of the vast changes in the technology, and the importance consumers place upon beautiful eyelashes as an essential part of their overall approach to looking good leading to feeling good, this chapter is worth the read.

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3.3.5.1 LASH ANATOMY:

Eyelash hair is cosmetically unique and different than the rest of the hair on our body. Eyelashes are much shorter than scalp hair. Lashes are approximately 4–14 mm in length, depending on location, race, and age. Eyelash follicles extend about 2 mm deep into the dermis. Due to thinness of the eyelid, sectioning reveals a thin epidermis with the absence of hypodermis.

The upper eyelid contains approximately 300–400 eyelashes arranged in multiple rows in triangular or pyramid shape. Lower eyelashes have shorter and fewer lashes, with generally about 100–150 lashes on the lower lid.

All eyelashes are characterized by a tendency to bend from the bulb to the tip of the shaft. The degree of curvature is dependent on the asymmetric distribution of keratin 38 in the hair bulb, resulting in a curved fiber. Curvature is noted most in the African race, followed by Caucasian and least in Asians.

In contrast to scalp hair, the eyelash is free of arrector pili muscle. There are roughly 50 glands in the upper eyelids and 25 glands in the lower eyelids. Two types of glands, known as Zeis and Moll glands, surround the eyelash follicles. Zeis glands are unilobular sebaceous glands. They are found at the margin of the eyelids, producing an oily substance into the mid-portion of lash follicles. Moll glands are modified apocrine sweat glands. Meibomian glands are sebaceous glands secreting lipid to form a tear film. Melanocytes are found in very high density in three areas: the basal layer of the eyelid epidermis, the ORS, and the eyelash follicle.

3.3.5.2 LASH GROWTH CYCLE:

Length of eyelash cycle is much shorter than scalp hair, with anagen one to four months and telogen four to eight months. By comparison, scalp hair anagen phase is two to six years and the telogen phase (the transition from resting into the active growth phase) is three months. During the anagen (growth) phase, average daily growth rate is 0.12 mm with range $\pm$0.05 mm. Approximately 60–85% of eyelash follicles are in the telogen phase, in comparison to scalp which is only 15%. Thus duration of the eyelash anagen phase is strikingly shorter, with

growth rate lower than that of the scalp hair.

It has been observed that the use of lash conditioners increases the percentage of lashes in anaphase from 20 to 90% and extends the duration from four months to two years. Although the exact cellular mechanism is not well defined, it has been suggested to be through the regulation of intracellular c-AMP levels but has not yet been proven (16).

3.3.5.3 LASH CONDITIONERS:

a. History:

Synthetic prostaglandin analogues, used for the treatment of glaucoma in the form of ophthalmic drops, have been shown to result in the desired cosmetic side effect of increased eyelash length, number, and thickness (2–6). This is likely due to follicles entering the telogen phase prior to entering the anagen phase.

b. Safety Assessment:

To achieve the desired cosmetic goal of eyelash beautification by means of increasing length, number, and thickness, the novel synthetic compound dechloro ethylcloprostenolamide was developed in 2007 and its safety was assessed.

Human exposure with Human RIPT (Repeat Insult Patch Test) in 209 individuals confirmed no dermal irritation or sensitization. *In vitro* toxicity in mammalian cell was assessed and demonstrated to be non-cytotoxic. Local lymph node assays (LLNA) indicated negative for evidence of delayed dermal sensitization. Ames test evaluation by independent clinical testing (unpublished) in five salmonella strains indicated dechloro ethylcloprostenolamide is nonmutagenic. Ocular safety testing at an independent IRB-approved testing site by an ophthalmologist was conducted with four-week use in contact lens wearers, noncontact lens wearers, and individuals with self-assessed sensitive eyes. Individuals underwent serial ophthalmologic exams and questionnaires. No ophthalmic or ocular adverse effects were observed. There was no measurable change in intraocular pressure. It was concluded that the test material did not demonstrate a potential for eliciting ophthalmic irritation and is clinically safe. Safety study revealed no change in intraocular pressure, lacrimal duct, and conjunctiva, and was deemed safe for contact lens wearers and those with sensitive eyes.

c. Study:

In Choy et al., subjective reports by users of MD Lash Factor to improve the appearance of eyelashes—especially in regard to increasing length—was assessed (1). A randomized, double-blind, clinical trial was conducted with 34 healthy volunteers randomly assigned to one of three different treatment groups. These groups consisted of varying concentrations of the key ingredient (dechloro ethylcloprostenolamide, a novel synthetic topical prostaglandin analogue) and a placebo group. **Results:** All treatment groups demonstrated a statistically significant increase in eyelash length after four weeks of daily use. Group A: 1.69 mm, Group B: 1.94 mm, Group D: 1.44 mm and Group C (placebo): 0.63 mm. Twenty-four percent subjectively reported an increase in the thickness or fullness of their eyelashes. There were no complications. Fifteen patients reported minimal initial transient irritation. No discoloration was noted. **Conclusion:** Subjective reports by users of improved appearance in their eyelashes were confirmed. Use of dechloro ethylcloprostenolamide had minimal side effects.

Table I. Summary of results.

	Pre-application	After 4 weeks of application			
	Mean lash length±STD	Mean lash length±STD	Change in lash length (range)	% Change (range)	p-Value
Control group A, $n = 34$	3.46 mm±1.03	5.15 mm±1.13	1.69 mm (0–4.3)	49% (0–145)	8.78×10^{-8}
Group C (Placebo), $n = 11$	3.10 mm±0.70	3.73 mm±0.64	0.63 mm (0.1–1.5)	20% (3–51)	1.0×10^{-2}
Group B, $n = 14$	3.69 mm±1.15	5.63 mm±1.25	1.94 mm (0.2–4.2)	53% (3–156)	9.50×10^{-6}
Group D, $n = 9$	3.42 mm±1.12	4.87 mm±0.95	1.44 mm (0.4–2.0)	42% (10–87)	1.32×10^{-5}

d. Discussion:

The hypertrichotic properties of topical prostaglandin analogues used in the treatment of glaucoma have been well described in the literature (2–6). Elgin *et al.* (6) describe a 0.67 mm mean increase of eyelash length in 20 adults after six months of latanoprost therapy. Similarly, Sugimoto *et al.* (2) report a significant quantitative increase in eyelash length (0.7–0.8 mm) after two to ten weeks in 17 patents treated with latanoprost for glaucoma. Johnstone and Albert (3) also describe a greater thickness of eyelashes and additional lash rows as well as an increase in the length of lashes with topical latanoprost. Prostaglandin analogues used in these studies were in the form of ophthalmic drops. In Choy *et al.* study (1), the novel topical prostaglandin analogue, dechloro ethylcloprostenolamide, was applied directly to the eyelash roots, resulting in a significant increase in eyelash length at different concentrations of the active product as well as a subjective enhancement of eyelash thickness in 24% or the patients. The absolute and relative increase in eyelash length observed with dechloro ethylcloprostenolamide was greater than that reported in the literature with ophthalmic preparations.

Iris pigmentation and darkening of the periocular skin are reported as rare side

effects of topical ophthalmic prostaglandin (7–10). Neither of these side effects was observed in patients using dechloro ethylcloprostenolamide. Abelson *et al.* (11) describe hyperemia with topical ophthalmic bimatoprost peaking one day after the first instillation and returning to near-baseline levels with continued use. Only one patient reported moderate irritation and discontinued the product, whereas 15 patients reported mild irritation with the use of dechloro ethylcloprostenolamide. However, this mild irritation was transient and in most cases resolved within a few day of use.

Latanoprost has been described in the treatment of alopecia areata for cutaneous use and eyelash formation (12, 13). One of the study patients had alopecia areata. This patient was in a prior study for four weeks using topical ophthalmic bimatoprost on lashes with minimal eyelash enhancement. After four weeks of topical dechloro ethylcloprostenolamide, a significant increase in eyelash length (100%) was observed. Furthermore, four months of follow-up data were obtained in 21 patients who continued to use the active product daily and exhibited a mean increase in eyelash length approaching an 80% increase from baseline. During first year of use of dechloro ethylcloprostenolamide in MD Lash Factor in 70,000 patients, no confirmed serious adverse reaction or iris discoloration was noted. The mechanism of action is unknown.

e. Newer-Generation Lash Conditioners:

Second-generation prostaglandin-free formulations have produced similar effects to an earlier prostaglandin formulation, with consumers generally noting longer usage duration needed to achieve noticeable efficacy. Active ingredients are cytokines/bioactive peptides, antioxidants, prostagladin precursors, and vitamins. Recently, a novel class of peptides derived from adipose-derived stem cells has been developed with no published data yet available on efficacy.

f. Observations with lash conditioner usage:

Users of lash conditioners will note new hair emerging from areas where one typically does not have lashes, such as the medial and lateral corners of eyes folds. Users with light lash colors will first note increase in length and thickness of lashes followed by color within the lashes. In our monitoring of 70,000 patients during the first year of use in all ethnicities, three cases of Hispanic users noted lashes falling out during first two months of use with a gradual return of lashes over a two-month period. This is likely due to catagen follicles entering the anagen phase. No confirmed iris pigmentation changes were

reported in 70,000 patients in 2009 by this author.

Similar hair enhancement has been noted from lash conditioner on brows and scalp. An independent clinical testing facility conducted open enrollment of 30 healthy subjects (50% male and 50% female) ages 18–45 with men with grade II–III Hamilton and women with grade I–2 to I–4 Ludwig classification. Dermatologists ruled out chronic skin issues, concurrent hair loss treatment, or treatment known to cause hair loss within six months. Follow-up was made at 42, 84, and 121 days. Data analysis of self-evaluation and photos was taken. Data analysis of self-evaluation involves establishing frequency tables to each qualitative question. Two percentages Z1 and Z2 are calculated as follows: Z1 = favorable opinion (Ex: "Completely agree" + "Somewhat agree"); Z2 = unfavorable opinion (Ex: "Completely disagree" + "Somewhat disagree"). The statistical difference in frequencies between favorable and unfavorable opinion is evaluated using the Chi-squared test at 5%.

g. Results:

1. 88% agreed that there were little to minimal discomfort while using the product.
2. 96% felt product was easy to apply.
3. 71% agreed product helped with hair growth with 17% noted improvement at four weeks, 38% noted at eight weeks and 8 % noted at 12 weeks.
4. 50% agreed product helped with thickness of hair.
5. 63% agreed improved volume of hair.
6. 46% agreed improved softness of hair.
7. 75% would recommend product.

CONCLUSION:

MD® Follicle Energizer showed subjective findings of improved hair appearance and user acceptance. This material may be considered as an adjuvant treatment to concurrent hair loss therapy.

FUTURE DEVELOPMENT:

Usage of proteins secreted by adipose-derived stem cells

A Question for Thought and New Directions in Hair Growth Products:

"Given the rich stem cells residing in hair follicles, is it feasible to

generate new hair follicles?"

A first attempt at arriving at an answer to this provocative question lies in the following quote from the American Journal of Cosmetic Surgery by *Hirotaro Fukuoka, MD, PhD; Hirotaka Suga, MD, PhD; Keigo Narita, MD; Rei Watanabe, MD; Satoru Shintani, DDS, PhD:*

"Scalp hair was noted to regenerate after topical application of such a protein."

The author's preliminary data on injection of stem cell–derived purified growth factors yielded interesting results after one treatment in four weeks in scalp hair. Such a regimen may be feasible for lashes and eyebrows as well.

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SUBSTRATE:
THE NAILS

Part 3.4
Substrate:
The Nails

PART 3.4

THE NAILS

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ABSTRACT

This chapter discusses the structure of nails and describes in detail various abnormalities that may occur. Since nails and their appearance are a significant

part of beauty/cosmetic care, it is important to understand the various issues that make nails unsightly and what can be done about them. The discussion also focuses on the potential effects of various chemicals used in nail beautification and provides critical thinking directions for improving nail appearance.

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3.4.1 INTRODUCTION -TOENAILS AND FINGERNAILS

The primary function of toenails and fingernails is to protect the distal aspect of the digits. They protect the bones and surrounding soft tissues from injuries.

They enhance tactile sensation to the digits. Nails are also used as a tool to increase precision gripping of small objects by human beings.

a. Fashion

Originally, polishing of the nails dates back to 3000 BC in China and later in Egypt. The color of the nail polish symbolized social classes [1]. The modern history of polishing the fingernails and toenails as a fashion statement dates back to the beginning of the 19th century. Oils, powders, and creams were applied to the nails and the nails were buffed, leaving them shiny [2]. Today, most nail polishes are derivatives of pigments in solvents.

b. Function

The nails are located at the distal ends of both the fingers and the toes on the dorsal aspect. Their maintained function is to protect the digits from trauma [3]. The curvature of the nail helps to define the shape of the distal end of the digits. Although there is a complete absence of nerve endings inside the nail, nails enhance the tactile sensation of the digits with pressure on the surrounding soft tissues and their nerve endings. This property, along with their useful ability to grip small objects, allows humans to have a more precise use of the fingers.

c. Anatomy

The "Nail Unit" consists of the following parts:

- Nail plate (*Corpus Unguis*)
- Nailbed
- Medial nail groove
- Lateral nail groove
- Distal nail groove (*Hyponychium*)
- *Epionychium*
- Posterior nail groove (Cuticle)
- Nail root (lunula, Matrix, Matrix Unguis)

The nail plate is the actual hard part of the nail. It is made of the tough protein keratin. *Unlike the keratin of skin*, the nails do not peel off in the form of scales. The keratin of nails also contains a much lower lipid content than skin, which allows much less water flux across the nail.

The nailbed in the skin below the nail plate contains living dermal tissue, which includes capillaries and glands. This vascular area gives the nail plate a pink appearance where it is adhered. At the distal end, the nail plate appears

whiter in color where it is separated from the vascular nailbed.

The medial and lateral nail grooves form the indentation on the lateral borders of the nail plate. They are sometimes called the nail folds.

The distal nail groove, called the hyponychium, is at the end of the digit where the epithelium of the skin meets the nail plate. It forms a seal that protects the nailbed from infection and trauma.

The epionychium is a thin band of epithelium that connects the nail plate to the proximal skin.

The posterior nail groove, called the cuticle, is a thin layer of epithelium that extends from the skin over the epionychium and the proximal end of the nail plate.

The nail root, also call the nail matrix, is the germinal tissue that is responsible for producing cells that become the hard nail plate. The nail root extends 5–10 mm proximal to the posterior nail groove. The visible part of the nail root beyond the skin is called the lunula and appears as a half-moon-shaped, lighter-colored region at the proximal part of the nail.

d. Development and Formation

The earliest signs of fingernail development appear in the ninth week in utero. Fingernails and toenails develop by the same process; however, toenail development is slightly slower and about four weeks behind that of fingernails.

Skin folds and grooves begin to form at the distal end of the fingers and will eventually define the structure of the nail unit. The first structure to appear is the matrix at week 11. By week 20, matrix cells exhibit adult keratinization.

At the week 32 a hard nail plate of the finger is formed. At birth, a long thin nail plate is present, which overhangs and curls over the distal digit.

In the discussion of nail formation, one must look at each component of the nail unit and what it contributes. The proximal nail fold is the wedge-shaped structure on the proximal edge of the nail unit. Its dorsal side differentiates to normal epidermis, while its ventral side differentiates to form the cuticle, which adheres to the dorsal surface of the nail plate.

The hyponychium is similar in that the distal portion contributes to the normal epidermis of the volar skin, while the proximal portion helps in adhering to the nail plate.

The matrix differentiates to form the nail plate as we know it. It occurs by specialized tissue kinetics, as described by Zaias [4], who showed this in

monkeys and rats by introducing a radio-labeled marker; this was later confirmed by Norton [5] in humans. These studies materially advanced our understanding of matrix kinetics.

The nailbed epithelium differentiates both in an upward and a lateral motion. The upward growth produces specialized epithelium, which adheres in a special interconnecting manner to the developing nail plate, thus aiding in the adherence of the nail plate to the nailbed.

The lateral growth of the nailbed epithelium is toward the distal edge and moves at the same rate as the nail plate formation. Other authors have suggested different theories of nail plate formation, included here primarily for historical reasons [6, 7].

The matrix lies flat in the proximal nail unit. It produces a sheet of onychocytes (corneocytes), which grow diagonally along its entire length and move distally in a contiguous fashion. This development of multiple sheets of onychocytes eventually forms the nail and produces the dorsal surface of the nail plate; the midportion of the matrix produces the midnail plate; and the distal portion produces the ventral surface of the nail plate. Based on this anatomical arrangement, it can be concluded that the thickness of the nail plate is directly related to the length of the matrix.

A disruption of any of the processes discussed earlier will produce specific diseases of the nail at specific sites, which will be discussed later in this chapter.

3.4.2 HISTOLOGY, ULTRASTRUCTURE, AND COMPOSITION

The epidermis of the proximal nail fold is similar to the normal epidermis dorsally except that it is devoid of adnexa (sebaceous glands, eccrine glands, hair, arrector pili muscle), while ventrally it produces the cuticle. The epidermis of the nailbed is similar to that of the skin, but has no stratum lucidum or stratum granulosum layers and no hair follicles or sweat glands. There is a unique arrangement of the dermal papilla and rete ridges, which form a parallel pattern in the nailbed and interconnect in a tight fingerprintlike pattern to the nail plate. The nailbed moves forward with the growth of the nail plate [4, 5]. The epidermis of the matrix is thick and passes into the substance of the plate, which is formed by changes similar to keratinization in the epidermis; however, the end product is nail plate instead of stratum corneum.

The appearance of the lunula probably reflects incomplete keratinization in the

matrix just before incorporation into the nail plate [8]. The nail plate is made up of impacted and adhering layers of flattened cornified cells (dead onychocytes) that have lost their nuclei. The cells contain hard keratin, similar to that of hair with a high sulfur content mainly in the form of cystine, which comprises about 9–12% of the weight of the nail.

The keratin fibrils are primarily oriented parallel to the nail surface from side to side. The nail typically contains about 7–12% of moisture and 0.15–0.76% of fat, a little more (1.38%) in infants. The composition of the nail plate varies slightly from the early *in utero* plate to that in adults. Baden [9] showed that water content of the nail plate is related to environmental relative humidity. Calcium constitutes about 0.02–0.04% of the weight and does not contribute to the hardness.

Other trace elements have been detected and measured in nail clippings. The nailbed and matrix have a rich supply of blood from two arteries that run laterally along the digits and form oxygen-rich capillary beds, which lie below the nail plate [3, 8].

3.4.3 RATE OF NAIL GROWTH

Nails, unlike hairs, grow continuously throughout life. The rate of growth of the nail plate is determined by the turnover rate of the matrix cells. Early measurement of the growth rate in undergraduates and schoolchildren were made by notching the nail about 2 mm from the margin of the lunula and recording its progressive displacement in each succeeding month. No differences were found within the age range of 19 to 23 years, no sexual difference, and only a very slight difference between hands, with growth on the right being faster. However, there were differences between fingers, growth being fastest on the third digit and least on the fifth finger. Bean [10] studied his own thumb for over 30 years and found growth to be faster during the second to third decades, followed by a slowing-down trend and then a leveling-out over time. The rate of fingernail growth varies between 0.5 and 1.2 mm per week. Fingernails grow faster than toenails which vary between .2 and .4 mm per week. Family tendencies favoring similar growth rates have been noted, as well as increased growth during the summer and diminished growth in cold climates. Certain conditions affect the rate at which the nail plate grows. On average, it is higher in psoriasis, pregnancy, and nail-biting trauma. Regrowth is temporarily depressed in infective diseases, particularly in viral conditions such as measles

or mumps, yellow nail syndrome, and starvation [3]. To replace a fingernail completely takes about five to six months; toenails require 12 to 18 months.

3.4.4 NAIL PATHOLOGIES

Abnormalities of structure and appearance of the nails are more than cosmetic. Many work hours are lost each year due to absenteeism of patients who suffer from nail disease. There are many causes of nail disease: genetic factors, hereditary factors, infection, inflammatory, environmental, traumatic, secondary to systemic disease, biomechanical considerations, and idiopathic as well as the effects of aging. Skin and nail changes begin to occur as people age. During the aging process, the fingernails and toenails have been shown to thicken and show a slowing ingrowth while becoming more susceptible to diseases [11]. Nail problems also occur during pregnancy and consist of transverse grooves, increased brittleness, softening, and no separation [12].

Diseases and disorders of the nails can be classified into conditions according to: maladies peculiar to the nails themselves, onychodystrophies, nail manifestations of dermatitis, nail manifestations of systemic disease, and congenital condition of the nails. Disease also can be grouped into different classifications according to the type of disease, including: infection, psoriasis, contact dermatitis, eczematous dermatitis, hypo-vitaminosis, tumor, trauma, and genetic disease.

Only the most common conditions are discussed here, but readers may consult complete texts such as Scher and Daniel [8] for a comprehensive account of the various conditions that affect the nail unit.

a. Absence of Nails (Anonychia)

Complete absence of nails from birth, anonychia, is rare and appears to be associated in several ways with other congenital, hereditary defects. Absence of nails may be individual, multiple, or total in some congenital conditions. Loss of nail plate may occur with some inflammatory conditions such as lichen planus and irritant dermatitis to artificial nails, or secondary to trauma.

b. Nail Shedding (Onychomadesis)

Nails can be lost either by loosening at the proximal end or by separation from the nailbed. Shedding can follow trauma or severe illness or can be caused by drugs. More serious loss with scarring sometimes follows trauma, defective

circulation, lichen planus, epidermolysis bullosa, or drug eruptions.

c. Nail Separation from the Nailbed (Onycholysis)

The separation of the nail from its bed is fairly common. There are many causes, but quite often none can be found. Nail separation may result from external damage both traumatic and self-induced, that is, overaggressive cleaning, fungal and yeast infections, acute and chronic dermatitis, or drug eruptions. Various conditions such as psoriasis, tumors, and many genetic disorders can also cause onycholysis [3, 8]. Of particular interest are reports of onycholysis caused by cosmetic products [13, 14] and the use of acrylic [15] or artificial nails that cause a contact or irritant dermatitis leading to nail separation [16]. Nail hardeners containing formaldehyde [13], nail varnish containing phenol, ultraviolet nail dryers [17], and similar agents have all been implicated. Once onycholysis is present it is not unusual for colonization by either a Candida sp. or Pseudomonas sp., giving the nail a yellowish or green color, respectively. Often onycholysis is treated by removing the distal part of the nail that is not attached to the nailbed. This is usually a painless procedure. Once the separated nail is removed, emollients are applied to the nailbed and distal end of the nail plate daily in order to soften the skin and nail and to get the nail to reattach to the skin as it grows distally.

d. Brittleness

Brittle nails are among the most common cosmetic complaints. The phenomenon tends to occur in advancing age. While sometimes they may be associated with some underlying conditions, no good data are available, and the causes are mostly not known but are known to be related to nutritional disturbances, thyroid disorders, and skin conditions. Environmental factors are certainly important. Nails are kept pliable by their moisture content, and long thin nails are especially susceptible to very dry climates. Frequent immersion of the hands in surfactant solution is conducive and some time ago it was believed that soaps and detergents removed the protective lipids from the keratin. The continual use of nail varnishes and varnish removers have also been blamed [8]. Almost 60 years ago the reactions of 25 businesswomen to the same brand of nail lacquer were studied. It was found that nearly half of them showed brittleness and splitting of the nails while the rest were unaffected. Thus, there is no support for attributing nail brittleness to the use of conventional nail lacquers. Some investigators have reported improvement with gelatin and application of essential oils, but no

consistently effective therapy is known. Other researchers recommend avoidance of frequent immersion and nightly use of emollients.

e. Striations (Onychorrhexis)

Longitudinal striations are common in healthy nails and become more prominent with aging, and other clinical or occupational conditions such as lichen planus, psoriasis, and trauma. More prominent longitudinal ridging is seen in median nail dystrophy, which is attributed to habit tics (self-induced behavior). Transverse, horizontal striations can occur as a developmental anomaly. Severe depressions, known as Beau's lines, indicate a period of severe systemic disease, such as measles, mumps, pneumonia, rheumatoid arthritis, myocardial infarction, pulmonary embolism, high fever, or coronary thrombosis. Drugs may also induce inhibition of normal matrix production of nail plate. They can also be caused by trauma. Thinning of the nail plate is seen as secondary to decreased peripheral circulation, lichen planus, iron deficiency anemia, and epidermolysis bullosa.

f. Spoon-Shaped Nails (Koilonychia)

In koilonychia or spoon nails, the nails are thin, soft, and concave in the center. The condition results from iron-deficiency, and is usually, but not invariably, associated with anemia. It can also be seen in onychomycosis and has been noted in motor mechanics, where, perhaps, it is due to softening as a result of the use of oils or soaps. This condition may also be seen as a temporary disorder in young children and is also a cemented congenital anomaly. Plummer-Vinson syndrome (a combination of koilonychia, dysphagia, and glossitis) is primarily seen in middle-aged women and is also called Plummer's nails.

g. Splitting (Onychoschizia)

The splitting of nails horizontally, parallel to the nail plate, so that pieces of the surface break away, is very common in women and in advanced age. The main causes are probably repeated immersion in water and the use of nail polish and polish removers. The suggested treatment is to rub emollients into the nail plate after bathing and avoid frequent use of nail polish and removers.

h. Pitting

Pitting of the nails is found most commonly in psoriasis. Pits represent punctuated or depressed sites in the nail plate secondary to irregular matrix

production. They can vary in size, shape, depth, and number. Pitting is the most common manifestation of nail psoriasis [21] but can also be seen in chronic dermatitis, fungal infections, alopecia areata, Reiter's syndrome, lichen nitidus [22], and lichen planus. Minor degrees of pitting may be seen in healthy nails, and when no other skin complaints are present, they are considered to be normal variants.

i. Leukonychia

Leukonychia is complete or partial whitening of the nail plate and can be divided into acquired and congenital types. Complete leukonychia occurs very rarely as an inherited abnormality; often, epidermal cysts on the glabrous skin are associated with it. Partial leukonychia is very common and may take the form of white spots or white transverse streaks. It may be associated with many types of illnesses, exposure to chemicals, and traumatic events to the matrix. A white appearance of the nails is not necessarily due to leukonychia; it may result from changes in the nailbed, not in the nail plate.

j. Onychomycosis

Fungal infections of the nail unit are the most common conditions affecting nails. There are four distinct types of clinical presentation: distal subungual, superficial white, proximal subungual, and candida albicans onychomycosis. Distal subungual onychomycosis is the most common type and has been shown to be an autosomal dominant inherited condition in the chronic dermatophyte syndrome. The discoloration begins at the distal edge of the nail and spreads proximally. Onycholysis begins with thickening and irregularities of the nail plate and causes it to separate from the nailbed; subungual debris begin to accumulate in secondary bacterial infections. Proximal subungual onychomycosis starts at the proximal nail fold. It often begins as a small white area located proximally on the nail plate and subsequently enlarges and spreads. Superficial white onychomycosis produces a white powdery appearance. Candidal onychomycosis is usually seen in patients with mucocutaneous candidiasis syndrome. It begins by directly invading the nail plate itself rather than extending onto the nail from the adjacent skin. It is much more prevalent in the toenail than the fingernails.

k. Paronychia

Paronychia is defined as inflammation of the proximal or lateral nail folds or a

combination of the two. Paronychia occurs after loss of the cuticle either by trauma or by aggressive cuticle trimming or cuticle pulling. It can be acute or chronic. Acute paronychia is caused by introduction of an infectious agent into the nail folds, usually through trauma, ingrown nails, cuticle pulling, or exposure to an irritant agent. Paronychia with bacterial infections often needs medical attention to drain the infection and remove the ingrown nail as well as treating with antibiotics. Chronic paronychia occurs over time due to continual exposure to an irritative behavior, chronic ingrown nails, constant exposure to water from hand washing, or a specific contactant such as food items or chemical irritants.

l. Discoloration

Nails may be discolored for a wide variety of reasons. External causes include hair and other chemical dyes, smoking, and chemical compounds such as mercury salts, dithranol, and picric acid. Tints may leak out of nail varnish and after repeated use may penetrate the nail [17–20]. The use of artificial nails and either acrylic or pre-formed plates can cause discoloration. Abnormal formation or very slow growth of the nail can also produce color changes. Psoriasis and onychomycosis, among other abnormalities, cause opaqueness and a yellowish discoloration. In the “yellow nail syndrome” the nails almost cease to grow and several months later become yellow or greenish. They may also be thickened and curved from side to side. Yellowish, green, or gray nails can also occur as growth of nails slows down in old age. Infection by *Pseudomonas aeruginosa* under the nail may cause a greenish, black, or blue discoloration. Systemic drugs can alter the color in many ways. Prolonged administration of tetracycline may occasionally turn nails yellow. Mepacrine or Zidovudine (AZT) makes them bluish black, and chloroquine may produce blue-black pigmentation of the nailbeds.

The pigmented discoloration or pigmented bands are the most important types to distinguish. Although most pigmented bands seen in nails represent benign lesions, such as nevi or traumatic or splinter hemorrhages, one must always be suspicious of pigmented bands that are malignant, such as malignant melanomas. Any new onset or rapidly changing pigmented band in the nail should be evaluated by a dermatologist for possible biopsy. Early diagnosis can save serious sequelae of malignant melanoma.

m. Subungual hematoma

Subungual hematomas may cause the collection of blood underneath the nail

plates. It is often associated with acute trauma. When associated with acute trauma, there is often severe pain from the pressure of the blood underneath the nail. Subungual hematomas can also occur more slowly with repetitive trauma. Often these are not painful at all. This is often seen in runners and dancers.

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SUBSTRATE:
THE NOSE

THE NOSE:

Accessing the Biology of Human Olfaction: New, All-Natural Fragrance Ingredients; Novel Consumer Fragrance Experiences and Applications

by

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ABSTRACT

Contemporary fragrance creation

Contemporary fragrance ingredient discovery and fragrance creation relies on sensory testing and evaluation methods by humans. Perfumers or experts discover new fragrance ingredients and they create new fragrance formulations while human sensory studies comprising panels of nonexpert or naïve human subjects are used for further evaluation. Whereas contemporary fragrance creation methods have been developed over decades and are considered the gold standard precisely because of their central reliance on humans, recent trends suggest that this process alone may soon become obsolete due to its lack of speed and throughput. First, consumer demand for products that are all-natural, economic, and sustainable is growing. In some consumer applications, synthetic ingredients have become a mainstay in the fragrance industry. At the same time, it is only becoming harder to identify natural fragrance ingredients that can be sourced reliably. Many natural ingredients are sourced from equatorial regions that are forecasted to suffer most severely from global warming and political instability. As a result, pressure for the rapid and reliable discovery of new, all-natural fragrance ingredients is increasing. In order to discover new fragrance ingredients for all-natural and sustainable products, new discovery methods that enable rapid and reliable testing of hundreds of millions of ingredients and extracts are required.

Next-generation fragrance creation

Borrowing from the fields of cell biology and drug discovery, the application of

cell-based high-throughput screening (HTS) methods stand to enable the rapid and reliable testing of hundreds of millions of ingredients and extracts to aid in new fragrance ingredient discovery. Biologically, the sense of smell in humans is mediated by a family of ~350 odorant receptors. In principle, laboratory cells can be created to comprise odorant receptors. These cells could then be used as miniaturized detectors for “turbo smell testing” in cell-based assays to detect new fragrance ingredients. Ingredients identified as active in the cell-based assays could then be evaluated in human sensory studies or by perfumers. Importantly, the success or reliability of any cell-based assay depends on how closely it is produced to mimic the natural or native biology of the receptor it is designed to comprise. Recent advances in the science of cell-based assay creation reported across receptor classes—including drug, taste and odorant receptors—suggest that it is now possible, for the first time, to produce these assays such that they mimic the physiology of their intended receptors to a much greater degree than had been previously possible. In the context of olfaction in which such an especially large number of receptors are implicated, the advent of physiologically relevant cell-based assays in combination with expert and naïve human sensory methods will allow more rapid and reliable fragrance creation.

In this chapter we review:

(i) the background on the biology of olfaction and the sense of smell

(ii) the basic design and principles of miniaturized cell-based assays for high-throughput odorant ingredient discovery

(iii) novel classes of previously inaccessible natural fragrance ingredients that stand to alleviate the emerging bottlenecks, including natural ingredients that augment, diminish, or otherwise modulate the sensory profile of fragrances, and the forward-looking and previously unknown applications that these enable, and

(iv) recent understanding of the biology of odorant receptors as it relates to the creation of a fragrance-discovery engine with unprecedented high fidelity to the natural sense of smell.

In addition to facilitating the discovery of new natural ingredients and combinations of ingredients with desired aromatic properties, a systematic cell-based discovery engine could produce new fragrance ingredients for more precise further investigation of the biology and basis of human olfaction as well as create novel consumer products and applications. All in all, the advent of a rapid and reliable odorant ingredient discovery engine could open the gates to a whole new world of aroma research and product development.

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The Biology of Olfaction and Fragrance Ingredient Discovery and Creation

3.5.1 ODORANT RECEPTORS

Odorant receptors (ORs) are the biological entities that mediate the detection of scent. In humans, a family of at least ~ 350 genes encoding ORs has been identified. (1) This family or group of ORs are G-protein coupled receptors, or GPCRs. GPCRs are a classification of protein molecules localized in the outer membranes of cells. The ~350 ORs are integral membrane proteins; they traverse the cell membrane and comprise domains that are intracellular, transmembrane, or extracellular. While it is thought that certain odorant molecules may penetrate the cell membrane to impart their activity by interacting with transmembrane domains of the ORs, it is generally thought that it is the extracellular domains of ORs that mediate the detection of odorant molecules, as these domains would be most accessible to volatile compounds in the nasal cavity.

The ~350 GPCRs that have been described as odorant receptors are defined as such based both on their sequence homology and relatedness as well as their expression in cells that line the nasal epithelium. However, not each of these ORs has been confirmed as an odorant receptor. In addition, it is possible that other receptors in the nasal cavity and elsewhere may also play a role in human olfaction.

It may be helpful to think about the large family of ORs as the keys of a piano keyboard, where some odorant molecules will hit or activate one single receptor, or key, essentially sounding off one single aromatic note, whereas other more complex ingredients or substances may hit an entire chord, or several odorant receptors, all at once. Depending on which ORs are activated and how strongly,

a different smell may be experienced or perceived in the brain.

3.5.2 CELL-BASED HIGH-THROUGHPUT ODORANT DISCOVERY'PRINCIPLES AND BASIC DESIGN

Cell-based high-throughput screening of ORs

High-throughput cell-based screening of ORs requires laboratory cells that are treated to comprise ORs. Typically, for any given OR, a gene encoding the OR must first be introduced into cells, and the OR that is encoded must be synthesized by the cell and transported to its outer (plasma) cell membrane, or cell surface. The preparation of cells used may be transiently transfected cells or they may be stable cell lines. Transiently transfected cells refer to populations of cells that are freshly introduced with the gene encoding the OR, where a new batch of cells is produced each time, as required for testing. Stable cell lines refer to clonal populations of cells where a single cell introduced with the OR gene is selected and used to create a clonally prepared population of genetically identical cells. The advantage of using transiently transfected cells includes speed, as the time required to produce a stable cell line is not necessitated, whereas the advantage of using stable cell lines includes identification of a population of cells with desired and well-characterized properties. There are many methods available to achieve either transiently transected cells or to produce stable cell lines, and these must be carefully considered given the host cell type used and the gene to be expressed in order to achieve the most physiologically relevant cells and assays. In this chapter, data for the use of stable cell lines are presented.

In addition, creation of laboratory cells that comprise ORs that function as they do in their natural setting *in vivo* is challenging. The cells of the nasal epithelium that typically express ORs *in vivo* are highly specialized, polarized cells that are not approximated by typical laboratory cell cultures, which comprise cells derived from various tumors or are otherwise immortalized. Current methods for the production of cells suitable for HTS of ORs are described in the last section of this chapter. Once cells comprising the OR are produced, a cell-based assay that can be used to monitor the activity of the OR must also be produced. The activation of many classes of GPCRs, including ORs, results in an influx of calcium into the cytoplasm or interior of the cell. Therefore, the use of fluorogenic dyes that can be used to report the presence

and concentration of calcium is common. Figure 1 shows cell-based assay responses for a panel of eight cell lines, each comprising a different native and full-length OR (See Figure 1).

Figure 1 A cell based assay was used to test the activity of a set of 8 different ORs in response to an activating compounds. Dose response curves are shown. For each OR, a compound with an EC50 value of less than 100 µM is shown. The assay response (Y-axis) over time (X-axis) is shown.

High-throughput screening and discovery of new fragrance ingredients

Once a cell-based assay is created, it must be miniaturized to high-throughput format, where typically 384-well plate or 1536-well plate microtiter well plates are used to interrogate the activity of many different samples on the OR at any given time. As the testing of a plate requires on the order of one to five minutes only, hundreds of millions of extracts and ingredients may be tested on a 24-hour basis using standard automated and scalable screening instrumentation.

In HTS assays, it is important that the assay response is sufficient and consistent for reliable discovery of modulators. That is, in each well tested with an activator of the receptor, the signal-to-noise ratio of the response generated in the cell-based assay compared to buffer-only control must be great enough to enable reliable detection of either a modulator or blocker. In addition, this assay response must be consistent from well to well, to enable comparison of testing results for different compounds and ingredients across a plate. One common measure of the suitability of a miniaturized cell-based assay for HTS is the value for the Z´ Factor of the assay. The Z´ Factor of an assay is derived using an equation that takes both the signal-to-noise ratio and the well-to-well consistency of the assay response into account, with values ranging from 0 to 1. (2)

Typically, a cell-based assay with a Z´ Factor value of 0.4 or greater is considered suitable for HTS. Figure 2 shows OR cell-based assays with Z´ Factor values suitable for the HTS for the same set of eight receptors as depicted

in Figure 1.

Figure 2 The same set of ORs as shown in Figure 1 were miniaturized to 384-well plate HTS assay format. Responses for the testing of the 384-well plate assays with an activating compound (dark shaded) or buffer-only control (light shaded) are shown. Z' values of at least 0.4 were generated for each OR.

Once cell-based assays with a suitable Z´ Factor are established, the first step in discovering new odorant ingredients for a given application is to determine which of the panel of ~350 known ORs are relevant for the desired application. To do this, a panel of cells representing the entire family of ORs is exposed to the aromatic substance and the ORs that are activated in response to it are identified. To identify the ORs that correspond to the aroma of a rose extract, for instance, the receptors that respond upon exposure to the extract are selected. Aromatic substances like rose extract often comprise hundreds or thousands of aromatic ingredients. It is the combination of the aromatic or sensory profile of each ingredient that produces the net or perceived sensory experience or fragrance. Often, the key aromatic notes of a composite fragrance are derived from one or a few of these ingredients. Careful testing and analysis must be used to select the most meaningful set of ORs for HTS for any given aromatic substance of interest. Figure 3 shows the response of 320 compounds and extracts tested across the same set of ORs as shown in Figure 1 using the same HTS assay as shown in Figure 2.

Figure 3 320 test compounds were tested against the same set of ORs

as shown in Figure 1 using the same 384-well HTS assay as shown in Figure 2. Responses of the ORs to each compound are shown. **The assay response (Y-axis) over time (X-axis) is shown.**

An important consideration for fragrance discovery is that certain closely related natural extracts may be differentiated from each other by one or a few ingredients or notes. For instance, the extract from a particular subspecies of rose that is especially desirable compared to other rose extracts may be differentiated by the presence of one or a few particular aromatic constituents. By profiling the OR activity that is elicited across the entire panel of ORs for various rose extracts, those ORs that correspond to the unique aromatic notes of the preferred rose extract may be identified. In a next step, high-throughput screening using cell-based assays comprising these ORs may be used to identify new, natural ingredients that can be used to help replicate the aroma of the preferred extract. It may be that a number of different ingredients are required to activate the entire complement of relevant ORs.

Typically, a high-throughput screen identifies hundreds of prospective “hits,” or compounds of interest. Further testing and evaluation are then required to confirm which of these impart the desired fragrance experience in human sensory studies.

Sensory confirmation, iterative screening, and optimization

Once initial compounds of interest are identified in a high-throughput screen, human sensory studies are required to characterize the sensory properties of the compounds. For instance, in a screen designed to identify compounds that mimic the scent of a rose, the identified compounds would be tested compared to the gold standard using human subjects. Typically, compounds identified directly in a first round of screening must be improved upon to meet desired final characteristics. Ideally, a high-throughput screen yields compounds a significant percentage of which are confirmed in such downstream testing as having the desired or expected properties that would be predicted by the screen. The correlation between cell-based high-throughput assays and downstream assays is greatly dependent on the quality of the cell-based assays and the degree to which they are physiologically relevant detectors. Reliance on OR cell-based assays that comprise only full-length, native, and unmodified receptors is critical for this reason; any alteration of the native ORs could greatly diminish the physiological relevance of the resulting assay.

Regardless of the quality of the assay, more often than not additional testing and work are required to result in compounds that meet all technical success criteria. Even given a fully physiologically relevant screen, the compounds that are identified may have additional off-target activity or effects, such as activity on untested receptors or side aromatic notes. Sensory testing is therefore an important tool in prioritizing the hit compounds. In order to improve upon the compounds, additional rounds of screening may be necessary. Here, a library of compounds that includes compounds structurally or chemically related to the initial hit compounds is assembled and screened. Similarly, in the case that the initial hits are extracts, a second round of testing can include extracts related to the initial extracts. Iterative rounds of testing and downstream sensory evaluation can be used to find optimized derivatives of the original hits that most closely or exactly produce the desired sensory effect.

By designing the screening library to include extracts and ingredients derived from natural sources of unlimited supply or ones that are renewable, a limitless number of new compounds can be made available for iterative cell-based and human sensory testing. Each aromatic plant or substance often comprises thousands of volatile ingredients. Various preparations of these can be used to amplify the number of test compounds in the library. For instance, a library can contain extracts derived from a plant species where the plant species is cultivated under multiple different conditions or in various locations or geographies, or different parts of the plant can be extracted separately. In addition, the extracts can be treated in a variety of ways, including by heating, exposure to acids or bases, or incubation with enzymes to create even further chemical diversity. We can begin by limiting the library to natural ingredients that have a past history of human consumption and limiting its treatment to steps that are not industrial in nature. In a manner similar to cooking, or any other reaction that can take place in a common kitchen, we can provide access to the advantage that the included test substances will be generally regarded as safe, based on existing protocols for the assessment of safety. This, in turn, facilitates downstream human sensory studies, compared to novel or human-made compounds that must be extensively tested before they can be studied using human subjects.

The Extra Challenge

First and foremost, consider that in the case of odorant receptor biology and discovery, no fewer than ~350 receptors are implicated, where most cell-based

drug discovery relates to one or a handful of receptors. To navigate this space effectively, there must exist not only a robust cell-based discovery platform that is as comprehensive and physiologically relevant as possible, but expert sensory testing and input must be used to as a guide to calibrate, or "tune," the cell-based platform. In this connection, the analogy to the complement of ORs as keys of a piano keyboard is helpful. Imagine a complex aromatic substance or extract, for instance jasmine or mint extract. These substances may well interact with a large number of odorant receptors. In addition, some of the individual compounds contained in each extract may hit a cognate receptor hard and punctually, whereas other ingredients may exert their effect over time and more softly. A complete mapping of these substances across the entire known complement of ORs in addition to their known sensory attributes will be key to effectively understanding how to interpret the cell-based data generated during any discovery process. Access to the entire human olfactory system is required for effective discovery. Any effort at cell-based discovery that aims to rely on a representative subset instead of on all the known receptors carries the risk of missing important interactions.

3.5.3 NEW INGREDIENTS AND APPLICATIONS

Natural substitutes

In some fragrance applications, reliance is made on natural extracts that are of limited supply and high demand. In some instances, this has necessitated a shift towards the use of synthetic compounds and is in direct opposition to the expanding trend of sustainability and responsibility described elsewhere in this book. Often, it is challenging to exactly replicate the aromatic profile of the natural aromatic using the mixture of synthetics. In some cases there is a need for improvement, as consumer demand for the natural sensory experience is high and remains unmet. Furthermore, for natural fragrances that are in demand but of limited supply, development of new related fragrances is often impeded.

In this connection, the OR discovery engine may be used to identify new natural ingredients that can substitute for the original ingredients. In a first step, ORs corresponding to the desired aroma would be identified by exposing the panel to the aroma or to its signature components, if these are known.

Next, this subset of cell-based OR assays would be used in high-throughput screening to test millions of natural extracts, ingredients, and combinations of

these, in order to identify additional new ingredients that mimic the gold standard. Those ingredients that are identified may also have undesired characteristics. For example, such characteristics may have activity on additional ORs or undesired aromatic notes that are detected in downstream sensory testing. In these cases, additional rounds of iterative screening can be used. In each successive round, one would include prior hits in the library to be screened as well as a new set of test ingredients related to the original hits. For instance, if a substance in wild lily is found to partially mimic the desired scent of a rose but there is an undesired aromatic characteristic also, then the same wild lily, but cultivated in a variety of ways, may be used to prepare new libraries of extracts for testing. Additionally, a desired scent may be replicated, for instance, by combining two or more identified ingredients—each of which acts to impart a part of the aromatic profile of the gold standard.

Fragrance enhancers and fragrance blockers

Perhaps the most intriguing ingredients that stand to be discovered using a cell-based odorant discovery engine are fragrance enhancers and blockers. By definition, these ingredients work to modulate the sensory profile of other fragrance ingredients.

Using the analogy of music, a fragrance enhancer is defined as an ingredient that may or may not have its own aroma, which at certain concentrations, works to amplify or “turn up” the volume of a given desired fragrance or aromatic note. Similarly, a fragrance blocker is defined as an ingredient that can decrease or “mute” the smell of an undesired fragrance or malodor. Anecdotal examples of such ingredients have been known, often discovered randomly when a perfumer finds that one ingredient alters the sensory profile of other ingredients to which it is added. However, systematic discovery of such ingredients using perfumery alone has been challenging, as it is difficult to test any large number of random ingredients in combination with aromatic ingredients in order to identify those rare ingredients that can work as fragrance enhancers or fragrance blockers. The difficulty of accomplishing this is compounded by the fact that *any* molecule active on the odorant receptors can likely be expected to have an intrinsic odor of its own, as it is likely to interact with one of the large number of known odorant receptors. As such, classical methods would require thousands of test ingredients to be assessed, one by one, and at various doses against a key aromatic sample the sensory experience of which is desired to be amplified or muted. While skilled perfumers work to create fragrances, they are not machines

that can be used to test thousands of samples. Thus, the cell-based platforms for high-throughput cell-based discovery provide an approach to accelerating the effectiveness of perfumers in the design of a virtually endless supply of new fragrances.

In order to rapidly discover fragrance enhancers or blockers of a given aromatic substance, first the odorant receptors that correspond to that substance must be identified. In a next step, these odorant receptors would be exposed to the aromatic substance to record the response of the receptor. Next, test compounds would be added to monitor changes in this response. Because of the nature of high-throughput screening, hundreds of thousands of test ingredients could be tested at different doses per day. Such a process would allow the identification of those ingredients that work to either amplify or diminish the response of odorant receptors to the aromatic ingredient. These initial hits would then need to be further evaluated as described.

This approach can include a second round of testing using the high-throughput cell-based platform, for instance, to run dose-response studies. An ideal enhancer or blocker would be identified as one that increases or decreases the receptor activity at increasing doses of the test ingredient. Figures 4 and 5 show dose-response curves for fragrance enhancers and blockers, respectively, discovered using cell-based HTS.

Figure 4 Dose response curves for two potentiators each active on at least two ORs are shown. To generate the dose response curves, increasing concentrations of the potentiating compound (color plot) or no compound (dark plot) were tested on the OR in the presence of the same concentration of activating compound. EC50 values for the potentiators were calculated.

Figure 5 Dose response curves for compounds with blocking activity are shown. In the top panel, two different blockers each of which blocks the activity of one OR are shown. In the bottom panel, two ORs each blocked by the same compound are shown. Dose response curves are shown. IC50 values for the blockers were calculated.

Next, sensory studies would be needed to confirm the activity of the ingredients identified in the screen in human sensory studies. Sensory testing would also work to identify any unintended or undesired intrinsic sensory characteristics of these ingredients, or doses at which none are registered. Finally, once ingredients that are confirmed as active in human sensory studies are identified, the process is repeated. In each round, this is done by sourcing additional ingredients that are structurally or chemically related to the initial positive hits for an iterative round of testing and are designed to identify related ingredients with improved sensory profiles and characteristics.

Even with a high-throughput discovery engine, the systematic discovery of fragrance enhancers and blockers is still expected to be challenging. This is because of the large number of odorant receptors. Any ingredient must have activity across the entire subset of target receptors such that the desired sensory profile is experienced by a human subject, and no activity on any unintended receptors produces an undesired sensory profile or off-note. To discover those rare ingredients that act across the array of odorant receptors in exactly the manner that results in the desired sensory profile is no small order. Because the nature of high-throughput screening is such that it allows testing of millions of ingredients and combinations of ingredients, it is possible to identify hundreds of initial hits such that a systematic process for the discovery of final ingredients that meet all required criteria can be envisioned.

Again, the likelihood of success in any such attempt is predicated on the fidelity of the cell-based discovery process; that is, the odorant receptors that are provided for testing using cell-based assays must be accurate in *in vitro*

correlates of native odorant receptor function and physiology *in vivo*. This had been a challenge to achieve, until now.

Novel applications

The advent of methods for the systematic and reliable discovery of fragrance enhancers and blockers ushers in a new era for fragrance creation and a set of novel applications. A number of these are highlighted for illustrative purposes.

First and most basic, a fragrance enhancer can work to decrease the amount of a fragrance ingredient that is used in order to impart a desired aromatic profile. If a certain aromatic is limiting, less of it may be used. In cases where synthetic chemicals have been relied upon to mimic the sensory profile of a rare or prohibitively expensive natural extract or essence, often these fail to exactly replicate the gold standard. Fragrance enhancers, which can be limited to natural ingredients themselves, could be used in this case to enable reliance on the actual natural extract or essence only. This solution not only satisfies consumer demand for the use of all-natural ingredients, it also accomplishes a cost-saving as less aromatic is required.

Second, the discovery of fragrance blockers that can be used to block malodors including body or bathroom odor can be contemplated. Such ingredients can displace the use of strong, human-made chemicals presently used to mask such odors. Simultaneously, a consumer-friendly goal is reached in that the use of excessive chemicals may be curtailed. Perhaps of greatest interest to the perfumer, blockers of the aroma of body odor and of ingredients such as alcohol can eliminate these smells such that perfumes can be appreciated unadulterated by malodor or alcohol solvents.

Future potential

Perhaps most intriguing, the advent of reliable high-throughput discovery of aroma ingredients opens up entirely new applications for the cosmetic and personal care industries as well as many others. It is difficult to even predict what these may be. To capture the imagination, a number of possible future applications are described, many of which depend on progress in unrelated fields. First, many experts and laypersons alike have wondered whether emotions or moods that can be triggered by a particular scent have a biological basis. Is the smell of chamomile soothing and calming, or are we accustomed to thinking that it is? It is possible that using ever-advancing methods of brain imaging, more data can be generated to help us address these questions: Do we

see the same brain activity patterns in response to smelling a soothing aromatic as we do when in a state of calm? Do multiple calming aromas all result in the same brain activity? Does the smell of a forest of greenery evoke the same brain activity as images that we associate with freshness?

Diverse studies will need to begin to explore these questions, including brain imaging and also physiological and sensory studies. Methodologies for each of these will need to be refined and developed. However, the advent of high-throughput discovery of odorant ingredients provides one previously missing key element: for the first time, it could be possible to identify aromatic ingredients that are characterized as activating just a single or a very small subset of odorant receptors. The availability of such ingredients where the biological basis for their activity is defined provides a toolkit of reagents to probe the human response. Theoretically, all those receptors activated by chamomile could be identified, as well as aromatic substances that selectively activate each of these. Use of such aromatics in brain-imaging studies could be used to test whether any of these produce the calming patterns described. The availability of a cell-based HTS platform could allow the identification of such aromatic ingredients that could aid in the understanding of the biology and physiology of human olfaction in this and other applications. Imagine if it would be possible to find aromas that make us feel fresh and in a state of wellness and well-being.

At a more practical level, consider how unavailable or unreachable the sense of smell has been to the average person: Whereas, until now, we can play notes and music, or paint brush strokes and paintings, it has only been the trained perfumer who can truly “touch smell.” The advent of fragrance enhancers could be used to enable consumer access to the art of fragrance creation. A home stereo system, if you will, could be imagined in every home, where at the touch of a button the consumer would call to order various notes, like mint or sea breeze, all contained in miniature chambers. Small amounts only of hundreds of aromatics would be needed, as enhancers could be used to increase the activity of tiny doses. One could paint the mood of their home or room. If one creates a new fragrance, one could email the recipe to a friend for them to conjure it up in their own home, or a new fragrance that is released could be downloaded from a perfume website for easy sampling at home.

3.5.4 METHODS

In the preceding sections, the basic principles and design for high-throughput odorant discovery were introduced, and novel classes of aromatic ingredients that could be discovered have been outlined. For the first time, cell-based access to odorant receptors in their physiologically relevant form exactly as they exist in nature has been shown. The figures presented demonstrate the utility of this technology to access a subset of odorant receptors that represent the broad diversity of odorant receptors *in vivo*. Most significantly, these data point to the need of working with cell-based assays that exactly mimic native receptor biology and physiology as the bedrock and fundament for any cell-based discovery engine that utilizes cell-based assays. Taken together, the following were demonstrated:

1) Cell-based assays comprising native full-length odorant receptors can now be created.
2) This has been done for a panel of odorant receptors representing the various classes.
3) Cell-based assays suitable for high-throughput screening were developed.
4) Fragrance enhancers were identified.
5) Fragrance blockers were identified.

In this section, the state-of-the-art will be reviewed. Briefly, the odorant receptor biology field is rich and diverse, with hundreds of laboratories both academic and commercial exploring various aspects of odorant receptor biology. The purpose of this section is to outline the one key bottleneck that had existed, and to review recent advances that have overcome this limitation: Until very recently, it had not been possible to express odorant receptors in their native and intact form, exactly as they exist in nature, in the context of laboratory cells required for use in HTS. It has been reported that this is because in these cells, any OR that is expressed becomes trapped in the ER compartment of the cell. (3,4)

To by-pass the difficulties that were encountered, most laboratories have largely relied on one of two methods to enable expression of odorant receptors in cells: Either the receptor is truncated, for instance by cleaving off residues from one or both ends, or the receptor is extended, by adding additional residues that facilitate its expression in laboratory cells.(5, 6)

Though never desirable, these alterations were necessitated to achieve any cell-surface expression at all. It had been hoped that these alterations were otherwise benign; however, absent native odorant receptor cell-based assays, the testing to confirm this was lacking. Moreover, it is not unreasonable to question

that alterations that make the gross difference of enabling a receptor to be expressed at the cell-surface in the first place have only this desired effect—to facilitate laboratory practices and no unintended consequences. The risk had always been that though accessible, these altered receptors represent mutants that have little if anything to do with native odorant receptor biology or function.

Conversely, consider that odorant receptors are normally expressed in highly specialized cells in the nasal epithelium. These cells are not the same as the ordinary laboratory cells that are commonly used in the creation of cell-based assays. Attempts to express odorant receptors in average laboratory cells may have been challenging because of the fact that while these cells are often cancerous cells of various tissues that are used because they are hardy and grow well in the lab, they may not provide important but as yet unknown factors for proper expression and function of odorant receptors *in vivo*. As laboratory cells are populations of genetically divergent and not genetically identical cells, however, it may be possible that they include rare and unique genetics that enable expression of receptors that are otherwise inaccessible in their native and full-length form.

Indeed, a technology was reported that enabled the rapid testing of millions of cells for the isolation of rare cells capable of expressing full-length and native receptors, including odorant receptors and other receptors that had been previously out of reach in their native and full-length form. (7)

The platform technology has been applied in a diverse range of fields spanning flavor ingredient discovery to drug discovery. In a number of these cases, the cell-based discovery systems that had been developed have successfully been employed to discover candidate molecules that work in downstream studies, including in some cases human studies and evaluations for molecules ranging from salt taste enhancers to blockers of pain perception.(8,9)

Looking Towards an Exciting Future

While these data are promising, much work remains to be done. These data demonstrate that for the first time, methods are coming online to access odorant receptors in their native form. This advance by-passes the need to rely on modified receptors, and can be expected to increase the reliability of the discovery platform and the likelihood of success in its use as part of a high-throughput aromatic ingredient discovery engine. Sensory confirmation and perfumery will always be required to identify aromatics that meet desired criteria in sensory applications; however, the ability to rapidly “turbo smell test”

millions of natural ingredients against natural odorant receptors adds a previously unavailable discovery tool.

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SUBSTRATE:
THE MOUTH AND ORAL CARE

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ABSTRACT

While much of cosmetic chemistry and formulation is directed at maintaining or enhancing the appearance of the external surface of the body, the formulator of products for use in the human mouth must consider a more complex set of issues. Many oral care products address multiple consumer concerns at once, and it is common for an individual product to deliver both drug and cosmetic benefits. Understanding critical elements of oral biochemistry and microbiology will greatly help the formulator design products to deliver these benefits.

In addition to presenting some fundamentals of oral anatomy, physiology, and microbiology, this chapter will focus on the biology behind a variety of oral care consumer needs, the health and cosmetic problems behind these needs, and some approaches to solving these problems that can be introduced into oral care cosmetic or over-the-counter (OTC) drug products. Some of these concerns are summarized in Table 1.

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3.6.1 THE TEETH AND THEIR SURROUNDINGS

The oral cavity contains the teeth, tongue, cheeks, palates, and gums (gingival tissues), and is bathed in saliva. The teeth and gums are major sources of consumer concerns, many of them related to dental plaque and other tooth deposits. However, oral care needs arise in other parts of the mouth as well. The following provides an overview of the biology of the oral cavity.

3.6.2 THE TEETH

The normal human dentition consists of 20 primary (“baby”) teeth, which erupt from six months to four years of age, starting with the incisors, and are replaced by 32 secondary (adult) teeth, starting with incisors at about six years of age and culminating with the third molars (“wisdom teeth”), which erupt (in people who have them) around 20–21 years of age. Tooth makeup and form are influenced by genetics, the preeruptive environment (notably nutrition), and the posteruptive environment. Dental anatomy and alignment as well as individual oral care habits and salivary chemistry can have a profound effect upon the quantity, quality, and pathogenicity of the dental deposits that form on the teeth. Different parts of the tooth exhibit markedly different structure and characteristics, which make them prone to different types of problems.

Consumer needs	**Underlying problems**	**Functional solutions**
	“Cosmetic” Indications	
• Attractive teeth/’’ bright smile”	• Dental stain/discoloration • Potentially unsightly dental deposits	• Tooth stain removal/prevention • Plaque/calculus reduction
• Fresh breath	• Intrinsic/ extrinsic oral malodor	• Antimicrobial malodor prevention • Malodor masking/neutralization
• Fresh/clean mouth feeling	• Food/microbial residues (?)	• Flavorants, astringents, remove dental deposits (?)
• Clean mouth • Uncomfortable dental visits	• Dental plaque, calculus, stain • Uncomfortable dental cleaning to remove calculus or stain	• Plaque/calculus reduction • Stain reduction/prevention
• Dry mouth	• Insufficient saliva flow or altered saliva composition	• Lubricating/moisturizing rinses or gels • Artificial salivas
	“Drug” Indications	
• Healthy teeth/strong teeth	• Dental decay	• Decay prevention • Fluorides • Aid salivary function • Remineralization • Saliva stimulation

		• Plaque acid reduction
• Healthy gums	• Gingivitis • Periodontitis (*not* an OTC indication)	• Plaque/gingivitis reduction and prevention
• Sensitive teeth	• Exposed, hypersensitive dentin	• Tooth desensitization
• Oral comfort	• Ulcers of the oral mucosa (e.g., canker sores)	• Topical anesthetics • Antimicrobial agents

Table 1

a. Tooth Anatomy and Structure

Figure 1. Simplified sketch of tooth anatomy The tooth is divided into two areas: the crown (the enamel-covered part of the tooth that extends into the mouth) and the root, which is normally held firmly in place by the bone of the upper and lower jaws (Figure 1). Tooth crowns are covered by dense, hard dental enamel, the hardest tissue in the body. Enamel is thickest at the tip of the tooth and tapers to its thinnest point at the cemento-enamel junction (CEJ), where the crown gives way to the tooth root, which is covered by a thin mineralized layer called cementum. Underlying the enamel and cementum is a less dense mineralized tissue (dentin) that surrounds the dental pulp, which is rich in nerves and blood vessels.

In most individuals, the teeth are closely spaced and often touch side-to-side; the spaces between the teeth are referred to as interproximal spaces. The tooth

roots are held firmly in place in the jaws through periodontal ligaments. In normal healthy teeth, the gums surround the teeth and come to an apex just above the CEJ, so that the roots are not exposed to the oral cavity. The physical shape of teeth varies with tooth type, from the chisel-shaped incisors to the blocky molars, which often have pits and fissures on their biting surfaces. As discussed later, tooth shape can have a profound effect on processes that can lead to various dental problems.

b. Dental Enamel

Dental enamel is composed mainly of a partially carbonated form of the calcium phosphate mineral hydroxyapatite [3Ca3(P04)2.Ca(OH)2], which accounts for about 98% of its composition. Minor amounts of collagen-like protein, remnants of the organic matrix mineralized during tooth development, also remain in the enamel structure. Hydroxyapatite is capable of ion exchange, and anions such as F^- and CO_3^{2-} may replace the $OH^{- -}$ group, while cations such as Zn^{2+}, Sr^{2+}, and Mg^{2+} may replace Ca^{2+}. Caries susceptibility may be influenced by this ion exchange; for example, the extent to which OH has been replaced by F^- has a protective effect on the vulnerability of the enamel to acid demineralization. The hydroxyapatite, of which enamel is largely composed, is present in the form of crystallites that make up rods oriented at right angles to the surface. Water and selected ions can penetrate a small distance into the dental enamel surface along the plane of the crystallites, which can facilitate ion exchange.

As with all calcium phosphate minerals, dental enamel is soluble in acid. A substantial body of data indicates that tooth mineral is highly stable above a pH level of approximately 5.5, referred to as the “critical pH,” and that tooth demineralization occurs when the pH falls below this critical level. The extent of enamel solubility is determined largely by the amount of acid present (measured as pH), the duration of exposure to acid, the amount of soluble calcium and phosphate ions in the fluid bathing the enamel, and the presence of other ions that influence the balance between mineral dissolution and crystallization. Thus the concept of “critical pH,” while useful, should not be considered to be an absolute value.

c. Dentin

The layer of material beneath tooth enamel and cementum is the dentin. It contains approximately 70% hydroxyapatite, with the balance of its composition being collagen. Unlike the structure of enamel, which is characterized by

densely packed crystals, the dentinal matrix is perforated by a number of tiny, fluid-filled canals (dentinal tubules) that radiate from the pulp cavity to the surface. Dentin can be exposed to the oral cavity through damage to the enamel or gingival recession and wearing of the cementum (the mineral layer covering the dentin of the tooth root). The presence of open dentinal tubules can lead to hypersensitivity, in which the exposed dentin may be painfully sensitive to various stimuli, including heat, cold, pressure, sugar, acid, and so forth. This is discussed in more detail later in this chapter.

Dentin, like enamel, is also acid-soluble. Because it has less mineral content, and possibly because its structure more readily permits penetration of bacteria and acid, exposed dentin is generally believed to be more susceptible to decay than enamel. This explains the rapid advancement of dental decay after it penetrates the enamel layer and the vulnerability of exposed tooth roots to decay.

3.6.3 THE GUMS

The gums (gingival tissue) surround the teeth and overlie the bony structure in which the teeth are anchored. In health, the gum tissues directly adjacent to the teeth (the gingival margin) are firm and pink, extend just above the CEJ, and form a crevice that is not more than 3 mm deep. If dental plaque is permitted to accumulate adjacent to the gingival margin and within the gingival crevice, the gum tissue can become irritated, inflamed, and liable to bleeding. In some cases this inflammatory state stabilizes as chronic gingivitis, while in other cases the inflammation can advance to the destructive disease periodontitis.

3.6.4 ORAL FLUIDS

a. Saliva

Saliva is a major factor in the maintenance of a healthy mouth. Saliva is continuously being produced, bathing teeth and oral tissues in a dynamic environment that serves to lubricate the mouth, remove harmful materials from the oral environment, and maintain the mineral balance of the teeth.

Saliva is produced by three pairs of major glands and the smaller glands of the oral mucosa (labial, lingual, buccal, and palatal). The secretions differ from one another in composition and may also differ according to the rate of flow, time of

day, and so forth. For instance, it is well known that salivary flow is reduced at night; this makes it extremely important to cleanse the teeth between that previous midnight snack and going to bed, as the protective effects of saliva are substantially reduced during sleep.

Saliva contains mucopolysaccharides, proteins, enzymes, and inorganic materials such as calcium, sodium, potassium, chloride, bicarbonate, and phosphate ions, and bacteria shed from the oral surfaces and a variety of their constituents. The organic constituents of saliva are thought to be responsible for the development of the acquired dental pellicle (as described later). The presence of calcium and phosphate ions and the bicarbonate buffer system are believed to be significant both in the control of dental caries and in calculus formation. The pH of resting saliva ranges from about 6.5–7.2, while that of stimulated saliva can range up to about 8.0; the pH increase reflects a higher bicarbonate content in simulated supply.

The importance of saliva in preventing tooth decay following eating was demonstrated in the 1940s by R.M. Stephan. He devised a miniature pH electrode from antimony and used it to determine the pH of dental plaque at various sites on the teeth. His experiments showed that the pH of dental plaque decreased rapidly from about pH 6.82 to about pH 5 within a few minutes after consuming a sugar challenge and slowly returned toward neutrality over the next 20 to 60 minutes. If saliva flow to the teeth was restricted, the pH would remain depressed below the critical pH for enamel demineralization for much longer periods. The importance of saliva in maintaining oral pH may partially explain why an inadequate flow of saliva resulting from head and neck irradiation, drug-induced hyposalivation, or pathology can result in increased caries susceptibility and other oral problems.

b. Gingival Crevicular Fluid (GCF)

GCF exudes from the epithelium within the gingival sulcus or pocket. It is more similar to blood plasma than to saliva and can serve as a source of nutrients to the bacteria growing within the sulcus and along the gingival margin; it can also carry a variety of specific and nonspecific host defense factors.

3.6.5 THE ORAL SOFT TISSUES

The oral soft tissues consist of the tongue, gums, cheeks, hard and soft palate,

and sublingual region. The oral soft tissues are covered by a variety of nonkeratinized and keratinized epithelia. The surface of the soft tissues, most notably the tongue, can harbor large populations of oral bacteria that can serve as a reservoir to recolonize the teeth after cleaning. The microbial flora of the tongue can also play a prominent role in the generation of oral malodor (bad breath). Additionally, certain oral soft tissues are prone in some individuals to develop painful sores or ulcers, for example, aphthous ulcers (canker sores), which are addressed at greater depth later in this chapter.

3.6.6 DENTAL DEPOSITS

As soon as a tooth erupts into the mouth, and subsequently after every cleaning, it is prone to coverage with a variety of dental deposits that can cause diverse conditions that concern consumers. The enamel of a freshly cleaned tooth is rapidly covered by a pellicle of proteins adsorbed from saliva, followed by salivary bacteria that form dental plaque. Dental plaque bacteria can, under certain conditions, release by-products that can be harmful to the teeth or gums. Dental pellicle and plaque are also liable to accumulation of stain and neutralized deposits (dental calculus or tartar).

a. Dental Pellicle

Immediately after cleaning, the enamel surface is covered very rapidly by a 1–3 µm thick film of proline-rich phosphoproteins, peptides, and glycoproteins adsorbed from the saliva; thus the presence of a truly “clean” enamel surface in the oral environment is a fleeting phenomenon. The adsorption of salivary peptides and proteins appears to be relatively specific, starting within minutes of a tooth cleaning and continuing for several hours. While the acquired enamel pellicle is free from bacteria, it has been shown to contain a variety of bacterial constituents including enzymes such as glucosyltransferase, soluble polysaccharides, and various bacterial cell wall constituents.

b. Dental Plaque

Dental plaque has been recognized for centuries as a sticky film that forms on the teeth and has been the subject of concern as far back as the ancient Greeks. Over the years, the composition of dental plaque was thought to consist of food debris, mucus, denatured saliva, or “evil humors.” It was Anthony van

Leeuwenhoek, the inventor of the microscope, who first observed “tiny animalcules” (bacteria) in dental plaque in 1677. Around 1895, W.D. Miller established quite convincingly that dental plaque contained bacteria that could form acids from foods and that the acids could dissolve tooth enamel. This chemoparasitic theory of dental disease evolved to the belief that the way to control dental disease was to eliminate dental plaque, if not all bacteria, from the mouth. However, it was not until the 1960s through the 1980s that oral microbiologists started to understand the complexity and sequence of dental plaque and how it contributes to oral health and disease. Understanding the ecology of dental plaque and its effect on the character and pathogenicity of the plaque flora can provide strong insight into how a healthy balance with our natural oral flora can be established.

A central concept in our understanding of dental plaque and its pathogenicity is that plaque is actually a complex and heterogeneous microbial biofilm that varies substantially from site to site within the mouth, changes in nature over time, and is sensitive to a number of ecological parameters that can affect both its quantity and its quality. Dental plaque grows in an ordered manner, starting with the attachment of certain classes of bacteria (Gram-positive cocci) to the salivary pellicle through a set of relatively specific binding reactions similar to the interaction of antigens and antibodies. In general, these early colonizers are not associated with dental disease. Further plaque accumulation is a process combining growth of the early colonizers, elaboration of a sticky extracellular matrix that helps the bacteria adhere to the teeth and each other, and attachment and proliferation of additional bacterial species. Just as a plowed field can over time undergo succession from bare soil to grasses and weeds to shrubs to various species of trees, culminating in a mature forest, dental plaque changes and becomes more complex over time, dental plaque changes and becomes more complex over time. While the initial colonizing bacteria on smooth tooth surfaces are generally tolerant of oxygen and relatively nonpathogenic, later colonizers (especially in the region along the gumline and between the teeth where plaque tends to be thickest) become much richer in oxygen-intolerant anaerobic bacteria that can be associated with gum disease. In contrast, the microbial flora of tooth sites with reduced access to saliva (e.g., in the pits and fissures of the molars or in the interproximal spaces where teeth are tightly pressed together) can become dominated by bacteria that are relatively resistant to acid and can continue to produce acid at levels that can lead to tooth decay. The importance of particular populations of oral bacteria to oral problems such

as dental decay, gum disease, and oral malodor will be discussed in more detail later.

For further reading on dental plaque formation and ecology, its relation to disease, and strategies for controlling its pathogenicity, see the reviews by Burne and by Marsh *et al.* [1, 2].

c. Dental Calculus

The term "tartar" was commonly used to describe the mineralized deposits formed on neglected teeth. The origin of the term was derived from the similarity of mineralized dental deposits to the crystalline deposits formed in wine. The most common term in use is *calculus*. It may occur both above (supragingival) and below (subgingival) the gumline. Although these two forms of calculus may differ somewhat in composition, they both appear to originate from the mineralization of dental plaque bacteria and extracellular matrix.

Dental calculus varies in composition but always consists of about 80% of inorganic material containing calcium, magnesium, phosphorus, and other elements. The calcium and phosphorus are present in early plaque as Brushite ($CaHPO_4 \cdot 2H_2O$) and octacalcium phosphate [$Ca_8(HPO_4)_2(PO_4)_4$]. Whitlockite [$Ca_3(PO_4)_2$] may also be found, but the ultimate stage is probably apatite [$3Ca_3(PO_4)_2 \cdot Ca(OH)_2$]. This transition, which is also characterized by increasing mineral hardness and tenacity, is helped by the presence of fluorine, which is present in calculus to the extent of about 400 ppm. The remaining 20% of calculus is an organic matrix containing carbohydrate, protein, lipid, and bacteria and their constituents.

Calculus is first observed when the plaque on the enamel surface begins to mineralize. Today the most generally accepted explanation is that calculus is formed by a seeding mechanism, followed by calcium phosphate crystal growth and maturation. Rates and amounts of calculus formation can vary widely among people and within the mouth. This is probably due to a variety of factors, including salivary chemistry, local pH, plaque accumulation, and oral hygiene.

3.6.7 MAJOR ORAL PROBLEMS AND THEIR REMEDIES OVERVIEW

The major oral care problems that concern consumers can be broadly categorized as medical or cosmetic, although some conditions may fall within

both categories. Medical concerns for oral health include dental decay and gum disease (both associated with distinct pathogenic dental plaque floras), dental hypersensitivity, and dry mouth. Cosmetic concerns include dental staining and tooth whitening, oral malodor, and dental calculus. Some of these problems are widely distributed in the population worldwide, while others are less common.

a. Dental Plaque

Dental plaque is not actually a problem, as it is ubiquitous in individuals exhibiting both dental health and dental disease. However, as discussed earlier, certain populations of microorganisms in dental plaque exhibit characteristics that play a major role in the causation of dental decay and gum disease. It is important to remember that the pathogenicity of dental plaque is related not only to its mass but also to relatively specific classes of microorganisms. For instance, individuals with active dental decay tend to harbor higher populations of plaque bacteria that can produce and tolerate higher levels of acids, which are responsible for the destruction of tooth enamel. In contrast, dental plaque associated with gum disease tends to be highly populated with anaerobic Gram-negative bacteria that produce toxic substances such as volatile sulfur compounds, short-chain fatty acids, or bacterial toxins. These substances may directly damage or irritate gum tissues or might cause an inflammatory response that can lead to pathological changes in the gum tissue. In many cases the microbiological character and pathogenicity of dental plaque can be markedly modified by behaviors, such as oral hygiene or dietary habits, which can have a profound influence on plaque ecology and the rise or control of pathogenic microbial communities in the plaque.

A wide variety of methods are used to study dental plaque in the oral cavity. Some clinical indices are used to record quantity of plaque on individual tooth surfaces whilst others are used to characterise plaque accumulation on different regions of the tooth such as on the gum margin or between the teeth. This variety of methods reflects the range of claims made for oral care products. For example, a toothbrush study may want to demonstrate superior cleaning on the gum margin or between the teeth, while an antiplaque toothpaste might demonstrate reductions in overall plaque accumulation on the tooth.

b. Dental Calculus

It has been recognized for some time that there is an association between the presence of calculus and the incidence of periodontal disease, although

enthusiasm for this concept waned considerably in the middle part of the twentieth century. The commonly accepted theory is that calculus irritates the gingival tissues and encourages the formation of a pocket between tooth and gingivae, in which food debris and bacteria may lodge. However, dental calculus is considered a cosmetic issue. Even in the absence of concern regarding pathogenicity, consumers have reason to care about controlling calculus. Its accumulation can be unsightly, and it is a frequent cause for uncomfortable visits to the dentist that are required for its removal from the teeth.

While mechanical treatment by a dental professional is the only means of removing calculus, it is possible to prevent or slow its formation by a number of physical and chemical means. Mechanical plaque control (i.e., brushing and flossing) can be very effective in reducing calculus formation by removing the plaque matrix required to initiate calculus calcification. For those individuals who require additional assistance in calculus control, there are a variety of oral care products, primarily toothpastes, that contain agents that inhibit calcium phosphate crystal growth and thereby calculus formation. The most common agents in tartar control toothpastes are pyrophosphate and zinc salts such as zinc citrate, although there are a variety of other calculus-inhibiting compounds in products and in the patent literature. At present there is one tartar control mouthrinse on the market, which uses zinc chloride as the antitartar agent. The current mechanism of action of anticalculus agents is thought to lie in their ability to interfere with the initiation or maturation of calcium phosphate crystals, resulting in less mineral mass and a less mature mineral structure that is softer and more easily removed by mechanical scaling. For further reading on dental calculus formation and control, see the reviews by Ciancio [3], Mandel [4], and Jepsen *et al.* [5].

c. Dental Caries (Tooth Decay)

Dental caries, commonly known as tooth decay, is a disease that is widely distributed worldwide and is associated with more frequent consumption of foods containing sugars or refined starches. Tooth decay used to be considered a disease of children, especially in affluent societies with plentiful access to sugary foods, since most tooth surfaces became decayed by early adulthood. In recent years, preventive measures such as water fluoridation, the use of fluoride toothpastes, and the placement of occlusal sealants has resulted in very significant reductions of childhood caries. While these reductions in tooth decay may carry over into adulthood, an intact tooth surface is at risk throughout the

life of an individual, and adults must also pay attention to caries prevention.

Dental decay occurs as the results of tooth demineralization by acids produced during carbohydrate fermentation by dental plaque bacteria. It is interesting to note that consumption of carbohydrates always results in the production of plaque acids but does not always result in tooth decay. This is because saliva can often neutralize and wash away plaque acids and deliver calcium and phosphate salts to remineralize enamel before irreversible damage is done to the tooth structure. Tooth decay occurs only if the magnitude and frequency of carbohydrate consumption cause decreases in plaque pH of magnitude and duration sufficient to overcome the natural reparative power of saliva.

Enamel decay usually starts in small circumscribed areas and spreads beneath the intact tooth surface. Early carious lesions, frequently referred to as “white spot” lesions, are characterized by a thin layer of intact dental enamel overlying partially demineralized dental enamel. A critical feature of these incipient lesions is that under the right conditions they may be reversed or arrested by the process of remineralization. This process is significantly enhanced by the presence of fluoride and extra calcium and phosphate ions in saliva and plaque fluid.

If the acid challenge is great enough in both magnitude and duration, the demineralization penetrates deeper into the enamel and spreads within it, roughly assuming the shape of a cone with its apex orientated toward the dentino-enamel border and the base still below the enamel surface. The lesions may still be reversed by conscientious use of fluoride or other remineralizing strategies. At this stage of the process there still may be no visible break or cavitation evident on the enamel surface. It is generally accepted that the dentin begins to demineralize or soften before an actual cavity becomes visible. Gradually the tissue softens sufficiently so that it breaks. At this point, the carious lesion becomes a cavity and can no longer be repaired by natural means, requiring restoration by a dentist.

Control of Caries

Reduction of Fermentable Carbohydrate Intake and Removal of Fermentable Debris from the Mouth A very large body of clinical and experimental evidence implicates the consumption of fermentable carbohydrates, especially sucrose, as a major causative factor in tooth decay. Reducing the consumption of fermentable carbohydrates, most especially reducing the frequency of intake, can markedly lower caries risk. A major cause of this reduction is almost certainly a decrease in the magnitude and duration of acid formation by dental plaque,

permitting more effective remineralization by saliva. Any action that accelerates removal of fermentable food residue from the mouth, such as rinsing and brushing of the teeth, can also shorten the duration of acid attack. Furthermore, a substantial number of studies indicate that stimulation of salivary flow by chewing a nonacidogenic substance such as sugarless gum can also accelerate the removal of fermentable residue and increase the rate of salivary buffering in the mouth. For further reading on the impact of diet and eating patterns on dental caries, see the review by Geddes [6].

Reduction in Bacterial Activity and Plaque Pathogenicity Bacteria present in the mouths of most individuals will ferment carbohydrates, especially sugars, causing a pH drop to levels ranging from about 5.5 to under 4.5, below the critical pH for enamel demineralization. This fall in pH is represented by the classic Stephan curve, which can be reproduced *in vitro* and *in vivo*. Certain microorganisms, including *Lactobacillus acidophilus* and *Streptococcus mutans*, have been identified as specific caries-causative pathogens. These microbes are particularly effective at producing acid and are particularly resistant to acid environments, which can lead to their domination in pre-carious and carious dental lesions. Thus practices that reduce the amount and duration of plaque acid production, such as less frequent snacking, may also produce reductions in these particularly cariogenic plaque bacteria. There is also evidence suggesting that some substances such as fluoride, chlorhexidine, or the sugar substitute xylitol may inhibit the metabolism of cariogenic plaque bacteria.

Tooth decay tends to occur more frequently on regions of the tooth that have relatively restricted access to saliva (e.g., in the pits and fissures on the tops of the teeth, or between the teeth) or on areas of exposed dentin, such as exposed tooth roots, which are more acid-soluble than tooth enamel. Thus measures that decrease bacterial access or accumulation to these restricted areas, such as placing occlusal sealants or flossing between the teeth, can substantially reduce their susceptibility to dental decay. There is also some evidence that placing occlusal sealants not only reduces caries but also may help reduce overall risk of dental caries by eliminating sites that are the primary ecological niches for S. mutans and other acid-tolerant, potentially cariogenic bacteria.

Decrease of Susceptibility of Tooth to Acid Attack It is clear that fluoride ions decrease the acid solubility of hydroxyapatite; the two strongest hypotheses for this protective effect are that fluoride may ion-exchange with the hydroxyl group in hydroxyapatite to produce more resistant fluorapatite, and fluoride ions present during acid challenge may decrease solubility at the enamel surface. This

effect on enamel solubility may be a principal mechanism by which water fluoridation and fluoride-containing toothpastes and mouthwashes exert their effect. In 1952, it was established in the United States that there was an inverse relationship between the incidence of caries in children and the fluoride content of the local water supply, and subsequent clinical and public health studies have firmly established the value of water fluoridation as a safe and effective way of reducing dental decay.

In the 1950s, fluoride toothpastes were introduced in the United States and are now available worldwide. A substantial body of experimental and clinical data clearly supports the highly significant clinical value of fluoride dentifrices and mouthrinses in the prevention of dental decay. The most common sources of fluorides used in oral care products are sodium fluoride, stannous fluoride, monofluorophosphate, and amine fluoride (amine fluoride is not approved for use in the United States). Anticaries fluoride products are regulated as drugs; in the United States, requirements for active ingredients, specifications, and testing of anticaries drug products for OTC human use are detailed in a final monograph (21 CFR Parts 310, 355, and 369, and Docket No. 80N-0042).

Remineralization

An additional approach to reducing dental decay is promoting the remineralization of dental enamel. As stated earlier, dental plaque acids act to dissolve dental enamel, and this is counterbalanced by the remineralizing action of saliva. Increasing the oral concentration of ions that enhance this reparative process may substantially reduce dental decay. Fluoride is well known to increase the rate of remineralization and the resistance of dental enamel to subsequent acid attack. A growing body of work suggests that increasing levels of available calcium may also increase the rate of remineralization. Approaches to increasing orally available calcium include methods as diverse as using two-phase systems to promote the deposition of calcium fluoride on the tooth surface and the use of calcium-and phosphate-rich milk proteins to speed remineralization. Currently additional remineralizing strategies to reduce dental caries are still under development. One issue that is still unclear is the potential effect of aggressive remineralizing strategies on the rate of dental calculus formation, which would be an undesired side effect. For additional reading on dental caries prevention, see the reviews by Featherstone [7], Edgar *et al.* [8], and Pitts [9].

In clinical studies of anticaries products the number of Decayed (D), Missing

(M) or Filled (F) teeth (T) or surfaces (S) occurring during a clinical trial are compared using the DMFT or DMFS indices, representing the sum of the Decayed, Missing, and Filled surfaces or teeth. Uppercase letters are used to designate the permanent dentition and lowercase the primary dentition. Historically these indices have been used to measure dental caries when they reach the stage of dentine involvement (cavitation), and such studies often involve many thousands of subjects over two or three years. More recently there has been a move to methods that consider remineralization and demineralization of early caries lesions (white spots). New caries classification systems such as those proposed by the International Caries Detection and Assessment (ICDAS) group [Pitts, 9] have been developed to assist in this process. There has also been a wide range of detection methods developed that enable monitoring of lesions such as those using Quantitative Light Induced Fluorescence (QLF, Inspektor, Amsterdam, Netherlands), Electrical impedance (CarieScan, Dundee, Scotland), and Red Fluorescence (Diagnodent, Kavo, Biberach, Germany).

d. Dental Erosion

Dental erosion is the dissolution of enamel and dentine by acid of either intrinsic or external origin. It is distinguished from dental caries in that the acid does not originate from fermentation of sugars by bacteria in dental plaque. The rate and severity of dental erosion is a result of the interplay between several chemical, biological, and behavioral factors. The various chemical properties of the acid like its pH, titratable acidity or buffering capacity and calcium-chelation ability determine its erosive potential. Biological factors like the various characteristics of saliva and acquired pellicle have also been found to be important in the development of dental erosion. The acquired pellicle may protect against erosion by acting as a diffusion barrier or a perm-selective membrane preventing direct contact between the acids and the tooth surface. Behavioral factors like the frequency and timing of consuming acidic beverages and food and abnormal eating and drinking habits have also been attributed to the development of dental erosion. Swishing of the erosive agent in the mouth increases agitation of the erosive agent and hence enhances the dissolution process because the solution on the surface of the hard tissue is readily renewed.

In the mouth the erosive process is complicated by mechanical forces from abrasion and attrition of the vulnerable softened layer.

e. Periodontal Diseases (Gingivitis and Periodontitis)

Teeth are attached to the basal bones of the jaw through the periodontal tissues and are surrounded by gingival tissues. In health, the gingival tissues are firm and the gingival crevices are relatively shallow. However, the gingival and periodontal tissues are susceptible to a variety of inflammatory diseases, generally classified as periodontal diseases. The most common of these are gingivitis and periodontitis.

Gingivitis is a reversible inflammation of the gums that is not accompanied by irreversible destruction of the periodontal support tissues. Gingivitis is associated with increased dental plaque accumulation and/or high populations of Gram-negative anaerobic bacteria in the plaque and is characterized by inflamed and easily bleeding gums. Gingivitis appears to be caused by substances produced in plaque that irritate the gum tissues and induce inflammation. There is a considerable body of evidence suggesting that, while gingivitis is not associated with a single pathogenic species, different groups of organisms are clearly associated with higher or lower capacity to promote gingivitis. Dental plaques dominated by species associated with early plaque colonization (e.g., Gram-positive streptococci) are more highly associated with gingival health, while more mature dental plaques dominated by Gram-negative anaerobic species such as *Fusobacterium nucleatum* and *Prevotella intermedia* are more highly associated with gingivitis. These Gram-negative species produce a variety of toxic or irritating substances including volatile organic acids (e.g., butyric or propionic acid), volatile sulfur compounds (e.g., H_2S or methyl mercaptan), various antigens, and endotoxins. These irritating substances are hypothesized to penetrate the gingival epithelium and provoke the body's inflammatory defense systems, which ultimately leads to the swollen and bleeding gums characteristic of gingivitis.

Gingivitis prevention has focused on mechanical and chemotherapeutic measures that reduce plaque mass and inhibit the emergence of more pathogenic microbial species. Many clinical studies have demonstrated that gingivitis can be reduced or eliminated by improved mechanical oral hygiene (i.e., better brushing and flossing), which reduces the amount of dental plaque and also selects for a less pathogenic, Gram-positive dominated plaque flora. As adjuncts to mechanical oral hygiene, a number of oral hygiene products containing antimicrobial agents have been formulated to help prevent gingivitis by chemical means. These compositions apparently exert their therapeutic activity by killing and inhibiting the growth and metabolism of plaque bacteria (thereby reducing plaque mass), slowing the progression of the plaque microflora toward

enrichment for more pathogenic Gram-negative anaerobes, and reducing the production of microbial irritants. Agents that have been clinically demonstrated to reduce gingivitis include chlorhexidine, a fixed ratio of essential oils (thymol, menthol, methyl salicylate, and eucalyptol), triclosan, stannous fluoride, and cetylpyridinium chloride. In 1998, the United States Food and Drug Administration concluded panel hearings on antiplaque, antigingivitis drug compositions. The panel voted and published in 2003 a recommendation to accept some of these agents as antiplaque, antigingivitis agents.

A number of other chemical interventions for plaque and gingivitis control have been proposed, although these technologies are not yet available. These approaches include measures to prevent colonization or aggregation of one or more species of plaque bacteria, measures to disaggregate plaque to make it easier to remove, and measures to reduce the host inflammatory response.

Periodontitis is a more serious form of periodontal disease characterized by severe inflammation, increased pocket depth, and irreversible loss of alveolar bone supporting the teeth. Current thinking about the etiology of periodontitis suggests that it results from an interaction between certain classes of particularly pathogenic microorganisms and the patient's specific and nonspecific inflammatory defense mechanisms. The pathogenic bacterial species associated with gingivitis and periodontitis overlap to some extent, although periodontitis-associated dental plaques contain some particularly pathogenic species such as *Porphyromonas gingivalis* and *Bacteroides forsythus*, which are not generally associated with gingivitis. Periodontitis is frequently preceded by and accompanied by gingivitis, but not all dental sites with gingivitis will progress to periodontitis. Periodontitis appears to progress in an episodic fashion characterized by short periods of rapid bone loss (possibly caused by a severe acute inflammatory episode) followed by extended periods of chronic inflammation and relative clinical stability. Periodontitis can only be diagnosed and treated by dental professionals, and there are no OTC oral care products specifically directed to reduction or prevention of periodontitis. However, prescription and OTC products clinically proven to reduce plaque and gingivitis are often included in supportive oral hygiene regimens after periodontal treatment. For further reading on the pathogenesis and treatment of gingivitis and periodontitis, see the reviews by Ciancio [3].

f. Dentinal Hypersensitivity

In healthy teeth the dentin of the crown and tooth root is generally covered

completely by enamel or gum tissue. However, when dentin is exposed by enamel decay or fracture or gingival recession that exposes the tooth root, it becomes susceptible to a variety of problems. Gingival recession can occur for a variety of reasons, including periodontal disease, gingival injury from improper brushing of the teeth, and so forth. Once exposed, the cementum covering the root can be quickly worn away to expose the dentin. In a substantial number of individuals, exposure of dentin can lead to sensitive teeth (dentinal hypersensitivity), in which a variety of stimuli (e.g., heat or cold, pressure, or high-sugar foods) can trigger discomfort ranging from a mild twinge to severe pain. It is estimated that one in seven people is affected by some degree of dentinal hypersensitivity. In its more severe forms this condition can be debilitating, affecting the dietary and oral hygiene habits of the patient.

While several theories have been advanced to explain the mechanism of dentinal hypersensitivity, the most accepted model is the hydrodynamic theory. This theory proposes that exposed, unblocked dentinal tubules provide a channel from the outer surface of the tooth to the nerves in the pulp. Physical and chemical stimuli are postulated to affect the pressure on the fluid within the dental tubules, and these changes in hydrodynamic pressure are transmitted to the pulp nerves and interpreted as pain signals. The elements of the hydrodynamic theory (i.e., open dentinal tubules and responsive pulpal nerves) suggest two possible approaches to the reduction of dentinal hypersensitivity: blocking the tubules or reducing the stimulation of pulpal nerves.

There are a variety of currently available professional and self-applied treatments for dentinal hypersensitivity. The OTC products are dentifrices that provide agents that either promote blockage of open dentinal tubules (e.g., strontium chloride, arginine—calcium carbonate or stannous fluoride) or reduce the sensitivity of pulpal nerves by depolarizing the nerve membranes (e.g., potassium nitrate, potassium chloride, or potassium citrate).

For further reading on the origin and treatment of dentinal hypersensitivity, see the reviews by West *et al.* [11] and Ciancio [3].

g. Dental Staining

While it does not contribute to dental disease, dental staining is of considerable concern to a large number of consumers who believe that stained teeth detract from their overall appearance. This concern is possibly most acute in the United States, although there is evidence that the desire for “cleaner, brighter” teeth is increasing in other parts of the world. Dental stain or discoloration can be

roughly categorized as either intrinsic or extrinsic. Intrinsic discoloration exists within the tooth structure itself and may result from discoloration laid down during tooth formation, thinning of tooth enamel (permitting the color of the underlying dentin to show through), or loss of tooth vitality. Extrinsic discoloration results from deposition of exogenous colored material (chromogens) on the tooth surface. There are a variety of theories concerning the chemistry of extrinsic tooth staining, which probably results from a number of causes. These may include binding of ingested chromogens (e.g., pigments from tea, coffee, or tobacco) to the tooth surface either directly or through mediation of “bridging” molecules, binding of metal ions such as tin or iron (which can form a variety of pigmented complexes) to the tooth surface, or the binding to the tooth surface of colorless substances that later undergo a chemical reaction that transforms them to colored compounds. For further reading on the chemistry and mechanisms of dental staining, see the review by Nathoo [12].

There are a variety of measures to reduce or prevent dental staining, which can be grouped into measures that remove extrinsic stain by mechanical or chemical means, measures that prevent extrinsic stain buildup, and measures that chemically bleach extrinsic or intrinsic dental stain. Consumer products that mechanically remove dental stains are probably the most abundant and are dominated by dentifrices containing a variety of abrasive systems. Some of these products, most notably some targeted to smokers, are more abrasive than “normal” toothpastes; care must be taken while formulating or selecting these products that a safe range of abrasivity is not exceeded. There are also a number of OTC dentifrices or mouthrinses that claim to bind to complex staining compounds and prevent their accumulation.

There are a growing number of professional and self-applied products that contain bleaching agents that can reduce the color of extrinsic or intrinsic dental stain. Bleaching products contain a variety of peroxides (e.g., hydrogen peroxide, carbamide peroxide) and chlorine compounds (e.g., chlorine dioxide), and exhibit a range of clinical effectiveness. Because it is possible that some of these bleaching agents could be harmful to the teeth or oral soft tissues, especially if misused, this class of products has come under increasing scrutiny from regulatory and professional agencies. For instance, oral care products containing peroxide are not permitted in Canada. While it is possible to make a variety of safe and effective bleaching products to reduce dental staining, the formulator is well advised to become familiar with applicable standards and regulations to ensure product safety and regulatory compliance.

h. Oral Malodor

Another concern of consumers of cosmetic oral care products is oral malodor (also known as bad breath or halitosis). Bad breath has been of documented concern since ancient Greek and Roman times and continues to be of concern. Oral malodor can be classified into two basic categories, intrinsic and extrinsic. Intrinsic oral malodor has its origin within the individual and is most commonly caused by substances produced by bacteria in the oral cavity. Malodorous substances of bacterial origin are primarily produced by anaerobic species (some, but not all, of which may be associated with gingivitis or periodontitis) and include volatile sulfur compounds, volatile organic acids, and a variety of other volatile malodorous compounds. A smaller, but still significant, source of intrinsic oral malodor is a variety of systemic diseases or disorders that result in the exhalation of malodorous substances in lung air.

Extrinsic oral malodor originates from the ingestion of substances (such as garlic, cheese, tobacco, etc.) that contain malodorous compounds. The duration of extrinsic oral malodor is proportional to the amount and frequency of ingestion of the offending substance and the amount of time required to “wash out” the malodorous substances.

While there is relatively little that can be done to alleviate intrinsic oral malodor arising from systemic disease or disorder short of correcting the underlying condition, there are many approaches and products directed at reducing extrinsic and intrinsic malodor of oral origin. These approaches can be categorized into those that remove or wash away the malodorous substances, those that mask or cover up malodorous substances with more pleasant-smelling flavors or fragrances, those that chemically bind and neutralize the malodorous substances, and those that prevent or reduce the elaboration of intrinsic oral malodors.

Mechanical means of removing oral malodor include rinsing the mouth and brushing or scraping the teeth and tongue. In addition to washing away malodorous substances, improved oral hygiene can affect the ecology of the microbial communities in dental plaque and on the tongue, reducing the populations of anaerobic bacteria that are responsible for a large portion of intrinsic oral malodor and for gingivitis and other oral diseases.

Numerous products are employed to cover up bad breath; these include a variety of cosmetic mouthrinses, toothpastes, mints, chewing gums, and breath sprays whose principal function is to introduce flavors (such as mint) that can

overpower or mask unpleasant mouth odors. Note that many of these approaches also increase the wash-out of odorous substances either directly (e.g., mouthrinses) or indirectly through salivary stimulation (e.g., chewing gums or breath mints). For these products, a formulator's concern is primarily directed at improving the delivery and residence time of the "reodorant" flavor systems.

Another very popular approach to reducing oral malodor is to interfere with the ability of oral bacteria to produce malodorous substances. The most common means of achieving this is through the use of products containing antimicrobial systems that either kill or metabolically inhibit substantial populations of bacteria that produce the offending substances. Since bacteria that are dead or injured are incapable or less capable of metabolizing and producing malodorous substances, this approach can be highly effective in reducing malodor originating from bacteria growing in dental plaques or on the tongue. Many of the antibacterial products used to reduce plaque and gingivitis (e.g., a mixture of four essential oils, chlorhexidine, triclosan, or stannous fluoride) may also be useful in controlling intrinsic oral malodor.

A fourth approach to controling oral malodor is to ingest materials capable of binding with volatile odorous compounds and either neutralizing them or rendering them nonvolatile. Examples of products embodying this approach include those that contain metal ions such as copper, zinc, or tin (i.e., stannous ion), all well known to bind with volatile sulfur compounds. Other products in this class contain baking soda, which is also claimed to neutralize a variety of volatile malodorous compounds. These anti-odor substances have been formulated into mouthrinses, toothpastes, chewing gums, mints, and other oral dosage forms. Another class of compounds that has been proposed to systemically inactivate malodor consists of herbs or herb extracts (such as parsley oil) that are consumed as powders, tablets, or capsules. These products, which claim to inactivate malodorous substances in the stomach and thereby lower oral malodor, have not been demonstrated to have any value in reducing oral malodor. For further reading on the causes and management of oral malodor, see the review by Scully *et al.* [13].

i. Dry Mouth (Xerostomia)

Reduced saliva flow leading to dry mouth syndrome (xerostomia) can originate from a number of sources, including systemic disease, destruction of salivary gland tissue by radiation or chemotherapeutic cancer treatment, and medicinal drug use. While systemic diseases (such as Sjogren's syndrome) that destroy

salivary function are relatively rare, induced dry mouth syndrome, especially that caused by drug use, occurs to some extent in a substantial percentage of the population. Total or near total destruction of salivary function such as that exhibited by patients with Sjogren's syndrome or head and neck irradiation for cancer treatment can have a devastating effect on the oral cavity. Rampant caries, severe fungal infections such as candidiasis (thrush), loss of ability to taste, discomfort due to a pronounced sense of dryness, and irritation of the oral soft tissues are common in patients affected by severe xerostomia. Dry mouth of lesser severity, for instance that caused by some drugs, can also result in some of these problems, although usually to a less severe degree. There are now over 200 prescription and nonprescription drugs known to reduce saliva flow, including antidepressants, antihypertensives, and antihistamines; therefore this is more than a minor problem, especially in older individuals taking multiple medications.

Management of patients with dry mouth generally involves first reducing their susceptibility to oral disease. This commonly includes daily use of fluoride mouthrinses or gels and the use of antifungal drugs when necessary. A number of other products generally classified as "artificial salivas" are also available to increase the patients' comfort by reducing the sensation of oral dryness. These products typically contain a variety of humectants and lubricants to increase the sensation of oral moistness, as well as buffers and sometimes fluoride.

For further reading on xerostomia evaluation and management, see the review by Eveson [14].

j. Aphthous Ulcers (Canker Sores)

While oral ulcers, most notably canker sores (aphthous ulcers), are not as widely distributed a problem as gingivitis, they still affect millions of patients yearly and can be very troublesome and painful to those unfortunate enough to suffer them. Canker sores most commonly arise on the tongue and buccal mucosa. Their cause is still a matter of some debate and has been variously attributed to bacterial, viral, and autoimmune factors, although the evidence for an autoimmune origin is currently the strongest. A variety of factors has been suggested to predispose susceptible individuals to a canker sore attack, including minor injuries or irritation to the tongue or oral mucosa, selected foods, sodium lauryl sulfate (in toothpaste), and the like. Canker sores are characterized by shallow crater-like lesions approximately 2–10 mm in diameter (although sometimes larger) with sunken, grayish centers and slightly raised borders. They

can occur singly or in multiple clusters. Once manifested, they last approximately five to ten days and can be quite painful. For review see Akintoye [15].

There is no known prevention for canker sores except to avoid things that the patient's experience suggest are predisposing factors. Treatment of existing canker sores is generally targeted at reducing discomfort or speeding healing. Several products aimed at reducing discomfort contain topical anesthetics such as benzocaine or lidocaine, while others include agents such as diphenhydramine or corticosteroids to reduce the inflammatory response. Antimicrobial mouthrinses, most notably mouthrinses containing essential oils or chlorhexidine, have been shown to speed healing or reduce the incidence of canker sores. Agents such as silver nitrate or sulfuric acid have also been suggested to cauterize canker sores; this approach is generally regarded as unsafe and not recommended. As additional information regarding aphthous ulcers becomes available, additional approaches to treatment and prevention can be expected.

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SUBSTRATE:
THE LIPS

LIP SKIN: STRUCTURE AND FUNCTION

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ABSTRACT

The lips are covered by a thin keratinized epithelium layer on a flexible, connective tissue with a rich blood supply. The *stratum corneum* on the vermillion zone of the lip contains the same types of ceramides, cholesterol, and fatty acids found in other regions of epidermis, but the barrier function is weak, permitting a relatively high TEWL. There are sebaceous follicles in the connective tissue that supply sebaceous lipids to the lip surface. The morphology of the lips changes abruptly with increasing age.

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3.7.1 VERMILION ZONE/VERMILION BORDER

The lips represent a specialized transitional region in going from the skin to the oral mucosa. The line where the facial skin meets the surrounding the red external lip is known as the vermilion border of the lip. This term has frequently been misused to refer to the entire external red portion of the lip. The entire external red portion of the lip is referred to as the vermilion or vermilion zone. This vermillion zone has an underlying connective tissue that is more flexible than the connective tissue of most regions of the skin and is covered by a thin

orthokeratinized epithelium [1]. The underlying connective tissue has a rich blood supply, which contributes to the red coloration [2].

3.7.2 TEWL/HYDRATION

The projected area of the corneocytes on the vermillion zone of the lip is slightly larger than that of corneocytes from the cheek, and the TEWL through the lip is greater than TEWL through the cheek [3]. The transition from the exterior keratinized epithelium of the vermillion zone and the internal nonkeratinized epithelium is abrupt; however, the portion of the vermilion zone closest to the oral mucosa is sometimes referred to as the submucosa. Surprisingly, the hydration of the submucosa is less than the rest of the external vermillion zone [4]. Hydration of the upper lip is significantly greater than hydration of the lower lip.

3.7.3 SEBACEOUS FOLLICLES

Within the connective tissue along the vermillion border of the lip are sebaceous follicles, which are sebaceous glands without associated terminal hairs. The composition of the lipid mixture produced by these sebaceous follicles is the same as that produced by the major sebaceous glands of the skin [5]. The composition of sebum in the lumen of the gland is 57% triglycerides, 25% wax monoesters, 15% squalene, 2% cholesterol esters, and 1% cholesterol [6]. As sebum flows to and over the skin surface, the triglycerides undergo partial hydrolysis to release fatty acids, some of which are antimicrobial [7]. The most notable antimicrobial fatty acids are lauric acid (C12:0) and sapienic acid (C16:1Δ6). Sebaceous follicles are not only found in the vermillion border of the lip and in the oral mucosa, they encircle every orifice of the human body [7]. This distribution strongly suggests a protective function. In general, sebum secretion rates in adults decreases with increasing age. TEWL through the vermilion zone also decreases with age, but hydration appears to be independent of age [8].

3.7.4 STRATUM CORNEUM LIPIDS

As with other regions of the skin, the major lipids found in the *stratum corneum* of the vermilion zone are ceramides, cholesterol, and fatty acids. These lipids are

important in that they determine the permeability barrier function of the lips. The ceramides found here, as in other regions, are a complex mixture of structural types. Ceramides consist of a fatty acid amide linked to a long-chain base. The amide-linked fatty acids can be normal fatty acids, α-hydroxyacids, or ω-hydroxyacids, and the long-chain bases can be sphingosine/dihydrosphingosine, phytosphingosine, or 6-hydroxysphingosine. When the amide-linked fatty acid is an ω-hydroxyacid, a normal fatty acid is ester-linked to the ω-hydroxyl group.

A ceramide nomenclature system has been proposed in which the amide-linked fatty acid and long-chain base are each indicated by a single letter (normal fatty acid, N; α-hydroxyacid, A; ω-hydroxyacid, O; sphingosine/dihydrosphingosine, S; phytosphingosine, P; 6-hydroxysphingosine, H) [9]. The presence of an ester-linked fatty acid is indicated by a prefix E. In this system, a ceramide containing an α-hydroxyacid amide-linked to phytosphingosine would be ceramide AP. All nine possible structural types of ceramides have been detected in *stratum corneum* from the vermilion zone, and their relative proportions are similar to those found in other regions of skin. Weight percents of the ceramides from the *stratum corneum* of the vermilion zone are: EOS, 4.4; NS, 10.5; EOP, 0.7; NP, 23.3; EOH, 2.6; AS, 11.3; NH, 20.9; AP, 8.8; AH, 17.4.

3.7.5 CHANGE WITH AGE

A series of studies have demonstrated that the upper lip measured from the bottom of the nose lengthens with increasing age, while the vermilion zone becomes narrower [10–12].

We note that the literature has been comprehensively reviewed in this brief chapter and that not much work has been done in this area. This is will be of special interest to cosmetic and personal care scientists and formulators since products for lip care vary widely from moisturizers to lipstick. As such, we believe that lip care and beauty can be advanced considerably by providing a deeper understanding of the differentiation between “normal” skin and that of lip skin.

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SUBSTRATE:
FEMININE REJUVENATION

Part 3.8
Substrate:
Feminine
Rejuvenation

PART 3.8

FEMININE REJUVENATION

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ABSTRACT

In this chapter we address head-on this rarely discussed substrate, in the best *Harry's Cosmeticology* tradition.

As we have seen in many other chapters in this book, the perception of beauty and its relationship to well-being and skin health are intimately connected. Recent work in the cosmetic and personal care field has now extended beyond mere ingredients and formulations to other factors that provide an impact on beauty in the wider sense.

With the growing advent of dermatologists, OB-Gyn professionals, and other medical researchers formulating their own product lines, we deem it appropriate and timely for a detailed discussion of the anatomy, function, dysfunction, and approaches to improve the perception and well-being of individuals who want to feel and project beauty in their world.

While many may begin with the thought that this body substrate is more intended for medical/drug intervention, the reader will see there is much opportunity for the development of cosmetic-based over-the-counter, natural approaches that offer enhancement of this category and are both needed and wanted by the world's female population.

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3.8.5 A New Frontier
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3.8.1 THE ANATOMY

Vulva includes female genital regions that are externally visible in the perineal

region composed of mons veneris, labia majora, labia minora, and clitoris.

- **Mons veneris,** overlying the symphysis pubis, is the fatty prominence covered by curly hair.
- **Labia majora** are composed of two longitudinal folds of skin extending in elliptical fashion to enclose the vulvar cleft. Contain abundance of adipose tissue, sebaceous glands, and sweat glands. Embryologically analogous to penile shaft of male genitalia and the skin covered by hair on their upper outer surfaces.
- **Labia minora** are thin, firm, pigmented, redundant folds of skin, which split anteriorly to enclose the clitoris and meet posteriorly by the vestibules. Skin of labia minora does not contain hair follicles and is poor in sweat glands but rich in sebaceous glands.
- **Clitoris** is a small cylindrical erectile organ at lower border of symphysis. It is made up of two crura, body and glans. Crura lie deeply, in close apposition to peri-osteum of ischiopubic rami. They join to form the body of the clitoris, which extends downward beneath a loose prepuce to be capped by the acorn-shaped glans. Only the glans of the clitoris is visible externally between the two folds formed by bifurcation of labia minora. The visible clitoris is one to two inches in length. It is attached to the pubic bone and separates into two crura that are each about two to four inches in length. Clitoral hood acts as a shield for the clitoral glans. The role of the clitoral hood is to provide protection from too much stimulation.

3.8.2 VULVAR INNERVATIONS: PHYSIOLOGICAL AND ANALYTICAL

Vulva and perineum are innervated by femoral nerve posterior and lateral margins of vulva along leg crease. Genitofemoral and ilioinguinal nerve from L-1 and L-2 innervate the mons pubis upper labia majora to the level of urethra. Perineal branch of pudendal nerve (S-2–S-4) covers the lobes of labia majora. A rich plexus of over 8,000 nerve endings covers the dorsal aspect of the glans clitoris from the deeper pudendal nerve. The inferior cuneal nerve from S-1–S-3 also covers the vulva.

Assessing Sensory Perception on the Vulva and on Extragenital Sites

Sensory perception on the vulva as well as extragenital sites occurs by means

of detection of transient light touch by Meissner corpuscles and transient deeper pressure by Pacini corpuscles. Slowly adapting receptors, such as Merkel cells and Ruffini receptors, are responsible in responding to more sustained touch, such as sensing texture or shape.

While perception of burning or itching appears to be unaffected with aging, vulvar sensitivity to mechanical stimuli declines after menopause.

However, of the various demographics (age, gender, ethnicity) and anthropometric variables (height, weight, size of the body), *the most significant effect on sensory perception is advancing age*. This is also true for sensitivity to vibration, thermal threshold, and mechanical stimuli. Ethnic differences in sensory perception also have been reported. For example, Japanese subjects were more sensitive to touch and pain on the cheeks and palm of hands than Caucasians. However, Japanese subjects rated pain as less severe on a subjective scale than Caucasians—despite being more sensitive at perceiving it. Cultural stoicism may have contributed to higher tolerance to pain (1).

A Canadian study of 40 premenopausal women found that the labium minora and the mucosa of the vulvar vestibule were less sensitive than the forearm to filament touch and pressure. Although the labium minora was more sensitive to pain than forearm (2), similarly, a study of 13 premenopausal women found vulvar vestibule to be less sensitive to filament touch and pressure than deltoid muscle, forearm, or thigh (3).

A Swedish study found the clitoris to be less sensitive to perception of vibration than dorsum of hand but more sensitive than dorsum of the feet (4).

A Turkish study on vibratory threshold found vulvar sites (labia majora and minora, clitoris and vaginal introitus) were comparable in sensitivity to the first and second fingers and to the nipples. In this study, lips and ears were the least sensitive to vibratory stimuli (5).

A study conducted in the USA of both healthy and neurologically impaired women (6) noted significant loss of sensitivity to pressure/touch in postmenopausal women, hypoestrogenic women, women with vulvar atrophy, neurologically impaired women, and women with impaired sexual function, all of whom showed clear effect of estrogen on vulvar sensitivity. Although vulva has lower density of estrogen receptors than vagina, the effect of estrogen on touch sensitivity appears profound (7). Estradiol treatment significantly increased sensitivity of vestibule to pressure/touch relative to placebo at four to

six weeks. Although the mechanism of action is not known at this time, the potential sensor neural targets may be C fibers of Merkel cells (8).

In a Canadian study of premenopausal women aged 18–45, it was found that sexual arousal had no effect on extragenital sensations, suggesting dyspareunia is not due to lack of arousal (9).

Thresholds to touch averaged over all vulvar sites (clitoris, labium minus, perineum, and anal verge) were noted to be 4.6-fold lower in normoestrogenic compared to hypoestrogenic women (10).

Because of the importance of sensation to female sexual well-being and the perception and projection of beauty, in its broadest sense, an objective of an appropriate vulvar skin regimen should include the maintenance of vulvar sensorium by preserving nerve sensitivity functions.

3.8.3 VULVAR ATROPHY: CAUSES AND PHYSIOLOGY

In the U.S., half of the population is expected to be over the age of 50 in the next 20 years. There is a misconception that skin disorder is only cosmetic. *Because physiological changes occur with skin, if the causes are left untreated, such disorders can lead to a vicious cycle of chronic irritation and pruritis. These changes affect sexual function, comfort, and self-esteem.*

Common physiological changes with age include:

lower elasticity and diminished tensile strength,
reduceda dermal thickness,
reduced vascularity and cellularity of dermis,
decreased sensory perception,
reduced hydration,
flattening of the dermal-epidermal junction,
reduced epidermal thickness,
increased permeability, and
slower wound healing.

An awareness of these facts will bring a new pathway to individuals and companies looking for new products, new categories, and a common wish to enhance all aspects of individual beauty. The intention here is to expedite work

in this area beyond the medical/drug approaches already in existence and to include the development, use, and application of cosmeceuticals, natural products, *etc.* that are having a positive impact on beauty enhancement.

Skin changes can be slow and progressive with loss of subcutaneous fat beneath mons veneris and labia majora. Skin becomes pale, dry, and frail with tissue of low elasticity and pliability. Minimal lubrication results, due to decreased blood flow. Vulva becomes increasingly shrunken in appearance while pubic hair becomes thin, sparse, and brittle. There is reduction in size of labia minora, clitoris, and prepuce. Vulvar tissue can be diminished, obliterated, or even fused, with irritation and erythema evident. Skin becomes thin, shiny, and inelastic. Microscopically there are decreases in epithelial thickness and vascularity, and decreases in nerve endings but increases in dermal fibrosis.

An increase in permeability with thinning of the protective epithelium can predispose the vulva to chronic atrophic vulvitis with inflammation and abrasion/fissures from repeated irritation. If uncorrected, this process can lead to progressive sclerosing atrophy with stenosis of the vaginal orifice and effacement of the labia minora and clitoris (Kraurosis vulvae).

Causes of Vulvar Atrophy:

By 2010, over 64 million women in the U.S. were over the age of 45. It is estimated that vulvar atrophy is a silent epidemic affecting up to 50% of postmenopausal women. Women suffer in silence with this chronic and progressive medical condition and its impact on quality of life, sexual function, and urogynecologic health. Left untreated, vulvar atrophy can cause personal and marital distress and contribute to female sexual dysfunction (11). Many are reluctant to discuss their concern due to embarrassment, cultural taboo, and uncertainty about treatment options. In our opinion, women’s quest for personal beauty goes far beyond how their face looks, what color and shape their hair is, and the degree of wrinkling they have.

Vulvar Changes:

These changes are the result of a loss of tissue elasticity and thinning as well as decreased vascularity and subdermal inflammation. After childbirth, darker pigmentation changes of vulvar region are also noted. Vulvar skin retracted with tissue demarcation between labia majora and minor becomes blunted. Fragile tissue becomes prone to cracking and splitting. Thinned vulvar skin loses much of its natural surface defense mechanism and is more likely to become and remain inflamed.

With chronic inflammation of the skin in this area, lichen sclerosis develops, involving skin and mucous membrane of vulvar and rectal area. With higher pro-inflammatory cytokines—interleukin-1, interleukin-6, and TNF alpha in older skin—inflammatory response is likely. The body responds to new inflammation with repair accompanied by vulvar and perineal itching, thereby causing a vicious cycle. *Further thinning of keratin layer of labia majora and minora decreases protection against bacteria and fungus, leading to chronic infection.*

Common causes of hypoestrogenic atrophy are natural menopause and surgical/chemo-or radiation-induced ovarian failure. Usage of maintenance endocrine therapies can also cause ovarian dysfunction and vulvar atrophy. One common example is aromatase inhibitor used by breast cancer patients.

Other causes of such atrophy include young women undergoing a gonadotropin-releasing hormone agonist or antagonist, which has induced a hypoestrogenic state. Breastfeeding women have diminished estradio level, causing atrophy and decreased libido. Furthermore, allergy sufferers with chronic antihistamine use and ones on parasympathomimetic or tricyclic antidepressant medications (12) will have similar symptoms. Still other causes include use of birth control pills, Sjorgen's syndrome, anorexia nervosa, bulimia, or infertility treatments.

3.8.4 TREATMENTS

American television is full of drugs designed to correct male erectile dysfunction. For women of such partners, there can be a significant downside if they suffer from vulvar atrophy. Coitus with vulvar atrophy can cause lower genital tract skin and mucous membrane laceration due to dryness and decreased elasticity. Pain will cause pelvic floor muscle contraction and reduced vaginal secretion. *Over-the-counter lubricants with propylene glycol and chemical preservatives can cause sensitivity to women with an inflamed mucous membrane, leading to more local inflammation and pain.* Other products can dry out over time with firm residual kernels to become an added source of irritation.

If progressive tissue changes of thinning and inflammation occur, effects of estrogen are less dramatic. Estrogen will accelerate healing and decrease inflammation but will not return the tissue to a premenopausal state. It is not a

fountain of youth but will halt further degeneration.

As developers of new cosmetic and personal care ingredients, formulations, and products, our goal is to alleviate symptoms/discomfort and improve the quality of life by minimizing or reversing physiological changes.

a. Over-the-Counter Treatments

A number of OTCs can be used by women with minimal symptoms who are concerned about hormone use or are not candidates for hormonal treatment. Definitive efficacy data are lacking for almost all of the OTCs. These products are vaginal moisturizers and lubricants, but none are designed for the vulva.

Some OTCs may contain irritating ingredients such as warming additives, dyes, perfumes, bactericides, spermicides, benzocaine, chlorhexidine, and preservatives (parabens and propylene glycol). Because petroleum-based products such as mineral oil and petroleum jelly can interfere with condom use, they are best avoided.

b. Pharmacologic Treatment:

Estrogen

Topical low-dose estrogen is considered first-line pharmacologic treatment. Progesterone is not indicated with low-dose topical estrogen. Although limited in data in randomized, controlled clinical trials, estrogen is noted to be effective. A position statement from North American Menopause Society (NAMS) provides an evidence-based conclusion and recommendation for local estrogen treatment. Furthermore, NAMS concludes that there are inadequate data to support annual endometrial sampling or transvaginal ultrasound in asymptomatic patients (13).

In a cohort study of 1500 previously treated patients with breast cancer, no statistically significant difference in disease-free interval between use and without usage of vaginal estrogen was noted (14).

Usage of vaginal ring and vaginal tablet estrogen showed no significant changes in endometrium (15). Similar results were confirmed by endometrial biopsy (16). Due to lack of long-term safety data, one needs to have well-documented informed consent to risk and benefits prior to prescribing local estrogen to a patient with a history of hormone-sensitive malignancy.

After publicity surrounding Women's Health Initiative Studies, there is less frequent use of Hormone Replacement Therapy (HRT) in the U.S. This NIH (National Institute of Health) sponsored study highlighted the potential danger of taking systemic HRT, with small increased risk of developing breast cancer in women taking HRT for five years or more. Greater chance of heart attack and stroke were also noted. This finding led to a precipitous drop in numbers American women using HRT. *Unfortunately, benefits were downplayed and risks were emphasized.*

NAMS recommendations for patients without hormonally sensitive tumors is that they should not be treated differently than routinely managed postmenopausal patients. To date, the FDA has not yet approved estrogen-based products for use in patients with hormonally sensitive malignancy.

c. Novel stem-cell-derived peptides

SERM (Selective Estrogen Receptor Modulator) Ospemifene

Recently, the FDA approved a non-estrogen oral medication known as ospemifene. Ospemifene is a selective estrogen agonist and antagonist depending on tissue type. It has been approved for dyspareunia. Common side effects reported include: hot flashes, muscle spasms, increased sweating, and increased vaginal discharge.

Compared with placebo in clinical trial, daily usage of ospemifene decreased incidence of dyspareunia by –1.55 from baseline standard deviation of 2.7 compared with decrease of –1.29 from baseline by placebo.

Because ospemifene can act as an estrogen in other organs, it carries a risk of endometrial cancer, stroke, and blood clots. It should not be used in breastfeeding or pregnancy or used together with coumadin, rifampin, ketokonazole, omeprazole, rifampin, or buproprion.

At higher dosages, ospemifene is associated with increase in thymoma, adrenal subcapsular cell adenoma, sex cord stromal tumors, granulose cell tumors, and luteoma tumors in animal studies.

Topical growth factors and peptides

As stated above, physiological changes occur with aging skin in this substrate as well. Issues we would like to address in formulating a cosmetic product include:

- Improved sensory perception
- Improved hydration

- Improved appearance in color and tone
- Improved elasticity and tone of skin
- Improved firmness and volume

Topical growth factors and peptides have played a role in reversing the aging process described above, although the main use of growth factors is in wound healing (56, 57). Kinetin was first isolated from Herring sperm DNA in 1955, with natural antioxidant properties.

Stem Cells: The New Frontier

In a manner similar to that described for Topical Growth Factors and Peptides, Adult Stem Cell Derived Cells have exerted in vivo *and* in vitro *effects that address the issues mentioned above.*

Stem cells are a population of immature tissue precursor cells capable of self-renewal and provision of multi-lineage differentiable cells for tissues. Adult stem cells have fewer issues compared with embryonic stem cells, such as difficulties in control of differentiation and issues relating to ethics. Stem cells provide a building-block function by moving to the injured area and differentiating into neighboring cells, and by replacing damaged cells. By secreting diverse growth factors, they activate the neighboring cells to exhibit diverse pharmacologic action (paracrine effect). Human subcutaneous adipose tissue is a promising source of adult stem cells that secrete various cytokines and growth factors. These in turn control and manage the damaged neighboring cells (18, 19). The figures below demonstrate these results, which include an increase in collagen and anti-wrinkle effects.

Anti-wrinkle effect:

Stem cells were injected subcutaneously into a UVB irradiation-induced -wrinkle. Reverse replica demonstrates wrinkle-diminishing effects.

a. control, b. 1 × 103, c. 1 × 104, d. 1 × 105 cells (23)

Figure 1 Stem Cells

Figure 2 Collagen-stained blue, demonstrating increase in collagen content with adult-derived stem cell. A: control, B: 1 × 10 3, C: 1 × 10 4, D: 1 × 10 5 (23).

Figure 3 Histopathology of pig skin 14 days after injection of ADSC (1×10 6. A denotes control; B represents treatment arm. Western blot noted 40× increase in collagen expression (22).

Stem cells are limited because collection is required and only autologous transplantation is possible. Injection is mandatory, with short life span out of body and a long time to manufacture. Adult-Derived Stem Cell secreted factors have advantages over ADSC in that ADSC can be difficult to handle, transport, and commercialize, and they have a short shelf life. There is also the threat of passing on viruses, diseases from other animal-source nutrients to cultured stem cells in the laboratory, uncontrolled growth, and misdirected growth, especially of embryonic cells.

Recently, novel processing has enabled mass production of quality-controlled peptides from adult stem cell growth mediums. Protein extracts from conditioned media are available with following key ingredients in sufficient concentration for efficacy:

TGF beta 1 (transforming growth factor)
PDGF (platelet-derived growth factor)
KGF (keratinocyte growth factor)
Basic FGF (fibroblast growth factor)
HGF (hepatocyte growth factor)
VEGF (vascular endothelial growth factor)
SOD (superoxide dismutase)

Figure 4

TABLE 1. Concentration of Cytokines and Extracellular Matrix Proteins	
Proteins	*Concentration*
PDGF	44.41 ± 2.56 pg/mL
bFGF	131.35 ± 30.31 pg/mL
KGF	86.28 ± 20.33 pg/mL
TGF-β1	103.33 ± 1.70 pg/mL
HGF	670.94 ± 86.92 pg/mL
VEGF	809.53 ± 95.98 pg/mL
Type I collagen	921.47 ± 49.65 ng/mL
Fibronectin	1466.48 ± 460.21ng/mL

Table 1, Reference (23)

These cytokines are needed in maintaining components of the dermis, promoting wound healing, stimulating collagen synthesis/secretion, regulating cell growth and division, promoting epithelial cell growth, promoting the formation of new blood vessels, stimulating and forming of new blood vessels, and providing protection of skin from oxidative damages.

In a clinical study using Adult Derived Stem Cell Protein Extract (AAPE; Proteomics Inc., Seoul, Korea) applied topically 12 times at two-week intervals for wrinkle indications, 47% of subjects showed good to excellent improvement, with 63% judged to have very good to excellent improvement. Cytokines were

injected intradermal into skin to evaluate efficacy in subsequent studies and exhibited confirmed efficacy.

One such peptide has been approved by U.S. CTFA as of September 2006 (Cosmetic Toiletry and Fragrance Association/ Personal Care Products Council) with the INCI name of Human Adipocyte Conditioned Media Extract (21343). Readers interested in learning more about peptides can consult another chapter on this subject in this text. Results for ADSC-derived factors are promising and might have potential in skin regeneration because can they can be manufactured in large scale with long-term stability while relatively devoid of safety issues (20, 21).

3.8.5 A NEW FRONTIER:

Adipose-derived stem cells and their secretory factors are a promising therapy for skin aging as reported in *Dermatologic Surgery* (Oct. 2008). Such products have been used on the face with good results in the author's clinical practice. Based on extrapolations from patients' subjective reports, and observation from past gynecological experience that the vulvar skin's rapid healing is due to a rich vascular supply similar to the face, formulations specifically designed for the vulvar region have been prepared containing such stem cells. *Initial results have shown a promising, positive, subjective response in restoration of sensitivity and skin quality. As such, these results represent a new frontier and direction for product developers interested in addressing the issues discussed in this chapter.*

Topical usage of adult stem cell secreted factor for wrinkle reduction. Daily application photos were taken four weeks apart. See Figures 5.

Figure 5 Topical usage of adult stem cell results

Figure 6 Improved firmness in periocular skin four weeks after injection of adult stem cell derived factor.

Figure 7 Improved firmness and color via injected adult stem cell derived factor four weeks after treatment.

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NINTH EDITION

Harry's Cosmeticology

Harry's Cosmeticology

9th Edition

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About the Editor-in-Chief

Meyer R. Rosen

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Meyer R. Rosen is President of Interactive Consulting, Inc. (www.chemicalconsult.com). He is a Thought-Leader and expert in the field of Technical Marketing and multi-industry Technology Transfer Applications including, but not limited to: cosmetics and personal care, applied rheology, applied surface and interfacial chemistry, polymers, organosilicones, professional editing and custom preparation of Mind-Maps® for the organization and presentation of complex information.

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I acknowledge the ongoing sense of calm thoughtfulness of Ben Carr, my wonderful publisher and his confidence and trust in my judgment while providing the special support that has meant so much to me, over the almost three years it has taken to produce the book you are reading today.

I also acknowledge and thank the many authors of this book for their commitment to making this the best Harry's ever written. They have taught me many things by their writings and provided superb networking contacts to people who had the background to write about the areas I saw as needing to be in the book. I am grateful for their growing friendship and relationship and providing me the opportunity to interact with many of the finest minds/people; and their thinking, as well as following my guidelines to achieve our goals.

I am also grateful for the many kind words from my authors and editors who liked my professional editing skills and sharpening them as I went along. Their patience in explaining- in writing- the answers to all of my seemingly endless questions contributed much to my understanding our industry in a way that was far more in depth than I brought to beginning this book. As I said to them, "If you can't explain it to me so I really understand it; then how will our readers understand what you are saying?

I give special thanks to my Editors who took my thoughts and ideas and worked with me to find authors who knew their subjects and could organize and combine the thinking of- the- many to produce a unified- whole. Special thanks also to Navin Geria, Howard Epstein, Chia Chen, Bruce Victor, Bozena Michniak-Kohn, Ruud Overbeek, Manuel Gamez-Garcia, Michael Prencipe, Chuck Warren, Lee Stapleton, Adam Friedman, M.D., Ray Rigoletto, Roger McMullen, Randy Wickett, Martha Tate and so many others who have contributed to this book.

Finally, I wish to thank my friend and colleague, Professor Doctor Johann Wiechers, former President of the IFSCC (International Federation of the Society of Cosmetic Chemists) who unfortunately, unexpectedly and oh-so-quickly passed from this life while on one of his numerous visits to countries around the world. Johann travelled to more countries than I can name, to encourage and support the IFSCC in its mission to bring beauty and health to the many through his clear

thinking, close, objective, ever-questioning and challenging examination of the "science" associated with cosmetics and personal care. Once upon a time, he told me that while travelling the world, he always stayed in the same type of room of his favorite hotel chain because "it always made him feel like he was at home."

A manager I once had told me that if "I had lemons, I should make lemonade". And so it was with Johann, who turned the enormous amount of time he spent in travelling to producing an incredible volume of questioning, challenging and probing scientific papers for us to read and think about for years to come.

Wherever you are, Johann, I want you to know that your work and critical thinking approach to cosmetic science has impacted us all- and we miss you greatly.

Dedication

This book is dedicated to my wife Selma, my Soulmate, Committed Listener and Partner in the Journey-of-Life.

She who knows, and reminds me, to put the past in the past in order to open the doorway to the creation of new possibilities and generate new directions for growth in areas we do not know that we do not know.

How remarkable it is
When mere words on paper
Grow together
Beyond themselves.

Such words as these
Are tracks in time.

Memories of a mind
Focused
A heart and soul
Ensconced.

—Meyer R. Rosen
July 4, 2014

Preface

Dear Reader:

This book is filled with highly technical and not-so-technical, but critical, information on the current state of the art in the cosmetic and personal care industry.

Before you jump into it, as I hope you will, for years to come, I take the liberty and license of providing some personal thoughts to let you know what motivated me in taking on this project, which has now been about two and a half years in the making.

If you had asked me if there ever would come a time that I would take on another major book project as large as the one I did about ten years ago (*Delivery System Handbook for Personal Care and Cosmetic Products: Technology, Applications, and Formulations*) . . . well, I would have gracefully declined.

However, as with many things in life, "everything has a season"—and there came a day that a soft-spoken, supportive man by the name of Ben Carr, Publisher of Chemical Publishing Company, offered me the opportunity to_be Editor-in-Chief of the widest-selling book in the cosmetic industry over the past sixty years! It was time to say yes, for in my heart, and for over thirty years, I had wanted to deepen my understanding, learn from, and then teach many of the brightest minds in the world about this intriguing area called Cosmetics. The reason it intrigued me is that the concept of creating beauty provides joy for us all in looking good and feeling good as a result.

Ralph Gordon Harry, FRIC, created the first edition of what later became *Harry's Cosmeticology*. At the time (1954) it was called *Cosmetics: Their Principles and Practices*. We believe he also authored the 2nd through the 6th editions as well. The 7th edition was published in 1982 and co-edited by J.B. Wilkinson, MA, BSc, CChem, FRSC, and R.J. Moore, BSc, CChem, MRSC, MIInfSc; followed by the 8th edition in 2000 by Martin M. Rieger, PhD, and now, the 9th edition by myself, Meyer R. Rosen.

The Preface of the first edition describes the evolution of the modern cosmetics industry, which was grounded in the needs of the military in World War II. Some of the areas Harry mentions as stimulants for cosmetic scientific and technological creativity included, but were not limited to, development of safe and efficacious

sun-screening agents for men marooned on liferafts or in the desert who might be subjected to very severe solar exposure without shelter; flashburn creams designed to protect exposed skin surfaces against burns ("commando makeup"); and anti-sunburn lipsticks. Soaps, shaving creams, toothpastes, and fragrances were also developed for the military.

On the home front, special toilet soaps and barrier creams were developed to reduce dermatitis and, at the same time, the value of cosmetics as a morale builder became recognized when it was claimed that they served to combat fatigue and that a dressing room in a factory might improve efficiency by as much as 10 to 15%. All these developments necessarily had repercussions in the cosmetic field and resulted in the synthesis and development of other cosmetic ingredients such as insect repellants, emulsifiers, detergents, antioxidants, preservatives, and more.

As we move forward sixty years to today's cosmetic needs and wants, there have, of course, been major shifts in what sources the development of cosmetics and personal care products. *It is this enormous shift that has motivated me to take on the job of Editor-in-Chief of the 9th edition of* Harry's Cosmeticology.

As I see things, the population of our world has increased dramatically—as has the breadth of its ethnic interchange. This trend is escalating exponentially. It now has spread from a women-only context to men as well. With it, the age of the population is increasing, benefited by the wonders of modern medicine, enormous breakthroughs in understanding the genetic code, and the beginnings of applying that understanding to further improving the health, well-being, and appearance of the old (at any age) yearning to be young(er). In fact, underneath this yearning, I assert, is the wish to live longer and perhaps, unspoken, to live forever.

I don't know if this will be possible someday, but I do know the yearning is there—recall, for example, the age-old search for the Fountain of Youth. What intrigues me is that nowadays, all the research on the skin to make it look younger is merely the surface of a deeper search to make our bodies younger inside as well as out.

If we put aside the regulations that separate cosmetics from drugs; if we put aside the ever-changing regulatory barriers that differ from one geographical area to another, we come, in my opinion, to the heart of the matter.

Whatever you want to call it, be it cosmetics, drugs, the search for medical breakthroughs etc., it is all the same need being expressed. We all want to reverse the "inevitable" impact of gravity and time on ourselves and our loved ones. My intention in becoming Editor-in-Chief of the current 9th edition of *Harry's* is to support that yearning in the best way I know how.

I believe we are at a tipping point in fulfilling this eternal search for youth. With the breakthroughs in understanding genetics, epigenetics, and biochemistry—as well as the enormous growing consumer pull, the need to develop approaches that really fulfill the longing to be young again is the opportunity of our lifetimes.

It is my pleasure to give you, in the best tradition of sixty years of *Harry's Cosmeticology*, the knowledge described and documented in this book. I give it to you for your study and to mentor future generations of beauty/youth-seekers in moving towards this goal.

Meyer R. Rosen

FRSC, FAIC, CPC, CChE

Editor-in-Chief: *Harry's Cosmeticology*, 9th Edition

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PART 1 IN THE BEGINNING

PART 1.1

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MBA Senior Lecturer Metropolitan State University Denver

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MULTI-FUNCTIONAL BOTANICALS FOR TOPICAL APPLICATIONS

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ANTIMICROBIAL PRESERVATIVES FOR THE COSMETIC AND PERSONAL CARE INDUSTRY

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PART 4.2.2

RHEOLOGY MODIFIERS AND CONSUMER PERCEPTION

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PART 4.2.4

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MARINE INGREDIENTS FOR SKIN CARE: AN OCEAN OF RESOURCES

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TOPICAL REDUCTION OF VISIBLE SKIN DETERIORATION DUE TO CELLULITE

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CYTOKINES, GROWTH FACTORS, AND STEM CELLS: NEWEST APPROACHES TO YOUNGER LOOKING SKIN

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PART 5 ANTI-AGING

PART 5.0

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PART 5.1

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SIRTUINS AND SKIN

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EPIGENETICS OF SKIN AGING

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PART 6.1

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NANOCOSMETICS

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PART 11 TESTING

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EFFECTS OF CLEANSING PRODUCTS

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IN VITRO ASSAYS TO MEASURE EPIGENETIC MECHANISMS INVOLVED WITH CONTROLLING GENE EXPRESSION

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PART 12 SUSTAINABILITY AND ECO-RESPONSIBILITY

PART 12.0

A GLOBAL APPROACH FOR THE COSMETIC AND PERSONAL CARE INDUSTRY 1990

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PART 12.1

DEFINING SUSTAINABILITY AND HOW IT CHANGES THE INNOVATION PROCESS

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PART 13 COSMETIC MANUFACTURING

PART 13.0

MANUFACTURE OF COSMETICS SECTION OVERVIEW

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PART 13.1

COSMETIC MANUFACTURING PROCESSES

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PART 14 PACKAGING

PART 14.1

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PART 4

INGREDIENTS

INGREDIENTS I

INGREDIENTS

Editor's Introduction to the Ingredient Section

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When Meyer approached me in July 2012 to be the Editor for the ingredient section of the 9th edition of *Harry's Cosmeticology*, I gladly accepted, not knowing how much work would be involved. How naïve of me.

It's been about ten years since the last (8th) edition, and we would point out that *Harry's* has been the largest-selling book to our industry over the past 50 years. In the last decade or so, there have been a number of key breakthroughs in ingredient development, and this section is a perfect place to introduce them. We have chosen to divide up the ingredients that have been widely employed or are relatively new, but promising enough that we have included them. You will find three Parts to our ingredient description. After a while, between Meyer and me, the number of authors and subjects somehow multiplied and the entirety just became "ours" and yours!

A few breakthrough technologies that are worth highlighting include: from land to laboratory (the use of biotech/fermentation to produce ingredients); plant stem cell technology; development and utilization of marine ingredients; breakthroughs in skin whitening; and the advancement in hyaluronic acid.

We started calling on friends in the industry to join us and help. We took this opportunity to put together a program as complete as possible to include ingredients from ingestible to topical, sea to land, and rare to sustainable (land to laboratory). In terms of functionalities, we have covered the various aspects of skin concerns to prevent aging—a major area to come as a result of the rapidly escalating population of seniors and their need, nay demand, for products to make them look younger and address their hope that some of these could actually make their skin behave as if it was younger.

Thanks to all the contributing authors, we finally pulled the whole program together and after 18 months of reading, editing, and revising (the scenery in my backyard went from luscious green to snow twice). It is finally ready for publication. Here we are: *Harry's Cosmeticology*, the 9th edition.

I was very fortunate to work with experts in the cosmetic industry who have devoted their lives to the profession (with 40+, 50+ years of experience). Editing the ingredient section is like taking abbreviated courses for a master's degree in Cosmetic Ingredients. Not only did I have the privilege to work with experts who specialize in certain types of ingredients I knew about, but I also worked with experts outside my field of training: preservation system, color, emulsifier/surfactant, and even dietary supplements.

My grateful thanks to all the authors who have graciously contributed to the ingredient section by putting their knowledge down on paper for those of you seeking such information in depth, but also for having to deal with the many edits and questions we both had as you taught us. Meyer and I believe that if you could teach us what you know, in the Harry's way—from the "simple" to the "complex"—that would indeed be a breakthrough. Our contributors have become my teachers, and hopefully lifetime friends. The ingredient section will serve as a summary and reminder for those of us working in related fields, and as a treasure for those of us unfamiliar with the topics. This section contains not only a basic know-how, but our vision is that it will also serve as a pathway to addressing accelerating skin concerns related to aging.

With the wealth of knowledge collected/found in this new edition, I can proudly say that *Harry's Cosmeticology*, 9th edition, is definitely more than a compendium or guide. I would like to thank all the contributing authors who worked with Meyer and me amidst their busy schedules—and my apologies for the harassing phone calls and emails. A special thanks to Meyer, who co-orchestrated this program with me to make this section possible. We hope the reader will enjoy the fruit of our labor as much as we enjoyed putting the ingredient section together.

SURFACTANTS: THOUGHTFUL, PRO-ACTIVE INTERVENTION AT THE INTERFACE OF MULTIPHASE DISPERSED SYSTEMS

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ABSTRACT

Often we are asked to dig deeper and not simply look for superficial answers to life's problems. While this is generally good advice, for the formulating cosmetic chemist, this is not always the best advice. For these creative specialists the answers lay on the surface and in the surface-active agents (surfactants) necessarily employed in most cosmetic and personal care products. Without surfactants, there is no cosmetic industry. We would be left with smearing oils and colors on our bodies, and relying on water alone to clean us up. What a mess!

Surfactants are not only the key to cleansing hair and skin, but are of paramount importance in producing the desired and required aesthetics of formulations of all types and in all categories. Personal care products provide an important function for acceptance in our culture in terms of achieving a person's cleanliness and perception of beauty. These functions are crucial elements of the role of personal care products in society, but there is much more. A cosmetic product, whether it be a shampoo, skin cream, or antiperspirant, is a luxury item. Luxury items are about an experience as much as, if not more so than, an effect. In order to be successful, a personal care item must not only function, it must excite! In view of this elemental truth, proper understanding and use of these small but powerful interfacial components is a key to the commercial success of a formulation.

Understanding all of the physical/chemical aspects of surfactants is not critical to successful use of these materials. However, a background and understanding of these aspects are interesting nonetheless, and can help unlock some of the mysteries of their impact and activity on product development approaches. This chapter will first provide an introduction to the world of surfactants, and then move quick-

ly into the specific functions that surfactants perform in key application areas. Following this, a step back will be taken to look at a more detailed description of the physical/chemical aspects of how surfactants work. The chapter will conclude with a more in-depth view of the chemistry of the more important surfactants in use today and provide a glimpse into their future in the personal care industry.

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4.1.10 SURFACTANT INTRODUCTION

a. History

Surfactants have been used for several millennia. Early mention of the use of soap, a naturally derived anionic surfactant, comes from the Akkadians around 2800 BCE. This surfactant was manufactured by boiling fats with ashes, and used for cleaning wool and cotton in textile production. Other early applications employed by Mesopotamians for soap was cooking utensil cleaning and treatment of skin diseases. Later the Phoenicians spread this surfactant-producing technology to Egypt and later to Gaul (today France). With the Roman conquest of Gaul, soap production became very important for use in the elaborate public baths. A full-scale soap-production facility (though no reliable production data exist) located in Pompeii was unearthed, dating back to the volcanic eruption that destroyed the city in 79 CE. Location of the facility was likely due to the ready availability of the raw material, ash, and the fact that Pompeii was a resort city.

With the fall of the Roman Empire, soap production appeared to fall considerably until around the tenth century. Not only were they the Dark Ages, they were also dirty. Soap was an item of commerce and trade, produced best by those in Italy and France, and sold around the world, though only the wealthy could afford this luxury item. In other areas, soap-making was a local or home craft. By the 17th century, many large towns had soap-boiling operations, and by 1850, commercial soap-making for the masses was established by such companies as Colgate (1).

The first nonionic surfactants came onto the scene in the 1930s, developed by the Atlas Powder Company in Delaware. Using waste sorbitol made as a by-product of mannitol production and reacting with long-chain fatty acids, the sorbitan ester surfactants were produced. In the 1940s, these oil-soluble materials were made more hydrophilic by the binding of a polyethylene oxide chain onto the molecule. For instruction on how to use these novel surfactants, which have a character much different than soap, William C. Griffin designed the HLB system. Since then, hoards of new anionic, amphoteric, and cationic surfactants have been invented and put into wide-scale use, making for a cleaner, safer, and more pleasant world.

b. General Function

The word "surfactant" is a shortening of the term "surface active agent." Surfactants' work is done at interfaces. The molecules are called amphiphilic, because they are attracted to two different characters of materials. Most often, one part of the molecule is hydrophilic, or water-loving. This portion is also call the polar head group. The other portion of this amphiphilic molecule is hydrophobic, meaning that it is uncomfortable in water. These surfactants break down the interfacial tension

between substances, one of them usually being water, and the other air, immiscible liquids, or solids. The ability of surfactants to have a dual affinity makes them uniquely placed to perform a variety of useful functions in personal care products.

c. Use

Creams and lotions are not formed or stabilized easily without the use of surfactants. Cleansing hair, face, and body is extremely difficult without the use of surfactants. Hair conditioners, makeup, and deodorants would be in a sad state without surfactants. It must be remembered that personal care items are much more than just technical items designed for a specific function on the skin or hair. All of these products are as much about the emotional experience as about the specific task. Thus personal care items are luxury goods, not unlike cars and electronics, regardless of price point. Who would buy (well, at least more than once) a lotion that stayed separated, a cream that felt terrible, or a shampoo that did not foam? All of these products could do the job technically, but would leave the consumer dissatisfied and looking elsewhere. A great many of the positive emotional cues we receive from use of our personal care products, whether they be performance or aesthetic, are the result of clever use of surfactants.

d. Types

There are hundreds of different surfactant chemistries from which to choose for a given personal care application. All of them fall into four basic categories, based on charge behavior (no, not how much the suppliers charge for these wonderful materials!). The original surfactants, soaps, belong to the greater category of anionic surfactants. These surfactants possess a net negative charge. The second group, mentioned in the History section, fall into the category of nonionic surfactants. These molecules have no charge, except when being purchased. A third group is called cationic, for they have a net positive charge. The last group is termed amphoteric, meaning the surfactant changes charge character based on the pH of the system (2).

4.1.11 FUNCTIONS

a. Emulsifying

This is likely the most complex of the surfactant functions. Recent research indicates strongly that the selection of emulsifier and emulsion stabilizer is of great importance to the overall aesthetics of a cream or lotion. Much work is involved in formulating an emulsion. Surfactants play a key role in the two main steps involved making these high value systems for the face, hand, and body. First, there is the step of droplet creation. This can be accomplished with mechanical energy

such as mixing, but aided greatly through the judicious use of surfactants, which reduce the interfacial tension between the immiscible oils and water. The second step is to stabilize these droplets against aggregation, creaming or sedimentation, Ostwald ripening, and coalescence. Density differences between phases and Van der Waals forces tend to work against these kinetically stable but thermodynamically unstable systems.

There are four main stabilization mechanisms used to produce personal care emulsions. The first is charge, or ionic stabilization. The second is nonionic, or steric stabilization. A third is stabilization based on the formation of liquid crystalline lamellar phases, and the last but not least is polymeric stabilization. Each places different demands on surfactants, and requires different formulation and manufacturing techniques. Each has advantages and disadvantages, and is seldom used in isolation.

With charge stabilization, an ionic double layer is formed around the emulsion droplet. This creates electrical repulsion with surrounding droplets, slowing emulsion instability. This can be accomplished with anionic surfactants like soaps or phosphate esters, or, less commonly, with cationic surfactants. This mechanism is almost exclusively used in oil-in-water emulsions. Very often charge stabilization is used along with steric, polymeric, and liquid crystalline stabilization. Soaps are generally formed during the formulation process through the neutralization of such long-chain fatty acids as stearic acid with sodium hydroxide or TEA. The pH of soap-stabilized systems must be above 7, which makes them irrelevant for emulsions containing alpha or beta hydroxyl acids. Phosphate esters and cationics have greater pH tolerance. As a general rule, cationic and anionic surfactants have the highest irritation potential when compared to other surfactant types, but low-irritation skin care emulsions can be formulated with judicious use of these materials.

Using nonionic emulsifiers to engineer steric stabilization of droplets requires the most formulation work to optimize, but these emulsions are generally best able to withstand the harshest formulation challenges placed on them. These challenges include low and high pH, high electrolyte levels, low and high internal phases, varying oil polarities, and combinations thereof. These emulsions must be designed such that there is a very consistent and flexible film of emulsifier around the droplets. The simple HLB system was designed to ensure maximum packing of emulsifier through tuning the ratio of hydrophilic to lipophilic surfactant based on the specific requirement of the oil phase selected.

Surfactants consist partly of the molecule that is hydrophilic, or water loving, and partly of the molecule that is hydrophobic. An HLB value is assigned to nonionic surfactants based on the weight percentage of the molecule that is hydrophilic, then dividing that number by five. For instance, a surfactant such as

Steareth-20 has a hydrophilic polar head group that is 75% of the weight of the total molecule, and the HLB value is deemed to be 15 (75 / 5 = 15). The higher the HLB, the more hydrophilic the surfactant is. Sometimes ionic surfactants are listed with HLB values, but these are of limited usefulness. Surfactants can be combined to give a composite HLB value for the system. The result is purely arithmetic. If an HLB 15 surfactant is combined with another at HLB 5, blended in equal parts, the composite HLB is 10.

Oils to be emulsified have HLB requirements. A simple test will show the required HLB of a given oil or blend of oils. Test blends of surfactants at defined HLB values are prepared, generally using sorbitan oleate (HLB 4.3) and polysorbate 80 (HLB 14.9) blended to even HLB values from 6 through 14. Two grams of each blend are mixed with 20 grams of test oil in a 50 mL glass jar with a top. Water is then added, filling each jar. The jars are capped and hand-shaken 20 times. The emulsion showing the least separation is the winner and recorded as the HLB requirement of the oil. If two of the even HLB blends appear close in stability, the odd-numbered value in between is tested.

Blends of oils have an HLB requirement equal to the sum of the HLB requirements of each oil multiplied by its percentage of the total oil phase. Once the HLB requirement for the oil phase is determined, various chemistries of nonionic surfactants can be evaluated for compatibility, cost, and effectiveness, with the optimum blend ratio already having been determined. For best emulsion stability, a blend of low and high HLB surfactants, each a few HLB units away from the requirement of the oil phase, is preferred.

The HLB system is designed for optimization of steric stabilization using nonionic emulsifiers. Other stabilization mechanisms can be used in addition to enhance emulsion stability. The system works independently of phase/volume ratio, total surfactant level, or chemistry type of ingredients.

Liquid crystalline lamellar phases are surfactant structures built in the aqueous phase either alone or around an emulsion droplet. They are generally made with nonionic surfactants, though some anionic and cationic surfactants have shown useful behavior as well. The structures formed are similar to the lipid bilayers found in the upper layers of the skin, and the surfactants used tend to have low potential for disruption of the skin moisture barrier. The resultant emulsions are reported to possess good resistance to wash-off, and offer enhanced moisturization, reduced evaporation rates, and superior delivery of water-soluble actives. Commercial examples of liquid crystal emulsifiers such as a blend of sorbitan stearate and sorbityl laurate are best utilized by adding the solid product, and any other waxy materials to be used in the formulation, to water heated to 80°C and stirring until a white uniform mixture is obtained. Heat is removed at this point with

stirring continued. A room-temperature oil phase can be added now or at any time during the cooling phase of the aqueous portion. These systems work independently of HLB, so no adjustment to the surfactant system needs to be made to account for the character of the oil phase. Small amounts of hydrocolloid should be added to enhance stability, though no additional surfactants should be used.

Surfactants are often used to help with the creation of droplets in systems that are stabilized primarily with polymers. Polymers stabilize emulsions mainly through building viscosity and a polymer matrix between the droplets of an oil-in-water emulsion. Most water-soluble polymers used to stabilize emulsions are not very interfacially active, even those that are hydrophobically modified, so as such can use the help of surfactants to reduce the amount of mixing energy necessary to create droplets, and can be further aided by the steric stabilization they provide. Generally nonionic surfactants of low to mid HLB are best. Polymers stabilize by building the rest, or zero shear viscosity of an emulsion, and allowing that viscosity to be reduced when shear is applied. These emulsions possess unique rheological and aesthetic qualities that have appeal for some applications. Polymers are very widely used to increase high-temperature stability of anionic, nonionic, and liquid crystalline stabilized emulsions.

b. Cleaning

In practice, cleaning involves wetting the soiled substrate, in case of hair or skin, removing the soil (oils, dirt, makeup) from the substrate, and suspending the soil and preventing its redeposition. Surfactants placed in water are uniquely qualified for this task. Soils are adhered to the substrate, so for good cleansing, the aqueous solution must have a greater tendency to wet the substrate than the soil. Surfactants make water wetter, in a sense. A surfactant will increase the affinity of the soil for the water. With surfactant, hydrophobic tails attached to the soil and polar head group out into the water, and the soil moves to maximize surface contact with the water. Surface area is maximized by the soil being lifted from the substrate and forming emulsion droplets. Anionic and nonionic surfactants are often blended for superior cleansing.

c. Foaming

Foam is a dispersion of gas in a small volume of liquid. The ability of a surfactant to stabilize foam depends on the ability to increase elasticity at the gas-liquid interface. Anionic surfactants generally provide the highest foam because the ionic charges and oxygen-containing functional groups help increase elasticity. Eventually, though, film drainage is too great and foams break by what is termed the Marangoni Effect.

Foam does not have a physical role in cleansing, but it is an important cue to the consumer that a shampoo or other cleanser is working. More importantly, large volumes of tight, rich, long-lasting lather are a sign of luxury to the consumer and greatly enhance the experience and chance of repurchase (3).

d. Solubilization

In the IUPAC Compendium of Chemical Terminology, micellar solubilization is defined as follows: "In a system formed by a solvent, an association colloid and at least one other component (the solubilizate), the incorporation of this other component into or on the micelles is called micellar solubilization, or briefly, solubiliztion." The term "solubilizer" has, in many parts, come to be synonymous with solvent. This causes great confusion and should be avoided. In a sense, though, such materials as water-insoluble fragrance oils dissolve in the clear micelle solution of cleanser surfactant systems. Nonionic surfactants are, by far, the best solubilizers, and are often added to anionic surfactant systems for this purpose.

Just as foam has no technical value in the cleansing process, neither does fragrance. Nonetheless, the scent of a shampoo or body wash may be the determining factor in deciding consumer acceptance and delight. Having the fragrance properly solubilized in a clear shampoo or skin cleanser is extremely important.

e. Conditioning

Hair and skin possess a net negatively charged surface. These substrates are conditioned through the use of positively charged cationic surfactants, which attach to the oppositely charged hair or skin. Hair conditioners use long-alkyl-chain cationic surfactants, which ease brushing and improve the overall appearance of the hair. Conditioning shampoos use cationic polymers, which are deposited on to the hair upon rinsing, often aided in this process by the use of nonionic surfactants in addition to the base-anionic surfactant system.

f. Dispersing

Dispersants are generally used to improve the appearance and performance of liquid foundation makeup and sunscreens containing inorganic particles. Low HLB nonionic surfactants are used to disperse solids in emollient oils. The dispersant must be able to attach to the particular particle surface or coating, and also be soluble in the dispersing media. Once the optimum dispersant has been selected, a dispersant demand study should be performed to determine the optimum level of dispersant. The optimum level will yield the lowest viscosity for the dispersion, with lower and higher levels of dispersant showing increased viscosity.

g. Lubricating

Surfactants can have a lubricating effect on the hair and skin. The surfactants as such, especially cationic surfactants, possess a lubricating effect, though this is effect is also seen in such anionic surfactants as phosphate esters. Other surfactants, especially in certain association structures, can have a lubricating effect.

4.1.12 APPLICATIONS: A LOOK AT SOME OF THE MANY

a. Creams and lotions

Surfactants are mainly used as emulsifiers in creams and lotions. Emulsifiers not only allow the emollient oils to peacefully coexist, but also have an impact on the aesthetics of an emulsion. Use levels are generally from 1% to 5%, and generally are anionic or nonionic, though cationics are used as well. Irritation potential can be a concern, as well as disruption of the skin barrier lipid bilayers, especially when using more hydrophilic surfactants. Soaps and phosphate esters are the primary anionic surfactants employed. Sorbitan esters, polysorbates, glyceryl esters, sucrose esters, PEG-esters, and ethoxylated fatty alcohols are the most popular nonionics.

b. Shampoos

Surfactants perform the cleansing and foaming of shampoos. In addition, the viscosity and flow characteristics are dictated by the selection and interaction of the surfactants. Shampoos generally start with an anionic sulfate surfactant for maximum foaming and cleansing, add an amphoteric or betaine surfactant to increase mildness and give mild conditioning, and follow with a nonionic amide for viscosity and flow improvements, fragrance solubilization, and foam enhancement. To these systems salt is added to increase viscosity. The anionic portion is generally from 6% to 12%, the amphoteric roughly 5%, and the nonionic around 2% of the formulation. Sulfate-free formulation brings on a host of challenges in terms of cost, viscosity, flow, clarity, and foam.

c. Skin Cleansers

Skin cleanser formulation is very similar to that for shampoos. Many times it is difficult to distinguish a shampoo ingredient list from that of a skin cleanser. Handwash formulations tend to use anionic sulfonates as well as sulfonates. Cost is generally lower, as is foam and viscosity. Facial cleansers tend to use milder anionic surfactants and higher levels of amphoteric and nonionic surfactants. Body washes very often take on an additional responsibility of skin moisturization, containing relatively high levels of emollients.

d. Conditioners

Most hair conditioners are built with a long-alkyl-chain cationic surfactant for conditioning, a fatty alcohol for viscosity, and a nonionic ethoxylated fatty alcohol for emulsion stability. These systems usually have less than 10% total solids, often more like 5%.

e. Color Cosmetics

Surfactants are used as emulsifiers and dispersants in such color cosmetics applications as liquid foundations and mascara. Many foundation makeup formulations are water-in-silicone emulsions, and as such use silicone-based nonionic emulsifiers.

f. Antiperspirants

Surfactants are not an integral element of stick antiperspirant formulations, but are at times used to enhance the washability of formulations from the skin and fabrics. Roll-on antiperspirants are a very difficult emulsion challenge, because they generally are of low viscosity, low pH, very high electrolyte content, low internal phase, and contain polar oils and fragrances. Blends of low and high HLB nonionic ethoxylated fatty alcohols are most widely used for this application.

4.1.13 HOW SURFACTANTS WORK

a. Surface tension effects

Surfactants lower the surface tension of water by reducing the energy at the air/water interface. This surface tension reduction allows water to better wet surfaces for effective cleansing action. The concentration of surfactants is higher at the surface than in solution.

b. Interfacial activity

There is considerable interfacial tension between water and oils, causing them not to mix. Strong hydrogen bonding forces keep water molecules isolated from the less polar oils. Surfactants work at the interface of oils and water due to their amphiphilic nature. Hydrophobic portions of surfactants associate with the oils while the polar head groups interact with the water, lowering the tension at the interface. Interfacial activity of surfactants is also involved in the cleansing mechanism as soils are emulsified and removed in the water. Emulsification is truly an interfacial phenomenon.

c. Micelles

Micelles are simple surfactant structures formed in aqueous solutions. When in water, surfactants will move to protect the hydrophobic tails by collecting in spherical

structures with those tails on the inside of the sphere. The surfactant concentration at which these structures begin to form is called the Critical Micelle Concentration (CMC). Solubilization takes place as oil is dissolved in the interior of the micelle.

d. Aggregation structures

Various surfactants at varying concentrations will form more advanced structures in water. As surfactant concentrations increase in water, cubic then hexagonal phases (and some lamellar) are formed. These phases are often seen in anionic cleansing surfactant systems and can be altered by the incorporation of amphoteric and nonionic surfactants to the mix, and the addition of salts (4). The viscosity produced by the manipulation of these variables can be considerable, and the resultant shear thinning rheology quite pleasing to the consumer. Lamellar phase liquid crystals formed by certain lipophilic surfactant emulsifiers, when heated above the Krafft temperature in water, are very useful in the development of creams and lotions with exciting aesthetic and performance attributes.

e. Charge effects

Surfactants carry various electrical charges in water. Those with a net negative charge on the polar head group are called anionic, and those with a net positive charge are referred to as cationic. The charge can be fairly localized or dispersed, changed by such materials as electrolytes or other surfactants (4). Nonionic surfactants possess no appreciable electrical charge. Structures surrounded by a certain charge will be repulsed by adjacent structures carrying the same charge. Amphoteric, or zwitterionic surfactants vary their charge behavior based on the pH of the system. At lower pH, these surfactants exhibit cationic behavior; in more alkaline situations, anionic. In addition, there is a narrow pH range in which amphoteric surfactants show nonionic surfactant character known as the zwitterionic point. Care should be taken when using amphoteric surfactants not to formulate at the zwitterionic point for a given material.

f. Foam generation

Foam is generated when air is trapped beneath a liquid surface containing surfactant molecules. The physical action of rubbing a surfactant solution on to the hair or body with the hands helps create the pockets of air. Anionic surfactant solutions create the greatest volume of foam. When amphoterics are used as primary surfactants, the foaming character is not great, but when combined with anionics the effect is quite good with regard to foam generation. Nonionic surfactants do not create large amounts of foam, though some can give an acceptable amount of foaming, even if the quality of the foam is not outstanding.

g. Foam stabilization

The various pressures exerted on a foam structure eventually cause it to be destroyed. When draining of the liquid causes the top wall of the foam to fall below a critical thickness, the bubbles break. Foams created with anionic and amphoteric surfactants are stabilized through the use of such nonionic surfactants as alkanolamides. Complex mixtures of surfactants allow for the generation of initial, or flash foam, large volumes of foam, and foam that lasts through the use of the product on the hair, hands, face, and body.

4.1.14 SURFACTANT CHEMISTRY

a. Anionics

Anionic surfactants possess a net negative charge (5). Primary use of anionic surfactants is for foaming and cleansing in cleanser systems. In addition, anionics are used as emulsifiers in creams and lotions. Of surfactants used in personal care products, anionics are used in the largest volumes. Skin and eye irritation potential can be high, but usually mitigated by low use levels and being combined with other surfactants.

Sulfates

The primary use of sulfates is in shampoos and body washes. Foaming capabilities are outstanding. Sulfates are produced by the sulfation of natural or synthetic alcohols or alcohol ethoxylates. Counter ions are typically sodium or ammonium. Ethoxylation of the alcohol decreases irritation and foam. Structure forned is $ROSO_2^-$ in which R is a fatty alcohol or alcohol ethoxylate.

Typical sulfates used include:

1. Sodium lauryl sulfate
2. Sodium laureth sulfate
3. Ammonium laureth sulfate
4. Sodium trideceth sulfate

Sulfonates

Sulfonates are used mainly in hand wash formulations and are manufactured by sulfonation of alpha olephins. Foaming capabilities are very good and the surfactant is stable over a wide pH range, though formulations can be difficult to build in viscosity. Chemical structure formed is RSO_2^- where R is an alpha olefin.

Typical sulfonates used include:

(1) Sodium C14-16 olefin sulfonate

Phosphate esters

Phosphate ester surfactants are used as emulsifiers in creams and lotions, especially sunscreens, and foamers in facial cleansing systems. They are produced

through phosphation of alcohols or alcohol ethoxyates. Emulsions are stable over a wider pH range than soap-emulsified systems. Phosphate esters produce thick, rich, lubricious foams in cleansers. Chemical structure is $ROPO(OH)O^-$ where R is a natural or synthetic alcohol or ethoxylate.

Typical phosphate esters used include:

(1) Sodium laureth-4 phosphate
(2) Potassium C12-13 alkyl phosphate
(3) Potassium cetyl phosphate (emulsifier)
(4) Dicetyl phosphate (and) Ceteth-10 phosphate (emulsifier)

Sarcosinates

Sarcosinate surfactants are used mainly in mild foaming cleansers. Foams produced are thick and rich, and are under consideration as primary foaming surfactants. They offer conditioning effects and are compatible with cationic surfactants. Production is through reaction of an acyl chloride with n-methyl glycine.

Typical sarcosinates used include:

(1) Disodium laureth sulfosuccinate
(2) Disodium lauryl sulfosuccinate

Glutamates

Glutamates are very mild surfactants used in skin-cleansing systems. They form thick foams and offer excellent feeling on the skin after rinsing. Glutamates are compatible with cationics and are considered acceptable for many natural formulations.

Typical glutamates used include:

(1) Sodium cocoyl glutamate
(2) Disodium lauroyl glutamate

Lactylates

Lactylate surfactants are used as emulsifiers in creams and lotions and as foam boosters and stabilizers in shampoos and skin cleansers, imparting softness and moisturization. In cleansers, lactylates can improve the salt-thickening response of cleansing surfactant systems. They are the reaction product of a fatty acid and lactic acid. Lactylates are seen as naturally derived.

Typical lactylates used include:

(1) Sodium lauroyl lactylate
(2) Sodium stearoyl lactylate (emulsifier)

Isethionates

Isethionates are mild surfactants used primarily in synthetic detergent cleansing bars, but are finding use in liquid cleansing products. Foam is slippery and

luxurious, and are the esters of isethionic acid and fatty acids. The methyl ester can be used in clear systems.

Typical isethionates used include:

(1) Sodium cocoyl isethionate
(2) Sodium methyl lauroyl isethionate

Taurates

Taurates are mild surfactants used in opaque shampoos and skin cleansers. They offer foam boosting and stabilization with light conditioning properties.

Typical taurates used include:

(1) Sodium methyl cocoyl taurate

Carboxylates

Carboxylate surfactants are mild and used to reduce the eye irritation of anionic surfactants. Chemical structure is $RCOO^-$ where R is an alcohol or, more typically, and ethoxyate.

Typical carboxylates used include:

(1) Sodium trideceth-7 carboxylate

Other anionic surfactants

Other anionic surfactant classes of note include:

(1) Alpha sulfo methyl esters
Sodium methyl 2-sulfolaurate (and) disodium 2-sulfolaurate
(2) Fluorosurfactants
TEA-C8-18 perfluoroalkylethyl phosphate
(3) Sulfosuccinates
Disodium laureth sulfosuccinate
(4) Sulfoacetates
Sodium lauryl sulfoacetate
(5) Secondary alkane sulfonates
Sodium C14-17 alkyl sec sulfonate

b. Nonionics

Alkanolamides and alkoxylated amides

Alkanolamides and alkoxylated amides are used extensively in all types of cleanser formulations. These materials help manage salt thickening, solubilize fragrances, modify foam feel and density, and enhance conditioning. Alkanolamides are generally produced through the reaction of a fatty triglyceride with such amines as monoethanolamine (MEA) and diethanolamine (DEA), though the latter have fallen into disfavor based on toxicological concerns. Alkoxylated amides generally

are produced by the addition of propylene oxide or ethylene oxide to an MEA amide to transform the solid amide into an easier-to-use liquid.

Typical alkanolamides and alkoxylated amides used include:
(1) Cocamide MEA
(2) Cocamide DEA
(3) PPG-2 hydroxyethyl cocamide
(4) PEG-5 cocamide

Ethoxylated fatty alcohols

Ethoxylated fatty alcohols are used primarily as emulsifiers in creams and lotions, roll-on antiperspirants, and hair conditioners, colors, and relaxers. In addition, certain ethoxylated fatty alcohols are used as solubilizers and gelling agents in hair-styling products. There are low and high HLB variants based on the fatty chain length and degree of ethoxylation.

Typical ethoxylated fatty alcohols used include:
(1) Steareth-2
(2) Steareth-21
(3) Laureth-23
(4) Isoceteth-20

Glyceryl and polyglyeryl esters

Glyceryl and polyglyceryl esters are used as emulsifiers in creams and lotions. Glyceryl esters are produced generally through transesterification of a triglyceride and glycerin. Polyglyceryl esters are reaction products of a fatty acid and a polymerized glycerin. Glyceryl esters are low HLB emulsifiers, often used along with PEG-esters. Low HLB polyglyceryl esters are very effective water-in-oil emulsifiers, whereas the higher HLB products can help form ethoxylate-free steric stabilized emulsions.

Typical glyceryl and polyglyceryl esters used include:
(1) Glyceryl stearate
(2) Polyglyceryl-3 diisostearate
(3) Polyglyceryl-10 oleate

PEG-esters

PEG-esters are used primarily as emulsifiers in creams and lotions. They are produced either through reaction of a fatty acid with ethylene oxide or polyethylene glycol. PEG-esters are normally high HLB emulsifiers, though specialized polymeric PEG-esters are powerful water-in-oil emulsifiers.

Typical PEG-esters used include:
(1) PEG-100 stearate
(2) PEG-40 stearate
(3) PEG-30 dipolyhydroxystearate

Silicone copolyols

Silicone copolyols are powerful water-in-silicone and water-in-oil emulsifiers used in liquid foundations, sunscreens, and facial treatment products. Ethylene oxide and often propylene oxide are added to the dimethicone.

Typical silicone copolyols used include:

(1) PEG/PPG-18/18 dimethicone
(2) Lauryl PEG/PPG-18/18 methicone
(3) Cetyl PEG/PPG-10/1 dimethicone

Alkyl polyglucosides

Alkyl polyglucosides are used as foamers and irritation reducers in mild cleansing formulations. More recently, longer chains are being marketed as liquid crystal emulsifiers for creams and lotions. They are formed through the reaction of a fatty alcohol with glucose. Alkyl polyglucosides are seen as naturally derived alternatives to ethoxylated nonionic surfactants.

Typical alkyl polygucosides used include:

(1) Decyl glucoside
(2) Lauryl glucoside
(3) Cetearyl glucoside

Polysorbates

Polysorbates are used in baby shampoos to reduce eye irritation and sting, and in creams and lotions as high HLB emulsifiers. In addition, they are used as solubilizers in gels, toners, and cleansers. Polysorbates are produced by the reaction of sorbitan esters with ethylene oxide.

Typical polysorbates used include:

(1) PEG-80 sorbitan laurate
(2) Polysorbate 20
(3) Polysorbate 60
(4) Polysorbate 80

Sorbitan esters

Sorbitan esters are used mainly as emulsifiers in creams and lotions. They are the reaction product of sorbitol and various fatty acids. Sorbitan esters are used as low HLB emulsifiers for steric stabilization and also can form liquid crystalline structures. Oleate esters are also used to produce water-in-oil emulsions.

Typical sorbitan esters used include:

(1) Sorbitan stearate
(2) Sorbitan sesquioleate

Poloxamers

These ABA type block copolymers are mainly used as solubilizers and gelling agents in oral care products. The A portion of the block is an ethylene oxide polymer, the B in the center is a propylene oxide polymer serving as the hydrophobic portion. Each block is varied to obtain differing degrees of hydrophilicity and phase behavior.

Typical poloxamers used include:

(1) Poloxamer 407
(2) Poloxamer 188

c. Amphoterics

Betaines

Betaine surfactants are very widely used in all sorts of cleansing formulations. Betaines are mild and foam well, contributing to viscosity of most formulations. Technically betaine surfactants are cationic surfactants, not amphoteric because they never are in an anionic state, but their performance is similar to other amphoterics so are generally classified as such. Betaine surfactants are formed from the alkylation of a tertiary amine with chloroacetic acid.

Typical betaines used include:

(1) Cocamidopropyl betaine

Amphoacetates

Amphoacetate amphoteric surfactants are found in mild shampoos and skin cleansers due to their irritation reduction properties, moderately good foaming in hard and soft water, and light conditioning effect. These products are produced through carboxymethylation of a fatty imidazoline and generally contain high levels of salt.

Typical amphoacetates used include:

(1) Sodium cocoamphoacetate
(2) Disodium cocoamphodiacetate

Propionates

Propionate amphoteric surfactants are used in many cleansing products because of their irritation reduction, moderate foaming, and light conditioning properties. Versions of propionates are considered salt-free, which can have advantages in certain formulations.

Typical propionates used include:

(1) Sodium cocoamphopropionate
(2) Disodium cocoamphodipropionate

d. Cationics

Quaternary ammonium compounds

Quaternary ammonium compounds are the primary conditioning agents found in rinse-off hair conditioners. Their positive charge adheres to the negatively charged hair. Quaternary ammonium compounds are synthesized by reacting a tertiary amine with an alkylating agent such as methyl chloride.

Typical quaternary ammonium compounds used include:

(1) Cetrimonium chloride
(2) Distearyldimonium chrloride
(3) Behentrimonium methosulfate

Alkyl amines

Alkyl amines are heavily used in hair-conditioning formulations. These materials are not true cationics as such, but are generally protonated in formulations with acids giving them cationic character below pH 7.

Typical amidopropyl amines used include:

(1) Stearamidopropyl dimethylamine
(2) Cocamidopropyl dimethylamine

Amine oxides

Amine oxides are used as foam stabilizers, viscosity boosters, and solubilizers in cleansing systems. At times these surfactants are listed as nonionic or amphoteric. Amine oxides are synthesized by oxidation of tertiary amines.

Typical amine oxides used include:

(1) Cocamine oxide
(2) Cocamidopropylamine oxide
(3) Dihydroxyethyl cocamine oxide

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INGREDIENTS FOR CREATING THE NEXT GREATEST LIPSTICK

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ABSTRACT

Lipstick is a color cosmetic product that most consumers take for granted probably because it is such a ”simple” product. Formulators and marketers do not take it for granted as it is a money maker. It is so small and compact. There are so many of them out there- in every perceivable color- and many consumers just pick a shade they like and purchase it without really looking to see what is actually in it. This chapter addresses key questions about this ubiquitous cosmetic product category. These include, for example: what makes one lipstick better than another? What really goes into making a lipstick? and what ingredients can go into a lipstick formula to make it the next greatest consumer product? It also covers exactly what a lipstick is, and how formulators can improve on what is already on the market and how to keep up and generate new product distinctions in a context of a rapidly changing product.

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4.1.2.1 INTRODUCTION

It may be small and simple but lipstick is one of the most important market segments in the color cosmetics market segment and even the entire cosmetics industry. It is probably the most varied cosmetic product in terms of SKUs. Of the over 50 brands of lipsticks currently on the market, there are almost 2000 different shade names. That's not counting some of the smaller unknown brands that just have local distribution. And most of these are just in the United States cosmetics market alone. Lipstick sales were *up* by almost 15% in 2011. This is significant growth and creates a huge amount of revenue for cosmetic marketers as well as increased market share for some brands. In a sluggish economy, the increase in lipstick sales helps companies improve their bottom line when they don't do well with expensive treatment products or high-end fragrances. As a cosmetic, some lipstick brands are even being targeted towards the men's market. But what makes lipstick such a unique staple in our industry?

A lipstick is a crystalline solid that is molded into a cylindrical shape and inserted into a swivel up cartridge for ease of application and storage. Various waxes and oils are melted and mixed together to create the sturdy and solid stick matrix. The various physical attributes that come from each type of lipstick are a result of the different fats and oils used in the formula. These physical attributes are responsible for the product's application, texture, slip, tackiness, shine, and length of wear. And these all can be changed by varying the ratios of the waxes, fats, and oils in the lipstick formula. But the ideal lipstick must have a very strong and sturdy crystalline structure.

When a formulator has come up with a suitable formula, the lipstick can be molded using a number of methods. They can be molded using metal book molds that may require flaming to eliminate seams from the molding process and any other surface flaws, giving the stick a smooth, even, and shiny surface free of defects. Another filling method involves the use of silicone molds, where the molten lipstick is poured into silicone inserts. These inserts allow for the filling of unusual shapes that were nearly impossible with conventional book molds such as sharp edges and sharp points. They can even have corporate logos embedded into the stick surface. The silicone molding process does not require flaming, as the sticks come out smooth with a shiny stick surface. However, the use of silicone-based ingredients must be used with caution in the silicone molding process because the silicones used in the formula may be too compatible with the filling process and cause problems. These different methods are standard for the filling of lipsticks in the cosmetics industry.

What sets one brand apart from another? What is the difference between a lipstick that wears for four hours and one that wears for eight to ten hours? These attributes have been sought after by lipstick formulators ever since the very first

lipstick was molded. And it has always been a challenge. In other words, it's not that easy. But lipstick formulas are relatively simple. So how exactly is it done? What ingredients go into making the next greatest lipstick?

Historically, a freestanding conventional lipstick is a combination of hard waxes, various lipids, and colorants. This combination allows for hardness, some flexibility on application, and adequate color payout. Waxes are the key component of any lipstick. The typical waxes used in a lipstick formula are Candelilla Wax (Euphorbia Cerifera), Carnauba Wax (Copernicia Cerifera), White Beeswax (Cera Alba), and some mineral-based waxes. You may also see other hard waxes used in a lipstick such as Japan Wax, Montan Wax, various amorphous Microcrystalline Waxes, Ceresin, high-melting-point Polyethylenes, and some others. Some even act as plasticizers to provide flexibility to the stick. Both Beeswax and Japan Wax are also available in synthetic grades. These are significantly cheaper to use than their natural counterparts but offer the same physical characteristics. These are the backbone of most lipsticks and are the main contributing factor to a strong crystalline structure. And there are different grades within each type of wax. These in combination with various lipids, fatty materials, and oils (polar solvents, vegetable-derived oils, and various esters) are the basis for most of the lipsticks that can be found at the cosmetics counter today.

Figure 1 Lipstick Ingredient Bases

When looking at the ingredient label for any of the lipsticks on the market, one will see that they contain many of the same ingredients. Those at the top of the ingredient list are the higher-concentration ingredients, and many at the bottom are incidentals. Somewhere at the top of this list you will see many of the same waxes

being used as well as oils and esters. The hierarchy remains quite similar. This doesn't make for much of a difference from formula to formula or brand to brand. Some may be slightly softer than others while others may have more color laydown. Some may exhibit more shine while others may seem to be tackier. All in all these are the characteristics that can be found in a conventional lipstick. However, when looking at the ingredients used, they are all relatively the same from formula to formula. Figure 1 shows examples of some common lipstick ingredients. These basic ingredients are all that is needed to create a lipstick. They may exhibit different characteristics; however, they are just all variations on the same theme.

All the textural differences mentioned above come from the variety of vegetable oils, mainly castor oil (*Ricinus communis* seed oil), esters, and other solvents that are available to the cosmetic formulator. There are many different vegetable oils, all with different feel characteristics. There are many different polar oils that can be used as a starting point in a lipstick aside from castor oil. Depending on the feel characteristics that are sought after, castor oil can be completely eliminated from the formula. Castor oil is used because it is an inexpensive polar solvent, it is convenient, and it works as a good pigment-wetting agent. But nowhere does it state that castor oil *must* be used to make a lipstick. Although it is the main solvent used to grind the pigments, other oils can be used.

4.1.2.2 WHAT ATTRIBUTES ARE WE LOOKING FOR IN A LIPSTICK?

First, what attributes are we looking for in a lipstick? Second, what new ingredients are out there and which of them will be suitable in a lipstick formula? And third, what ingredients will deliver these desired attributes? The main attributes that a consumer looks for in a lipstick are that it must be applied with comfort and maintain this comfort as long as it is on the lips, it must have and maintain a high degree of shine, and it must wear for a long time—no less than four to six hours. While these attributes may sound quite easy to achieve, in reality they are very contradictory. It is very easy to create a lipstick with high shine and nice comfortable feel, but to make it long wearing at the same time becomes the biggest challenge.

Shine is a function of melt, and melting means the product is wearing off because it is *liquefying*. Increasing the wear involves the use of polymers and film formers, and these are usually stiff and uncomfortable on the lips. Increasing wear can also involve increasing the powder loading, which may tend to dry out the lipstick, again affecting the comfort level of lipstick wearing. Conventional lipsticks cannot be made to contain all these attributes together in one product, what everyone in the industry calls "The Holy Grail of Lipsticks." Many formulators have used this phrase and it turns out to be quite true. It is sort of a balancing act, getting the most out of the ingredients to achieve the maximum level of positive attributes.

You can never perfectly achieve everything. I say positive, because along with positive you have negative. The negatives are what formulators try to combat in the product, such as tack, drag, and dullness. That's the tradeoff you get trying to achieve all the positives. So what new technology can be used to achieve all the positive attributes we are looking for in a lipstick? We will work up to that as we move forward in this chapter.

4.1.2.3 TRADITIONAL INGREDIENTS USED IN CREATING A LIPSTICK

The most common ingredients found in lipsticks are esters. There are thousands of esters that can be used in a lipstick formula. These range from light penetrating esters to heavy viscous liquids and pastes. The only limits to the number of esters that can be used are the different acids and alcohols that are used to create them. With so many different variations, the different feel characteristics that can be exhibited in a lipstick formula should be virtually limitless. And more esters are being developed, as the industry always requires them.

But by using the same ingredients over and over again all we are doing is creating an overabundance of "me too" type products on the market, differing only in the combination of ingredients used and not so much in new technology. This new technology can involve new types of ingredients, the science used to create them, as well as methods for manufacturing; but it is science that will play a key role in the next generation of lipsticks. It is this new technology that we will focus on in this chapter in order to create the next greatest lipstick. It is always technology that creates the point of difference in new products. Otherwise we will just wind up with another product in the "me too" category. It is quite easy, but certainly not considered groundbreaking.

Technology—what is it and how does it relate to lipsticks? After all, the cost of ingredients to make a simple lipstick is just a 5–10¢ combination of waxes, oils, and some other simple components. What can technology possibly do here? Since technology can involve a whole lot of different things, the bottom line is to do something entirely different. And the obvious place to start is with the ingredients. You don't need many ingredients in a formula to make it feel elegant. It is just the right choice of ingredients that will do the trick.

Of the different types of esters that can be used there is one group called Sugar Esters of Fatty Acids. These are a little different than conventional esters in that they are based mainly on the disaccharide Sucrose (common table sugar which is only derived from plant sources) as a starting point and reacted with a fatty acid to form the ester. Common sucrose fatty acid esters used in cosmetics are sucrose distearate, sucrose palmitate as well as a number of others. They can also be made from fatty acids derived from specific plants: sucrose cocoate, sucrose

polycottonseedate, sucrose polypalmitate, sucrose pentaerucate, or sucrose polysoyate, but any fatty acid can be utilized in the esterification process. There are a number of others, but they all cannot be listed here. Their functionality is dependent on the specific fatty-acid chain utilized. They contribute to feel and moisturization owing to their sucrose carbohydrate backbone. They are considered natural ingredients and are approved as direct food additives and even considered natural emulsifiers in some markets. The sugar aids in reducing water loss and helps with adhesion to the lips. Speaking of sugars and carbohydrates, sweeteners (both natural and artificial) are seeing more use in lip products along with different types of fragrances, flavorings, and maskants, and most are GRAS (Generally Recognized as Safe). You see these ingredients mainly in food products, as they are edible.

And speaking of fatty acids, a lipstick formulator can use some natural oils that are high in essential fatty acids. Depending on the oil selected, these provide various amounts of omega-3, -6, and -9 fatty acids. Some plant-derived oils, mainly from the seeds and nuts of the plants, are high in both omega-6 (linoleic acid) and omega-3 (linolenic acid) fatty acids. These are considered unsaturated fatty acids. So a formulator does not have to use fish oils to get the benefit of all three essential fatty acids. Not only do they possess excellent aesthetic properties, they can also be used to make skin care claims with restorative properties as well. For example, kukui nut oil is high in omega-3 and omega-6 fatty acids, so it should see benefits in cosmetics. Oleic Acid, a major component in olive oil, is an omega-9 fatty acid. There are also omega-7 fatty acids. These are also considered unsaturated and the site of unsaturation is seven carbon atoms from the end of the carbon chain. Rich sources of omega-7 fatty acids include macadamia nut oil and sea buckthorn oil.

The next step when using conventional esters of fatty acids is to look for molecules that have longer chains with unique substitutions and branching such as behenates, arachidonates, and erucates. These can add some unique properties to a lipstick formula such as creaminess, cushion, and body. They can be based on saturated or unsaturated fatty acids. This just creates more of a variety of esters based on the fatty-acid starting point. These higher-molecular-weight ingredients perform unlike any of the esters we are currently familiar with and can offer new and unusual feel properties to the lipstick. High-viscosity alky esters such as bis-diglyceryl polyacyladipate-2 offer creaminess and skin protection due to their film forming capabilities. Various butters (hydrogenated vegetable oils) can also serve this purpose.

Figure 2 is a chart that shows what ingredient ratios can be used to create different types of lipsticks. As we stated earlier, a lipstick is a combination of waxes and oils in sufficient ratios to produce a freestanding structurally sound stick. The final selection of waxes will be dependent on their individual melting points, hardness values, and sometimes solubility with the other ingredients in the formula.

While the choice of individual waxes may be the same (according to their INCI names), it is the grades of these waxes that may vary. As they are extracted from the plant, waxes have various fractions that have different hardness values and melt points. Different grades of the same wax may even differ in color and odor. This chart shows how the same ingredients can be used to create different types of lip products; most of them are sticks. Essentially there is very little difference in the ingredient selection to create these lipsticks. What if the choice of waxes were to change? What other waxes are available to the formulator that can be used to create a lipstick?

	LIP GLOSS PLOT	CREAMY LIP STICK HIGH SHINE	LONG WEAR LIP STICK	TRANSFER PROOF LIP STICK	LIP LINER STICK
WAXES	5–10%	10–15%	15–20%	10–15%	15–25%
VOLA TILES	NONE	NONE	15–20%	35–40%	VARIES
OILS AND SEMI-SOLIDS	70–80%	60–70%	35–45%	10–25%	40–50%
PIGMENTS/ POWDERS	5–10%	10–15%	10–15%	10–20%	15–25%

Figure 2

There are some new waxes that have been presented by wax manufacturers to the formulating community. These are neem wax, esparto wax, bayberry wax, sunflower wax, various polyester waxes (based on castor oil), jojoba wax, rice bran wax, and other naturally derived plant waxes that can create a suitable crystalline structure. These in combination with other conventional waxes can add a point of difference to a lipstick. There are silicone waxes that can be used in conjunction with the conventional waxes, which will add significant feel attributes as well as providing hardness to the stick itself. We still need the carnauba and candelilla for strength and shine and we still need to have beeswax for stick shrinkage and mold release. There are yet other waxes based on different chemistries that can be used to create a luxurious lipstick formula.

Hard waxes have a fairly narrow melting point range (around 3–5°C) and usually show a sharp transition when going from the solid phase to liquid phase. Soft waxes on the other hand have a much wider melting point range (around 10–12°C). These undergo a relatively gradual viscosity change around the melting point. This will be evident when running a drop point on the lipstick. The drop point is a relative measure of a product's melt viscosity but is

not exactly the same as its melt point. While the melt point may be sharp and happens quickly, the drop point will take longer for the product to move (drop) or *flow*. Rheology plays an important role in lipstick formulation. Rheology is very important in a lipstick, as one still wants to inhibit product *flow* especially at elevated temperatures. Even though a stick is a solid at room temperature and doesn't really flow, this will help maintain stick integrity when the temperature does start to rise. The stick may get soft as the temperature goes up but we don't want it to actually melt in its case. We will talk a little more about rheology later on in this chapter.

Silicone waxes act in very much the same way as typical hydrocarbon waxes (various grades of ozokerite or paraffin) in that they go through a phase transition from the solid phase to a fairly viscous liquid phase over a well-defined and predictable temperature range and can be controlled as the silicone molecule is synthesized. This phase transition usually occurs at or slightly above room temperature. What this means is that when a lipstick is applied, the user gets the benefit of a luxurious creamy laydown. Most silicone waxes are compatible with paraffin waxes, making them work well with many of the hydrocarbons we formulate with. Silicone waxes are unique because they have a much higher molecular weight than paraffin waxes and usually have a higher viscosity when they are liquids. Again, depending on the chain length, this can be controlled.

Silicone chemistry is becoming a very large and integral part of cosmetic formulations, and not only in lipsticks. They offer a wide variety of characteristics that are just not available from conventional ingredients. The worst part of using silicones is trying to pronounce some of the names and making sure they are spelled correctly on the ingredient label. Different silicone manufacturers are experimenting with their own silicone technology, knowing what the industry and cosmetic formulators are looking for. The addition of different oil-soluble groups to the silicone backbone results in materials that are highly desirable in cosmetic formulations. This creates a whole new class of ingredients with a huge variety of sought-after properties. Unlike plain dimethicone, alkyl dimethicone compounds are soluble in many oils, including mineral oils and petrolatums, esters and other oil-soluble materials due to the presence of the alkyl group. Alkyl silicones are extremely hydrophobic and are even insoluble in dimethicone. They are unique in that as the alkyl group increases, the hardness and melt point increase. For example, the alkyl chains of C32 and higher can have a melt point as high as 60°C or more. Conversely, as the silicone portion of the molecule increases, the softer the material becomes.

Silicone elastomers and blends are available as gels made with elastomer particles dispersed in a silicone-based carrier fluid or as a dry powder that can

be dispersed in a suitable solvent by the formulator into the product. These elastomer blends can vary greatly from high viscosity to low viscosity, from high tack to very low tack—all the properties that a formulator may look for in a lipstick ingredient.

Ingredients such as silicone polyglucosides. polymethylsilsesquioxanes, silicone polyvinyl acetates, stearoxymethicone/dimethicone copolymers, acrylate copolymers/hydroxypropylmethicone and polysilicone-11[1] along with many other different silicone compounds will add the positive feel and performance characteristics that lipstick formulators are looking for in a product. Some of these are silicone elastomers, which were first used in the medical field, are excellent film formers and easy to work with. If they were acceptable in that field, they certainly were safe and permitted for use in cosmetics, as safety testing would have been conducted on these materials as well.

Along with the waxes, there are the other main ingredients that go into making a lipstick. Here too, silicones can play a significant role in the outcome of the lipstick formula. Modifications in the silicone molecules can yield new ingredients with unique and novel feels and application characteristics. The remarkable thing about these silicone polymers is that modifying the chain with known positive properties, they can be made to feel comfortable on the lips, unlike the films derived from conventional polymers. Some prime examples are alkyl modified silicone compounds, silicone waxes, and other silicone derivatives currently being synthesized and investigated by today's silicone chemists. Depending on their molecular weight and chain length, these can be hard waxes or soft gels that offer a very wide variety of performance characteristics. Their compatibility and solubility with other ingredients is surprisingly quite good due to their chemistry.

Phenyl modified silicones such as trimethylsiloxyphenyl dimethicone have higher a refractive index than conventional dimethicones. That means they offer more shine than a regular dimethicone fluid. Since most silicone compounds are not noted for providing high shine, these can help alleviate that problem to some degree. They are clear and colorless like dimethicone and are available in various viscosities from 20 to 1000 cps. These are also very compatible with many different esters, some longer-chain alcohols, and other silicone compounds but not so much with hydrocarbons (which do not offer that much shine). They also can reduce the tackiness that is associated with acrylic acid polymers, which can be used to extend wear.

Different silicone manufacturers have different approaches to new silicone technology. So the lipstick formulator should "shop around" to see what is being developed by the various manufacturers and determine if there is a place for any of this new technology in a new lipstick formulation.

Conventional waxes have even been modified with silicones. There is siliconyl beeswax, siliconyl carnauba wax, siliconyl candelilla wax, and siliconyl polyethylene and even synthetic waxes[2]. The advantage of using siliconyl waxes is that it improves the compatibility and solubility of other silicones in a formula that would normally be very difficult to combine and work with. As science progresses, there may be other waxes based on silicones that will be made available to formulators. As the need for additional and different waxes increases, the development and introduction of these waxes will also increase.

There may also be synthetic waxes that can be based on chemical reactions. These waxes may be the by-product of the chemical reaction. One must be careful, as any chemical process has costs associated with it and if the process is refined or modified to cut costs, then the by-products may be reduced or even eliminated as a result. This means that the synthetic wax you have been using may have limited or no availability down the road. This also means that reformulation will be necessary no matter how fabulous the ingredient was. Most manufacturers will know this ahead of time and give sufficient lead time for the reformulation. Needless to say, the reformulation may not be that easy because the wax may have been that unique. That's probably why the wax was used in the first place.

No matter what the choice of waxes, it should be remembered that a suitable and stable crystalline structure should maintain a melt point no higher than 75°C and no less than 65°C. Stability at elevated temperatures is very important as lipsticks are sold in every market around the world and temperature conditions vary greatly throughout the year. The same formula can be sold in locations on the equator in the summer as well as northern Canada in the winter. So the same "global" formula must pass under *all* product stability conditions.

The next major hurdle to overcome is selecting the remaining ingredients that will contribute to all the positive attributes in the lipstick. Lipids, fats, oils, esters, soft waxy materials, etc. will all be needed to achieve the desired qualities one is looking for in the lipstick. But using all the same ingredients will only bring you back to that "me too" scenario. So we go back to new technology to achieve all of the desired attributes we are looking for. What is very important is to maintain stability and compatibility in the lipstick formula. The correct balance of polar and nonpolar solvents must be used in order to maintain stick structure and integrity, but polarity and pigment wetting are equally as important. Figure 3 shows how various emollients are categorized and classified in the world of cosmetic ingredients. This can be used as a gauge for compatibility of different ingredients in the same or similar formulas.

Figure 3

Most ingredients used in a lipstick are on the polar side but this polarity can vary from ingredient to ingredient. Nonpolar ingredients must be kept to a minimum in the formula as there are compatibility issues to be concerned with, which can sometimes lead to other problems such as *sweating* in the lipstick. This is something every formulator wants to avoid. Sweating can happen as the temperature of the lipstick rises and causes molecular movement. The liquid droplets usually reabsorb (leaving no marks on the stick surface) when the temperature equilibrates or comes back to room temperature. The worst situation is when a lipstick sweats at room temperature with no rise in temperature. This may be a sign of ingredient incompatibility and definitely will be very unappealing to the consumer. Sweating can also be treated and avoided by adding clays to the formula in the form of master gels.

Bentonite and hectorite clays pre-dispersed in a solvent can be added as a gel to the lipstick formula. The addition of these clays (in the form of gels) helps reduce and slow down molecular movement at elevated temperatures and can reduce or eliminate the incidence of sweating in the lipstick. As previously mentioned, rheology is a very important parameter in a lipstick. These clays tend to alter the rheology of the lipstick system and reduce flow characteristics. Within the lipstick's crystalline matrix the clays and other powders fill in the gaps between waxes and oils and prevent the liquids from seeping through when the temperature elevates. They are mainly used in cosmetic products for rheology and viscosity control. Since these clay master gels can be difficult to prepare in the lab (ionic placement is critical for proper viscosity build), it is much better to use them pre-made in the

form of the gels. There are many solvents that these gels are already prepared in, so the formula has a wide variety to select from.

Probably the most important attribute that is sought after by consumers as well as formulators is wear and longevity. Let's talk about this first, since it may be the most difficult attribute to deal with. While it may be easier to make a lipstick shiny and feel nice on the lips, making it wear long is not as simple as one would think. The ideal lipstick should last on the lips for at least six hours. The average lipstick has about four hours of wear, or less. This is extremely subjective because each wearer has different wear habits and may need to apply the lipstick regularly. Many people drink a lot, eat a lot, talk a lot, and just move their mouths, causing the lipstick to wear off sooner. Some people just enjoy applying lipstick. The object is to reduce this product movement or "transfer" by creating a film that does not wear off that fast. To get more than six hours is quite the challenge. But that is what marketers and formulators are striving for.

Feel and shine are the next most important lipstick characteristics. There are many ingredients available to the formulator to give a stick a very nice creamy feel and application as well as provide an abundance of shine on the lips. Some esters have a very high refractive index and provide a great deal of shine. Aside from polybutene-type ingredients, there are many new ingredients being introduced to the cosmetics industry, many from unusual sources, which can provide high shine.

While polybutenes and their derivatives provide extremely high shine, they can be extremely tacky on the lips and are synthetic in origin. Hydrogenated polyisobutenes maintain their shine but are much less tacky. Both the polybutenes and hydrogenated polyisobutenes come in various grades that have increasing viscosities. Some pour freely and some are almost solids and do not flow easily at all. These must be solubilized in compatible materials in order to make them usable in the formula.

4.1.2.4 TRENDY AND EXOTIC INGREDIENTS FOR LIPSTICK

A whole new group of ingredients is being made available to the cosmetics industry. These are being sourced from various rainforests throughout the world. They can come from the Amazon Basin in Brazil or as far away as equatorial Africa and Asia (see Figure 4). These ingredients, while offering unique feel attributes, are also quite functional. "All natural" is one of the directions the cosmetics industry is heading towards. Indigenous peoples and ancient civilizations used these as staple ingredients in their normal lifestyles because they worked so well in a variety of ways. This functionality is being transitioned into today's cosmetics and personal care products. What people routinely used these ingredients for in their everyday lives, cosmetic formulators are routinely making claims for in their products.

Figure 4

Ingredients such as copaiba balsam, andiroba oil, sange de drago (dragon's blood resin), açai butter, buriti oil, maracuia oil, and a host of others with strange-sounding names are becoming a very strong trend in many cosmetic products, including color cosmetics and lipsticks. These ingredients will add moisturization and emolliency to the lipstick as well as making the stick feel nice and creamy and at the same time providing shine. Included in this category would be various oily extracts derived from plant sources. While being a bit more costly than conventional ingredients, they can add some interesting claims to a product, which in many cases pays for itself in quality—especially for some of the major prestige and high-end brands.

Many peoples around the world used (and still use) the forest and what it offers as a source of all their daily needs (and livelihood): for food and nourishment, health and healing, cleansing and beauty, for barter and trade. Many of the characteristics we try to include in today's cosmetic products are based on what these ingredients have to offer. That is why there is such a concern over the decrease in the world's rainforests. As the rainforests disappear, so do the sources of these ingredients. Some of these ingredients can only survive in these specific environments and is sometimes their *only* source.

So we have all these new oil-soluble ingredients: solids, liquids, pastes. These can be added to a formula to create a great lipstick. But a formulator must know what ingredients to use in order to achieve a specific attribute, especially esters, which can and do vary greatly. Adding the wrong ester may not improve shine or increase creaminess on the lips. There are ways that this can be measured so that the correct ingredient is selected.

Figure 5

Spreadability is an important gauge in determining if an ingredient will be smooth when applied to the lips. So spreadability is important in determining how a lipstick will apply and how it will feel. It can be measured by comparing the different "spreading coefficients" that each ingredient possesses. With many ingredients, spreadability increases with polarity, increased molecular weight, and the degree of branching on the molecular chain. Figure 5 examines some common cosmetic ingredients and the difference in spreadability they provide. The lower the number, the "dryer" the ingredient will feel and the less it will spread. While spreading coefficient calculations and equations involve the spreading of an oil onto the surface of another liquid (usually water), relative to one another the liquids cited can still be compared against one another. After all, there is some thin layer of moisture on the lips when a lipstick is applied. For example, for a lotion with mineral oil used as a base, in order to spread freely and evenly on the skin (which contains some moisture if not really *dry* skin), its polarity and spreading coefficient can be increased with the addition of surfactants or emulsifiers in order to help it blend into the skin.

4.1.2.5 MISCELLANEOUS

Mineral oil and many other hydrocarbons and petroleum-derived ingredients can feel dry on application and have very little slip. So they may not offer as much creaminess or shine to a lipstick formula as a moderate molecular-weight ester

such as triisostearyl citrate (TISC) can. While they may be great for occlusivity and moisturization because of the films they generate on the lips, hydrocarbons add very little in terms of positive aesthetic qualities. While they are necessary in a stick formula to maintain good crystalline structure, stick formulations based on hydrocarbons and mineral waxes exclusively are put into an entirely different product category such as concealers and foundation sticks. Plus many formulators are staying away from mineral oil in many of their formulations. Light esters can feel dry as shown by their low spreading coefficients while different silicone oils begin to have higher slip by exhibiting spreading coefficients of 35 and higher. The use of volatile fluids should be avoided unless the formula actually calls for them. Here the package must be taken into consideration and must be airtight to prevent the loss of solvents. This may ultimately add additional cost to the product but it is something that cannot and should not be overlooked. There are times when special packaging may be required when a new product is developed.

The combination of ingredients with high-slip and oil-soluble film formers may also help increase wear. Such film formers that come to mind are the family of Ganex™ polymers (or their new name Antarons™). We have Ganex™ V220 (PVP/eicosene copolymer), a waxy solid, Ganex™ V216 (PVP/hexadecane copolymer), a liquid, and Ganex™ WP660 (PVP/triacontene copolymer), another waxy solid[3]. These were originally used mainly in hair care products for shine but can be used to create a shiny film on the lips. They are very compatible with many lipstick ingredients and high use levels are not that necessary to create an adequate film. What also can be investigated are anhydrous adhesives as long as they produce a flexible breathable film on the lips. Their adhesive properties should extend the wear of the lipstick. Once again, low levels should be used in order to avoid the product being too sticky on the lips.

The use of powder fillers can also add some unique feel properties to a lipstick. Nonabsorbent spherical silicas, boron nitride (BN), polymethyl methacrylate (PMMA), polytetrafluoroethylene (PTFE), and other specialty powders and polymers can provide a ball-bearing effect, greatly affecting the application characteristic of the lipstick. They provide a smooth slippery feel when applied and the lipstick tends to feel like it is gliding onto the lips. These ingredients come in various grades (opacity and particle size), so it is easy to make them "fit" into the formula, but the correct grade must be chosen in order for the lipstick to perform properly. A formulator can also use the various grades of synthetic nylon (nylon-6 and nylon-12) and natural silk powders (derived from silk protein) to enhance the feel and application properties. Many ceramic powders are used in cosmetics, especially for these unique feel characteristics.

Ceramic powders such as boron nitride require high heat and pressure in their manufacturing process. A reaction starts out with natural borax and a high-temperature

nitrogen source. Unlike spherical powder, these result in a lamellar structure where the powder particles glide over one another instead of roll. As these molecules do not exist in nature and must be synthesized, they cannot be considered natural materials even though their names imply that they may be mineral in origin. For a consumer, this can be very deceptive for a product's package and label copy. Since many ingredients are synthesized, some cosmetic feel characteristics can be as well.

Borosilicates are another class of effect pigments that due to their very smooth and flat particle surface and shape can also enhance the feel of a lipstick, whereas plain micas and other effect pigments have irregular shapes with jagged edges with different platelet thicknesses, and the larger particles can be felt on the lips. Calcium aluminum borosilicate and calcium sodium borosilicate are two such effect pigments in this category. They are a form of glass bead with a very controlled surface. There are other fillers that have a nice slippery feel, such as lauroyl lysine, an amino acid powder that is also used as a texture modifier. Yet there will still be other fillers that owing to their particle shape will enhance the feel of a lipstick. That's just the way our industry and new cosmetic ingredients have evolved. It is up to the formulator to come up with just the right combination of ingredients, such as the ones mentioned in this chapter, to get the optimum performance characteristics and the best positive properties out of the lipstick formula.

Particle size is very important, as it relates to feel. For instance, bronze and copper metallic powders have a very small particle size (and a large surface area) while glitter pearls, which are actually plastic polymers (polyethylene terephthalate or PET), can be very large. These glitters will most likely be felt on the lips. Glitter pearls are also hygroscopic and absorb moisture from their surroundings, including the lips. There are some particle-size restrictions for mica-type effect pigments (no higher than 150 microns) in some other cosmetic eye products. For non-mica pigments, good judgment should be used when using larger particles in any cosmetic product. Larger particles will impact the feel of the product. Pearls at 150 microns may feel raspy on the lips and for people with sensitive skin or lips, this is an absolute no no and should be avoided.

This brings us to the actual color additives, the ingredients that make a lipstick what it is—a product to deposit bright vivid color on the lips. But care must be taken here as well, as some colorants are highly absorbent and can feel somewhat dry or draggy on the lips. Colorants such as D&C Red Nos. 6 or 7 Lakes and Yellow Iron Oxide have a fairly high oil absorbance and will suck up a lot of liquid whereas colorants like FD&C Blue No. 1 Aluminum Lake and Titanium Dioxide do not suck up as much liquid as the colorants previously mentioned. This is evident as some dispersions will pour at a given color concentration and others will not at the same color concentration. The oil absorbance of all the powders in a lipstick formula can be calculated. There is a standard ASTM method for

this. These differences can be overcome by balancing the formula with a highly absorbent ingredient like alumina hydrate or an ingredient with low absorbance such as barium sulfate. In all cases colorants must be ground in a suitable solvent using a ball mill or three-roll mill. They can also be purchased from suppliers as pre-ground dispersions, so no grinding is necessary. This is ideal for cosmetic manufacturers that do not have grinding capabilities.

One of the more conventional ways of extending wear is to use staining pigments based on bromofluorescein dyes (such as Lakes of D&C Red Nos. 22, 28, and Orange No. 5) as colorants to the lipstick formula. These colorants, as with all other synthetic organic colorants, are used in the form of insoluble lakes. They start out as water-soluble dyes that are pH sensitive and will stain the lips bright pink well after the actual lipstick has worn off. These colorants are beautiful and create wonderful shades but they do have their drawbacks. Care must be taken when using them, as they are hygroscopic and absorb moisture from the lips. Using these pigments at too high a concentration may tend to dry out the lips, which again brings us back to the comfort factor.

Lipstick Pigment Levels

Light Coverage	4 - 6%
Medium Coverage	6 - 12%
High Coverage	12 - 18%
Super HIgh Coverage	18% (or higher)

Figure 6

Figure 6 shows the approximate use levels of colored pigments that can contribute to the various coverage levels in different types of lipstick formulations. The total level of color in the final product should always be considered, as the increase in color concentration usually has to be taken from other ingredients somewhere else in the formula, usually the oils, making the formula potentially dryer feeling. This doesn't take into account the pearls, which will also affect the lipstick feel when used at high levels. Pearls must be used in order to achieve a desired shade or specific effect.

The total amount of color additives, pearls, and other fillers used in the formula should be looked at very closely, as this will ultimately affect the final feel of the lipstick. While higher payoff may be a desirable attribute, this can also be accomplished by making the lipstick formula a little softer, which will also make it a little creamier and this should improve feel and shine at the same time. But excessively high pigment loading may also interfere with the lipstick's crystalline

structure and make it softer or even mushy. You go from crystalline to amorphous. While this may be acceptable for fingertip or brush application, it is highly unacceptable for a freestanding stick. It will just break too quickly, usually at its weakest point, the cup line, if too much stress is applied during application.

One way to check if a lipstick has sufficient crystallinity and hardness without the use of sophisticated equipment such as a Chatillon is to crush it between the thumb and index finger. While this is very qualitative, it does give a person a very good idea of exactly how a lipstick is supposed to feel when it breaks. When applying force, the lipstick should have resistance when squeezed harder and harder until it finally breaks. It should *snap* when it breaks, and this is a unique feel between the fingers. It should not crush and feel soft and mushy. This snap is something that is easily recognizable compared to a simple flat crush. A simple flat crush means the stick would be too soft with not enough crystallinity. The crystallinity is for lipstick strength and compatibility with a host of functional ingredients. It should also be strong enough (but obviously not too hard) for when it gets into the hands of the consumer, who will subject it to a myriad of stresses and strains.

The use of unconventional ingredients can result in a lipstick that has the desired qualities and still maintains a strong crystalline structure, making for a very stable stick. While there still may be a balancing act to overcome, achieving these goals by using ingredients that are not normally used should make it easier to balance feel, shine, and wearability without greatly sacrificing any of them. While there still may be a need for the use of conventional ingredients and polar solvents such as castor oil, which is used to wet out and grind pigments, a bulk of the formula will be made up of new and unique ingredients—getting away from the "me too" products that are on the market.

Also, a lot of the information presented here in this chapter comes from years of hands-on experience. Many of the basic concepts regarding lipsticks are written in books such as this, but the rest come from actually making batches at the bench. This also allows the formulator to understand what a lipstick is supposed to feel like and what combinations of ingredients to use in order to get there. While this may sound trivial, a formulator must have an acceptable stick structure that can be modified as needed with a wide variety of ingredients. Once the formulator is satisfied with the structure of the lipstick, the aesthetic properties of shine, feel, and longevity can then be adjusted. All the cosmetic ingredients cited in this chapter are readily available from multiple suppliers and distributors in the cosmetic industry.

Partnering with cosmetic ingredient suppliers and distributors can sometimes help the formulator in obtaining new and unique ingredients that will contribute to ideal aesthetic properties. Suppliers are searching the globe for new and sometimes

provocative ingredients that will enhance the properties of today's cosmetic products, including lipsticks. On a global scale, lipsticks are the one cosmetic product that consumers *cannot* live without. They are highly prized and very personal. You will always find a woman carrying at least *one* lipstick (and a mirror of some sort) in her purse or carry bag at all times. They don't go anywhere without it. In fact, women get very upset when their favorite lipstick shade is discontinued or no longer available.

It is hoped that the lipstick market segment sales figures will surpass and go far beyond 2011's 15% growth mark. The more novel and useful the ingredients are, the better the chance of creating that next greatest lipstick. If the product works, people will buy it. Good formulating skills and some experience will make this process a bit easier. With a little creativity and the understanding of how ingredients work, the next greatest lipstick won't be that far off. And that lucky formulator who creates it will really have something to be very proud of. I say lucky, because it will require a lot of work and searching for those special ingredients. The effort that a cosmetic formulator puts into creating the next greatest lipstick will truly be worth it. But which market will this great lipstick come from? Only time will tell, but it shouldn't be that far away.

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HYALURONAN (HYALURONIC ACID) – A NATURAL MOISTURIZER SKIN CARE

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ABSTRACT

This chapter describes the chemical and physical properties of hyaluronic acid and its cleaved, lower-molecular-weight fragments as well as its derivatives relative to their interesting and useful topical application to moisturize skin in anti-aging products.

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4.1.3.1 STRUCTURE AND SELECTED PHYSICAL-CHEMICAL PROPERTIES OF HYALURONAN

Hyaluronan (HA) is a naturally occurring linear polysaccharide consisting of alternating β-1,4-linked units of β-1,3-linked glucuronic acid and *N*-acetyl-D-glucosamine (Figure 1.1). HA is a main component of the extracellular matrix in connective, epithelial, and neural tissues and is known to play an important role in the tissue hydration and water transport mainly due to its enormously high water-binding capacity. The high water-binding capacity of HA can be attributed to high density of fixed negative charges in HA chains from carboxyl groups, which causes the osmotic pressure and draws the water molecules into the tissues containing HA.[1] For example, HA within skin layer holds up 1000 times its weight in water molecules.[2] Additional biological functions of HA include maintenance and viscoelasticity of liquid connective tissues such as joint synovial fluid and eye vitreous, supramolecular assembly of proteoglycans in extracellular matrix, and numerous receptor-mediated roles in cells, mitosis, migration, tumor development, and metastasis and inflammation.[1]

Figure 1.1 Hyaluronan (HA) structure.

The extensive repertoire of HA biological functions suggests the existence of different conformations and specific binding interactions.[3] It is likely that the conformation is affected by the local environment, including ionic strength and specific ion interactions, and the presence of interacting species including proteins and lipids. In the extracellular matrix, under normal physiological conditions and ionic strength, HA is generally believed to exist as a crowded random coil molecule.[4] A change in HA conformation is suspected under specific pathological conditions. Lowering the pH results in protonation of carboxyl groups and increased solution viscosity. In contrary, there is a polyanion contraction with rising ionic strength

due to the increased electrostatic shielding of HA. This latter effect is accompanied by a decreased solution viscosity. The HA solution viscosity is also temperature dependent and is a function of the overlapping parameter, molecular weight and concentration.

Some applications involving HA as an active ingredient require filtered HA solutions. The question that arises is whether filtration changes the physical-chemical properties of HA or not. According to our unpublished results, when proper dilution is used, filtration of HA (from 15 kDa up to 1.7 MDa) solutions through 0.22 μm nylon filter had no impact on molecular weight, polydispersity, and solution viscosity. The filtration impact on biological properties has not yet been established.

The major disadvantage of native HA is its low resistance against enzymatic degradation and short half-life after internal administration due to natural scission by enzymes and degradation by radicals. For this reason, at the present time the main challenge is the design of new HA materials that retain or increase the hydration properties of the polysaccharide and at the same time last longer in the application point. In order to increase the biological resistance of HA towards enzymatic degradation, modifications are being introduced in HA structure.[5,6] Another challenge is to synthesize HA derivatives with altered and specific physical-chemical and biological functions. Since the biological properties are connected with the HA molecular weight, there is also a great interest in HA degradation and evaluation of biological behavior of HA fragments.

4.1.3.2 PREPARATION OF HYALURONAN FRAGMENTS, ISOLATION AND CHARACTERIZATION THEREOF, CHARACTERIZATION OF DEGRADATION PRODUCTS

Disintegration of the HA β-linkages leads to formation of hyaluronan fragments of different sizes, identified by a name that indicates the number of monosaccharide units of which they are composed, e.g., a tetramer consists of four monosaccharide units—two glucuronic acids and two *N*-acetyl-D-glucosamines (GlcNAc). The name of the other fragments is created analogically. Fragments comprising more units are characterized by average molecular weight in Daltons. Thus, an eicosamer having 20 subunits is in literature specified as 20-mer, but it is also possible to say HA of molecular weight 4 kDa.

Mechanisms of the HA cleavage into its smaller fragments comprise enzymatic, free radical, thermal, ultrasonic, and chemical methods such as acid and alkaline hydrolysis.[7] Non-enzymatic reactions proceed randomly, and by this means mixtures of HA saccharides with different molecular weight can be obtained. By contrast, enzymatic cleavage is selective and allows preparation of HA

oligosaccharides with specific structure. Enzymatic digestion is catalyzed by two main types of enzymes—hyaluronate lyase and bovine testicular hyaluronidase (BTH).[8] Both enzymes produce fragments that contain an even number of saccharide units with GlcNAc at the reducing end. While HA oligosaccharides obtained by BTH are fully saturated, hyaluronate lyase produces oligosaccharides with the double bond between C-4 and C-5 position of uronic acid at their nonreducing end. Both saturated and unsaturated HA oligosaccharides lose under alkaline conditions GlcNAc from the reducing terminus with formation of odd-numbered oligosaccharides.[9]

During non-enzymatic cleavage, HA can be partially transformed into undesired degradation by-products exhibiting unwanted biological properties like carcinogenesis. Their identification is important so as to suggest ways of their elimination and to offer a final fragmented HA product free of toxic impurities. Until now only degradation by-products arising from acidic and alkaline hydrolysis have been established. Cleavage of hyaluronan under alkaline conditions gives a mixture of stereoisomers (*E*)-3-dehydroxy-3-en-D-GlcNAc and (*E*)-3-dehydroxy-3-en-D-ManNAc,[8] while acid hydrolysis of hyaluronan leads to creation of furan-like derivatives and derivatives of cyclopentanone.[10] These degradation by-products can be removed from the reaction mixture by ultrafiltration through a membrane of suitable cut-off.

Physical-chemical properties and biological activity of native HA significantly differ from its low-molecular-weight fragments. While high-molecular-weight HA is a space-occupying material and contributes to the structure of the extracellular matrix and tissue integrity, smaller-sized HA fragments are involved in a wide spectrum of complex biological mechanisms. From the cosmetic perspective, the most important properties of HA fragments involve stimulation of angiogenesis and supporting the proliferation of fibroblasts. Pro-angiogenic properties are exhibited especially by short HA oligosaccharides comprising from 6 to 20 saccharide units. HA oligosaccharides are mitogenic for endothelial cells, support their migration, and induce multiple signaling pathways.[11] Their good biocompatibility and low toxicity make them an ideal therapeutic agent for the situations when stimulation of new blood vessel growth is needed. *HA oligosaccharides are also components of dermatological compositions for combating hair loss and stimulating hair growth*. In the field of reproductive medicine, HA oligosaccharides may help fertilization.

Hyaluronan fragments can also be used for treating and preventing wrinkles, expression lines, fibroblastic depletion, and scars. HA oligosaccharides (with molecular weight <5 kDa) contribute to the suppression of wrinkles in both layers of the skin. At the epidermis, they stimulate the endogenous synthesis of high-molecular HA, which has a positive effect on hydration. At the level of the dermis,

HA fragments decrease the creation of pro-inflammatory interleukins, which are responsible for generation of free radicals capable of damaging both components of the extracellular matrix and the skin cells themselves. The most ambitious application of HA oligosaccharides is treatment of cancer. Anti-tumorigenic effects of HA oligosaccharides are based on their ability to sensitize cancer cells to treatment. The binding of oligomers to the HA receptors also inhibits the pro-tumorigenic signaling of high-molecular-size HA. Some low-molecular-weight HA molecules are believed to release strong immune reaction to the cancer cell.[12-14]

Analytical Techniques for Characterizing HA Molecular Weight

Since the length of HA polysaccharide chain is closely related to its biological functions, it is necessary to determine its molecular weight properly. A range of analytical techniques has been employed to assess the sizes of HA fragments. These were originally determined from the ratio of total glucuronic acid concentration to that of the reducing end GlcNAc group. More recently, other methods including viscosimetry, electrophoresis, mass spectrometry, and liquid chromatography have been used.[15] Electrospray ion mass spectrometry (ESI-MS) is mainly used for molecular weight characterization of short HA oligomers (up to 40-mer) derived from enzymatic digestions.[16] Enzymatic degradation provides a complex mixture of even-numbered HA oligomers, which must be fractionated using suitable techniques such as electrophoresis or liquid chromatography before ionization. ESI-MS utilizes a flowing stream containing the analyte and provides gentle ionization. Proper care must be taken in setting the cone voltage that accelerates the ions into the mass analyzer because high values of the cone voltage may result in an artifact of an increase of charged species. By contrast, low cone voltage leads to insufficient ions. At lower cone voltage it is possible to observe odd-numbered HA oligomers as artifacts in ionization.

As in the ESI-MS technique, it is not possible to avoid fragmentation of larger HA oligomers; it is advisable to use MALDI-TOF. This technique is suitable for characterization of polydisperse mixtures; it presents tolerance towards salts and buffers and is highly sensitive.[17] However, MALDI-TOF cannot be considered a quantitative method because desorption of HA fragments differs according to their size extensively. These complications can be overcome by using size-exclusion chromatography (SEC) coupled with multi-angle light scattering detector (MALS) and refractive index (RI), which provides detailed information of molecular weight, molecular weight distribution, molecular size, and solution properties in a wide range of HA fragments.[18] A limitation of the technique is that an SEC column cannot separate HA fragments sufficiently. Hence, two SEC analytical columns packed with an appropriate resin and connected in series are the possible solution for the separation of HA fragments. In this set, the length of the analysis is still acceptable.

4.1.3.3 PREPARATION OF CHEMICAL DERIVATIVES OF HYALURONAN AND THEIR CHARACTERIZATION

As it has been mentioned earlier, native HA suffers from its low resistance towards enzymatic digestion. This means that native HA has a high rate of *in vivo* turnover, which limits the period of time during which HA may act in the place of application. For this and other reasons, chemical modifications of HA intended for cosmetic or pharmaceutical applications are recommended. Chemically modified HA derivatives are also usually more mechanically robust and thus they are more suitable for final products manufactured using higher temperatures, pressures, or shear stress. *Modified HA derivatives usually have stronger binding capacity to the skin*, and as a result they are not washed off as easily as unmodified HA. An extensive research has been developed for chemical modification of HA. The strategies employed can be divided into two categories. The first one involves chemical attachment of pendant groups to change the properties of the polysaccharide and modulate the hydrophilic character of HA. The usual aim of these modifications is to lower the hydrophilicity of HA, so that the modified biopolymer will be able to blend with hydrophobic materials typically used in cosmetic products.

The second strategy for chemical modification of HA involves cross-linking in order to prepare insoluble derivatives of HA or hydrogels.[19] In fact, almost all of the commercially available HA products are stabilized by cross-linking to obtain materials with longer residence times and increased mechanical robustness.

Regardless of the modification strategy, the new, modified HA materials should remain biocompatible and biodegradable, as well as retaining the moisturizing properties. A critical point in the synthesis of HA derivatives is the preservation of the original chain length, since HA lubricant, shock-absorbent, and space-filling properties are closely correlated to its high viscosity, which is directly related to the initially high molecular weight of the biopolymer. From our personal experience, degradation is always inevitable during chemical modification processes.

Chemical modification of HA

Such modification can be carried out by the addition of the following functional groups to the polysaccharide backbone: carboxyl, primary or secondary hydroxyl groups, or the amino group after deacetylation. The chemical modification of carboxyl group is often preferred because it is the recognition site for HA natural receptors and hyaluronidase.[20] This method of modification may decrease the HA susceptibility towards enzymatic degradation.[21] However, the biological behavior of the polysaccharide, including its biodegradability, may change after such derivatization. Thus, the effect of each modification on the biocompatibility of the polysaccharide must be investigated in order to characterize the result on its properties.

HA coupling via its carboxyl group requires its previous activation to make it reactive to nucleophiles. The modification of carboxyl groups produces new amide or ester bonds in HA backbone. The typical reagents are the same as those used in common peptide synthesis, such as water-soluble carbodiimides (EDC and derivatives), carbonyldiimidazole (CDI), carbonyltriazole (CDT), N-hydroxysuccinimide (NHS), *p*-nitro-phenol, p-nitrophenyltrifluoracetate, and ethyl chloroformate (ECF). Chemical modification of HA is primarily performed in water or in organic solvent. To carry out modification in organic solvents such as DMSO, DMF, or formamide, a previous conversion of sodium hyaluronate to its acid form has to be done. However, this conversion drastically degrades the polysaccharide. Thus, the modification in water is always preferred. Previously enounced methodologies are in general patented and very well known by people skilled in the art. These strategies have been used for the synthesis of nano-emulsifiers,[22] films,[23] hydrophobized HA,[24] or hydrogels.[25]

On the other hand, the modification of the hydroxyl groups may involve the formation of ether bonds by reaction with bis-epoxides.[26] An example is the commercially available **Juvederm®**, a commonly used filler in cosmetic surgery. This product is chemically cross-linked by divinyl sulfone. Hydrophobized HA can be synthesized via formation of carbamoyl bonds after activation of hydroxyl groups.[27] The oxidation of the primary hydroxyl has been used for bioconjugation with lipids and subsequent hydrophobization.[28] However, some allergic reactions have been recently reported for commercially available products,[29] and so new challenges have appeared for scientists devoted to HA chemistry.

In the case of the formation of insoluble derivatives of HA (hydrogels), the capacity to retain water by the polysaccharide should be always determined by measuring the degree of swelling. *The denser the hydrogel is, the lower its water uptake capacity and swelling ratio.* In fact, regarding HA, most of the experimental data indicate that high swelling ratio values correspond to the lowest modification of the biopolymer. Low degree of substitution usually results in the formation of hydrogels with low cross-linking density, resulting in larger absorption of water and thus high swelling ratio. Conversely, high degree of substitution helps to form homogenous and more compact hydrogel network with lower water uptake capacity. Compression tests are often used to determine the smoothness of the hydrogel. Additionally, rheological studies are essential to evaluate the effect of derivatization or crosslinking.[30] Typically, the rheological behavior of HA and its derivatives is characterized by non-Newtonian behavior. The mechanical integrity of HA hydrogels is usually determined by measuring shear modulus (G), the larger the G value, the more resistant network towards deformation. The G value usually increases with increasing HA modification. After chemical modification it is always necessary to evaluate the effects of the derivatization on the physical, chemical,

and biological properties of the polysaccharide, as well as possible degradation during the process.[31]

4.1.3.4 HYALURONAN PENETRATION INTO THE STRATUM CORNEUM AND INTO THE SKIN

Skin is the largest reservoir of HA in our body, since it accounts for 50% of the total body HA content. Under ideal conditions, HA is found in all the layers of the epidermis and dermis. In the epidermis, HA is most prominent in the upper spinous and granular layers, where most of it is extracellular. By contrast, the basal layer of the skin also contains HA but it is predominantly intracellular. Presumably, basal keratinocyte HA is involved in cell-cycling events, whereas secreted HA in the upper outer layers of epidermis is part of the mechanism for dissociation and eventual sloughing of cells. The HA content in the dermis is far greater than that of the epidermis, and this accounts for most of the HA content in skin. The HA of the dermis is in equilibrium with the lymphatic and vascular system, while epidermal HA is not.[2]

Both epidermal and dermal cells are able to synthesize HA throughout our lifetime. However, *the skin cells lose their ability to produce optimal amounts of HA during the aging process. That is why the skin of babies is very rich in HA and is so soft, full, smooth, and supple, while with increasing age dehydration occurs; the skin thins and wrinkles appear.* The decline in HA production is also accompanied by a decreased suppleness, reduced elasticity, and loss of skin tone. The most dramatic histochemical change observed in senescent skin is the marked decrease in epidermal HA. In senile skin, HA is still present in dermis, whereas the HA of the epidermis has disappeared entirely. In fact, recent studies have indicated that the total level of HA in dermis remains constant with aging. However, at the same time, the proportion of total glycosaminoglycan synthesis devoted to HA is greater in the epidermis than in the dermis, and the reasons for precipitous fall with aging is unknown at the present time.

In order to retain the aesthetic appearance of the skin, and treat the signs of "dermatological" aging, it is recommended to keep "refilling" skin with HA from adolescent age onwards. It is already known that HA taken orally does not show any benefit to skin, because skin cells are not able to extract HA from the bloodstream.

The more reliable HA skin refill is by applying HA topically. Topical application of high-molecular-weight native HA (>500 kDa) is not recommended, because it does not efficiently penetrate the deeper skin layers mainly due to its large size. Instead it forms films that act as barriers against moisture loss.

Conversely, HA fragments sized smaller than 500 kDa are claimed to permeate and moisturize the skin. An efficient uptake of HA (357 kDa) by epidermis is indicated in Figure 4.1, where an isotopically labeled ^{14}C-HA was incubated with full thickness porcine ear skin using Franz diffusion cells at 37°C for 48 hours. *However, at the same time there was hardly any HA uptake deeper into dermis layers.* This *in vitro* penetration test into the porcine skin should resemble what will, with high probability, occur *in vivo* in humans. The results suggest that HA of 357 kDa applied topically is useful for the replenishment of HA levels in epidermis but is still too large to efficiently penetrate into dermis. Smaller HA fragments are then needed for more efficient penetration when dermis targeting is required.

Figure 4.1 Penetration of HA Fragments: ^{14}C-HA activities within 200 μm skin slices (pigs ear) after 48 hours penetration at 37°C.

4.1.3.5 MOISTURIZING PROPERTIES OF NATIVE HIGH-MOLECULAR-WEIGHT HYALURONAN AND HOW THE MOISTURIZING PROPERTIES OF HYALURONAN CHANGE AS THE MOLECULAR WEIGHT IS REDUCED

Due to the unique hydration, viscoelastic, and biocompatibility properties of HA and in view of a range of molecular-weight materials' ability to penetrate skin, *HA is being extensively used in cosmetic products as moisturizer, thickener, and stabilizer*. Since HA is a water-soluble product, it can be easily incorporated in the water phase of cosmetic formulas.

Due to its negative charge, HA is incompatible with cationic compounds or proteins at certain concentrations. A typical concentration of HA in cosmetic products varies between 0.01 and 0.2%[32]. *HA is suitable for application in a wide range of cosmetic compositions devoted to skin care and designed for topical applications.* Typical cosmetic products supplemented with HA molecules include serum,

moisturizers, creams, shampoos, conditioners, and bath oils. The benefits of HA being in the formulation are usually connected with reduction of the dermatological aging, reduction of the depth of lines and wrinkles (including fine lines), decrease of skin fragility, amelioration of skin atrophy, improvement of skin firmness and texture, decrease in pore size, restoration of skin brightening, and improvement of skin barrier function.

In hair-care and hair-treatment formulations, HA restructures the keratin fibers "inside" the fibers, improves elasticity of hair, and increases the washing resistance of colored hair.

The advantage of HA over other widely used moisturizers (glycerin, propylene glycol, and sorbital, polyethylene glycol) resides in the fact that its moisturizing properties are not significantly influenced by relative humidity. Unlike the other moisturizers, HA is able to keep good water retention capacity both at low and high relative humidity. In contrast, common moisturizers at low humidity and/or under xerotic conditions (dry skin, deep wrinkles, and squamous skin) absorb moisture from the inner skin rather than from the air, which in turn causes the skin to dry out. Together with other substances, HA belongs to Natural Moisturizing Factor (NMF) substances, which are hygroscopic, act as a surfactant, and buffer the skin thereby preserving its aesthetic appearance.

The moisturizing properties of HA are related to its hydration, often referred to as an average number of water molecules affected by the presence of HA anion in aqueous solutions. HA hydration was studied using several approaches including NMR,[33] viscosimetry, ultrasonic velocimetry, and thermal analyses.[34] Among these, thermal analyses, namely differential scanning calorimetry (DSC), is able to estimate i) non-freezable bound, ii) freezable bound, and iii) free water due to their different thermodynamic properties caused by the presence of HA (Figure 5.1). The non-freezable bound water is the fraction of total water content that is in close contact with HA surface and it is supposed to be either hydrogen bonded to HA molecules or is restricted by junction zones formed by HA chains. The freezable bound water is the next layer, which interacts weakly with HA molecules and non-freezable water layer. Free water is not influenced by HA and its properties resemble pure water. Therefore, it is mainly the amount of non-freezable and freezable bound water, which is considered as the number of water molecules hydrating HA structure. The number of bound non-freezable water molecules in 650 kDa HA is 7.6 per disaccharide HA unit. In terms of freezable bound water, the hydration increases up to 43 molecules per HA disaccharide unit.[35] In terms of grams, this means that 1g of 650 kDa HA can strongly hold from 0.8 to 2g of water. Further DSC results indicate that the water-binding capacity is independent of molecular weight from 30 kDa up to 750 kDa. Increased hydration (for about 0.12 g of water per g of HA) was detected for 1.4 MDa HA.[36]

Figure 5.1 Depiction of freezable-bound, non-freezable-bound and free water molecules in the presence of HA

To estimate the effect of different molecular-weight HA on skin hydration properties, a group of volunteers (n = 8) applied for 60 days (1 time per day) an o/w emulsion containing 0.005% native or fragmented HA. The hydration results reported in Figure 5.2 indicate that in the case of smaller HA fragments, namely 4.6 and 17 kDa HA, there is a significant skin hydration capacity increase after the first month of emulsion application. This result is in agreement with easier and faster penetration of smaller-sized molecules into the skin and with the fact that at epidermis small HA fragments stimulate the endogenous synthesis of high-molecular HA and in this way positively affect skin hydration properties. However, the more efficient hydration tendency of small-sized HA fragments was completely suppressed after another two months of emulsion application, where the best results were obtained for the native 1.66 MDa HA. As it was discussed above, the high-molecular-weight HA did not penetrate the skin but formed an efficient barrier against moisture loss. The results of the three-month study indicate that the skin hydration capacity is both HA molecular size and application time dependent.

Figure 5.2. Changes in hydration capacity of skin after the treatment with 0.005% HA in o/w emulsion.

4.1.3.6 SELECTED DERIVATIVES OF HYALURONAN AND THEIR EFFECT ON SKIN MOISTURIZING

Although a great number of HA derivatives have been synthesized in the last 15 years, there is a scarcity of literature devoted to the impact of HA modification on its moisturizing properties.

In general, it is believed that increasing the hydrophobic character of HA will enhance its penetration through the hydrophobic stratum corneum into the epidermis. Then, due to the fact that the permeability of substances through skin is not only related to their lipophilic character but is also inversely related to their size, small-sized hydrophobic HA fragments should theoretically favor transdermal transport.

To approve or disapprove the theoretical prediction, hydrophobized HA (50 kDa HA modified with 50% of hexyl functional groups) was tested *in vivo* for moisturizing effect. Similarly as in previous case, hydrophobized HA was integrated in o/w emulsion, which was daily applied by a group of eight volunteers. The changes in skin hydration capacity were compared to other two groups of volunteers using placebo and 17 kDa HA (Figure 6.1). In this case, the results indicated that the hydrophobized HA enhanced skin hydration capacity comparably to unmodified HA fragment. However, the effect on skin-moisturizing properties of tailor-made hydrophobic HA derivatives is unknown and still remains to be elucidated.

Figure 6.1. Changes in hydration capacity of skin after the treatment with 0.005% unmodified and hydrophobized HA in o/w emulsion.

Brown and colleagues[37] found that topical unmodified HA significantly enhanced the partitioning of both diclofenac and ibuprofen into human skin when compared to an aqueous control, pectin, and carboxymethylcellulose. This suggests that glycosaminoglycans, when allowed to interact with water, can enhance the percutaneous penetration of some drugs and cosmeceuticals useful in cosmetics and personal care. The details of their interaction remain to be elucidated.

In this matter, hydrophobized HA may represent even more efficient transdermal drug delivery systems because of two main reasons. First, as it was mentioned earlier, the partial hydrophobicity should increase the permeation rate of HA through the stratum corneum. Second, the hydrophobized HA contains both hydrophilic and hydrophobic functional groups, which under certain conditions may enable formation of micro and nano vehicles. These are able to increase the stability of poorly soluble lipophilic components and nutrients. The transformation of HA molecules into liposomes, microemulsions, or polymeric micelles can be used in transdermal research as a better alternative method to enhance permeation of components through skin. In fact, penetration of small hydrophobic drugs from such delivery systems into the epidermis can be increased more than two times.[38] Unlike other polymeric based delivery systems, HA-based polymeric micro and nano structures have major advantages due to excellent biocompatibility and biodegradability of the backbone. Thus, the feasibility of native HA and its derivatives for the development of new materials for applications in cosmetic, biomedical, and pharmaceutical fields is still under review.

4.1.3.7 COSMETIC APPLICATIONS FOR VARIOUS MOLECULAR WEIGHTS OF HYALURONAN

The skin hydration process proceeds through a very complex mechanism. Regarding HA, the whole range of molecular weights of HA is related to skin moisturizing. However, as it was described in details above, the exact moisturizing mechanism differs with HA molecular weight. For this reason, the choice of HA fragment for cosmetic composition should be always considered in context with intended reason of use and application. Simplifying the problem of Mw choice, the larger the Mw of hyaluronan the more predominant are physical-chemical properties, while biological properties will prevail in case of smaller Mw fragments.

For this reason, high-molecular-weight HA (about 1 MDa) is usually added in cosmetic formulation in order to increase composition viscosity and improve the stability of composition film when applied on skin. In this way, high-molecular-weight HA has positive effect on hydration of upper epidermis layers, which is manifested by lower trans-epidermal water loss (TEWL). Penetration properties (and thus anti-aging effect) of high-molecular-weight HA can be improved by combination of HA with skin penetration enhancers.

HA with molecular weights around 250 kDa enables deeper skin hydration due to its deeper penetration, where HA interacts with skin cells and extracellular matrix components. It is efficient in skin texture enhancement and wrinkles reduction.

Very small HA fragments (50 kDa and below) are very useful in anti-aging because after their penetration into deeper skin layers they serve as signal molecules for skin cells to synthetize new HA molecules. In addition, small HA fragments are

easily hydrophobized and in this way they penetrate deeper into epidermis and dermis, enhancing skin hydration capacity and enabling deep transdermal transport of complexed or bound hydrophobic actives from formulation.

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GLOSSARY

Angiogenesis: The development of new blood vessels.

Anti-tumorigenic: Tending to suppress tumors.

BTH: Bovine testicular hyaluronidase, EC 3.2.1.35.

DMF: N,N-Dimethylformamide

DMSO: Dimethyl sulfoxide

Endothelial cell: A thin, flattened cell, a layer of them lines the inside surfaces of body cavities, blood vessels, and lymph vessels, making up the endothelium.

ESI-MS: Electrospray Ionization Mass Spectrometry. An analytical technique that can provide both qualitative (structure) and quantitative (molecular mass or concentration) information on analyte molecules after their conversion to ions.

GlcNAc: N-acetyl-D-glucosamine

HA: Hyaluronic acid, hyaluronan.

Inflammation: The end result of the different bodily processes, in response to an injurious agent.

Interleukins: A variety of naturally occurring polypeptides modulating inflammation and immunity.

MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization –Time Of Flight. A method of mass spectrometry in which laser radiation of analyte-matrix mixture results in the vaporization of the matrix which carries the analyte with it. Ion´s mass to charge ratio is determined via a time measurement.

MALS: Multi-Angle Light Scattering. An analytical technique for determining absolute molar masses, sizes and conformation of all types of macromolecules.

Mitosis: The process where a single cell divides resulting in generally two identical cells, each containing the same number of chromosomes and genetic content as that of the original cell.

Mitogenic: Causing mitosis.

NMF: Natural Moisturizing Factor

Pro-angiogenic: Tending to promote angiogenesis.

Pro-inflammatory: Tending to promote inflammation.

Proliferation: The reproduction or multiplication of cells.

SEC: Size exclusion chromatography. A method in which molecules in solution are separated by their size**.**

AYURVEDA IN PERSONAL CARE

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ABSTRACT:

Ayurveda is the ancient Indian system of medicine for well-being and longevity. Ayurveda, roughly translated as the "knowledge for long life" is one of the oldest forms of traditional medicine based on the Hindu principles. The practice of Ayurveda dates back to 1200 BCE, focusing on plant-derived ingredients for medicinal benefits. The philosophies of Ayurveda address the overall well-being of an individual and the principles are therefore aligned to cure the cause of an ailment and not the symptom. Ayurveda combines the wisdom of medicine and plant-based natural ingredients to provide a natural remedy for ailments, by a combination of oral and topical applications. The values of Ayurveda are ever more pertinent for personal care applications in industrialized countries. In particular, consumers from Europe and North America are trending towards sustainable and green personal care. Eastern philosophies of Ayurveda using natural, botanical extracts offer a holistic approach to personal care that is both green and sustainable. In recent years, a strong body of scientific evidence suggests that Ayurvedic treatments are just as effective in personal care applications for cosmetic benefits compared to traditional chemistries. Ayurvedic herbs have been used in skin care for a variety of benefits like brightening, sun-protection, anti-aging, anti-inflammatory, and antioxidant. Recipes created with Ayurvedic herbs can also be found in hair care application with scalp-health enhancement, prevention of hair-thinning, and hair fail benefits. This section focuses on the variety of commonly used Ayurvedic botanical extracts, their proof of concept, and their claims in personal care. This section will also address the critical factors that influence the future success of Ayurvedic ingredients for topical applications.

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4.1.4.11 BACKGROUND AND FOLKLORIC USE OF AYURVEDIC MEDICINE

The origin of Ayurveda dates back to 1st century BCE in what is present-day India. Ayurveda was among one of the three oldest known forms of medicine, similar to Greek and Chinese medicine. The term "Ayurveda" is a Sanskrit word that embraces two facets, *Ayus* or *Ayur*, which refers to life principle, and *Veda*, the knowledge of how to live a long and healthy life (1). Like most ancient forms of medicine, Ayurveda is synonymous with holistic medicine and focuses on all facets of the individual for harmonious well-being. The primary means of treatment is by using phyto-active plant ingredients to cure various ailments. Ayurvedic practitioners have broad knowledge of plant biology and studied extensively the 1200 plants described for pharmacological applications (2). The medicinal practices are incredibly long and complex, and involve several aspects as described below (3):

1. Kayachikitsa (General Medicine)
2. Samhita (Basic Principles)
3. Vikriti Vigyan (Pathology)
4. Dravya Guna (Pharmacology)
5. Rasa Shastra (Pharmacy)
6. Shareer Kriya (Physiology)
7. Shalya Tantra (Surgery)
8. Kaumara Bhritya (Pediatrics)
9. Panchakarma (Bio-Purification Measures)

Ayurvedic therapeutic principles focus on treating a disease's cause and not just mitigating the symptom. In addition, the ideologies of Ayurveda focus on developing customized medicine and therapy for each individual, as opposed to mass-produced pharmaceutics practiced in western medicine. This is accomplished by creating a unique assessment for each individual based on body type, mainly categorized as "doshas" or basic energy types. There are three main energy types as described below (4):

- **Vata –** (air) energy that controls bodily functions associated with motion
- **Pitta –** (bile) energy that controls the body's metabolic systems
- **Kapha –** (phlegm) energy that controls growth in the body

Ayurvedic texts describe each individual with basic energy types following the above-mentioned principles. Imbalance of energy occurs due to a variety of factors such as genetics, stress, and unhealthy diet (1, 4). Much of the practice of Ayurvedic medicine emphasizes the need to balance energy for longevity. One might argue that knowledge and practice of Ayurveda is one of the oldest pioneering philosophies of the "beauty from within" concept that is widely accepted globally by the present-day cosmetic and beauty industry. This topical treatment was augmented in combination with an oral supplement, much like the cosmetic market today.

4.1.4.12 INGREDIENTS APPROPRIATE FOR COSMETIC/TOPICAL USE

Dermatological therapy using Ayurvedic ingredients has been practiced extensively in South Asia since the origin of Ayurveda. "*Panchakarma*," or bio-purification, uses oil or water based extracts of Ayurvedic blends to address dermatological concerns. These customized blends were taken in conjunction with oral supplements and applied as poultices for daily applications. Ayurvedic herbs become commonplace in the Western world around the 19th century, coinciding with the end of British colonization of India. The Ayurvedic recipes consist of simple botanical extracts, standardized ingredients, or purified actives, and can be of water or alcohol extracts based on the claim of the product. Different preparations have been used in cosmetic formulation as an ingredient or found in liposomal preparation. Some ingredients are also formulated as nutritional supplements to work synergistically with topical products (5). A brief summary of compounds used for Ayurvedic practices is given in Table 1 (End of Chapter). The following section describes in detail the most commonly used Ayurvedics in the beauty and personal care industry.

Ayurvedic ingredients with antioxidant potential

The formation of reactive oxygen species (ROS) results in cellular aging at different levels (19). ROS can react with various cellular components like the DNA, proteins, lipids, and saccharides, causing significant damage. ROS are important signaling molecules as part of the normal cellular processes and play a vital role in signaling and cellular cascades. Due to the exposure from the environmental insults (pollution, UV, stress, and smoking) the levels of ROS can increase dramatically and be a major factor contributing to aging. Skin, being the primary defense against environmental assault, is further damaged by ROS generation. Chronic exposure to ROS results in decline or even loss of skin's normal function. Damages, which can happen at different levels, include: RNA/DNA damages, impaired

lipid homeostasis, cellular inflammation, and oxidation of proteins and lipids. All of which lead to premature aging (19).

Almost all cells have endogenous protection systems, mostly in the form of enzymes to mitigate and convert the reactive singlet oxygen, the most important enzyme being *superoxide dismutase*. Another approach is to protect human skin by the use of antioxidants from herbs. Several Ayurvedic herbs that have found success in the cosmetic industry are primarily of interest for their antioxidant benefits (7). It has been well documented scientifically that folkloric medicine, particularly Ayurvedic medicine derived from plant extracts, has significant benefits in mitigating exposure to ROS.

The most successfully used Ayurvedic antioxidants include Amla (*Phyllanthus emblica*), Ashwagandha (*Withania somnifera*) Guduchi (*Tinospora cordiofolia*), Vidarikanda (*Pueraria tuberosa*) and Yashtimadhu (*Glycyrrhiza glabra*). These botanicals have rich sources of Vitamin C, phenolic acids, bio-flavonoids that act as strong anti-oxidants (7).

Amla (*Phyllanthus emblica*), a well-studied Ayurvedic botanical, is one of the richest known sources of Vitamin C. It also has high amounts of tannins, which not only have antioxidant benefits but is also anti-inflammatory in its effects. The active components of Amla are primarily gallic acid and ellagic acid. Amla has been used successfully formulated in cosmetic formulations to mitigate photo-damage as a result of UVA and UVB exposure to skin (7).

Ayurvedic ingredients with anti-inflammatory potential

Inflammation is part of a complex cellular response involving various cellular retaliators and multifaceted, interrelated biological processes (20). Most commonly, the inflammation stage is characterized as acute or chronic, based on severity of the inflammatory response. A variety of factors can trigger inflammation, not limited to environmental effects, stress, and infection (20). Figure 2 illustrates the inflammatory cascade in skin triggered by exposure of environmental stressors such as UVA or UVB and demonstrates the direct link of inflammation and skin aging processes, particularly with regard to breakdown of several critical extracellular matrix proteins such as collagen and elastin, and an increase in matrix metallo-proteases. In addition, a direct cause of inflammation is the impaired stratum corneum function of the skin. Stratum corneum plays a critical role in maintaining optimum function and barrier homeostasis. In addition, ROS generated by either acute or chronic exposure to various environmental pollutants also increase the inflammation response in skin and leads to premature aging (Figure 1).

Figure 1: Inflammatory cascade triggered by reactive oxygen species (ROS)

The most commonly prescribed anti-inflammatory medicine for is Aspirin, a nonsteroidal anti-inflammatory drug. For topical applications, the use of standardized and purified plant-derived compounds has proven to be successful. Figure 2 lists the purified compounds from Ayurvedic herbs used for cosmetic and therapeutic applications.

Clofibrate

Bakuchiol

Shatavarin-1

Himachalol

(1S,6R)-γ-himachalene **Guggulsterone-Z and Guggulsterone-E**

Figure 2: Chemical structure of ingredients used in ayurvedic practices

An example of an Ayurvedic plant studied extensively by scientists all over the world is turmeric. Turmeric a well-known Ayurvedic herb that has been used for millennia as part of the South Asian diet and is ascribed with anti-inflammatory and antiseptic benefits. Presently noteworthy research is being done with a purified secondary metabolite from turmeric, which has been identified as curcumin (8). In particular, curcuminoids have the ability to suppress certain cancers. For cosmetic use, curcuminoids act as a strong anti-inflammatory and modulate the expression of key inflammatory mediators such as TNFα, NFkB, COX2, and IL1 (9). Also, curcuminoids have the ability to protect extracellular matrix proteins such as collagen, elastin, and inhibitor of metalloproteinases when tested in skin-equivalent models (10). In summary, curcuminoids have the ability to be multifunctional, capable of mitigating the effects of free radicals and reducing inflammation, which makes this ingredient attractive to the cosmetic market.

Other noteworthy Ayurvedic ingredients with anti-inflammatory benefits include Bhrami (*Bacopa monnieri*), Bhrinjraj (*Eclipta alba; Eclipta erecta*), and Chirata (*Swertia chirata*).

Ayurvedic ingredients with antimicrobial potential

Modulating infection and regulating microbial consortia are of considerable interest for the cosmetic and beauty industry. Two of the most commonly occurring disease states, *acne* and *dandruff*, are a direct result of microbial infection. In the case of acne, the pathogenic microorganism is *Propionibacteria acnes*, while *Malassezia furfur* is primarily responsible for infecting the scalp and causing dandruff (11, 12). In addition, antimicrobial compounds are of huge interest for maintaining the integrity of cosmetic formulation as robust preservatives. There is enough evidence that Ayurvedic botanicals and blends have significant effect on controlling microbial population (13).

The bark of Neem (*Azadirachta indica A. Juss*) has been well documented for its antimicrobial potential, particularly in applications for oral care (14, 15). Neem plant has been the focus of several interdisciplinary research efforts for therapeutic applications worldwide. Over 100 active-compounds from Neem have been purified, and the biological activity associated with these compounds focus on skin care, oral care, and dental care applications (15). In fact, studies have demonstrated that Neem extracts are effective to control most common pathogenic microbes such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger* (16).

Current research focuses on developing blends of Ayurvedic plant extracts and identifying the antimicrobial efficacy in standardized tests for therapeutic benefits. An example of a blend of Ayurvedic herbs is Triphala, a combination of three plants Haritaki (*Terminalia chebula*), Bibhitaka (*Terminalia belerica*), and Amalaki (*Emblica officinalis*). Triphala is readily prescribed as a tonic to control microbial infection.

Much of the effort focuses on identifying the mode of action of these compounds to develop formulations for enhanced efficacy. Several double-blind clinical studies are being conducted to evaluate the efficacy of Ayurvedic products to mitigate disease states such as acne, dandruff, and others (17, 18).

4.1.4.13 CONCLUSION

There is a consensus among the scientific community globally for the need of a holistic, sustainable medicine. The global megatrends of aging population in particular support this need. Ayurvedic philosophies for customizable medicine tailored to individual need are also one of advantages. The challenge for Ayurvedic medicine is to have randomized and controlled efficacy evaluation for a large population. Considerable scientific effort is being focused on validating the efficacy of Ayurvedic medicine to support and broaden its applicability.

Consumer product companies are able to channel this ancient knowledge by offering Ayurvedic ingredients to the cosmetic market. Current Ayurvedic research focused on the development novel biological testing with standardized active constituents will help bridge the gap between Eastern philosophies and Western medicine.

As the cosmetic industry navigates towards sustainable and natural preservatives, Ayurvedic ingredients could be of high-value with well-established efficacy. The medicinal benefit of Ayurvedics will open up for global application.

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TABLE 1

Common Name	Latin name	Part used	Mode of action	Claim
Chakund; Pumar; Panwar	*Chrozophora prostrata*	Seeds	Anti-inflammatory action	Skin inflammation
Neelkanthi	*Sphaeranthus indicus in combination with Grewia sp.*	Leaves and Tender branches	Anti-inflammatory	Dermatitis
Bakra; Jamrasi	*Elaeodendron glancnnl*	Leaves	Anti-inflammatory	Eczema
Gular	*Ficus glomerata, Roxb.*	Bark and Leaves	Anti-irritant	Eczema
Chameli	*Lawsonia inermis*	Leaves	Anti-irritant	Eczema
Kamila; babchi	*Psoralea corylifolia (Babchi)*	seeds		Dermatitis and rheumatic joints
Ashwa-gandha	*Withania Somnifera*	Root	Antioxidant and T-cell activation	Anti-aging
Guduchi	*Tinospora cordifolia*	Leaf and stem	Anti-inflammatory, antimicrobial	Anti-aging
Neem	*Azadirachta indica, A. Juss.*	Leaf and stem	Antimicrobial, anti-inflammatory	Skin care and oral care
Amla	*Phyllanthus Emblica*	Leaf, stem and fruit	Antioxidant, anti-inflammatory	Skin care and oral tonic
Guduchi	*Tinospora Cordiofolia*	Leaf and stem	Antioxidant, anti-inflammatory	Anti-aging
Vidarikanda	*Pueraria Tuberosa*	Leaf and fruit	Antioxidant, anti-inflammatory	Anti-aging
Yashti-madhu	*Glycyrrhiza Glabra*	Root	Antioxidant, anti-inflammatory	Anti-aging
Bhrami	*Bacopa monnieri*	Root	Anti-inflammatory	Anti-aging
Bhrinjraj	*Eclipta alba; Eclipta erecta*	Root, stem	Anti-inflammatory	Hair and skin care
Chirata	*Swertia chirata*	Root	Anti-inflammatory	Anti-aging
Haritaki	*Terminalia chebula*	Fruit	Anti-inflammatory	Skin care and oral tonic
Bibhitaka	*Terminalia belerica*	Seeds	Anti-inflammatory, antioxidant	Hair growth stimulation

Table 1: Summary of commonly used ayurvedic ingredients (4, 6)

PROBIOTICS IN TOPICAL PERSONAL HEALTHCARE: A NEW UNDERSTANDING . . . A BRIGHT FUTURE

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ABSTRACT

Probiotics are beginning to be employed more in topical skincare because of recent science indicating live organisms are not required to elicit a positive bioactive response. The individual molecules believed responsible and the possible mechanisms of actions are reviewed. Recent evidence that keratinocytes possess immune activity similar to bowel epithelial cells connects observed activity of both oral and topically applied probiotics.

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4.1.4.21 INTRODUCTION

"Probiotic" is an old term with lots of definitions. The World Health Organization has one that is fairly standard: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host." We have used probiotics internally for thousands of years. Once we knew microorganisms were involved, we assumed these "good" bugs helped protect us from "bad" bugs. We also ascertained that the mechanism of action had something to do with the digestive tract, as these were all taken orally.

The use of probiotics as additives in topical personal healthcare products has been restricted by the obvious apparent need for "live" organisms, and we did not have any knowledge of the mode of action. We are all aware of the numerous foods that purport to offer probiotic ingredients and benefits: yogurt, buttermilk, miso, sauerkraut, kefir, etc. Many of these foods have been around for possibly thousands of years. We knew the result but not the mechanism. We also learned long ago that certain bacteria and yeasts could provide both a preservation component and a health benefit and, critically, not produce pathology.

Over the years the following organisms have become considered probiotic:

Selected strains of *Lactobacillus including: acidophilus, casei lactis, Plantarum, reuteri, rhamnosus, and helveticus*
Selected strains of *Bifidobacterium*
(new genus for *Lactobacillus bifidus*)
Selected strains of *Saccharomyces boulardii*
Selected strains of *Streptococcus thermophilus*
An argument can be made for: Selected strains of brewers' yeast *Saccharomyces cerevisiae*

These fermented foods did not spoil as fast and seemed to make us less prone to illness. Later we would say these seemed to help our immune system in a direct manner. Most of these products also appeared to work whether the organism was still alive or had become inactivated (e.g., from low pH, ethanol level, or pasteurization).

This would imply that the first part of the WHO definition needs a little alteration: "Live or dead microorganisms which when administered in adequate

amounts confer a health benefit on the host." This is not unexpected, as we can use either live or dead viruses in a vaccine to confer immunity. At this point a preferred term would be Probiotic Derived Bioactive (PDB), assuming the isolated ingredient from the total organism could be shown to be bioactive—i.e., possess immune stimulating properties, anti-inflammatory properties, etc.

4.1.4.22 OVERVIEW

Many researchers over the years have demonstrated that numerous biomolecules can be isolated from probiotic organisms (11–21) that are immune-stimulatory without any pathological characteristics.

So it would appear you do not even need the intact dead organism but rather only a particular part of the organism to produce a PDB. This is interesting but still does not tell us how the body "sees" these molecules or how it knows they are foreign, and what it produces that helps our immune system. These derived probiotics were thus, at least partially, a function of the outer coating of these organisms—since the inner parts of the probiotic organism were not needed.

For about 70 years we have known and used Beta-1,3-D-glucans (see Figures #1 and 4) associated with nonpathological yeast cell walls, as nutraceuticals and skincare additives. In the 1940s Zymosan was isolated from yeast cell walls by Dr. Louis Pillemer and determined to have immunological stimulating properties. By the 1970s Dr. Nicholas DiLuzio at Tulane University had identified the active biomolecule as Beta-1,3-D-glucan, and its use in treating human cancer and other conditions had begun.

Numerous patents and scientific articles cite (1–6) the use of Beta 1-3 containing D-glucans for immune stimulation, etc. In skincare, the "Glucan Glow" was advertised even if we did not know the cause. Numerous manufacturers now offer Beta-1,3-D-glucans from different sources. In all cases, if the appropriate molecule is present the same stimulation is observed independent of the source.

As with all topical bioactives, the delivery system employed to apply the topical PDB is critical to efficacy. The Beta-1,3-D-glucans have been used for many years, yet only recently has quantitative Polymerase Chain Reaction (qPCR) technology allowed the actual *in vivo* testing of human host peptide upregulation by PDBs.

Figure 1. The complex nature of the yeast cell wall, which not only includes the Beta-1,3-D-glucans but also glycoproteins, which also have immune-stimulating potential. Many of the commercial Beta-glucan preparations have been treated to remove all but the Beta-glucans. This includes the removal of all proteins, which can produce antigenic or overstimulation of the immune system, resulting in negative responses.

Figure 2. Probiotic *Lactobacillus Acidophilus* Cell Wall

As can be seen in Figure 2, the Probiotic organism *Lactobacillus Acidophillus*, a lactic acid producing organisms responsible for yogurt and buttermilk, has several well-known molecules associated with immune stimulation such as lipoteichoic acid and peptidoglcans (17). These molecules also were isolated and demonstrated to elicit stimulation of various immune-related cells in cell culture.

What's the common mechanism tying these together? The story gets a little bit more complicated at this point. In the mid 1980s a breakthrough occurred with the discovery of the defensins (1–4). These powerful antimicrobial peptides were found to be produced by our epithelial cells. Whether these epithelial cells were in the trachea, bowel, or epidermis of skin, they all had the ability to produce these host defense peptides. This was the key; this is the first line of defense against foreign organism invasion. The human host defense antimicrobial peptides can kill any bacteria, yeast mold, or virus attempting to enter through our skin, nose, lungs, digestive trachea, vagina, urethra, or skin.

The next discovery in the mid 1990s determined the mechanism by which the defensins were produced. Pattern Recognition Receptors (PRR) were found on epithelial cells that "recognized" specific chemical components from bacteria, yeasts, viruses, etc. One category of these was given the name "Toll-Like Receptors" (TLR's), which when activated, directly caused the production of antimicrobial defensins. So far, about 11 have been characterized—although all their functions are not yet known. These toll-like receptors were found on all epithelia including the mouth and nasal cavity, as well as in keratinocytes and Langerhans cells found in skin.

This innate system does not require any previous exposure to the organism. Therefore, these molecules can directly attack new foreign organisms and appear to also stimulate cellular parts of the human immune system into a second-line macrophage immune defense. The defensins also have numerous secondary characteristics besides antimicrobial properties. The literature reports that in addition to the antimicrobial properties, both proliferative and anti-inflammatory properties have been observed.

In the case of B-glucans it was also discovered by Brown et al. (2003) that the surface receptor Dectin-1 was also needed for the full response to fungi. The response to the B-glucans in the fungi wall is the production of TNF (tumor necrosis factor) and the defensins.

Figure 4: Unusual Biomolecules Found in Pathological and Probiotic Microorganisms Not Common To Humans

We now have a basic picture of how this ancient defense system works. It evolved over millions of years to allow mammals a first line of defense—without the requirement of immune cells being present or acquired immunity to have formed. All the cells, which come in contact with the natural environment, were given the ability to respond to foreign life forms (i.e., bacteria, yeast, molds, and viruses). All our surfaces inside and outside are one big immune system.

The toll-like receptors and other PRRs have evolved to "see" biomolecules that are foreign to us. We do not produce these molecules within our bodies. These biomolecules are outlined in Figure 4 (11–21). Probiotics are nonpathological

microorganisms that produce these molecules yet do not produce pathological symptoms—they are nontoxic, benign microorganisms, which look like bad guys but are not. They stimulate the toll-like receptors but cause no harm.

Like so many discoveries, our ancestors learned by trial and observation how probiotics could stimulate this ancient defense system without producing an actual illness. We could produce topical, oral, vaginal, and nasal products that could stimulate the local immune system to directly combat bacteria yeasts and viruses without actually knowing the exact mechanism.

However, knowing the exact mechanism helps in the next step for formulating with probiotics: How do you determine *in vitro* what works and what doesn't? Knowing that the defensins are the agents produced now leads to the next technology. In the past, only small animal testing (Rosette Test) could determine if an ingredient activated the immune system, and it did not directly measure the defensins.

Companies such as Mattek in the United States and Skin Sensitive in Europe have developed epithelial cell models that mimic the lining of the oral and nasal cavities, which produce defensins when stimulated with the appropriate molecules outlined in Figure 3.

Figure 3: Components In Fungi, Bacteria and Viruses That Activate Defensin Production Via Toll-Like Receptors

Commercial ELISA can monitor the defensin levels in these cell cultures before and after applying the test candidates. The results are at least semi-quantitative and give direct evidence of the potential efficacy of a topical, oral, vaginal, or nasal probiotic ingredient. This allows a rapid, reasonably priced (at least for large companies) alternative to animal testing that gives unequivocal data as to probiotic ingredient efficacy for product formulation.

Numerous raw material suppliers have made various probiotic-derived ingredients available for many years. For obvious reasons most commercial skincare or nasal or oral care products cannot be based on live organisms, which may continue to expel gas or consume other components in the formulation. Therefore, we must either kill the organism and use the whole organism as the stimulant or fractionate the organism via mechanical, chemical, or enzymatic methods.

In reality all these methods are being employed to enhance the bioavailability of the critical molecules, which are associated with probiotic activity. In many cases one could not determine the particular effects of these without human or animal testing. This makes it very difficult as a formulator to choose an appropriate probiotic ingredient.

However, the use of newer *in vitro* analytical technologies described previously allow these ingredients to be classified as true Probiotic Derived Bioactives. While the Beta-1,3-D-glucans have been the primary PDBs, numerous INCI-named ingredients are appearing in products with the scientific names of one or more of the organisms listed on the first page followed by "ferment," "extract," or "lysate" or a combination of these terms.

4.1.4.23 CONCLUSION

With the advent of testing biotechnology, the future is bright for probiotic ingredients and especially Probiotic Derived Bioactives. Being natural, with the capability of being certified organic in many cases, probiotic ingredients offer safe, green, and effective ingredients for the future. Aging is the loss of the ability to repair and maintain a healthy immune system. The ability to stimulate immunity and self-repair is a dream as old as probiotics. Yet the discovery that the defensins are overproduced in certain conditions, such as psoriasis, indicates that not all topical probiotic formulations are suitable for all consumers.

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BIOACTIVES GREEN AND SUSTAINABLE INGREDIENTS FROM BIOTRANSFORMATION AND BIOFERMENTATION

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ABSTRACT

Historically, fermentation is defined as a natural process of converting complex carbohydrates into alcohols using bacteria or yeast. The use of fermentation for food precedes human history. Evidence of traditional wine and beer fermentation with yeast has been documented since 3000 BCE. Since the discovery of antibiotics by fungi in the 1920s, biotransformation of ingredients via fermentation has been explored for pharmaceutical applications. Fermentation and cell culture encompass a wide range of organisms including bacteria, fungi, plants, algae, and mammalian cells. Harnessing the power of biotransformation of organisms has been exploited for a multitude of industrial benefits including pharmaceutical, nutritional, bio-energy, and cosmetic applications. The biotransformation techniques employed for cosmetic and personal care traditionally focus on natural methodologies using safe microbes and plants. In addition, much of the process of fermentation and cell culture can be done in a controlled environment and elicited to express secondary metabolites and novel bioactive ingredients of interest. These ingredients expressed during fermentation can be difficult to isolate, and not found in nature in significant quantities. Therefore, fermentation and cell culture become sustainable means of producing unique ingredients with tremendous potential for personal care applications. For example, cell cultures of rare and economically challenged plants are a sustainable means to express important bioactive components such as carotenoids and flavonoids. Biofermentation and plant-tissue culture are definitely more economical and sustainable compared to extracting carotenoids and flavonoids from field-grown plants. Current industrial practices for personal care

focus on exploring novel extracts and components generated by biotransformation that are both natural and recombinant. These novel ingredients can be peptides, proteineaceous extracts, enzymes, small molecules, and phytoactive components. The following section focuses on describing the novel ingredients generated from biofermentation of organisms (microbes, plants, and algae). The section will discuss the mode of action and efficacy of these novel ingredients, then applications in the personal care industry. The section will conclude with the future outlook for bioactives in personal care generated by fermentation and cell culture.

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4.1.4.31 RISE OF GREEN AND SUSTAINABLE COSMETIC INGREDIENTS FROM FERMENTATION

Fermentation-based products have a long history of use for cosmetics and beauty product applications. Whether it is the application of yogurt for scalp health, applying leftover grape-musts for skin care, or the use of fermented foods for beautification, the benefits of fermentation-based ingredients transcend cultures and geographies. Historically, most of the fermented products used for cosmetic mimic the nutritional diet referenced in a particular geographic region. The combination of fermentation and folkloric botanicals was often explored for enhanced benefit for health and beauty with continued success. The scientific community of consumer products realized the true potential of exploiting organisms for creating the next generation of ingredients for consumer products.

The focus of biotransformation efforts of the 19th and 20th century capitalized on the diversity of organism species available for fermentation. Fermentation efforts included a wide variety of bacterial species, yeasts, fungi, algae, plants, and unique organisms found in extreme environments (extremophiles). Coupled with the advances in the field of biotechnology for pharmaceuticals and drug discovery, the advent of using organisms (in particular microorganisms) as “biological factories” allowed scientists to generate novel compounds for beauty and wellness.

The essence of fermentation is the biotransformation of simple nutrients (sugars) to create novel compounds (secondary metabolites, bioactive compounds, vitamins, enzymes, antibiotics, peptides, and growth factors, etc.). The most commonly used fermentation and cell-culture applications for cosmetic applications broadly follow the principles of "submerged" fermentation and "solid-state" fermentation methods. Submerged fermentation is the fermentation using liquid media in an enclosed tank (typically made of stainless steel). In this type of fermentation, the media are utilized relatively quickly and active compounds are typically secreted in the media or can be extracted from the organisms (13, 16). The advantage of using submerged fermentation allows organisms that require high nutrients to grow robustly, and the purification of products is relatively easily. In solid-state fermentation, the nutrients utilized are carbon-rich raw materials (such as bran, paper pulp), which are utilized slowly. During solid-state fermentation the breakdown of nutrients takes place at a slower rate and the extraction process of involves multiple steps. The advantage of solid-state fermentation is the ability to recycle the nutrient source, therefore enabling a sustainable process (10, 13, 16). Solid-state fermentation is very common for fungal organisms and in utilizing food-waste biomass for the purposes of generating high-value compounds. The general schematic of a solid-state and submerged fermentation is shown in Figure 1. Recent advances in chemical engineering focus on modifying either the submerged fermenter or solid-state fermenter for most applications commonly used in cosmetic and consumer product industry (examples include air-lift bioreactors for algae, adapted with specific light sources; bioreactors with detailed air controls and mixing targeted for plant cell culture).

Schematic of fermenter typically used for solid-state fermentation

Schematic of fermenter typically used for solid-state fermentation

Figure 1: Solid-state and submerged fermentation examples (10):

For cosmetic and topical applications, fermentation and cell culture of a variety of organisms have been explored broadly. The most commonly utilized microorganisms are inspired by the nutritional and food industries, namely *Saccharomyces cerevisiae* (brewer's yeast) and *Lactobacillus sp.* (probiotic microorganisms). In addition, fungal species such *as Aspergillus niger* and *Aspergillus oryaze* (mold used for Koji rice fermentation) are well-developed fermentation vehicles. Algal cultures from macroalgae (kelp) to microalgae such as *Spirulina sp.* and *Ulkenia sp.* have been fermented in the bioreactor for specific secondary metabolites. Lastly, plant meristem cultures or the ability to harvest a broad variety of plant stem cells has garnered much success in cosmetic formulations. Furthermore, small-molecule fermentation of compounds such as resveratrol and other polyphenols have shown to be beneficial in cosmetic formulations and are bridging the gap between nutrition and personal care. Recombinant techniques have been employed to genetically modify organisms for the sustained expression of very specific secondary metabolites and compounds. It is also important to note that much of the work done in the area of fermentation and biotechnology incorporates multiple organisms and plant-based biomass.

The use of "stressed" lysates in cosmetic products is well established, and it has been demonstrated that these derivatives can help accelerate potential skin care activity in skin (7). For example, it has been found that organisms such as *Saccharomyces cerevisiae* and plant-cell cultures such as red rice callus culture can be grown in a bioreactor in the presence of external stresses. The response of organisms to external stresses, such as heat shock, peroxides, and ultraviolet light, is to provide enhanced production of novel active compounds that could have potential benefits for topical applications (1, 4). Figure 2 describes the various criteria that can be manipulated for fermentation of organisms. Current research also suggests that "dual fermentation," the ability to grow multiple organisms simultaneously for bioactive expression, has potential anti-aging benefits.

Figure 2: Factors influencing fermentation of ingredients for cosmetic and beauty products

In general, safety of the organisms is paramount for commercial success and market acceptance. The fermentation products can range from crude extracts, mixtures, and standardized active ingredients, to highly pure compounds (>99% purity). The success of biotransformation of organisms and the rise of fermentation can be attributed to several factors (11):

I. Biological diversity of species
II. Ability of organisms to express exclusive compounds that would otherwise be unavailable
III. Bioprocess conditions in a fermenter, allowing flexibility and controlled processing
IV. Ability to create stress-environments for organism, exposure by manipulating bioprocessing parameters
V. Technologies enabling expression of large quantities of desired active ingredients without the need to harvest large areas of biomass
VI. Enabling utilization of waste biomass to generate new compounds
VII. Reduced waste generation by using microorganism as the factory compared to significant amount of toxic byproducts made with chemical synthesis.
VIII. Chemical engineering, molecular biology, and microbiology advances allowing the scalability of several organisms from benchtop scale to industrial applications

4.1.4.32 THE IMPACT ON THE ENVIRONMENT

The natural trend in cosmetic industry has become a mainstay in consumer-product innovation. Consumer-product companies such as L'Oréal, Procter and Gamble, and Johnson and Johnson are evaluating every aspect of a product from packaging, supply chain, to manufacturing (Unilever, 20). In recent years raw material manufacturers are embracing fermentation as a way to ensure sustainable production of green ingredients for cosmetics and consumer-product applications. The market need for bio-based ingredients for cosmetic applications are captured in Table 1. It is estimated that a growth in the number of applications reaching commercial-scale production using fermentation could reach revenues up to €153bn by 2012 (21).

Table 1: Biotechnology options for cosmetic ingredients (21)

Polymer	Market (USD Billion)	Bio-based natural option
Polyurathene	~27	Soy based polyols
Polyester resins	~13	Maleic acid from succinic anhydride (manufactured by fermentation)
Nylon 6	~13	Caprolactam by fermentation
Acrylonite butadiene styrene	~11	Butadiene by succinic acid

The advantages of using biotechnologies versus conventional chemistries are enormous, based on industry's best practices for ingredients such as succinic acid, propanediol, hyaluronic acid, and others. The advantages of biotechnological innovation using fermentation to generate novel compounds compared to traditional chemistries are several (12).

I. Environmental benefits
   - Positive impact on CO_2 emissions
   - Ability to use renewable biomass waste generated from agriculture
   - Limited use of fossil fuels for manufacturing
   - Limits the need for pesticides and herbicides (particularly applicable for plant-cell culture)

II. Novelty of bioactive compounds via fermentation
   - Easy to generate significant quantities of novel ingredients
   - Prevents depletion of wild-biomass that may be scarce
   - Enables growth under conditions otherwise unattainable
   - Higher concentration of active components
   - Opens frontier to new active components
   - Easier way to procure uniform ingredients

4.1.4.33 ACTIVITY IN COSMETICS

Historically, the popularity of use of fermentation-based products in skin and hair care applications resonated with two microorganisms, *Saccharomyces cerevisiae* and *Lactobacillus sp*. Traditionally, live yeast cell derivatives (LYCD) from the yeast *Saccharomyces cerevisiae* have found the greatest use in personal care applications (1). The reason for this success is because *Saccharomyces cerevisiae* is a well-known, safe microorganism that has found use in multiple industrial applications such as beer brewing and baking. The organism is easy to grow and products can be isolated with relative ease; although color and odor control are key challenges in product development. For example, the application of lysates derived from *Saccharomyces cerevisiae* have shown to have an effect in increasing tissue respiration and accelerated wound healing (2, 4). In addition, the peptide fractions derived from yeast extracts can help stimulate skin-cell renewal and cell turnover through increased metabolic activity in the growing skin layers (14). Extracts from *Lactobacillus sp*. have significant applications in skincare such as the expression of beta-defensin production for anti-aging benefits (17). The extraction of standardized lactic acid from the fermentation of *Lactobacillus sp*. shows promise in skin-lightening benefits when tested on *in vitro* skin-equivalent models. The expression of bacteriocin peptides (short-chain antimicrobial peptides) is currently used as a management of *acne* (10, 14).

Numerous compounds from marine algal extracts have gained much importance for cosmetic applications due to its success in skin care applications. Marine algal compounds such as phlorotannins, sulfated polysaccharides, and tyrosinase inhibitors have shown applications towards a variety of skin-related topical formulations. *In vitro* studies on the extract from marine alga *Corallina pilulifera* have revealed the ability to prevent UV-induced oxidative stress and also the expressions of matrix metalloproteinase (MMPs) -2 and matrix metalloproteinase-9 in human dermal fibroblast cells (19). . Fucoxanthin isolated from *Laminaria japonica* has been reported to suppress tyrosinase activity suggesting broader applications in skin lightening (Figure 3). It is well documented that photo-damage to human skin by UV radiation leads to significant skin damage and ultimately premature aging. Skin damage by oxidants may lead to increasing MMPs expression and collagen degradation. Extracts of *Chlorella* have been shown to play some biochemical functions as well as *in vitro* inhibition of MMP1 activity (15). Micro-algae *species (Ulkenia sp.)* are well known for its ability to produce important lipids and the administration of the microalgae extracts have shown to significantly influence the effect of skin lipid synthesis (particularly cholesterol).

Figure 3: Tyrosinase inhibitors from algal extracts for potential skin-lightening applications (19)

Plant-cell cultures (meristem) are refined extracts of a selected plant and can be used for sustainable production of high-value compounds (3, 5). Red rice meristem extracts when used in cosmetic formulation suggest influencing epigenetic factors of aging, by influencing the (CPG promoter sequence) pro-collagen 1 and dermatopontin expression *in vitro.* In particular the red rice callus culture extracts are shown to improve Procollagen-1 and dermatopontin, which are extracellular proteins present in skin that deteriorates as we age. *Malus domestica* (apple) meristem cultures have shown promise in improving longevity of skin stem cells by delaying senescence of essential cells as a means to combat chronological aging. Influencing these proteins can have a noteworthy anti-aging effect and therefore is suitable for a variety of cosmetic applications. Plant-cell culture can also be a sustainable means to cultivate high-value compounds such as carotenoids and phytochemicals for skin and hair care applications. Figure 4 describes various plant-derived high-value compounds typically used in cosmetic formulations. One such group of high-value compounds are flavonoids (over 5000 plant-derived compounds with a flavane nucleus containing at least two benzene rings). Flavonoids can be extracted from several plant-cell cultures and have been reported in free radical scavengers; they are UVA absorbent, cyto-protective, and anti-inflammatory. They are also said to inhibit DNA damage and to affect cellular signaling pathways. Predominant dietary flavonoids include isorhamnetin, catechin, kaempferol, epicatechin, quercetin, and epigallocatechin-3-gallate (EGCG) (Figure 4) (5, 6, 22).

Figure 4: Chemical structure of high-value compounds obtained from plants (5)

Recombinant technologies are often exploited for the expression of specific peptides for cosmetic applications. Depending on the size of the peptides, there have been documented scientific benefits of using peptides in skin care formulations ranging from repressing the up-regulation of MMP-1 by skin fibroblasts (tetra-peptides), inhibiting tyrosinase (for skin lightening), or improving the function of skins structure by influencing TGF_{β}, (tri-peptides and their derivatives) (7, 22, 23). It would be remiss not to capture the significant contributions of recombinant technologies (genetically modified organisms) in the cosmetic and OTC realm. A good example is the successful commercialization of hyaluronic acid (traditionally found in rooster combs) in recombinant bacterial systems, enabling consistent large-scale production of an essential molecule for skin aging (18).

CONCLUSIONS

There remains a strong need for fermentation-based innovation as a solution to develop new active ingredients for cosmetic applications that are sourced sustainably. The popularity of fermentation-based products in the consumer-product arena is primarily a combination of three factors—namely safety, efficacy, and cost competitiveness. There is a consensus among the scientific community globally for the need of biotechnology-based ingredients whether from microorganisms, algae, or plants as molecular toolkits to generate new products. The market need is to develop products that are effective as well as green. Organizations should choose the biotechnology programs judiciously, based on market need and technology capabilities. The continued success of biotechnology will depend on demonstrating safety of ingredients from fermentation, public acceptance of such technologies, and continued investment in technology innovations to support long-term growth and value creation.

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MULTI-FUNCTIONAL BOTANICALS FOR TOPICAL APPLICATIONS

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ABSTRACT

Today both consumers and manufacturers are looking for "greener alternatives" for use in cosmetic and personal care products. Infact, the herbal or natural-based cosmetics are one of the fastest-developing segments in the personal care segment. Major cosmetic brands are shifting towards incorporating natural compounds in their products and consumers can find ever-growing choices in "green" cosmetics.

Use of botanicals in skin or personal care products is not new; they have been used in cosmetics for centuries. In Ayurveda, the Indian traditional medicinal system, which is heavily based on botanicals, there are several examples of plant-derived products for alleviating skin problems or enhancing the skin beauty.

In many instances, modern science has provided the scientific evidence for the activity of botanicals. Structural elucidation using the latest technology has helped to identify the active phytochemicals present in such botanicals and understand their mechanism of action. Botanical extracts are no longer the crude extracts used in the traditional system, but have evolved into highly pure and standardized extracts, their efficacy established with modern cell- and enzyme-based assays.

In the present chapter we look into various key elements that are involved in developing botanicals as cosmeceutical actives. We also describe different extraction techniques that are commercially available for obtaining the extracts of choice, and the role of standardization in developing these botanicals extracts as cosmeceuticals with consistent quality and efficacy. We also would present examples of multifunctional botanicals with focus on their activities, standardization, and applications in cosmetics, along with any clinical study done on them. Apart from this, we discuss the very important subject of sustainability of natural resources for use in cosmeceuticals.

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4.1.5.1 INTRODUCTION

Today we live in the age of green consumerism, and the personal care market is no different. Over the last one decade, the natural cosmetic market has shown tremendous growth and has been the fastest-developing segment in the personal care industry. In this consumer-driven market, the shift towards natural cosmetics can also be seen from the fact that many major cosmetics companies are now opening up for including natural products in their product line. The use of natural products in beauty and makeup is not new; examples of it can be seen in several civilizations. Use of oils such as thyme and rosemary on skin by Egyptians is well depicted hieroglyphically. Japanese used safflowers as pigments for rouge and lip makeup for ages (www.jcia.org), and in Indian culture examples of natural cosmetics such as dyeing hair with henna are well known (Auboyer 2002). The following sections are dedicated to discussion on the natural actives or natural cosmeceutical actives.

4.1.5.2 NATURAL AND NATURALLY DERIVED ACTIVES

Though definitions may vary for natural and naturally derived actives, it is important to understand the difference between naturally occurring and naturally derived cosmeceutical actives. Naturally occurring compounds when provided in

their natural form can be called natural products; hence epigallocatechin gallate when provided in an extract can be considered a natural active.

On the other hand, naturally derived actives do not occur in nature, but can be synthesized from naturally occurring sources. Examples of such compounds will be discussed in a later part of this chapter.

4.1.5.3 EXTRACTION TECHNIQUES

Since the natural compounds exist in a natural matrix of plant tissues and cells along with thousands of other coexisting compounds, to use them in conventional topical formulations it is important to extract them selectively from their natural matrix and purify them. These natural compounds may be present in certain parts of plant or herbal material, such as leaves, rhizomes, fruits, bark, or heartwood. For extraction of raw material, the process should be carefully chosen. The main requirements of the extraction process are:

a. Should be compatible with the plant material
b. Should be capable of selectively extracting and purifying the compound
c. Should be environmentally friendly
d. Should not be degenerative to compound or plant material
e. Should be economically feasible

Based on the raw material, there are various extraction processes that are commonly used in industry. Some of the major extraction processes are discussed below.

(1) Solvent extraction process
(2) Freeze-drying process
(3) Supercritical fluid extraction process

Solvent extraction is the most commonly used process. This process uses a variety of solvents, from a polar to nonpolar range, based on the particular compound of interest. Ethanol, methanol, isopropyl alcohol, ethyl acetate, acetone, and hexane are commonly used for extraction in industrial quantities. These solvents extract selective compounds based on the polarity of the compounds, thus enriching them in the extract.

The freeze-drying process is used for more sensitive and heat-labile compounds, which cannot withstand high-temperature treatment for long period of times, as in the case of solvent-extraction process. Freeze-drying is carried out in three steps: (1) freezing, (2) primary drying, and (3) secondary drying. The temperature of the freezing step is maintained at –40°C, converting most of the water present in the raw material into ice. Primary and secondary drying is carried out at elevated temperature to remove most of the unfrozen water (SM Patel 2010).

Supercritical fluid extraction is a process where supercritical fluids such as carbon dioxide is used for extracting the material from a solid or liquid matrix. Carbon

dioxide is the most commonly used supercritical fluid in this type of extraction. Carbon dioxide at its critical temperature (31°C) and critical pressure (74 Bars) tends to behave both like a gas and a liquid. This type of extraction process requires an expensive setup and has limitations in the of types of raw material that can be extracted. Carbon dioxide, being a nonpolar itself, can extract compounds of similar polarity and is thus limited to nonpolar compounds present in oils and oleoresins. Use of modifiers can to some extent increase the range of compounds that can be extracted (He et al. 2005).

4.1.5.4 ROLE OF STANDARDIZATION

One thing that can decide the efficacy of a product is the amount of active inside; thus it is of prime importance that the product should be properly formulated to contain the required amount of active inside. In natural products where the extracts may contain several hundred compounds present in macro- to micro- and even in nano-quantities, it is of utmost importance to have a proper standardization of the extract. Standardization is defined by the American Herbal Product Association (AHPA) as "the body of information and controls necessary to produce materials of reasonable consistency. This is achieved through minimizing the inherent variation of natural product composition through the quality assurance practices applied to agricultural and manufacturing processes" (Gaedcke et al. 2003). The first step in standardization of an extract is identification of the active compound. Once the active component is identified, it can be used as a reference for the quality of an extract and also its dosage levels for formulation. For a good batch-to-batch efficacy, the product should contain the same amount of actives. One of the major tasks in natural product formulation is preparation of a reference library of compounds that are used for standardizing an extract.

Apart from consistency in quality, standardization also helps to preserve the authenticity of the natural extracts. Proper identification of the marker compounds and chemical fingerprinting of the extracts using the latest chromatographic tools help ensure that extracts provide the desired biological activity and are free from any adulterants.

4.1.5.5 MULTIFUNCTIONAL BOTANICALS

a. Saberry®

Saberry® is the trade name for the Indian gooseberry extract marketed by Sabinsa. Saberry® is standardized to 10% of the active compound Beta glucogallin. Indian gooseberry or Amla is regarded as a tonic herb or *rasayan* in Ayurveda. It is also known as a detoxifying herb and is used to correct the doshas in Ayurveda. Apart from its use in oral Ayurvedic formulations, Amla is also used in topical beauty

care. Amla oil is known to be one of the world's oldest natural hair conditioners. Amla fruits have also been studied for their skin-lightening activity and MMP-1 inhibitory activity.

Amla fruit were long considered a rich source of vitamin C, which also accounts for its high antioxidant activity. However, the studies carried out on Amla in the last few years have raised questions on the presence of ascorbic acid in the fruits.

In order to unfold the nutritional and cosmeceutical benefits of gooseberry, a proper identification of a valid biomarker was required to resolve the confusion and authenticate the extract. Extensive work done by the research group at Sabinsa using modern isolation and hyphenated techniques like HPLC, flash chromatography, and spectroscopic method LCMS and NMR led to the identification of biomarker compound Beta glucogallin (Figure 1).

Fig 1: Structure of Beta glucogallin

Amla extract biostandardized with Beta glucogallin revealed the true cosmeceutical potential of this superfruit from India (Majeed et al. 2009).

Cosmeceutical potential of Saberry® (Indian gooseberry extract)

Antioxidant Activity

Brunswick Labs in Wareham, MA evaluated the ORAC activity of Saberry® using state-of-the-art ORAC tests. The ORAC* assays—ORAC, HORAC, NORAC, SORAC, and SOAC—are validated tests to measure broad-spectrum antioxidant power. The ORAC activity is expressed in micromole standard per gram. ORAC analysis (Table 1) showed that Saberry® has excellent ORAC activity in hydrophilic media. As expressed in the table below, Saberry® showed combined ORAC activity of 3586 micromole Trolox Eq per gram.

ORAC Activity

ORAC Hydro (µmol TE/g)	ORAC Lipo (µmol TE/g)	ORAC Total (µmol TE/g)	HORAC (µmol CAE/g)	NORAC (µmol TE/g)	SORAC (Kunits SODeq/g)	SOAC (µmol VItE/g)
2678	4	2682	345	904	102	1351

Table 1: ORAC activity for Saberry®

ORAC* Activity or Oxygen Radical Absorbance Capacity is an assay to measure the antioxidant activity in biological samples. AOAC International recently approved the ORAC method as a first action official method for measuring antioxidants in foods. (http://www.aoac.org/News/AOAC_SPS-FAM-01042013-2.htm). The various ORAC activities mentioned above are part of the ORAC activity assay, namely, $ORAC_{hydro}$ measures the activity against peroxyl radicals in hydrophilic medium. $ORAC_{Lipo}$ measures the antioxidant activity against peroxyl radicals in lipophillic medium. HORAC measures the activity against hydroxyl free radicals, while NORAC measures the antioxidant activity against peroxynitrite radicals. SORAC measures activity against the superoxide free radicals, and SOAC measures the antioxidant activity against the singlet oxygen.

1. Skin-lightening activity

The skin-lightening activity of Saberry® Amla extract standardized with 10% Beta glucogallin was studied by the research group at Sabinsa Corporation using nonanimal test models. Saberry® was tested for its melanogenesis-inhibitory activity using B16 F1 melanoma cells. The melanin production in these melanoma cells is induced by α-MSH and cAMP cells in two different samples. These cells were then treated with varying concentration of Saberry over a period of nine days with test substance (Saberry®) treatment repeated at regular intervals for three days (Figures 2, 3).

The melanin thus produced in the cells was treated with 1N NaOH, and absorbance was read at 405 nm in a microplate reader. The study was carried out with comparison to kojic acid.

Saberry showed IC_{50} of 14μg/ml in both cAMP induced and α-MSH–induced melanogenesis inhibition. Kojic acid in the same test showed IC_{50} of 100μg/ml (Sami Labs Report 09-223).

Figure 2: Inhibition of camp-induced melanogenesis by Saberry® in B16 F1 mouse melanoma cells

Figure 3: Inhibition of α-MSH–induced melanogenesis by Saberry® in B16 F1 mouse melanoma cells

2. UVA and UVB protection by Amla extract

Amla extract is known for protecting the human dermal fibroblast cells against oxidative stress. The studies have shown its protective effect on human skin fibroblasts, especially for production of pro-collagen. In a study published in *J Ethnopharmacology* (2008), it was found that Amla extract stimulated the proliferation of fibroblasts and helped to induce the production of pro-collagen in a dose-dependent manner. Study also showed inhibition of MMP-1 enzymes from the fibroblast cells (Fuji et al. 2008).

MMP-1 or matrix metalloproteinase (MMP) is responsible for the dermal photo-aging in human skin. UV irradiation actually up-regulates the production of UV-induced collagenases such as MMP-1 from dermal fibroblast cells (Quan et al. 2010).

Scientists at Sabinsa studied the cell viability of Swiss 3T3 mouse fibroblast cells in the presence of UV exposure with and without Saberry®. The Swiss 3T3 mouse fibroblast cells were placed in a microplate and treated with varying concentrations of Saberry and exposed to 5J cm-2 UV dosage and 0.05J cm-2 UVB dosage. After the exposure, the cells were incubated in a CO_2 incubator for 48 hours and developed by NRU staining techniques to analyze the cell viability (Figure 4).

Saberry® showed IC_{50} of 14µg/ml for protection against UVA radiation and 42µg/ml against UVB radiation (Sami Labs Report 09-223).

Sami Labs Report: 09 223 Cosmeceutical potential of Saberry®

Figure 4: UV protection by Saberry® in Swiss 3T3 mouse fibroblast cells

Cosmeceutical applications of Saberry®

Saberry® is standardized with a unique biomarker—Beta glucogallin. It is a beige-colored extract and completely water soluble. It can be used in anti-aging formulation, skin lightening or skin fairness, and sun-protection formula. It is free from any preservative.

Formulation guidelines

1. Solubilize Saberry® before adding it to emulsions below 40°C.
2. It forms a hazy solution with water (1:10) at 40°C.
3. Maintain the pH of the formulation as slightly acidic (5.0–6.0).
4. Saberry® can be gelled using thickeners such as carbomer, acrylates copolymer, and lecithin.
5. Protect the finished product from direct exposure to light and heat and use opaque packing.

Suggested level of use

Skin-lightening formulations: 0.1–0.3% w/w

Anti-aging: 0.05–0.2% w/w

Suncare/ after suncare: 0.2–0.3% w/w

Depigmentation: 0.1–0.2% w/w

b. Ellagic acid (from pomegranate)

Ellagic acid (Figure 5) is a naturally occurring polyphenol present in a variety of plants such as cranberries, raspberries, walnuts, grapes, and pomegranates (Usta et al. 2013).

Figure 5: Ellagic acid

Pomegranate as a source of ellagic acid has been of much interest for both nutraceutical as well as cosmeceutical benefits. Pomegranate fruits have shown antioxidant properties due to the presence of ellagic acid, which is one of the main polyphenols in the pomegranate fruit (Bell et al. 2008). Ellagic acid has been shown to have anticarcinogenic, antioxidative, and anti-proliferative activities.

Skin-lightening activity

Ellagic acid has also been studied for its topical benefits. Ellagic acid, being a polyphenol, is able to interfere with oxidative steps in the melanin synthesis, which is catalyzed by the tyrosinase enzyme.

Furthermore, due to its antioxidant activity it can also scavenge the reactive oxygen species, which can also play important role in melanin synthesis (John et al. 2005). Another mechanism proposed for its skin-lightening activity is related to its higher affinity to copper present on the active site of the tyrosinase enzyme (Shimogaki et al. 2000), which catalyzes the oxidative conversion of tyrosine to dihydroxy-phenylalanine (DOPA) and further from DOPA to DOPA-quinone.

To understand the underlying mechanism of tyrosinase inhibition by ellagic acid, Shimogaki et al. studied tyrosinase inhibition by ellagic acid in the presence of copper. While the tyrosinase enzyme activity was reduced or inhibited in presence of ellagic acid, this was partially recovered following the addition of copper. This suggested that the ellagic acid inhibits the tyrosinase activity by affecting the copper present at the active site of the enzyme (Shimogaki et al. 2000).

By incubating the cells with ellagic acid in the presence of copper compounds, researchers were able to show that ellagic acid inhibits the tyrosinase activity by affecting the copper present at the active site of the enzyme.

UV-protective activity

In the animal study done by same group (Shimogaki et al.), the protective activity of ellagic acid against skin pigmentation induced by UV rays was studied and compared to kojic acid and arbutin.

The study was carried out on brownish guinea pigs, which were exposed to UV-B irradiation once a day for eight days on a shorn test site on their backs. The test substance (ellagic acid) was applied topically every day for six weeks. After the six weeks the animals were sacrificed and skin samples were studied. It was found that ellagic acid plays a key role in preventing skin pigmentation following UV irradiation. The melanin content of skin was found to be reduced with ellagic acid application, not only in the basal layer, but also in other layers such as the stratum spinosum, stratum granulaosum, and stratum corneum.

Comparative data showed that ellagic acid at 1% was more efficient than kojic acid and arbutin at same percentage in reducing pigmentation on the skin.

Cosmeceutical application of ellagic acid

Ellagic acid containing extracts can be used for skin lightening, for management of hyperpigmentation, UVB protection, and antioxidant activity. As ellagic acid is not soluble in water, solvents such as PEG 300 and BG can be used to dissolve the active in the formulation stages. Suggested level of use is 0.1–0.5%.

Quasi-drug status of ellagic acid in Japan

Ellagic acid is one of the approved quasi-drug ingredients for management of hyperpigmentation disorder in Japan. Skin-lightening quasi-drug is a category of cosmetics that can be regarded as functional cosmetics, and can prevent or improve hyperpigmentation disorders such as melasma and chloasma. Quasi-drugs require a premarket approval by Japanese ministry of Health, Labor and Welfare (MHLW) (Ando et al. 2010).

c. Tetrahydrocurcuminoids (Turmeric extract)

Figure 6: Structure of tetrahydrocurcumin

Tetrahydrocurcuminoids are the major metabolites of the naturally occurring curcuminoids in the turmeric rhizomes. The curcuminoids are the active compounds present in turmeric. curcuminoids have been focus of several research studies and clinical trials for their anti-inflammatory, anti-cancer, hepatoprotective, antioxidative activities etc. (Osawa 2007).

Curcuminoids have also been traditionally used on skin for skin fairness and for healing of wounds. Paste of turmeric and neem is used for ulcers and scabies (Phillip 2011, 2nd ed.). However, due to their dyeing nature, the use of curcumin or turmeric based topical products on skin is quite cumbersome. Application of curcumin on skin can lead to staining that may last for a couple of days, not ideal for conventional cosmetic application for Caucasian skin.

Scientists at Sabinsa found a novel way to introduce the goodness of curcumin/turmeric in conventional cosmetics without including the staining nature of the curcumin.

Tetrahydrocurcuminoids (Figure 6), as mentioned above, are the major metabolites of curcuminoids. Tetrahydrocurcumin is obtained by hydrogenation of the double bonds existing in the curcumin structure. Due to loss of conjugation in the structure (Figure 7), the tetrahydrocurcumin absorbs the light in UV area and appears white in color. Tetrahydrocurcumins are not only free from color, but also from staining nature of curcumin.

Figure 7: Conversion of curcumin to tetrahydrocurcumin

Tetrahydrocurcumin as an antioxidant

Tetrahydrocurcuminoids are obtained from curcuminoids, which are well known for their antioxidant activity, and hence the studies have been carried out to compare antioxidant activity of both curcumin and its metabolites—tetrahydrocurcuminoids. Studies carried out by Osawa et al. found that tetrahydrocurcumninoids had the strongest antioxidant activity among all the curcuminoids tested in different assay methods.

Osawa et al. selected curcumin, demethoxycurcumin, and bis-demethoxy curcumin and their corresponding hydrogenated tetrahydrocurcuminoids for comparison by using linoleic acid as substrate in an ethanol/water system. They also used *in vitro* systems such as rabbit erythrocyte membrane ghost and rat liver microsomes for determining the antioxidant activity of these compounds by measuring the TBARS (thiobarbituric acid reactive species) generated by the oxidants (Osawa et al. 1995).

DPPH Free Radical Scavenging activity

Free radical scavenging activity of tetrahydrocurcuminoids was determined using DPPH assay (diphenyl picril hydrazyl radicals). DPPH radicals show a decrease in absorbance due to their quenching by antioxidants.

Different concentrations of test substance are incubated with 0.1mM DPPH methanolic solution for 30 minutes. The reduction in absorbance of DPPH radicals is then studied at 516nm and scavenging activity is expressed as SC50.

Using the above method to determine the free radical scavenging activity, tetrahydrocurcuminoids was compared with resveratrol (95%). The results are given in the table below.

Tetrahydrocurcuminoids were found to have more than twice antioxidant activity than the resveratrol, the major polyphenols present in grapes skin and red wine (Sami Lab Test Report 201).

Product	**Scavenging Activity SC_{50}**
Tetrahydrocurcuminoids 95%	1.14
Resveratrol 95%	3.24

Table 2: DPPH free radical scavenging activity

Skin-lightening potential of tetrahydrocurcuminoids

In view of traditional use of turmeric on skin for increasing glow and radiance, tetrahydrocurcuminoids were evaluated for their skin-lightening potential using an enzyme-based method involving tyrosinase inhibition as well as an *in vitro* method using melanogenesis inhibition in B16 F1 mouse melanoma cells.

In the enzyme-based assay, tetrahydrocurcuminoid compared favorably to several other skin-lightening natural compounds such as kojic acid, vitamin C, and Arbutin (Sami Labs Report No. 09, 222). The table below gives the tyrosinase inhibition activity in terms of IC_{50} (Table 3).

Product	**Inhibition Capacity IC_{50}**
Tetrahydrocurcuminoids 95%	2.0
Kojic acid	3.0
Vitamin C	9.33
Arbutin	33.0

Table 3: Tyrosinase inhibition activity

In another test on skin-lightening potential using the B16F1 melanoma cells, tetrahydrocurcuminoids have shown great potential in reducing the conversion of tyrosine to L-DOPA and thus formation of melanin in the cells. The melanogenesis activity was compared with the kojic acid, vitamin C, and arbutin. In this cell-based assay (Sami Labs Report No. 09 222), tetrahydrocurcuminoids showed significantly better melanogenesis inhibition potential compared to kojic acid, vitamin C, and arbutin (Table 4).

Product	Melanogenesis Inhibition Capacity IC_{50}
Tetrahydrocurcuminoids 95%	3.5
Kojic acid	100
Vitamin C	25
Arbutin	100

Table 4: Melanogenesis inhibition activity

In another study done by researchers at Sabinsa, skin-lightening potential was compared with hydroquinone using melanogenesis inhibition model with B16F1 mouse melanoma cells. The results (table) showed that although the hydroquinone inhibits melanin more effectively, it was toxic at those levels. Due to its toxicity, hydroquinone use is restricted.

Conc. (mcg/ml)	*% inhibition of melanin*	
	THC CG	**Hydroquinone**
0.125	32	12
0.25	40	18
0.5	45	48
1	52	**toxic**
5	80	**toxic**
10	toxic	**toxic**

Table 5: Melanogenesis inhibition with toxicity

d. Sabiwhite® - 955 tetrahydrocurcumin extract

Sabiwhite® (INCI: Tetrahydrodiferuloylmethane) is a color-free natural extract derived from turmeric roots with proven luminosity-boosting and skin-lightening effects. It is an effective skin-lightening agent with multifunctional topical benefits and no sensitization side effects. SabiWhite® works on multiple targets that cause undesirable pigmentation, including free radicals, UV exposure, melanin production, aging, etc.

In vitro studies antioxidant studies on Sabiwhite®

1. Reactive oxygen species scavenging in Swiss 3T3 fibroblast cell line- IC_{50} 1.44µg/ml
2. ORAC Activity – 10,815µg/ml Trolox eq/g
3. HORAC Activity – 3,152µMol gallic acid eq/g

Clinical Study on SabiWhite®

In a randomized, double-blind, placebo-controlled comparative study—tetrahydrocurcumin (Majeed et al. 2010), the major metabolite in the tetrahydrocurcuminoids, was compared for its safety and efficacy against hydroquinone.

This study was carried out in the Philippines by the Research Institute for Tropical Medicine.

In this study Sabiwhite®, Sabinsa's brand for tetrahydrocurcumin, was compared with hydroquinone over 50 human subjects in a randomized, placebo-controlled four-week study. The concentration of H\hydroquinone was used at 4% while 0.25% of tetrahydrocurcumin was selected for comparison for safety as well as efficacy. The study was carried out in two phases. In Phase 1, the 48-hour IQ chamber closed patch test was carried out on 50 healthy subjects (aged 21–45). The subjects who developed no adverse reaction were included in Phase 2 of the clinical trial.

The Phase 2 trial was carried out as a randomized, double-blind placebo-controlled comparative study of 0.25% tetrahydrocurcumin (brand name Sabiwhite®) against 4% hydroquinone formulation for depigmenting activity.

The results showed that 0.25% tetrahydrocurcumin was more effective than 4% hydroquinone in reducing the pigmentation as demonstrated in the four-week trial. Further, the 0.25% THC showed 100% compliance with no patient dropout or any incidence of adverse reaction, while in the hydroquinone group 50% of the subjects developed an adverse reaction, thus demonstrating the benefits of THC as an effective and safe alternative to hydroquinone.

Cosmeceutical potential of tetrahydrocurcuminoid

Tetrahydrocurcuminoid is an off-white to cream-white powder; it can be used in skin lightening, fairness formulations, management of hyperpigmentation, and in age-defying products. The toxicity studies have shown no irritation potential on skin. *In vitro* dermal irritation studies have shown no irritation, while the ocular irritation studies have shown minimal irritation potential.

Patents on Tetrahydrocurcuminoids

1. Majeed, M. et al. (2003). Cross-regulin composition of turmeric derived tetrahydrocurcuminoids for skin lightening and protection against UVB rays. **US 6653327.**
2. Majeed, M. et al. (2006). Use of tetrahydrocurcuminoids to regulate physiological and pathological events in the skin and mucosa. AU 2006235807.
3. Majeed, M. et al. (2007). Use of tetrahydrocurcuminoids to regulate physiological and pathological events in the skin and mucosa. **EP 1171144 B1.**

4. Majeed, M. et al. (2008). Use of tetrahydrocurcuminoids to regulate physiological and pathological events in the skin and mucosa. **NZ 514884.**
5. Majeed, M. et al. (2009). Process of making and method of use of tetrahydrocurcuminoids to regulate physiological and pathological events in the skin and mucosa cells. **EP 1328263.**

e. Cococin™ (Freeze-dried coconut water)

Cococin™ is freeze-dried extract of green coconut water. Coconut water is a liquid endosperm of *Cocos nucifera*. This endosperm is present in greatest amount in a green coconut. As the coconut matures, this liquid serves for growth of solid endosperm and the embryo. Coconut water or liquid endosperm serves as a natural reservoir of nutrients to promote the tissue growth. The coconut water in a green coconut is rich in proteins, amino acids, vitamins, and growth hormones essential to promote the tissue-growth minerals (Awua et al. 2011; Tulecke et al. 1961).

Coconut water is regarded as a reservoir of nutritious compounds present in the coconut. Traditionally it has been used as refreshing beverage replenishing the minerals lost from the body and has been often used as a natural electrolyte rehydration solution due to its isotonic nature. Coconut water is a quite popular ingredient in sports nutrition drinks with high potassium and low sodium content.

Manufacture of coconut water solids - Cococin™

Cococin™ is manufactured using the freeze-drying process. The young green coconuts are carefully selected and the liquid endosperm is collected from these coconuts. This liquid endosperm is then microfiltered to filter out any extraneous substance that could be introduced in the extraction process of endosperm from the coconut. The freeze-drying process under controlled conditions ensures that the coconut solids obtained from the process retain their biological activity. The amorphous nature of the freeze-dried material protects the protein components during pulverization and storage. During storage, the material transforms into the more stable crystalline state, which is also less hygroscopic (Majeed et al. 2007; U.S. Patent 7,300,682).

Applications

Apart from the nutritional benefits, coconut water has many potential cosmeceutical applications, where it can be used on skin as well for hair care. In the next few paragraphs we will discuss the potential applications of the coconut water (Cococin™) in hair care as well as skin care.

Cococin™ in hair care

Coconut water as mentioned previously is a reservoir for nutritious compounds, some of which promote cell and tissue growth. Cytokinins are one such plant-growth substance present in the endosperm of green coconut, which are involved in cell growth and differentiation (Kobayashi et al. 1995). In view of the role of coconut water solids in supporting cell growth, coconut water (Cococin) can be used in applications supporting the growth of human tissues such as hair follicles. Thus the potential use of Cococin can be found in hair-growth products such as hair gels, shampoos, conditioners, etc.

A study to evaluate the effect of Cococin™ on cell growth was carried out by the biological research group at Sabinsa (Sami Labs Report 208).

Cell proliferation enhancement by Cococin™ was studied by inflicting a wound over the fibroblast (Swiss 3T3) monolayer and fibroblast proliferation was monitored (Figure 8) in media containing varying concentrations of Cococin™.

Figure 8A: Control (Day 0)

Figure 8C: Cococin (Day 0)

Figure 8B: Control (Day 1)

Figure 8D: Cococin (Day 1)

Figure 8: Cell proliferation enhancement by Cococin™ in Swiss 3T3 fibroblast cell line

Results from the cell proliferation was observed and compared with fetal bovine serum. The comparative proliferation can be seen in the graph below (Figure 9).

Figure 9: Proliferation of fibroblasts using FBS- and Cococin-containing media

Study showed that the coconut water solids in Cococin™ support cell growth. Further, Cococin™ may be used in the application to support the growth of human tissues such as hair follicles. Cococin™ can be used in hair care formulations.

Cococin™ patents

U.S. patent (No. 7,300,682) The patent is titled "Method of preparation and use of coconut water in mammalian tissue nourishment growth and healthy maintenance." The patent covers the derivation process, composition, and intended uses of Sabinsa's branded ingredient Cococin™, a freeze-dried powder form of green coconut water.

Cococin™ is covered by **European patent (No. EP1341547)** for its process, composition, and use in the European market.

The newly patented SABERRY®+COCOCIN™ synergistic formulation protects dermal papilla cells from stress signals. Dermal papilla cell clusters are mesenchyme cells, which perform several important functions that include:

1. Being reservoirs of multi-potent stem cells, which are critical assets in the area of regenerative medicine;
2. Having the physical inductive influence on the cells of undifferentiated epidermis to push into the dermis as part of processes involved in skin appendage formation;
3. Acting as body's antidote mechanisms for preventing unwarranted inflammatory reactions; and
4. Producing useful antimicrobial proteins.

Cococin™ for skin care

As mentioned earlier, coconut water has extremely good rehydrating activity and hence can be used as a topical moisturizer and improves the skin elasticity and skin tone.

In an unpublished (22 female subjects, aged 20–35 years) double-blind placebo-controlled clinical study (Research Report Sami Labs Ltd 2005), 200mg of cream containing 1% coconut water solids (Cococin™) and other natural ingredients was applied daily for eight weeks on the left arm of each subject and cream base was applied on the corresponding area of the right arm to serve as control.

The reduction in skin roughness was evaluated with skin visiometer, and dermal elasticity was measured using a cutometer.

Results (Table 6) showed that treatment with cream containing Cococin™ significantly improved the skin elasticity, which was manifested in decreased skin roughness and improved skin tone. Thus coconut water solids in Cococin™ nurtures keratinous tissue and supports tissue integrity, thereby potentially inhibiting the appearance of signs of aging and the manifestation of wrinkles.

Figure 10: Change in elasticity on application of Cococin-containing cream

Cosmeceutical applications of Cococin™

Coconut water solids in Cococin nurture keratinous tissue and support tissue integrity, thereby potentially inhibiting the appearance of signs of aging and the manifestation of wrinkles.

Cococin can also be used in hair care for its hair-growth potential.

The recommended usage levels are 1–5%; it is completely dispersible in water.

f. ForsLean (*Coleus forskohlii* rhizomes)

Coleus forskohlii belongs to the family *Lamiaceae*. Coleus is one of the most versatile plants that grow in arid and semi-arid climates. It has been well known in Ayurveda, the traditional Indian medicine system, for treating conditions such as insomnia, intestinal spasm, heart and lung conditions, etc. (Schaneberg & Khan 2003). *Coleus forskohlii* is one of the species of coleus that is unique as it only contains the compound forskohlin. Forskohlin is a direct activator for adenylate cyclase, which is involved in generation of cyclic AMP (Shoback and Brown 1984), a secondary messenger that plays a key role in activation of lipase involved in breaking the fat. This has led to use of coleus in weight management. Sabinsa introduced patented coleus as a dietary supplement for weight management under the brand name of ForsLean®.

Coleus forskohlii roots are the main source of forskohlin. The roots, apart from forskohlin, are also rich source for coleus oil, which has good skin care activity.

Coleus oil

Coleus forskohlii contains an essential oil that has a spicy note; it can be used in cosmeceutical industry. The main component of the oil are 3-decanone, bornyl acetate, gamma eudesmol, and Beta-sesquiphellandrene (Paul, Radha, and Kumar 2013). The coleus oil can be extracted using a supercritical fluid-extract process. The rhizomes of *Coleus forskohlii* after sun drying are powdered and loaded in the extractor. Using carbon dioxide as the supercritical fluid, the rhizomes are extracted to give light-yellow-colored oil with a strong citrus odor. The oil is standardized with Alpha-pinene, Beta-pinene, bornyl acetate, and decanal. The coleus oil can be used as an antimicrobial ingredient and a perfumery ingredient, as it has a spicy, musky odor.

Antimicrobial activity of *Coleus forskohlii* oil

In house studies done at Sabinsa (Sami Labs Report 198) have shown that coleus oil is more effective than tea tree oil in inhibiting growth of the skin pathogens such as

-*Propionibacterium acnes* – Associated with acne on the skin

-*Staphylococcus aureus* – A bacterial strain found in infected wounds and skin eruptions, including acnes

-*Staphylococcus epidermis* – A bacterial strain occurring in a variety of opportunistic bacterial skin infections

In-house study data (Figure 11) at Sabinsa shows that coleus oil at 2% concentration is as effective as tea tree oil or clindamycin. Its aroma is also an advantage over the tea tree oil, as it does not require odor masking in the formulation.

Figure 11: Comparison of antimicrobial activity against *P acnes* between coleus oil, tea tree oil, and clindamycin.

The suggested level of use of coleus oil in skin care formulation is 5–10% and in aromatherapy or perfumery level can be 0.5–1.0%.

Patent on coleus oil

US patent 6,607,712 Majeed M., Prakash S.: Composition and methods containing an antimicrobial essential oil extracted from *Coleus forskohlii*.

Coleus extract 95% (containing 95% forskohlin)

The coleus rhizomes as mentioned earlier, apart from the oil are also a source of active component forskohlin. This compound is exclusively found in only *Coleus forskohlii* species. Forskohlin is a direct activator for adenylate cyclase, which is a transmembrane enzyme with key regulatory roles in some of the human tissues including fat cells. Sabinsa pioneer "ForsLean®" is a coleus extract standardized to the active compound forskohlin, which is a direct activator for secondary messenger (cAMP), this direct activation of cAMP takes place without activating the adrenergic receptors. This mechanism plays a key role in inducing lipolytic activity in fat cells without causing any unwanted side effects associated with activation of receptors as seen in the case of ephedra.

Topically the forskohlin extract can play a significant role in management of cellulite, due to its mechanism of action as explained above. Cellulite, also known as gynoid lipodystrophy (Small et al. 2006) is a condition that affects millions of women around the world. Though the presence of cellulite is not a disease, it remains an issue of concern to a great number of women.

Cellulite is the visible fat deposited in pockets under the skin, giving an orange peel effect on the skin in areas around hips, thighs, and buttocks due to excessive development of volume of adipocytes. Collagen fibers that connect the fat to the skin may stretch, break down, or pull tight, allowing the fat cells to bulge out.

Coleus forskohlii extract standardized to 95% forskohlin can be used in skin care as a conditioning and anticellulite agent. Since it is involved in the enzymatic breakdown of fat in the adipocyte cells, it can be useful in the management of cellulite. Forskohlin has shown to potentiate topical fat loss in combination with yohimbine and aminophylline (Bagchi and Preuss 2013).

ForsLean 95% can be used in the form of skin lotion, creams, and patches with a suggested level of 2–5% in the formulation for the management of cellulite.

Clinical results on coleus extract containing forskohlin

In a double-blind study, six women were treated with 25X10E-5 mol/L forskohlin in an aquaphor base on one of the thighs, while the other thigh was treated with base alone. The study was carried out for four weeks with the treatment repeated five days per week. The subjects were also placed on an 800 Cal/day diet and encouraged to walk (Greenway et al. 1995).

After the four-week study period, fat loss under the skin was measured. Results showed that in four women who completed the study, there was significant decrease in the treated thigh girth as compared to the untreated thigh.

Figure 12: Change in thigh girth (Greenway et al. 1995).

Sabinsa also performed a single-centered, randomized, double-blind study to evaluate efficacy of the ForsLean 95% as a novel topical agent for the management of cellulite (Study report: Protocol no. CPL/16/ CELR/ MAR/ 08, 2008).

The study, which was carried out for a duration of four weeks on 54 female subjects with cellulite, showed potential of the ForsLean-containing formulation in reducing cellulite, with a reduction of 9.37% in mid-thigh circumference, and 9.28% reduction in body fat content. In superficial fat reduction, the ForsLean group showed significant difference in all four parameters measured by

ultrasonography. In the anterior-transverse, anteriorlongitudinal, anteriormedial-transverse, and anteriormedial-longitudinal groups, nearly 23.9%, 21.7%, 34.2%, and 34.1% reduction from baseline was observed.

g. Cosmoperine® (*Piper nigrum* fruits)

Cosmoperine® is a patented extract for enhancing topical bioavailability. It is obtained from the hydrogenation of extract obtained from black pepper fruits. The fruits are extracted to obtain an enriched extract containing piperine. This extract is then further hydrogenated under controlled conditions to obtain a 95% tetrahydropiperine containing an off-white-colored extract. Cosmoperine is used in cosmetics for enhancing the penetration of active compounds topically (Ahmed et al. 2012).

Laboratory studies on the Cosmoperine have shown its role in improvement of topical bioavailability of compounds such as betamethasone dipropionate, green tea polyphenols, forskohlin, and other active compounds used in skin care.

Study with betamethasone dipropionate

Bioavailability enhancement effect of Cosmoperine was observed with betamethasone dipropionate in an experiment designed to determine the transdermal diffusion of BMDP through hydrated skin mounted on a Franz diffusion cell. Betamethasone DP is a topical anti-inflammatory ingredient that has a poor absorption potential. It was observed that in the presence of Cosmoperine, the drug completely diffuses through the skin in about five minutes of application. This effect of Cosmoperine can improve the absorption of BMDP, thus making it act faster in the skin and providing the benefits (Sami Labs Report 2000).

Figure 13: Improvement in transdermal absorption of BMDP in presence of Cosmoperine

Study with Green Tea Polyphenols

Cosmoperine® was studied for its bioavailability enhancement activity for three botanical extracts—green tea polyphenols, *Coleus forskohlii* extract, and tetrahydrocurcumin extract (derived from curcuminoids). These three extracts represent different functionalities in cosmeceuticals (antioxidant effect from green tea polyphenols, skin-conditioning effect from *Coleus forskohlii*, and skin-lightening activity from tetrahydrocurcuminoids) and also different solubilities (water-soluble green tea extract and lipid soluble forskohlin and tetrahydrocurcuminoids).

Cosmoperine® enhances the permeation of the green tea polyphenols across the egg membrane by about 30% on average). Cosmoperine® enhanced the permeation of forskohlin by about 40% in the first 30 minutes, when used at the level of 5% of the forskohlin concentration. The antioxidant action of the tetrahydrocurcumin was enhanced by adding Cosmoperine® (Sami Labs Report 2000–2003).

In case of the tetrahydrocurcumin, the free radical scavenging efficacy of tetrahydrocurcumin was evaluated in the absence and presence of Cosmoperine®. The antioxidant activity of tetrahydrocurcuminoids was found to be enhanced by tetrahydropiperine.

Human safety trials with Cosmoperine®

A 48-hour repeat insult patch test was used to evaluate the skin-irritation potential of Cosmoperine®. Cosmoperine® at a level of 0.01% and 0.1% (an effective dose range for the compound), was used in the skin patch test in a petroleum vehicle. The test was conducted on 50 human subjects for 48 hours. Results showed that both the applied dosages were safe and neither dose caused any skin irritation. These results indicate that Cosmoperine® does not act as a skin irritant in a dose range considered effective for topical nutrient delivery (Majeed et al. 2005).

Above study was conducted by US FDA accredited BioScreen Testing Inc Lab.

Clinical Study on Cosmoperine®

Cosmoperine® was evaluated for its topical bioavailability enhancement activity in a combination containing tetrahydrocurcumin formulated for management of photo-aging. The study was performed by Clin World as a three-month prospective, randomized, double-blind, non-crossover, comparative clinical trial with 20 subjects suffering from mild to moderate photo-damaged skin.

While Cosmoperine® by itself does not have any antioxidant properties, its effect on antioxidant activity of THC was evaluated. Antioxidant activity is important for management of photo-aging. The two formulations compared had an equivalent amount of THC (0.5%) while one of them had an additional 0.1% Cosmoperine® for bioavailability enhancement.

The parameters assessed were hyperkeratosis, collagen density, and lymphocyte infiltration.

Hyperkeratosis

The efficacy of creams was evaluated for reduction in hyperkeratosis, and results revealed that the cream X (containing 0.1% Cosmoperine®) was more efficacious in this function.

Figure 14: Comparison of efficacy hyperkeratosis with cream X (containing 0.1% THP and 0.5% THC) with cream Y (containing (0.5% THC).

Collagen Density

Collagen histochemistry has also shown that there was a significant reduction in collagen density with both cream X and Y; however, the comparison of efficacy between the two creams revealed that cream X (containing 0.1% Cosmoperine®) is more effective.

Figure 15: Comparison of collagen density at end of the study

Effect on lymphocyte infiltration

The comparison between the two formulation showed that there was statistically significant reduction in lymphocyte infiltration with both creams X and Y, while there was significant reduction with cream X (containing Cosmoperine® 0.1%) compared to that of cream Y.

Figure 16: Comparison of % efficacy of cream X and Y in lympocytic infiltration

This three-month study showed that tetrahydrocurcumin was effective in reversing the photo-damage to the skin. The comparative efficacy of the cream containing THC (0.5%) and Cosmoperine® (0.1%) was found to be more than the cream containing THC alone. The faster response to the Cosmoperine-containing cream was attributed to the bioavailability enhancing effect of the Cosmoperine® or tetrahydropiperine. The tetrahydropiperine enhances the transdermal bioavailability of the co-administered nutrient or actives. It was concluded from the study that the inclusion of Cosmoperine® in the THC cream was more beneficial in accelerating the reversal of photo-damage on the skin (Sami Labs Report 2006).

The mechanism of Cosmoperine®

Cosmoperine®, which is a lipophyllic compound, may possibly increase the solubilization of the intracellular lipid moiety in the skin, making it more permeable to the applied nutrient or drug.

h. ARTONOX® (*Artocarpus lakoocha* wood)

Artonox® is the brand name for the *Artocarpus lakoocha* extract standardized to 95% of oxyresveratol. *Artocarpus lakoocha* is a large deciduous tree widely distributed in the Southeast Asian region including Thailand, India, Sri Lanka,

Myanmar, Vietnam, etc. *Artocarpus lakoocha Roxb., moraceae* belongs to same genus as jackfruit (*Artocarpus heterophyllus*). This tree is well known for the traditional medicine "Puag haad" (Wongsawad et al. 2005; Palanuvej et al. 2007).

Oxyresveratrol

Figure 17: Structure of oxyresveratrol

Oxyresveratrol (Figure 17), analogous to resveratrol structurally, is a tetraoxygenated stilbene found in mulberry, grapes, red wine, and peanuts. Recently oxyresveratrol has shown activities such as antioxidant, antiviral, anti-inflammatory, neuroprotective, and topically it has been found to have tyrosinase-inhibitory activity (Likhitwitayawuid et al. 2006).

In 2009, research on *Artocarpus lakoocha* extract detected the presence of oxyresveratrol in its heartwood extract, thus providing a new source of this stilbene compound (Maneechai et al. 2009).

Artocarpus lakoocha

Figure 18: Heartwood of *Artocarpus lakoocha*

Recent studies on the *Artocarpus lakoocha* have confirmed the presence of oxyresveratrol in heartwood (Figure 18). Further studies have also revealed the potential use of Artocarpus as a skin-whitening agent (Singhatong et al. 2010).

Development of Artocarpus extract: Artonox®

Scientists working on polyphenols at Sabinsa evaluated the raw material from various species of Artocarpus tree, grown in the Indian subcontinent, such as *Artocarpus heterophyllus*, *A. communis*, *A. chaplasha*, and *A. Lakoocha*. Study showed that *Artocarpus lakoocha* was good a source of oxyresveratrol in its heartwood. Alcoholic extract from the heartwood of Artocarpus was taken up for purification followed by crystallization to obtain a highly enriched Artocarpus extract standardized with oxyresveratrol 95% by HPLC (Figure 19) Artonox®.

Figure 19: Chromatogram of Artonox®

Antioxidant potential for Artonox®

Antioxidant strength of Artonox® was evaluated by both the DPPH and ORAC assay system.

- DPPH Assay

The antioxidant activity of Artonox was initially evaluated by using DPPH radicals. Diphenyl picryl hydrazyl (DPPH) radicals are color stable, free radicals that lose color rapidly in the presence of antioxidants. It is a reliable method for evaluating free radical scavenging activity. Artonox showed remarkable free radical scavenging activity in DPPH assay as compared to other stilbene compounds such as resveratrol, pterostilbene, and kojic acid.

Artonox® (95% oxyresveratrol) exhibited strongest free radical scavenging activity, with IC_{50} of 2.7µg/ml, followed by resveratrol (IC_{50} 3.01µg/ml) and trans-pterostilbene (IC_{50} 4.3 µg/ml). Kojic acid showed the lowest activity, with IC_{50} 500µg/ml (Figure 20) (Sami Labs Research Report—Unpublished).

Figure 20: Antioxidant activity by DPPH assay

- **ORAC activity assay**

Oxygen Radical Absorbance Capacity assay has become a gold standard for antioxidant evaluation. Though primarily used for evaluation of antioxidant capacity of nutritional supplements and foods, today ORAC can be used in the cosmetic field for measuring the antioxidant potential of cosmeceutical ingredients against various free radicals present in the skin environment.

ORAC activity of Artonox was compared with some stilbenes and other antioxidants (Sami Lab Report—Unpublished). Results as shown in Figure 21 below indicate that Artonox® can act as an anti-aging product as well as providing antioxidant cover to the formulation to increase its shelf life.

Figure 21: ORAC activity of selected antioxidants

Skin-lightening potential of Artonox®

The main active compound of Artonox®-oxyresveratrol belongs to family of stilbenoid compounds. Stilbenoid compounds have been reported to possess the tyrosinase-inhibitory activity, with level of oxygenation of stilbene structure clearly affecting the tyrosinase-inhibitory activity. Thus tetra-oxygenated stilbene such as oxyresveratrol displayed ninefold stronger tyrosinase-inhibitory activities than resveratrol, which is a tri-oxygenated stilbene (Likhitwitayawuid et al. 2002).

Skin-lightening activity of Artonox® was evaluated by two methods.

1. Enzyme based tyrosinase-inhibitory assay
2. Cell based melanogenesis-inhibitory assay

1. Tyrosinase-inhibitory assay

The tyrosinase-inhibitory assay is an *in vitro* assay using commercially available mushroom tyrosinase enzyme. It is based on the principle that tyrosinase is a rate-limiting enzyme in melanin biosynthesis and it acts on L-tyrosine to form a pink-colored complex. The progress of the test is measured spectroscopically at 475nm. Skin-lightening agent or tyrosinase inhibitor will inhibit the formation of the complex and thus quench the pink color intensity in the test.

Artonox standardized with 95% oxyresveratrol showed strong tyrosinase-inhibitory activity in the test with IC_{50} of 0.049 µg/ml. This activity was 140 times stronger than kojic acid (Figure 22), confirming the superiority of stilbene compounds over kojic acid for skin-lightening application (Sami Labs Report).

Figure 22: Tyrosinase-inhibitory activity of Artonox and kojic acid

Oxyresveratrol, being a target for the tyrosinase-inhibitory activity, has also been studied for its inhibition mechanism. The studies showed that oxyresveratrol was a noncompetitive inhibitor of mushroom tyrosinase with respect to L-DOPA (Kim et al. 2002).

Sami Labs Report Unpublished.

2. Melanogenesis-inhibitory assay

Studies have shown that some cytokines and growth factors also play an important regulatory role in melanogenesis. α-MSH (melanin-stimulating hormone) is one such factor. It binds to its receptor on melanocytes (cells producing melanin in the skin) and stimulates melanogenesis via cAMP-MITF (microphthalmia-associated transcription factor). Thus agents that block the signal pathway also exhibit the depigmentation against melanocytes (Ding et al. 2011).

Hence the melanogenesis-inhibitory assay was carried out on the Artonox® to estimate its depigmentation activity (Sami Labs Report).

Sami Labs Report Unpublished.

The melanogenesis-inhibitory assay is a cell-based assay where intracellular melanin in B16 F1 mouse melanoma cells is treated with various concentration of sample. The melanin thus formed is later extracted and estimated in a microplate reader.

Artocarpus showed a strong inhibitory activity on melanogenesis with IC_{50} of 12µg/ml as compared to kojic acid, which showed IC_{50} of 100µg/ml.

The melanin is induced by MSH in melanoma cells and treated with varying concentrations of Artonox. The slides below show the dose-dependent inhibition of melanin formation in the presence of Artonox® (Figure 23).

Figure a. MSH-induced melanogenesis in control melanoma cells

Figure b. 32% inhibition of melanin in Artonox-treated cells (10µg/ml)

Fig c. 50% inhibition of melanin in Artonox-treated cells (12µg/ml)

Figure 23: Melanogenesis inhibition by *Artocarpus lakoocha* extract 95%

Anti-inflammatory effect

Artonox® was also evaluated for its anti-inflammatory effects against the carrageen-induced model of inflammation in rats. Artonox significantly reduced the

paw edema. The results suggest that the anti-inflammatory properties of oxyresveratrol may be correlated with inhibition of iNOS expression through down-regulation of NF-KappaB binding activity and significant inhibition of COX-2 activity.

Test	**Artonox® (Oxyresveratrol 95%)**
Elastase inhibition (IC_{50})	120µg/ml
Collagenase inhibition (IC_{50})	92µg/ml

Cosmeceutical application

Artonox® (Oxyresveratrol 95%) acts on some of the major targets that cause undesirable pigmentation, age spots, freckles, and other related skin conditions. Artonox® (oxyresveratrol 95%) has shown good efficacy in various *in vitro* studies conducted by Bio Research Div of Sami Labs Limited. Artonox® (oxyresveratrol 95%) was found to possess significant antioxidant, anti-inflammatory, and skin-lightening potential.

Potential applications can be as:

a. Skin lightening (natural tyrosinase and melanogenesis inhibitor)
b. Anti-aging
c. Antioxidant

i. *Eclipta alba* (Bhringraja)

Eclipta alba is a well-known Ayurvedic herb that has been known for its pharmacological actions including antibacterial, antihepatotoxic, antioxidant, anti-haemorrhagic, and anti-hypeglycemic properties (Pandey et al. 2012). Its use as a hepatoprotectant is quite popular and is a part of many herbal formulations for management of liver diseases.

Eclipta alba grows is widely distributed in the Southeast Asian countries such as India, Thailand, and China. *Eclipta alba*, commonly known as a Bhringraj in Ayurveda, belongs to the family *asteraceae* (Neeraja et al. 2011). A wide range of phytochemicals are present in the *Eclipta alba* including the wedelolactone in the leaves, which is considered as main active in this herb.

Apart from its benefits on oral consumption, it has also been reported to improve hair growth on topical application (Kirtikar and Basu 1975).

Eclipta alba has been evaluated for its potential use in alopecia or hair loss. Alopecia is regarded as a dermatological disorder where the subject suffers from hair loss from the scalp or other parts of the body. It can occur in both sexes and has a lot of psychological implications.

Studies have been performed to look into the possible role of *Eclipta alba* in management of alopecia and hair fall.

In one of the studies (Roy et al. 2008), the researchers evaluated the petroleum ether and ethanolic extract of *Eclipta alba* for their effect on promoting hair growth. The extracts of *Eclipta alba* were incorporated in creams and applied topically on the skin of albino rats. The hair growth was compared with minoxidil 2% solution.

Results showed that the petroleum ether extract showed a greater number of follicles in the anagenic phase of the hair growth, which can be regarded as an active-growth phase of hair follicles, during which the hair root divides rapidly and adds to the hair shaft. The rats in the *Eclipta alba* cream group also showed less time required for the complete hair growth. Overall, the results obtained for the hair growth were better than the positive control minoxidil.

In another study done at Dabur Research Foundation (Dutta et al. 2009), the *Eclipta alba* methanolic extract was evaluated for hair-growth activity in pigmented C57/BL6 mice. These mice were preselected for their characteristic telogen phase of hair growth. The extract was topically applied. The results from the immunohistochemical study performed on the mice showed that *Eclipta alba* was able to induce the anagen phase in the mice. The study showed that *Eclipta alba* can be used as a hair-growth promoter.

Sabinsa manufactures the *Eclipta alba* extract with 50% wedelolactone, which is the active component in the leaves of the plant. This extract can be used for formulation of hair care products.

Figure 24: Structure of ursolic acid

j. Ursolic acid (*Salvia officinalis* leaves)

Ursolic acid occurs in nature in several edible fruits such as apples, basil, cranberries, peppermint, and rosemary. Chemically, ursolic acid is a pentacyclic triterpenoid acid, whose anti-inflammatory activity and its activity in inhibiting various types of cancer have been well studied. Studies have shown that it may inhibit the proliferation of cancer cells and also induce apoptosis (Shishodia et al. 2003; Wang et al. 2011).

It is found often along with another triterpenoid—oleanolic acid, which is structurally very similar to ursolic acid.

Anti-inflammatory activity is found in several triterpenoid, and ursolic acid is no exception. Ursolic acid has shown anti-inflammatory activity against mouse ear edema caused by 12-o-tetradecanoylphorbol-13-acetate. The mechanism of the anti-inflammatory activity has been suggested to be through histamine release from mast cells, reducing production of arachidonic acid by inhibiting enzymes such as cycloxygenase and lipoxygenase. Inhibition of inflammatory enzymes such as elastase was also thought to play a role in the anti-inflammatory activity of ursolic acid (Liu 1997).

Apart from the anti-inflammatory activity of ursolic acid, which can play an important role in the anti-aging formulations, ursolic acid has also been found to increase the ceramides and collagen in the skin. In a published study, a liposomal preparation of ursolic acid was found to increase ceramide content of cultured human keratinocytes as well as increase in collagen content of cultured human dermal fibroblast cells. The research also claims that the said liposomal preparation also increased the ceramide content in human skin over an 11-day period (Yarosh and Brown, 2000).

In 2003, Nishimori et al. found that ursolic acid on topical application shows marked improvement of collagen bundle structure and shows reduction in wrinkling without any side effects. The degenerative changes in collagen bundle structure can cause the skin wrinkles and loss of elasticity in the skin.

Use of ursolic acid in cosmeceuticals

Extracts containing ursolic acid can be used in the anti-aging formulation for the repair and anti-wrinkling effect on skin. The suggested level of the use is 1–5%.

k. Boswellin® CG (*Boswellia* extract)

Boswellia serrata is also known as Indian frankincense or salai. Boswellia finds description in Ayurvedic literature for its application as an anti-arthritic supplement. Boswellia has been used since ancient times in many civilization for preparation for incense, medicine, cosmetics, and perfume.

Boswellia extract is obtained from the gum resin of the Boswellia tree. The gum resin is collected from the tree by scraping away the bark of the tree and collecting the exudates from the tree.

The gum resin thus obtained is known for several pharmacological activities, such as anti-diabetic and anti-rheumatic; it is also used for treatment for diarrhea, neurological disorders, mouth sores, and hepatic stimulant.

In Ayurveda, Boswellia has been used for the management of several inflammatory conditions both internally as well as topically. The anti-inflammatory properties of Boswellia can be attributed to the presence of Boswellic acids (Majeed et al. 1996). Boswellic acids are unique, as they inhibit both 5-lipoxygenase and human leukocyte elastase enzymes (Safayhi et al. 1997). 5-lipoxygenase enzyme catalyzes the conversion of arachidonic acid into inflammatory molecules such as leukotrienes.

Anti-inflammatory activity of boswellic acids is one of the most sought-after pharmacological properties—and is also responsible for its cosmeceutical applications.

Anti-inflammatory activity of boswellic acid is directed towards two inflammatory enzymes.

1. Lipoxygenase enzyme
2. Human leukocyte elastase enzyme

Human leukocyte elastase is a serine protease enzyme that initiates injury to the tissue. This injury then triggers the inflammatory process. The tissue injury thus releases the HLE enzyme, which then starts digesting or breaking down the elastin and collagen type IV in the skin, causing the loss of elasticity and texture.

Boswellic acids present in the Boswellin® CG can help to reduce the inflammatory enzymes present in the skin and thus can reduce or slow down the aging process. The dual inhibition of 5-lipoxygenase and human leukocyte elastase is unique to boswellic acids.

Sabinsa performed a clinical study (Mainkar et al. 2003) on Boswellin® CG to determine its application in reducing the puffiness and dark eye circles. Twenty-one female subjects aged 21–35 years were selected for the study.

The subjects were divided into two groups; one group was provided with "Under Eye Cream" containing 1% Boswellin® and the other was given placebo cream. The cream was applied under each eye twice a day for period of four weeks.

At the end of the study the dark circles and puffiness were assessed by a dermatologist.

The results showed that there was 42% reduction in the dark eye circles at two weeks and at the end of the study there was 86% reduction in the dark eye circles. Boswellin CG was found to be safe and effective in reducing the dark eye circles (Figure 25).

Figure 25. Efficacy of under-eye cream vs. placebo treatment

Boswellin® CG has been clinically evaluated as a topical agent in analgesic formulation (Figure 25) (Research Report Sami Labs February 2000).

In an open field study, a topical cream Chilisin® consisting of boswellic acids, capsaicin, and methyl salicylate was evaluated in 12 volunteers monitored by several independently working chiropractors. This study was coordinated by Synergy Wellness Clinics, DeSoto, Texas.

The study showed that Chilisin® cream applied regularly on a daily basis for atleast two weeks resulted in significant improvement in all parameters tested, with pain score diminished by half as a result of treatment with Chilisin®.

Figure 26: Mean scores of arthritic symptoms as evaluated in an open field study of boswellic acids containing topical analgesic Chilisin®.

4.1.5.6 SUSTAINABILITY OF BOTANICALS

Today the demand for the herbal and natural actives in the market is growing at a tremendous pace, which requires careful planning towards growing, harvesting, and procuring the raw material. The natural resources are limited and increasing

consumer demand puts pressure on the natural resources. To meet these demands, proper utilization of the natural resources is a must. Crop rotation, replantation, and improved high-yield variety can be useful tools in creating the sustainable natural resources.

CONCLUSION

In this chapter we have described the various multifunctional natural/nature- derived extracts for skin and hair care. Plant-derived ingredients are gaining increasing acceptance both from the formulators and consumers. Green products are safe and are more skin friendly, and their use results in fewer chemicals and toxins being released into the environment. Use of such ingredients is an imperative in today's world since we have now embraced the concept that the use of natural resources must be carried out responsibly, and in eco-friendly manner.

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INGREDIENTS TO STRENGTHEN SKIN BARRIER INTEGRITY: From Algal Protective Exoskeleton to a Protective Barrier for the Epidermis

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ABSTRACT

The skin epidermis serves numerous functions, but its primary purpose is to act as a protective barrier for the body from the external environment. It is composed of keratinocytes piled in layers lying upon each other thanks to specific complex junctions maintaining cohesion and communication among them. With aging this "young" architecture becomes disorganized, and this leads to a weaker protection and poorer communication of information signals between cells.

Some living organisms have developed a protective mechanism to this disorganization by an adaptation to their environment. For instance, *Padina pavonica*, a brown alga, builds an organized external deposit of calcium carbonate in the form of aragonite crystals for its protection.

As calcium bioavailability is necessary for the synthesis of essential epidermis structures, a high-tech active extract of *Padina pavonica* was developed and studied. This study highlights the activity of the extract for maintaining bioavailable calcium and thereby stimulating the synthesis of calcium-depending structures, crucial for the epidermis-protective function.

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INTRODUCTION

The primary function of the epidermis is to act as a protective barrier for our body from the external environment. Maintaining its integrity and resistance is crucial for proper functioning of the skin. In view of this function, and our present understanding of it, there is considerable ongoing research on skin to improve its strength and to find new active ingredients with innovative modes of action that improve its integrity.

4.1.6.1 SKIN BARRIER AND EPIDERMIS ORGANIZATION OVERVIEW

Skin barrier function resides primarily within the *stratum corneum* (SC), the top layer of the epidermis. It plays a vital role in forming a protective barrier and helps to prevent percutaneous entry of harmful pathogens in the body.

Epidermis is the upper layer of the skin tissue, just below the *stratum corneum*. It is composed of several layers of keratinocytes that undergo continuous

morphological changes reflecting their underlying keratinization as a protective barrier of the epidermis (both mechanical and chemical).

This epidermal differentiation begins from the depth to the surface of the epidermis cells and enables the distinguishing of four layers on a skin cut: the germinal layer (or basal), the spiny layer (or spinous), the granular layer and horny layer, or *stratum corneum* (first compacted then desquamating). Each stage of epidermal differentiation is characterized by the expression of specific proteins.

Therefore, maintaining a good level of proliferation and differentiation of keratinocytes from within the epidermis is crucial for the proper functioning of the skin barrier and for its integrity. Thus, the most important function of keratinocyte differentiation is the establishment and maintenance of the *stratum corneum*, since the latter consists of corneocytes and extracellular lipids secreted by differentiated keratinocytes, which together function as a barrier against the entry of chemicals and microbes from the environment and protect the body from dehydration (Figure 1).

Figure 1

a. Epidermal cells structures for skin resistance and cohesion

The mechanical strength of the epidermis depends on the integrity of the epidermal cell skeleton, which primarily consists of cytokeratins. Keratinocytes are piled in layers of cells, lying on each other, in which cytokeratins are synthesized in high quantities. Cytokeratins are specific proteins building the cytoskeleton of keratinocytes and responsible for their resistance. In each layer, daughter cells only differ from mother cells by the maturation of cytokeratins, which undergo modifications from the deepest layer of the epidermis to its surface. The most external layers are made of corneocytes (final form of the keratinocytes' differentiation) that no longer contain a nucleus and are filled with almost only a framework of modified cytokeratins.

The structural integrity of the skin also depends on dynamic junctions between cells called desmosomes. They are special membrane-associated structures of the epidermis equivalent to press studs that attach or detach depending on the cell needs. Indeed, the formation of desmosomes mostly takes place in the basal layer, the deepest one in the epidermis, where it is favored by the palissadic arrangement in perpendicular layers of keratinocytes placed very near one to the other. Having a particular structure at the junction of the basal layer with the dermis, where they are called hemi-desmosomes, desmosomes evolve from one layer to the other to end up in corneo-desmosomes at the *stratum corneum* level, where enzymes break them to favour the desquamation process.

Desmosomes are highly organized and build an intercellular adhesion point strongly anchored by cytokeratin filaments that penetrate deeply into the cytoskeleton, thus ensuring cohesion for the functioning of the epidermis.

The resulting "clipped" architecture of the epidermis explains its resistance and cohesion but also favors good intercellular signals, which are necessary for its proper function (Figure 2).

Figure 2

b. Cell structures depending on calcium

Indeed, desmosomes and cytokeratins are closely associated with each other, ensuring the junctions between keratinocytes and the cohesion between epidermis and the underlying dermis. It is therefore important to emphasize the critical role of both structures for the consolidation of the mechanical function and the integrity of the epidermis.

Cell communication is ensured by chemical messengers passed from one cell to another, a process that also depends on desmosomes and cytokeratins cell structures, which are calcium-dependent.

Indeed, calcium is involved in the maturation of cytokeratins as well as in the adhesion of the desmosomial proteins. Calcium ions are transferred from the blood to the most superficial cells by channels specialized in ionic transport in cell membranes. Calcium is then attracted to the structures on which it must act, particularly cytokeratins and desmosomial proteins.

c. Calcium's information-signaling properties

Calcium is an excellent information aid, as it can combine with a number of acids and be included in many minerals. The same salt with the same chemical formula can result in different minerals, depending on the atomic arrangement. Indeed, oyster shell, for instance, is covered by an outer layer, the thickest one, made out of an amorphous (thus nonorganized kind of calcium carbonate) and a thinner layer of shining calcite, itself covered with mother-of-pearl; the two latter minerals are crystallized (thus organized) forms of calcium carbonate. The soluble form of these minerals releases calcium ions with distinct solubility coefficients in the surrounding media and are able to pass on electric potentials at different levels. This process allows for the circulation of information. In mammals, the role of calcium is essential for blood coagulation, muscle contraction, nervous influx transmission, and heart rhythm regulation.

Signaling information associated with calcium in the skin is observed at two levels: the maturation of cytokeratin proteins that set up mechanisms of apoptosis, program cells' death for protection purposes (renewal of the epidermis), and the organization of skin's immune defense. Indeed, the terminal differentiation of epidermal keratinocytes, linked to the maturation of cytokeratins, is an essential process for the renewal of keratinocytes and ends in the formation of enucleated "dead" cells: the corneocytes. Therefore, *calcium is a key regulator in epidermis homeostasis of keratinocyte proliferation and differentiation.*

Many studies highlight the signaling function of calcium ions on keratinocytes as promoting cell-cell adhesion, and differentiation-related genes. Some studies (1–2) provide evidence that keratinocytes deficient in the calcium-sensing receptor fail to respond to calcium stimulation and to differentiate, indicating a role for the calcium-sensing receptor in transducing the Ca^{2+} signal during differentiation. It has also been observed that calcium is involved in recovery and wound-healing. Immediately after a scratch, there is a wave of increased calcium ionic transport outward from the damaged area that remains elevated in a peripheral layer of cells around the damaged area for several minutes and then disappears gradually. These studies confirm a signaling pathway that appropriately directs the wound-healing process in epidermis (3). Calcium signaling via gap junctions might be involved in the induction of calcium waves in response to mechanical stress at the upper layer of the epidermis (4).

4.1.6.2 SOLUTION FOR ALTERATIONS IN EPIDERMIS

Skin aging is characterized by a variety of morphological and physiological alterations of the epidermis including a lesser communication between keratinocytes, a flattening of the dermal-epidermal junction, a loss of cell cohesion, and a slow-down of the cells' turnover rate that leads to subsequent slowing of the wound-healing process.

a. Epidermis structures responsible for epidermis resistance

Studies on skin aging highlight targeted action on key epidermis structures involved in skin barrier strength, cohesion, and integrity. Such epidermis structures are tight-junctions, dermal-epidermal junction, epidermal growth factor, etc.

Tight junction (TJ) is recognized as a second barrier of the skin. Evidence from some studies suggests that altered expression of (TJ) proteins leads to an abnormal permeability barrier and that the TJ could be affected by the status of the *stratum corneum* barrier. Moreover, there is increasing evidence now that TJ in skin, which localizes in *stratum granulosum*, also contributes to epidermal barrier formation. Among TJ proteins, claudin-1 and claudin-4 have been demonstrated to have a role in barrier function of the skin (5). Other studies on specific cell-adhesion proteins (such as beta-catenin, which provides stability to epithelia under stress) suggest that beta-catenin helps to fortify *adherens* junctions under mechanical stress, which in turn leads to the strengthening of tight junctions (6).

Aging leads to changes in the dermal-epidermal junction (DEJ), including a decreased thickness of the junctions and a reduction of the cytoplasmatic cell strands consisting of basement keratinocytes in the epidermis. DEJ is a complex anchoring structure that connects epidermis and dermis layers together. The epidermis basal keratinocytes are attached to the DEJ basement membrane through hemi-desmosomes. This basement membrane consists of the lamina densa and the lamina lucida. The latter is the area where the molecules associated with adhesion (e.g., integrins, laminin 5, fibronectin) are found. These allow for the binding of epidermal cells to each other. Collagen IV is the most important component of the DEJ lamina densa and acts as a basic support for the other components such as laminins, which represent, as such, the most important component of the basement membrane after collagen IV.

The result of a reduction in the surface of the DEJ (unfolding) leads to a reduction of resistance of the tissue, a reduction in skin firmness, an increase in the formation of wrinkles, and a stretched appearance of epidermis less resistant (7). All of these phenomena are, of course, "symptoms" of aging.

- Filaggrin is involved in epidermal differentiation and skin barrier formation. It specifically interacts with intermediate filaments, particularly keratins. Filaggrin binds to keratin intermediate filaments, causing their aggregation into macrofibrils which are aligned in tightly packed parallel

arrays. This process contributes to cellular compaction and permits extensive cross-linking of keratin intermediate filaments to form a highly insoluble keratin matrix. This matrix acts as a protein scaffold for the attachment of cornified envelope proteins and lipids that together form the *stratum corneum*. Filaggrin protein normally assists in cytoskeletal aggregation and formation of the cornified cell envelope, providing additional strength and structure. It is required for normal lamellar body formation and content secretion. Therefore, it has been highlighted that a fillagrin mutation contributes to a disrupted epidermal barrier, increased water loss, and inflammation (8).

b. Improving calcium bioavailability

Another solution to reinforce skin barrier integrity is to improve the skin's natural function and to enhance calcium bioavailability.

Epidermis (human exoskeleton) contains a high quantity of calcium (a hundred times more than in the dermis), which is involved in cell proliferation and differentiation. With aging, disruptions of mechanisms involved in the calcium metabolism (transport and fixation) appear, and a noticeable decrease of its mobilization results. This process leads to a decrease in the bioavailability of calcium, which in turn disturbs the epidermis homeostasis. This occurs in spite of the fact that its level in the blood remains remarkably stable. *The aging process is thus connected with a significant loss of the bioavailable calcium in the organism* (Figure 3).

Figure 3: level of calcium fixed in the keratinocytes is proportional to the quantity of HFP added in the culture medium

The direct consequences of the process above is a poorer communication between cells, and a lessened resistance of the epidermis. Indeed, the quantity of cytokeratins in the epidermis decreases as general protein synthesis slows down and degradation enzymes are activated.

The degradation described leads to a weaker maturation wave in the epidermis. Keratinocytes lose their perfect organization and cell connections are less effective as they are not in an optimal environment. They are no longer synchronous and communicate poorly. The epidermis cohesion is disrupted. This phenomenon follows the upward migration of the keratinocytes and is visible within the whole thickness of the epidermis, up to the *stratum corneum* which presents a more or less disrupted aspect. As a result, the skin surface becomes rather rough and fluffy (Figure 4).

Figure 4

Improving calcium bioavailability in the epidermis is therefore a key factor for proper functioning of the keratinocytes. This is possible, especially since a stable pool of calcium is available in the vascular environment of the skin and the blood.

4.1.6.3 A NATURAL INGREDIENT TO STRENGTHEN SKIN BARRIER INTEGRITY INSPIRED BY ALGAE PRIMITIVE BEHAVIOR

A knowledge of nature is a pool of information that has inspired the cosmetic industry to find natural solutions to improve the human skin physiological mechanisms. For instance, the unique strategy of adaptation of the *Padina pavonica* alga to its environment is a source of inspiration for the development of effective skin care ingredients.

a. A primitive strategy involving calcium

Padina pavonica, a brown alga belonging to the *Dictyotaceae* family, has developed a set of mechanisms to preserve its vegetative structure to its environment, thereby allowing it to survive in most extreme conditions encountered in the Atlantic Ocean and Mediterranean Sea. This alga builds on the surface of its thallus an organized fan-shaped deposit of calcium carbonate in the form of aragonite crystals (Figure 5a and 5b).

Figure 5a

Figure 5b

The formation of this exoskeleton needs messenger molecules to be sent from the deepest cells of the alga to its surface to initiate the crystallization of aragonite, as this does not occur naturally in seawater. Indeed, this aragonite bio-crystal cannot be formed spontaneously in seawater. It is usually formed under the influence of biological agents, under considerable and very precise conditions of temperature and pressure.

b. An ingredient with a unique mode of action

Knowing the crucial role of calcium in epidermis structures and skin resistance led to an interest for the development of a *Padina pavonica* extract able to mobilize calcium metabolism by human keratinocytes.

Underwater farms and a manufacturing unit have been set up in Malta for reasons related to the culture of the alga itself that grows nearly all the year round in the southern Mediterranean.

A technique for the extraction of the active fraction of *Padina* was developed to produce extract of *Padina pavonica*: HFP (hydrophilic fraction of *Padina*). The

result of the manufacturing process is a hydrophilic fraction of this alga prepared in a glycerin- and preservative-free carrier. After having extracted the active molecules, expected to be messengers synthesized by *Padina*, tests were carried out on human keratinocytes and skin explants.

Efficacy tests highlight an action on the skin by improving cell communication and optimizing cytokeratins and desmosomial proteins synthesis strongly implicated in the cohesion and strength of the skin structures. Moreover, in the presence of pollutants (acid industrial, smoke, tobacco, exhaust gas, . . .) HFP helps the skin to fight against destructuring agents and thus prevents premature aging of the skin.

These results lead to a conclusion that HFP contains water-soluble messenger molecules responsible for the crystallization of aragonite on the padina's surface. The active extract is thus able to act on the calcium-depending cell structures of the epidermis: it actively mobilizes calcium, generating a considerable increase in the synthesis of desmosome and cytokeratins. Thanks to the neo-formation of desmosomes and cytokeratins, the intercellular communication is restored at a good level of efficacy. *The improvement of calcium metabolism restores integrity in the epidermis and helps keratinocytes to recover all their functionalities.*

Skin was found to recover its original "young" cohesion properties responsible for its mechanical and biological resistance. This, of course, makes it the most important protective organ of the organism. As a result, skin looks firmer and younger. Even under the stress of environmental pollution, the HFP protects the skin and maintains a good level of epidermis structures.

c. Stimulation of calcium-depending cell structures

1. Cytokeratin synthesis

Cytokeratins belong to the intermediary filaments family and build up not only the framework of the epithelial cells but also pass on the transduction of signals from the environment to the cell nucleus. This is why their role is so important for the cellular cohesion and communication. A decrease in the synthesis of these proteins is observed with aging and is amplified in the presence of pollutants.

A test was carried out on human keratinocytes HaCaT put into wells and incubated in a culture medium at 37°C during a single night. Cultures were then rinsed with a sterile buffered solution and treated with 50µg/ml HFP.

The dosage of cytokeratins was made by means of the ELISA immune-enzymatic technique, which is based on the fixation of an antibody specific to the synthesized cytokeratins. The complex formed is revealed with a second antibody in solution, combined with an enzyme that reacts with a last substrate. The absorbance of the solution is measured at the specific wavelength of the final complex. It is proportional to the quantity of cytokeratins that is expressed in ng/ml.

(Figure 6) This assay shows a sizeable stimulation of the cytokeratins synthesis in presence of Hydrophilic Fraction of Padina: about 85% versus the control.

Figure 6: Stimulation of cytokeratin synthesis by HFP

Non –treated Keratinocytes (control) Keratinocytes treated with HFP (20µg/ml)

2. Desmosome formation

Desmosomes are disk-shaped junction structures that allow keratinocytes to adhere one to another and resist mechanical tractions: they ensure the epidermis's resistance. With age, their number lessens, which is one factor of the skin aging process.

A test is carried out on human keratinocytes HaCaT treated with increasing concentrations of HFP, incubated for 72 hours at 37°C, and rinsed. Cells are then fixed and kept at 4°C. Desmosomial proteins are made visual with the indirect immunofluorescence technique (SIGMA).

(Figure 7) The fluorescence intensity depends on the concentration in HFP. This means that the expression of desmosomes is HFP dose-dependent.

Keratinocytes treated with HFP (50µg/ml) Keratinocytes treated with HFP (100µg/ml)

Figure 7: Stimulation of the expression of desmosomial proteins by human keratinocytes under the effect of HFP

d. Restoring intercellular communication, a key factor of the epidermis's functioning

As in every organ, cell communication is an essential functioning mechanism. It allows all cells to get information about any occurrence that could disturb the organism's balance and to react properly and quickly. With age, this information network loses its efficiency and the skin defense process decreases.

A fluorescent marker, Lucifer Yellow (LY), is used. It does not spontaneously penetrate into cells but can diffuse from one to another through specific communication junctions (gap junctions) by migrating in intercellular spaces.

A test is carried out on keratinocytes previously grown to confluence, both in the culture medium control and in the culture medium containing HFP. After one night of incubation at 37°C, cell cultures are cut in their middle and the solution of LY is added. After incubation, they are fixed and observed with a fluorescence microscope (Figure 8).

Control culture with LY Culture impregnated with LY and treated with HFP (50µg/ml)

Figure 8: cell communication restored between keratinocytes

HFP favours cell communication by improving the transfer of messengers from one cell to another. Calcium-signaling via gap junctions might be involved in the induction of calcium waves in response to mechanical stress at the upper layer of the epidermis (4).

e. Improving skin barrier integrity: a better protective shield against pollution

HFP active cosmetic ingredient is able to restore the epidermis structure, even in aggressive conditions. Despite the presence of destructurating agents, tests carried out on skin explants highlight induction of the expression of the cell element involved in the solidity and cohesion of the epidermis.

The destructurating agents used for the tests were sulfuric acid and nicotine. Sulfuric acid is a polluting agent contained, for instance, in exhaust gas and industrial smoke. A number of scientists connect it to the premature aging of the skin in cities. Nicotine is a component of tobacco pollution. It would have a deleterious effect on the skin by activating enzymes responsible for the degradation of the intercellular cement and by damaging the junction structures of keratinocytes.

Tests were carried out on skin explants from plastic surgery implemented on a 51-year-old woman. The control explant is immediately deep-frozen at –70°C. The other explants are placed in a culture medium, the epidermis upwards, and incubated for eight days while treated every two days with the creams to be tested. They are then deep-frozen at –0°C, cut into 5µm fine pieces with a cryostat and stored at –70°C until histological staining and immune-marking procedures are made.

Skin explants were treated with a cream containing 1% of a destructurating agent (sulfuric acid or nicotine) alone or with 4% of HFP in a cream. Desmosomial and cytokeratin proteins were made visual on skin sections by the indirect immunofluorescence technique.

The test carried out proves that HFP alleviates the deleterious effects of sulfuric acid and nicotine. It helps the skin to keep a normal metabolism.

(Figure 9) The intensity of fluorescence expresses a higher synthesis of desmosomes in the presence of HFP.

Skin at D0 (Control) Cream with 1% nicotine D8 Cream with 1% nicotine + 4% HFP D8

Figure 9: Expression of desmosomial proteins in presence of nicotine

(Figure 10) In the presence of a powerful pollutant such as sulfuric acid, the fluorescence is only observed in the deepest layers of the epidermis. On the contrary, when the skin explants are treated with the cream containing HFP, the fluorescence is more intense and distributed in the whole thickness of the epidermis.

Skin at D0 (Control) Cream with 1% sulphuric acid D8 Cream with 1% sulphuric acid + 4% HFP D8

Figure 10: Expression of cytokeratins in presence of sulphuric acid

This proves that HFP protects the structure but also the organization of the major cytoskeleton components in all cell layers of the epidermis.

Observations of histomorphological skin sections treated with 1% nicotine show that HFP protects epidermis in an aggressive environment (Figure 11).

Skin at D0 (Control) Cream with 1 % nicotine – D8 Cream with 1 % nicotine and 4 % HFP - D8

Figure 11: histomorphological views of skin sections

Epidermis of skin explants treated with the cream containing only nicotine is considerably thinner compared to the control, and presents only two to three cell layers. When the explants are treated with the cream containing HFP, the structure of the epidermis doubles versus nicotine only.

CONCLUSION

Nature offers a wide variety of potential solutions for customers' needs provided by the cosmetic industry. This study highlights the benefits of the active hydrophilic

fraction *Padina pavonica* alga to restore skin ability and its metabolism to maintain and reinforce its integrity via calcium-depending structures. It is a new ingredient to strengthen skin barrier with a unique mode of action targeted and highlighted with efficacy tests. Some other studies, described elsewhere (9), discovered the power of this alga to act also on the fixation of calcium within the extracellular matrix of osteoblasts, giving another potential opportunity against osteoporosis.

GLOSSARY

- **Dermal-Epidermal-Junction (DEJ):** also named cutaneous basement membrane. It is an acellular zone that is between the dermis and the epidermis. It binds tightly the epidermis to the dermis. It determines the polarity of the basal keratinocytes, the spatial keratinocyte organization and the epidermal architecture; the epidermal stratification proceeds with the proliferating keratinocytes remaining attached to the basement membrane and the daughter cells migrating into the upper layers. It serves as a selective barrier allowing the control of molecular and cellular exchanges between the two compartments. It has also a fundamental role during the wound re-epidermization, by serving through its constitutive glycoproteins (mainly laminins) as a substrate for adhesion and migration of keratinocytes.
- **Fibronectin:** a glycoprotein of the extracellular matrix that binds to membrane-spanning receptor proteins called integrins. Similar to integrins, fibronectin binds extracellular matrix components such as collagen, fibrin, and heparan sulfate proteoglycans
- **Integrin:** a large family of homologous transmembrane, cell-matrix adhesion receptors mediating the attachment between a cell and its surroundings, such as other cells or the extracellular matrix (ECM). It transmits information about the chemical composition and mechanical status of the ECM into the cell. Therefore, in addition to transmitting mechanical forces across otherwise vulnerable membranes; they are involved in cell signaling and the regulation of cell cycle, shape, and motility.
- **Lamina lucida:** a component of the basement membrane. It is 20 to 40 nm thick and is traversed by anchoring filaments rich in laminin-5 and laminin-6 and which interact with the extracellular domain of α6 β4 integrin at the cell surface of keratinocyte to form an adhesion structure with the hemi-desmodomes.
- **Lamina densa:** a component of the basement membrane below the lamina lucida. It has a thickness that varies with age from 30 to 60 nm and is mainly composed of type IV collagen but contains also laminin-10

and laminin 7, nidogen, and heparan sulfate proteoglycans, a major one being perlecan. It constitutes the intermediate anchorage zone for the anchoring filaments originating from the epidermis and the anchoring fibrils issued from the fibrillar zone of the dermis.

- **Laminin 5:** major component of *lamina lucida* and one of the key components of the anchoring complex.
- **Thallus:** vegetative system (body) of algae and fungi.
- **Tight-Junction (TJ):** multiprotein complexes that mediate cell-cell adhesion and regulate transportation through the extracellular matrix. Because tight junctions encircle the cell and attach it tightly to its neighbors, these junctions act as a barrier preventing molecules from diffusing across an epithelial sheet between adjacent cells.

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ANTIMICROBIAL PRESERVATIVES FOR THE COSMETIC AND PERSONAL CARE INDUSTRY

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ABSTRACT

Most of the cosmetic and personal care products and ingredients our industry produces are subject to degradation. This occurs from a variety of sources such as contamination from microorganisms – bacteria and fungi – or, from a chemical perspective, UV light, oxygen reduction, heating and cooling (1). **We know, then, that substances degrade, but can the length of time prior to product degradation be extended to make it acceptable in the market?** This is the heart of the matter of this chapter. It is essential reading for individuals involved in the technology, marketing and business aspects of producing new products which will be re-purchased by consumers- for no matter how good the product is, no matter what the breakthrough and claims are, if the product degrades to something different than when it was made, it simply wont work anymore and no one will buy it.

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4.1.7.11 THE USE OF PRESERVATIVES IN COSMETICS, A BRIEF HISTORY

The Food and Drug Administration, established in 1931, enforced the Food and Drug Law of 1906, which was revised in 1938 to become the Food, Drug and Cosmetic Act, officially adding cosmetics. Research on preservatives began, in response to microbiological problems, specifically visible mold growth on cosmetics. At this time, parabens became well known for their antifungal activity and became the preservative of choice.

Microbiologists of the 1950s conducted efficacy studies on the biocides to determine their spectrum of activity.

However, antibacterial compounds do not necessarily convey preservative properties. Even the annual FDA list of antibacterials used in products does not differentiate between actives and preservatives. Antimicrobials that are active ingredients primarily kill gram-positive flora on body surfaces. Antimicrobials that are preservatives primarily kill molds, yeasts, and gram-negative flora in products. Despite a product containing a biocide, one should never expect the antibacterial active to also act as a preservative or vice-versa.

Our first focus in this chapter is preventing spoilage by microbial contamination. It should be recognized that bacterial growth might be occurring, but the product does not show this visibly. However, if the product is used, it may cause an infection to normal skin surfaces, or it may even invade skin areas that have been damaged, cut, or broken and cause an infection. Notice that, while microorganisms are ubiquitous to our surroundings in the normal environment, when they get into a product that has the nutrients they require, they can grow in rather large quantities.

If this happens to one of your products, then all of the marketing, advertising, and promotion will be useless, as people stop purchasing your product. If an infection occurs, you can face a large legal claim.

What is also needed are effective preservatives that greatly slow the degradation of a product and do not compromise the product's effectiveness.

4.1.7.12 TRADITIONAL PRESERVATIVES

Preservatives

The first area to discuss is the microorganisms—bacteria and fungi; they require certain minerals, sugars, and lipids in order to grow. Many can get them from products. To grow successfully, microorganisms require suitable temperatures, pH levels, and water activity, and the growth medium must be free from ionizing radiation (Hansen & Shaffer 2001). Most products we will be discussing contain these.

These microorganisms are widely distributed in nature and are able to utilize a wide range of materials as sustenance for growth. Most contamination is a combination of bacterial and fungal species, combined (Fassihi 2001). For example, a fungi may break the compound into the substances that it needs to live, creating by-products that are suitable for bacterial growth. Microbial growth, however, is not the only thing that preservatives are used to prevent. The ingredients of many products are not in a stable condition. As time passes, the product goes into a more unstable condition, until finally, the product no longer works as expected. The fungi can also produce extracts (e.g., penicillin) that will inhibit the growth of bacteria but allow the specific fungus to grow (Moore & Jaciow 1979; Ryan 2010).

The importance of choosing the proper preservative is important. Suppose one is making a hair shampoo to be sold in the USA. The product was a huge hit, and the manufacturer decided to expand the markets to include Japan and Canada. Unknown to her or her team was that both these countries have different views of the product, and the preservative could not be sold in these countries.

This is an obvious simplification, but this is the way various countries react. So, both the ingredients and the preservatives must be assured to be satisfactory in other countries if the product is to be sold in them.

Let me give the preservative and its frequency of use in the U.S. and Canada for 2010 (Steinberg 2012).

INCI Name	U.S. 2012	Canada 2010
Methylparaben	13434	54668
Propylparaben	10421	34075
Phenoxyethanol	8878	34075
Butylparaben	5289	20451
Ethylparaben	4869	18511
Isobutylparaben	2693	10542
Methylisothiazolinone	2408	9068
Methylchloroisothaizolinone/ Methyliosthiazolinone	2235	8445
DMDM Hydantoin	2035	7434
Imidazolidinyl Urea	2007	7998
Benzyl Alcohol	1991	6344
Caprylyl Glycol	1712	4466
Diazolidinyl Urea	1644	9212
Sorbic Acid/Potassium Sorbate	1037/1456	3224/6257
Benzoic Acid/Sodium Benzoate	604/1334	1788/5123

INCI Name	U.S. 2012	Canada 2010
Chlorphenesin	1065	3493
Dehydroacetic Acid/Sodium Dehydroacetate	948	1531/3470
lodopropynyl Butylcarbamate	834	3373
Ethylhexylglycerin	712	2580
Penetylene Glycol	705	1882
Triclosan	494	1589
Sodium Methylparaben	465	1754
Quaternium-15	389	1156
Isopropylparaben	336	1745
Chlorhexidine Dihydrochloride	224	293
Chlorhexidene Digluconate	195	832
1,2 Hexandiol	162	383
2-Bromo-2-Nitro-1,3-Diol	158	1320
Sodium Propylparaben	139	379
Citrus Grandis (Grapefruit) Seed Extract	108	755
Benzalkonium Chloride	97	260
Polaminopropyl Biguanide	96	254
Methyldibromo Glutaronitrile Sodium	93	1036
Hydroxymethylglycinate	90	1050
o-Cymen-5-ol	84	302
Phenethyl Alcohol	63	343
Lactoperoxidase	61	305
Chloroxylenol	58	282
Formic Acid and its salts	48	122
Hexamidine Isethionate	45	1
Phenxyisopropanol	29	35
Benzethonium Chloride	26	163
Triclocarban	26	48
5-Bromo-5-Nitro-1,3-Dioxane	23	74
Piroctone Olamine	20	124
Dichlorobenzyl Alcohol	15	49
Climbazole	13	23
Hinokitol	11	61
Polymethoxy Bicyclic Oxazolidine	10	165
Chlorhexidine Acetate	9	0

INCI Name	U.S. 2012	Canada 2010
Glutaral	9	21
Quaternium-73	8	32
Methenamine	6	36
Thimersal	6	0
Silver Chloride	5	12

Each of these preservatives acts in ways that prevent bacteria and fungi from growing and causing degradation of the final product. The reason why there are so many preservatives is there are vastly different chemistries in product formulations, and the preservatives cannot break down the product or its formulation. It is also very important that the preservatives do not cause any toxicology problems. (Toxicology is important in testing, but it is not the focus of this chapter.)

The United States has prohibited or severely restricted (Steinberg 2012):

1. Hexachlorophene
2. Mercury compounds
3. Bithional
4. Halogenated Salicylanilides

Canada has established a "hot" list of cosmetic ingredients that are either prohibited or restricted (Steinberg 2012).

Let us turn our attention to six factors that encourage microbial growth in products. They are:

1. Water content
2. Available nutrients
3. pH values
4. Osmotic pressure
5. Surface tension
6. Temperature

Water Content

It would seem that honey would be an ideal growth medium for bacteria and fungi in that it contains much water and sugar. The interesting point is that honey does not need preservatives. The reason is that the sugar and water are tightly bound to each other, so that they are not available to microorganisms for growth. So it is important that one distinguish between water content and available water or water activity in a solution.

Water activity can be written as "a_w" and defined as the ratio of water vapor pressure of a product compared to pure water at the same temperature (Steinberg 2012).

$$a_w = \frac{p}{p_0}$$

where:

p = vapor pressure of the solution

p_0 = vapor pressure of pure water

The water activity of a solution can be determined by (Steinberg 2012):

$$a_w = \frac{n_2}{n_1 + n_2}$$

where:

n_1 = number of molecules of solution

n_2 = number of molecules of water

Because this is a ratio of vapor pressures, activity is not a strong function of temperature. It is defined as the vapor pressure of a liquid divided by that of pure water at the same temperature; therefore, pure distilled water has a water activity of exactly one (Steinberg 2012).

Equilibrium relative humanity (ERH) is:

$ERH = a_w \times 100$

Measuring Water Activity

There is no device on the market at this time that can measure water activity directly. Presently, it is measured from the relative humidity in the air surrounding the sample after both the air and water samples are at equilibrium (Steinberg 2012).

Water activity is one of the most critical factors in determining the quality and safety of goods marketing to consumers across a wide range of market concerns (Steinberg 2012). Water activity affects the shelf life, texture, flavor, and the odor of products used in the cosmetics industry. While temperature, pH, and other factors can influence if and how many microorganisms can grow in the product, water activity is an important factor in spoilage.

Most bacteria do not grow in water activity below 0.91, and most molds do not grow in activity below 0.80 (Steinberg 2012). By measuring the water activity, it is possible to predict with some degree of accuracy which microorganisms will and will not be a potential product contaminate.

Water activity, not water content, can deliver the lower limits of available water for microbial growth. It can also play a significant role in determining the activity of enzymes and vitamins in the finished product (Steinberg 2012). This can have a big impact on the color and aroma of the product.

Typical Water Activity

Product	a_w
Shampoos	0.97
Conditioners	0.96
Liquid Soap	0.91
Hand Cream	0.86
Hand Lotion	0.98

Microorganisms		a_w*
Fungi	*Aspergillis niger*	0.77
	Candida albicans	0.87
Bacteria	*Staphylococcus aureus*	0.86
	Escherichia coli	0.95
	Pseudomonas aeruginosa	0.97

* Minimum needs

Some microorganisms seems to growth with water activity (a_w) as low as 0.70, so it is a good rule that the water activity needs to be below this level or the product will need some chemical preservatives (Steinberg 2012). As can be observed, most products have a higher a_w than fungi, and a chemical preservative is necessary.

Available Nutrients

If there are available nutrients, such as carbohydrates, proteins, and phospholipids for microorganisms, then preservatives must be utilized. Many microorganisms hydrolyze the linkages or certain monoionic surfactants that then allow nutrients to be ingested and the bacteria or fungi to grow (Forbes, Sahm, & Weissfeld 2002; Joklik, Willett, & Amos 1984). The rule of growth can be very high, depending upon the microorganisms that contaminate the product; usually, it is multiple microorganisms, both bacteria and fungi, if there is no preservative.

Anionic surfactants are also able to act as sources of food for microorganisms. The chemical structure of the components in the finished product contributes to the susceptibility for certain microorganisms that are capable of oxidizing terminal methyl groups to carboxyl groups in the system (Forbes, Sahm, & Weissfeld 2002).

Many vegetable gums that are utilized as product thickeners can be converted to nutrients by microorganisms (Lundov, Moesby, Zachariae, & Johansen 2009).

pH Value

Microbial growth occurs optimally at a neutral pH (pH = 7). Most bacteria and fungi can grow between pH 5–8 (Lundov, Moesby, Zachariae, & Johansen 2009). If the product constituents can be increased to pH 9 and above, many microorganisms become impaired. The same thing happens when the pH becomes more acidic, or lower than 7.

The pH value of a product's various attributes affects the degree of ionization of useable materials. This can influence the electrical charge of the fungi and both gram-negative and gram-positive bacteria. It also has the ability to modify enzyme production and activity, which regulates the growth of the cell structure.

The growth tolerance limits of pH differ widely in microorganisms. The pH should not be considered a sole cause of microbial growth but a contributing factor. The gram-negative *Pseudomonas spp*. can grow over an extremely large range of pH values, from 3 to 11 (Joklik, Willett, & Amos 1984). Most fungi can exist over a wide range of pH values; some have been known to live in pH 9.

Osmotic Pressure

The permeable membrane surrounding all cells can be ruptured by changes in osmotic pressure. Changes in osmotic pressure also lead the cell membrane in shrinkage and dehydration of the cell (Varvaresou et al. 2009). Osmotic pressure is the availability of water and is described by a_w, which is very important.

Concentrations of 40–50% glycerin and sorbital are inhibitory to almost all types of bacteria and fungi that are of importance in cosmetic products. Very concentrated aspects of products are more self-preservative, meaning their physiochemical properties do not permit growth. If a product is diluted with water, it may become susceptible to spoilage. Shampoos that are frequently sold to professional hairdressers can become degraded.

Surface Tension

Many gram-negative bacteria, coliform in particular, grow very well in the presence of surfactants. Most gram-positive bacteria do not grow well at surface tension levels below 50 dyn/cm. Gram-negative microorganisms such as *Pseudomonas spp*. and coliforms (e.g., *Cornebacterium sp*.) may grow well in shampoos and conditioners (Al-Hiti & Gilbert 1980). Cationic surfactants are toxic to many microorganisms, but anionics to just a few. Nonionics are not lethal to microorganisms.

Microorganisms that contribute to cosmetic product spoilage often are aerobic and anaerobic, depending upon the availability of oxygen for their metabolism (Lambert & Stratford 1999). The oxygen tension of most cosmetic products promotes sufficient oxygen for growth of microorganisms.

Temperature

Temperature plays a significant role in microbial growth. Most cosmetic products are not stored in ideal conditions, often in growth-promoting temperatures (Beales 2004). For example, mascara may be put in a purse or left in a car. If it is in a hot climate or stored in a car that can really heat up, spoilage by chemical breakdown and microorganism growth can easily occur. The ideal temperature for many bacteria is 30–40°C and, for fungi, 20–25°C.

Necessity of Preservatives

In previous times, many microorganisms have been found in cosmetic products both new and unused, and used (Beales 2004). It seems that it was not a bacterial or fungal infection, but a degradation of the product that caused preservatives to be used.

The main requirements for selecting a preservative are (Paulson 2013):

1. Noninterference of the cosmetic product's action or mode of activity
2. Freedom from causing negative attributes, such as products' odor, color, and feel
3. Does not cause toxic or irritating effects to the end user
4. Can cause longer storage than the product alone
5. Is stable to moisture, heat, and dryness
6. It must have activity at low concentrations.
7. It must be effective over a wide range of pH values.

When a compound is manufactured, its various components are unbalanced to give it, for example, cleaning ability. If preservatives are not present, the compounds break down into stable but less active structures. The product then loses its potency. This is also why preservatives are necessary.

4.1.7.13 PROBLEMS WE FACE WITH THE USE OF PRESERVATIVES

To situate preservatives in the industry and society, let us first discuss the quadrant system, which will explain our individual and social perspectives.

The Quadrant System

To better understand this topic, let us overlay this onto a four-quadrant system (Paulson 2002; Wilber 1995). You might ask, "What is the four-quadrant system?" It consists of the complete sphere in which you live, from the personal to the world of consumers in the subjective and objective components (2, 3). The four-quadrant system, then, consists of the objective and subjective components of you and the consumers who purchase your product. This is normally seen in four different paradigms (Kuhn 2012). At least two quadrants (the subjective quadrants) are suppressed

from our lives, but they exist and contribute to a product's success. Science has disassociated the entire subjective viewpoint, stating that only the physical is real. We will reintegrate these four paradigms into one megasystem—the quadrant view.

You

Subjective	- Your likes and dislikes - Prejudices - How you live your life	- Your objective view
	- Culture (what they like, will buy, and why they buy)	- Society (Rules, laws, and legislature)

Objective

Consumers

The top two components, consisting of "you," subjective and objective, are what I will first discuss. The top layer could also be another person, depending on the perspective. But as this chapter is read by you, it will be from "your" perspective. The upper right quadrant is how you can touch, see, and view the objective world. It is what is called "reality" for many people, for they do not see any of the other quadrants. It is their conscious scientific attitude. The other three quadrants are collapsed—unknowingly—into this one quadrant in our modern world, where the subjective, cultural, and societal quadrants are ignored. However, in reality, the four quadrants occur simultaneously in our lives.

The upper-left quadrant is the one in which we have our subjective experiences. For example, it tells us what we like and do not like. It tells us what we fear, what we appreciate, and describes our need for companionship with others close to us (Campbell, 1976; Mitchell & Black 1996). It contains the conscious, preconscious, and unconscious aspects of our selves. Our conscious aspects are those of which we are aware. Also, they are the areas that we prefer, such as the scent of a cologne, the feel of a skin moisturizer, and what we buy to feel secure. Our preconscious is our monitor. It does not let things that are not considered acceptable by our culture into our conscious. The unconscious is what is suppressed, repressed, and denied. It is also the quadrant that determines if a person will buy the product based on subjective experience. This includes ease of use, odor, color, size, and feel of the product, etc.

The lower-left quadrant represents the culture. It is extremely important, but often it is ignored. It contains the cultural beliefs shared by a society, and the shared values, such as the feel or fragrance of a product preferred by others, which has great influence on customers. These values are generally of two types: manifest

and latent (Newman 1997). Manifest are surface values that are conscious to the consumer. For example, your product may be on sale, so it is discounted and that is a reason for purchasing it. But deeper, more fundamental values are also present. These are referred to as latent values, which are generally unconscious to the consumer. The smell of the product may activate a memory of the person being younger and in love. Therefore, s/he buys the product with these unconscious needs as the driving force. What consumers believe to be true, then, is not necessarily grounded in reality. This should be remembered when using preservatives. If your product is degraded and spoiled at the time of use, it can affect future product sales.

The lower-right quadrant represents society. A society has rules and laws to follow—regulations that go into making your product. These societal requirements include conforming to regulation agency standards, which include the Food and Drug Administration (FDA), Federal Trade Commission (FTC), and the Environmental Protection Agency (EPA), as well as the rules, laws, and regulations they enforce. So before designing a product, it is important to fully understand the current legal regulations governing the product preservative components and their maximum levels.

The four-quadrant approach is important also, in that it is viewed as a system of four components that occur in the four areas of the preservative system. For example, let us look at a shampoo product. In the upper-right quadrant, we use it to clean our hair. If the preservative was not present, the shampoo could begin to break down. The first thing that could happen is color and odor change, while its basic cleaning chemistry components would not be as precise.

The antimicrobial aspects could begin to break down, allowing microorganism growth in the formula (Fassihi, 2001). A person would begin to notice a difference; it is not the same.

From a personal, subjective perspective, a person would now question the product's value. Perhaps a consumer would ask questions like "Does it smell the same?" "Does it feel different?" This will go on until the person finally says, "Something is wrong with this product." Instead of getting the aesthetic of "feeling springtime fresh," the person feels as the "winter doldrums." The values shared with others, both manifest and latent, are constantly changing. The conscious aspects—manifest values—of springtime freshness are changing. But the deeper, more fundamental value of being clean, and therefore being accepted and valued as a worthwhile person, are being unconsciously challenged. Maybe they support the notion that the person is now unclean and will be rejected as a worthwhile person. This causes the person to be unconsciously open to purchasing other shampoos.

This plays into how a person judges the things s/he buys based upon the culture. The lower-left quadrant contains all the information on which we act and should act in a culture from that culture's perspective (Paulson 2002; Wilber 1995). We know that this is where we place the feelings that we have compared to what our

culture has determined. So when we feel a "springtime fresh feeling," we compare it to coolness, the brilliance and lasting good smell and the renewed feeling we enjoy from the springtime. However, if the product and our conception of it are at odds, we feel bad.

The product failed to meet our cultural standards. We do not feel valued, loved, or appreciated by the people who are close to us or others. We must find something else to make us feel the connection.

The lower-right quadrant is the society with its rules and regulations (Paulson 2002; Wilber 1995). The Cosmetics, Toiletries, and Fragrance Association (CTFA) and the Soap and Detergent Association (SDA) are the two big regulators in this area. But there are others. For example, the FTC and interstate laws govern these product supplements as well. If the products' ingredients decomposed, a firm could be vulnerable to a lawsuit. For example, the product could be said to have caused facial damage or damage to the consumers' children.

These quadrants are all important, but for the sake of brevity, we shall focus on the lower-righthand quadrant in this chapter. In this quadrant, the percentage of a preservative and the exact components are provided. Preservatives are included in products to greatly slow the products' spoilage rate and microbial contamination (Fassihi 2001). The aesthetic appeal and functionality are also addressed.

CONCLUSION

In this chapter, the basics of preservatives have been discussed. This is not enough to finish a successful product, but it will help as you begin to look at a potential preservative and then make choices.

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ANTIOXIDANTS: EXTENDING THE SHELF LIFE OF YOUR PRODUCTS

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ABSTRACT

Antioxidants are being used for several purposes in the personal care industry, ranging from prevention of rancidity in formulations to sun protection and prevention of skin aging. These materials are generally used to neutralize free radicals, which may result from improper storage of the formulation or environmental stress on the skin.

Oxidation in formulations is a result of the generation of free radicals that convert unsaturated fatty acids in the contained oils into undesirably odoriferous molecules. These free radicals can be generated by heat, light, and metal ions. Antioxidants quench the formation of these free radicals, thereby reducing the rate of oxidation and subsequently increasing the shelf life of the product.

Traditionally many synthetic antioxidants have been used for the above-mentioned applications. However, due to heightened consumer awareness and toxicity questions, many of these synthetic ingredients have come under scrutiny. The cosmetic and personal care industry has responded to these issues by seeking and finding materials better suited to address these issues. There are several plant-based antioxidants available that can perform as well or better than their synthetic counterparts in these applications. This chapter will focus on various applications of natural antioxidants in extending shelf life of formulations.

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4.1.7.21 INTRODUCTION

Oxygen, despite being necessary for sustaining life, is also detrimental in many aspects. The damaging effects caused by the ubiquity of oxygen have been classified as "oxidation reactions." Some of the common negative observations attributed to oxidation include: browning of fruits and vegetables over time, rusting of iron, and even aging. One of the principle causes for the reduction in shelf life of cosmetic products is oxidation of unsaturated oils. Unprotected cosmetic products, when exposed to environmental factors such air, light, and high temperatures, are susceptible to oxidative degradation. This process results in discoloration, off-odor, and also physical instability of the products.

The oxidative-degradation products include peroxides and hydroperoxides. These products are not the primary source of the debilitating effects observed in the formulations, but rather, it is their degradation products such as aldehydes and ketones that cause undesirable visual and sensorial changes in the formulation. [1] [2] Although the mechanisms responsible for lipid oxidation have been studied extensively, as of yet they are still not fully understood. The rate at which oxidation of lipids occurs and the pathway it follows depends upon several factors such as the degree of unsaturation, the presence of other components in the system, and the physical state of the system. [3]

Antioxidants have become an indispensable group of additives to cosmetic formulations. [4] They not only effectively retard the onset of oxidation in products but can augment the consumer's natural topical defense system against oxidation. Most antioxidants are phenolic compounds, which have the ability to donate a hydrogen free radical easily and in turn get oxidized. [5] Both natural and synthetic antioxidants have been used in cosmetic products to improve their shelf life. [6] Many currently used synthetic antioxidants have been under extensive scrutiny due to concerns over their safety. In addition to this concern for safety, consumers

are becoming more aware of the sources of ingredients being used in their personal care products and are demanding that they be obtained from natural, sustainable, and safe sources. Plants produce a variety of complex phenolic compounds to protect their internal "machinery" from oxidation. Some of these plant-based molecules exhibit remarkable antioxidation capabilities rivaling those of their synthetic counterparts.

Several key factors need to be taken into account to choose the right antioxidant for a particular system. The most important ones are: the oil component of the system; polar or nonpolar oils; potential impurities in the ingredients used, such as metal ions; physical state of the formulation; gels, oils, emulsions, etc.; desired shelf life of the product and manufacturing process; heated or cold processing.

Numerous assays have been developed to determine efficacy of antioxidants. These assays vary from measuring the kinetics of lipid peroxidation to measuring the primary and secondary oxidation product. Since many of these assays are not conducted in the final formulation, care should be taken in interpreting the obtained data. Regardless, these assays are good tools in providing insights concerning feasibility of the antioxidant.

The overall goal of this chapter is to provide the reader with an understanding of oxidation, how it affects a formulation, and how antioxidants can be used to improve the shelf life of a cosmetic or personal care product.

4.1.7.22 OXIDATION

The phenomenon of oxidation is defined as loss of electron by a molecular species. The rate for a molecule to undergo oxidation depends on the activation energy needed to abstract an electron or a hydrogen free radical from it, which in turn depends on its molecular structure.

Mechanism of Lipid Oxidation

The basic mechanism of lipid oxidation has been very well characterized. This process can be divided into three distinct steps: Initiation, Propagation, and Termination. [3] [7]

Initiation

Auto-oxidation of unsaturated lipids is thought to be initiated by free radicals, which can be generated by heat, metals, or other impurities; and enzymes and light in presence of oxygen. [8] In this process, the a-carbon atom to the double bond loses a hydrogen free radical and forms a lipid radical (Figure 1).

Figure 1. Generation of lipid free radical by abstraction of hydrogen free radical from unsaturated lipids.

It is well characterized that polyunsaturated fatty acids are more susceptible to free radical oxidation than monounsaturated fatty acids. Table 1 illustrates the amount of energy required to abstract hydrogen from different carbons of linoleic acid. Hydrogen from position 11 is most easily removed due to the double bonds on either side of that c-atom. [8]

Table 1. Energy required for removal of hydrogen atom from different positions of c-atoms in linoleic acid.

Hydrogen Removal Position	Energy Required (kcal/mol)
	50
	75
	100

Lipid radicals can also be generated by addition of free radicals to the double bonds of unsaturated lipids (Figure 2). [9]

Figure 2. Generation of lipid radical by addition of free radical to unsaturated lipids.

Light-induced initiation or photo-oxidation occurs in the presence of oxygen and a photo-sensitizer molecule such as chlorophyll or other electromagnetic radiation–absorbing species. In photo-oxidation, light excites the sensitizer molecule to its triplet state (^{3}Sen*). This excited molecule then induces oxidation by one of the two mechanisms known as Type I or Type II. Figure 3 illustrates the general pathway for the two mechanisms. In Type I, the excited sensitizer can directly interact with the lipid molecule to generate lipid radicals which in turn starts a free-radical chain reaction, where the lipid radical can react with triplet state oxygen ($^3O_2$) to form peroxyl radicals (ROO*). In this case, the rate of reaction is dependent on the concentration and the type of sensitizer and substrate i.e. lipid molecules. In Type II, the excited sensitizer can interact with molecular oxygen, which is in triplet state, to form high-energy singlet oxygen species ($^1O_2$). The singlet oxygen can then react with lipid molecules to form hydroperoxides (ROOH). The rate of reaction for Type II pathway is primarily dependent on concentration and solubility of oxygen in the system. The resulting hydroperoxides can further dissociate into free radicals. [8]

Figure 3. Light-induced initiation and reaction with the lipid molecule via Type I and Type II pathway.

Metal ions, such as Fe^{+2}, Cu^{+2}, and Co^{+2}, can also initiate formation of free radicals by reacting with lipids (Figure 4). The generated free radical can then initiate a chain reaction. [10] [11]

$$RH + M^{+2} \longrightarrow R^{\bullet} + H^{+} + M^{+}$$

Figure 4. Metal ion-induced initiation and reaction with lipid molecules.

The activation energy for generation of free radicals can also be supplied by heat or by the presence of enzymes. High temperatures during processing of oils can initiate the free-radical chain reaction and can lead to oxidative degradation of oils. Also the presence of enzymes in a system such as lipoxygenases or any hydrolytic enzymes can reduce the activation barrier for generation of free radicals.

Propagation

The free radicals generated during the initiation step react with other molecular species in the system to form new free radicals in the propagation phase. This sets off a chain reaction where multiple species of free radicals are generated in the system (Figure 5). Lipid free radicals can react with oxygen to form lipid peroxyl radicals (ROO*), which in turn can react with other lipid molecules (R_1H) to form lipid hydroperoxides (ROOH) and lipid free radicals (R_1*). Hydroperoxides are unstable and can degrade to produce other free radicals such as lipid oxy radical (RO*) and hydroxyl radical (OH*). [3]

Figure 5. Generation of multiple free radical species during the propagation phase.

The propagation reaction is repeated many times and continues as long as unsaturated lipids are available. The chain reaction nature of this phase results into accumulation of hydroperoxides. The hydroperoxide species generated in the system are dependent upon the molecular structure and the unsaturation level of

the lipid. Lipid hydroperoxides are primary products of oxidative degradation and are odorless. [12]

Termination

Lipid free radicals are structurally unstable and are highly reactive species. Since the free radicals have an unpaired electron, they are constantly seeking to pair that electron with other molecular species. During the propagation phase the number of unsaturated lipids is decreasing, as they are being consumed by free radicals to form new free radicals. This results in a free radical–rich system where the free radicals bond with one another to form astable nonradical species, which results in termination of the propagation cycle. Figure 6 illustrates the different ways in which lipid free radicals are terminated.

Figure 6. Multiple reaction pathways by which free radical reactions are terminated.

The stable species generated during the termination step are considered as secondary products of oxidative degradation and most often are carbonyl compounds (aldehydes, ketones, or fatty acids). It is the presence of these reaction products that affects the sensory profile of the product, most often detected as off-odor and off-color. [12]

4.1.7.23 ANTIOXIDANTS

A simple definition of an antioxidant is a compound that opposes oxidation. Antioxidants are sacrificial molecules that undergo oxidation and slow down the oxidative damage done to the other molecules. [12] They tend to slow down oxidative reactions by inhibiting or retarding any or all of the three steps of auto-oxidation: initiation, propagation, and termination. The use of antioxidants is prevalent and necessary in cosmetic formulations, particularly the ones that use lipids. The shelf life of a product can be improved substantially by using the right antioxidants.

Antioxidants can broadly be classified under two categories: Primary and Secondary antioxidants. Molecules that are capable of quenching free radical species and thereby delaying or inhibiting the initiation step or slowing down of the propagation step during the auto-oxidation cycle are termed "primary antioxidants." Secondary antioxidants are compounds that slow down the oxidation reaction but by means other than just free radical quenching. [12] [11]

Figure 7 depicts the various free radical species that primary antioxidants (AH) can react with to form stable molecules and hence slow down the chain propagation and eventually the overall oxidative damage to the system. Primary antioxidants are typically phenolic compounds that can donate a hydrogen radical to the free radical species. In this process they reduce the primary radicals to non-radical species and in turn get converted to oxidized antioxidant species. Efficacy of an antioxidant depends on its molecular structure. Efficacious antioxidants can not only donate hydrogen radicals but can themselves form radicals that have low reactivity and low possibility of reacting with lipids. [11]

Figure 7. Reaction of antioxidant (AH) with a variety of free radical species generated during the auto-oxidation of lipids.

4.1.7.24 PRIMARY ANTIOXIDANTS

Synthetic Antioxidants

One of the most common synthetic antioxidants used in cosmetic formulations is butylated hydroxytoluene (BHT). [11] The stability of the free radical–reacted antioxidant depends on its structure and the number of resonating free radical structures it can form to stabilize that free radical. [13] Figure 8 illustrates the structure of BHT and the different resonating structures it forms during the stabilization of the free radical. The large number of resonating structures that BHT can form with free radicals makes it a potent antioxidant in an "oil-heavy" formulation.

Figure 8. Resonating structures that butylated hydroxytoluene (BHT) forms to stabilize free radical.

In addition to BHT, butylated hydroxyanisole (BHA) and *tert*-butylated hydroquinone (TBHQ) (Figure 9), are other synthetic antioxidants that have also been used in personal care formulations to prevent rancidity. [7] BHA is a mixture of two isomeric forms that are produced during its synthesis.

Figure 9. Structures of two isomers of butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ).

Over the past two decades, there has been a shift away from synthetic ingredients towards more natural products. This shift stems from the need of the consumer to understand sources and the environmental impact of the ingredients that are being used in the products that they consume. Another popular belief, though not always true, that everything natural is safe, has led to the ingredient research in finding natural alternatives to synthetic ingredients. This shift has also led to the decline in the demand of many commonly used synthetic ingredients, such as BHT. In addition to the consumer trend, there are also some regulatory limitations being imposed on the use of certain synthetic ingredients. These events have led to an increase in the use of natural ingredients, especially the ones derived from plants.

Natural Antioxidants

Nature is considered to be a highly efficient "chemist," with plants being its reaction vessels, producing molecules with such a high degree of complexity that they could never be reproduced economically outside of those plants. Plants can be considered as "factories" that can produce molecules that have applications in variety of fields. Fine chemicals produced from plants can truly be considered as renewable products because plants harvest light, air, and water to produce these molecules. Once the molecules of interest are extracted out of the plant, the spent biomass can be returned to the environment and recycled. Several botanicals have been used in cosmetic formulations that have been known to prevent lipid oxidation. [14] Two of the most common ones are nonpolar extract of rosemary and tocopherols obtained from vegetable oils and their derivatives.

Tocopherols are a class of compounds that have a chromanol ring and a phytyl chain. They exist in four different forms (Figure 10). [15] [16] Vitamin E activity of tocopherols has been mostly attributed to a-tocopherol. [17] Among

the different homologues of tocopherol, the antioxidant activity is in the following order: d-tocopherol > g-tocopherol > b-tocopherol ≈ a-tocopherol. [18] [19] [20] Use of a-tocopherol or vitamin E has been successful in preventing lipid oxidation at low concentrations; at higher concentrations it was found to be either neutral or pro-oxidant. [21] [22] [2] This dualistic behavior of a-tocopherol can be attributed to its inability to stabilize free radicals for long enough periods before the molecule itself becomes a free radical donor. The pro-oxidant mechanism of a-tocopherol is depicted in Figure 11. The a-tocopheroxyl radical formed can donate a free radical to other lipid molecules (RH) or lipid hydroperoxide (ROOH), where it can re-initiate the auto-oxidation of lipids and thereby act as a pro-oxidant. It is important to note that even though vitamin E might be used in a formulation for its skin benefits, if used at improper concentration it can reduce the shelf life of the formulation by pro-oxidizing the lipids. The pro-oxidant effect of vitamin E at high concentrations can be nullified by using co-antioxidants in the system.

Derivatives of tocopherols have also been used in cosmetic formulations. Most common derivatives are tocopheryl acetate and tocopheryl palmitate (Figure 12), where the hydroxyl group on the chromanol ring is esterified with a fatty acid. Esterification of the hydroxyl group stabilizes the tocopherol in the formulation but also strips away any proton-donating antioxidant activity of the molecule. It is believed that the ester hydrolyzes over time once it is absorbed in the skin and frees the tocopherol. [23]

Figure 10. Structures of different forms of tocopherol.

Figure 11. Pro-oxidation mechanism of a-tocopherol in a lipid system.

Figure 12. Ester derivatives of a-tocopherol.

Rosemary extract has been commonly used in variety of food matrices to prevent lipid oxidation. [3] Its usage in cosmetic formulations has been increasing over the past few years. [24] The extract is obtained from the leaves of the plant *Rosmarinus officinalis*. Most industrial-scale processes use organic solvents such as acetone, hexane, or methanol to extract the key antioxidant molecules from the leaves. Due to increasing trend towards natural products and certifications, many of the listed solvents are being replaced by supercritical carbon dioxide because of its acceptability as an extraction solvent for naturally processed ingredients. The nonpolar extract of rosemary leaves contains a class of molecules called phenolic diterpenes. [25] [26] The antioxidant activity of the rosemary extract has been mostly attributed to the two most prominent phenolic diterpenes: carnosic acid and carnosol (Figure 13). [27] The primary purpose for the production of these antioxidant molecules in the plant is to protect the photosynthetic pathway of the plant from oxidation. [28]

Figure 13. Structures of carnosic acid and carnosol found in rosemary extract.

Rosemary also yields an essential oil fraction, obtained by steam distillation, which is used for its fragrance and aromatherapy. [29] [30] This fraction has little to no contribution towards preventing lipid oxidation. Essential oils primarily consist

of terpenes such as camphor, 1,8-cineole and verbenone (Figure 14). [29] Terpenes are volatile compounds that contribute to the odor of the plant and are devoid of hydrogen radical donating property such as that found in other effective antioxidants.

Figure 14. Common terpenes found in rosemary essential oil.

Phenolic diterpenes are known to sequester free radicals efficiently because they can form other g-lactone phenolic diterpenes that have antioxidant activity. Carnosic acid on interacting with a free radical can undergo rearrangement and form carnosol, which is also an antioxidant. [31] [3] [9] The oxidation of carnosol results in the formation of rosmanol, which can then further oxidize into gladosol (Figure 15). The formation of successive antioxidants by oxidation of these phenolic diterpenes renders them an exceptional antioxidant capacity that can rarely be matched by any synthetic antioxidant where, upon oxidation, synthetic antioxidants are converted into an inactive form. The complexity and redundancy that nature has put into such molecules to counter oxidation can rarely be found outside of the plant kingdom.

Figure 15. Rearrangement pathway that carnosic acid undergoes during an oxidation cycle that produces subsequent antioxidants.

One of the challenges of using natural plant extracts in cosmetic formulation is the impact they can have on the organoleptic properties of the product. The herbal odors and colors imparted by the natural product can often limit their inclusion levels in the final product to ineffective amounts. For example, an organic solvent–extracted rosemary product will also have an essential oil fraction of the plant. To remove this odor, a secondary processing step needs to be included, such as distillation. But the resulting product will still have substantial color that can have an impact on the final product. Recently, a few low-odor and low-color rosemary products have emerged in the market that have low to no impact on the organoleptic profile of the final product. To achieve such a product, a secondary process is implemented that concentrates the molecule of interest in the extract and removes inactive molecules such as terpenes and chlorophyll. To develop such processes it is important to understand and identify the active molecule for the intended application in the formulation.

4.1.7.25 SECONDARY ANTIOXIDANTS

Secondary antioxidants reduce the rate of oxidation of lipids by several pathways, such as binding of metal ions, neutralizing singlet oxygen species, and scavenging of oxygen. [12] [11] One of the biggest limitations of using only the secondary antioxidants is that their mechanism of action is very specific while auto-oxidation of lipids is a combination of several different types of reactions. They are most effective when used in conjunction with primary antioxidants.

Metal ion chelators such as ethylenediaminetetraacetic acid (EDTA) or citric acid are extremely important in preserving the systems that have high water activity such as oil-in-water emulsions, soaps, and shampoos. In such systems, metal ions—which are more soluble in the polar phase—may diffuse into the water/oil interface and can initiate auto-oxidation of lipids. Chelation of metal ions results into neutralization of their charge and subsequently their ability to initiate auto-oxidation of lipids. [32] Figure 16 depicts general reaction schemes for EDTA and citric acid with metal ions.

$$M^{+2} + [EDTA]^{-4} \longrightarrow [M\text{-}EDTA]^{-2}$$

$$M^{+2} + [CITRATE]^{-3} \longrightarrow [M\text{-}CITRATE]^{-1}$$

Figure 16. General reaction scheme for chelators reacting with metal ions.

Alternatives to synthetic ingredients such as EDTA are being sought due to the rise in demand for plant-based natural ingredients. Citric acid, although it is a natural chelator obtained by fermentation, is not as strong as EDTA, which limits its use. Phytic acid or its salt form, sodium phytate, obtained from bran of rice,

wheat, or corn, is a strong chelator. [33] It is an inositol hexakisphosphate with six phosphate units bound to an inositol ring (Figure 17).

Figure 17. Structure of phytic acid (R) and its sodium salt (R').

Another natural chelator that has been used extensively in cosmetic formulations is gluconic acid and its derivatives (Figure 18). These molecules have been found to chelate di- and trivalent metal ions. [34]

Figure 18. Structures of various gluconic acid derivatives.

One of the most common oxygen scavengers or reducing agents used in cosmetic formulations is vitamin C or ascorbic acid. [35] Ascorbic acid can react with oxygen to form dehydroascorbic acid and hydrogen peroxide (Figure 19). [36] [37] This formation of hydrogen peroxide can further exacerbate the oxidative stress on the system. The three carbonyl units on dehydroascorbic acid render it highly reactive and can participate in a Maillard reaction and impart a brown color to the formulation. [38] To avoid complete oxidation of ascorbic acid, it is often used along with a primary antioxidant that can regenerate ascorbic acid from its oxidation products. [39]

Figure 19. Oxidation of ascorbic acid to dehydroascorbic acid.

Another class of compounds that have been used as secondary antioxidant is carotenoids. [40] They can quench high-energy singlet oxygen. [8] In the presence of carotenoids, singlet oxygen will preferentially transfer its excess energy to the carotenoid species present in the system to produce a triplet-state carotenoid. The excess energy of the triplet-state carotenoid is then given off as heat as it returns to ground state (Figure 20). Carotenoids are effective quenchers of singlet oxygen. One carotenoid molecule can quench numerous singlet oxygen molecules.

Figure 20. Singlet oxygen quenching by carotenoids.

The ability of carotenoids to quench singlet oxygen is directly related to the number of double bonds in the compound. Carotenoids with more conjugated double bonds are more effective quenchers. [41] Some of the common carotenoids used are b-carotene, lutein, lycopene (Figure 21). [42] Since all these molecules have conjugated double bonds, they have an intense orange to red color that is imparted to the final formulations. This coloring ability limits the type of formulations in which these molecules can be incorporated, being primarily color formulations.

In addition to the singlet oxygen quenching, these molecules have several skin benefits. For example, lutein has been shown to improve skin moisturization and elasticity, and also provide photoprotection. [43]

Figure 21. Structures of common carotenoids used in cosmetic formulations.

4.1.7.26 ANTIOXIDANT ASSAYS

Antioxidant ability of ingredients can be evaluated by several means. Some assays measure the relative ability in just oils, since oils are the primary component of a formulation susceptible to oxidation; others measure retardation of the oxidative damage in a finished formulation. In this section, few of the common methods to assess the efficacy of antioxidants in improving the shelf life of the formulation will be discussed.

There are also other methods, not discussed in detail, particularly 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Oxygen Radical Absorbance Capacity (ORAC) assays that are used for screening antioxidants. [44] These assays can provide relative antioxidant capacities of an ingredient, but disputes have arisen due to the lack of correlation between these assays and their ability to predict performance of an ingredient in a finished formulation. The primary reason for this disagreement stems from the fact that the matrices for cosmetic formulations are complex in comparison to the ones used in these assays. [3] The antioxidant activity depends on several factors such as media, temperature, nature of oxidants, and microenvironments in the formulation and not only on free-radical quenching ability of an antioxidant, which is what DPPH and ORAC primarily predict. These assays cannot predict the pro-oxidant effect of antioxidants. It is well known that certain antioxidants exhibit a concentration-dependent pro-oxidant effect in a lipid-rich matrix. Since the matrix used in DPPH and ORAC assays is an organic solvent to dissolve the dye, the potential for pro-oxidant effect of an antioxidant cannot be determined using these assays. Extreme care should be taken when using these

assays for predicting antioxidant ability in a formulation, and the observed effects should be corroborated with the more traditional assays described below.

Oxidative Stability Index (OSI)

OSI, also known as the Oil Stability Index, is an official method of American Oil Chemists' Society (AOCS) [45] and is well accepted throughout the industry in predicting antioxidant ability of an ingredient in oil. [46] The basic principle of this technique involves measuring the time it requires for a sample to undergo rapid oxidation under accelerated conditions. This time point is then referred to as Induction Period (IP).

In this assay, the sample is heated to a set temperature and is held at that temperature for the duration of the study. To accelerate the oxidation, purified air is bubbled in the sample, which results in oxidation of the sample. The sample then starts to produce oxidative degradation products such as peroxides first, followed by aldehydes and ketones, which are secondary degradation products. Upon further oxidation, the samples produce volatile fatty acids (VFAs). These VFAs are then carried by the air into a water reservoir, where they dissolve and change the conductivity of water.

A conductivity probe immersed in the water reservoir is then used to continuously measure the change in conductivity. The time point at which the rate of change in conductivity increases rapidly is known as the IP. The IP depends on several factors such as the oil or blends of oils used in the study, the unsaturation level of the matrix, the presence of any impurity, presence of an antioxidant or a pro-oxidant in the oil, and the temperature at which the study is being conducted. To study the effect of an antioxidant on the oil(s), a control sample without any additive is also subjected to the test along with the antioxidant treated samples. An ingredient that is an efficacious antioxidant will have a longer IP than the control sample, while a pro-oxidizing ingredient will have a shorter IP. The ratio of IP of a treated sample to that of the control is often used for comparative purposes and is called as Protection Factor (PF). A control will always have a PF=1, while an efficacious antioxidant will have a PF > 1 and a pro-oxidant will have a PF < 1.

As most OSI studies do not last for more than 30 hours, an OSI study could be used as a preliminary test to time-consuming stability studies. OSI could be conducted either on the individual oils that make up the formulation or on the blend of oils. The results from these studies could be used for determining the stability of the different oils, predicting improvement in shelf life of oil and hence the formulation and cost-benefit analysis of adding an antioxidant.

Peroxide Value (PV) Test

Peroxides and hydroperoxides are generated during the propagation phase of auto-oxidation. Peroxides are considered to be the primary

oxidative degradation products (Figure 5). The measurement of peroxides in a system is a good indicator of the oxidative stress a system is undergoing. Unlike OSI, which is used only for oil samples, PVs can be determined in both the oil and/or in a final formulation. The oxidative stress could be imparted in real time or accelerated by incubating the samples at higher temperatures (30–45°C).

PV is typically determined by Iodometry, which is a standard AOCS method (Figure 22). [47] In this method, peroxide (ROOH) is reacted with iodide ion (I^-) to generate iodine. The generated iodine is then titrated with sodium thiosulfate ($S_2O_3^{2-}$) with starch solution as an indicator where it changes from blue/black to colorless, marking the endpoint of the titration.

$$ROOH + 2I^- + H_2O \longrightarrow ROH + 2OH^- + I_2$$

$$2S_2O_3^{2-} + I_2 \longrightarrow S_4O_6^{2-} + 2I^-$$

Figure 22. Reactions involved in iodometric titration for determining peroxide value.

PV is defined as peroxide oxygen per kilogram of fat or the sample and has been traditionally expressed in the units of milliequivalents per kilogram (meq/kg) of the sample.

Generation and accumulation of peroxides and hydroperoxides do not contribute to the off-odor and off-color of a formulation. It is the secondary products such as aldehydes and ketones generated from peroxides and hydroperoxides that cause a system to turn “rancid” and affect the organoleptic profile of the formulation. [3] Since peroxides are precursors to organoleptic failure of a product, tracking them is a good indicator of onset of rancidity. However, care should be taken in interpreting the data, for in a system that has undergone substantial oxidative damage one may not be able to detect any peroxides because they might have been converted to secondary degradation product. Measuring PVs at that point will serve no purpose in predicting oxidative failure of system, and carbonyl products would need to be measured at that point. Figure 23 illustrates a hypothetical time profile for accumulation of primary (peroxide and hydroperoxide) and secondary (aldehydes and ketones) oxidative products generated in a system. If the measurements for PV were made only at time point A and B, it would give an impression that the system is not undergoing any oxidative stress. However, in reality there is accumulation of secondary products, which would change the organoleptic profile of the system. Hence it is important that frequent PV measurements are made in the initial stages of the study.

Figure 23. Time profile for generation and accumulation of primary and secondary oxidation products in a system.

Measurement of Secondary Oxidation Products

Most of the secondary oxidation products are carbonyl compounds, which are highly reactive towards nucleophiles. Several compounds have been developed to react with these secondary oxidation products, resulting in formation of chromophores which can be measured spectroscopically. A few of these molecules are listed in Figure 24. [48] [49] [1] For many of these assays validated kits can be obtained from various vendors.

Figure 24. Molecules used for detection of secondary oxidation products.

CONCLUSION

Oxidation is one of the major adversities that a cosmetic or personal care formulation faces during its lifetime. It is important to understand the source and mechanism of oxidation and the specific ingredients that are susceptible to this

phenomenon. Since oxidation is a foreseeable thermodynamic eventuality, it can only be slowed down but never stopped completely. Choosing the right antioxidant can help in warding off oxidation for a sufficient period of time and improve the shelf life of the formulation. In addition to choosing the right antioxidant, it is equally important to choose the right assays to measure oxidation and the effect of antioxidants on a system. The chosen assays should provide enough information to understand the right oxidative stress points in the system and also be pertinent to the final application for which the formulation is being developed. For example, if a nonaqueous formulation is being developed, then conducting OSI on a blend of the oils with an antioxidant might be sufficient. If an oil/water emulsion is being developed, then it might be prudent to conduct both OSI on the oils and PV on the final formulation.

There is a concept called the "polar paradox" which states that hydrophilic antioxidants perform better than hydrophobic antioxidants in nonpolar systems such as bulk oils; while hydrophobic antioxidants perform better than hydrophilic antioxidants in polar systems such as oil/water emulsions. [50] [3] Although not completely understood, the explanation for this behavior stems from the belief that oxidation is a surface phenomenon where it is initiated at the interface of the two phases. For bulk oils it is initiated at the oil-air interface, and for oil-in-water emulsions it is initiated in the water phase surrounding the oil droplets. Having an antioxidant at the interface, and not in the bulk, would improve its chances in retarding oxidation of the lipids in the system and hence protect the lipids from oxidative damage. The "polar paradox" does not always predict an antioxidant's performance but is too valuable to be ignored and may help in understanding the anomalous behaviors of antioxidants in a given system.

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INGREDIENTS II

NATURAL AND SYNTHETIC POLYMERS: DESIGNING RHEOLOGICAL PROPERTIES FOR APPLICATIONS

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ABSTRACT

There are many rheological additives a formulator may select to obtain the desired properties of the final formulation. Often several rheological additives would perform well in a particular formulation and the formulator must make some choices. Considerations regarding the product application, labeling, consumer targets, and pricing come into play. However, more important considerations are the requirements of processing and the stresses the formulation will encounter. Therefore, the pH of the formulation, the processing temperature and shear rate, the packaging conditions, and the consumer's handling of the product are extremely important. The formulator must also consider the desired appearance and properties of the final formulation.

Most rheological additives contribute more than controlling basic flow and viscosity properties. Many also provide flow properties, suspension properties, emulsion stabilization, stability to shear, heat, pH, or freeze/thaw cycles, etc. The rheological additives that can be utilized to achieve these properties in various applications are discussed in this chapter, as well as an overview of each of these rheological additive categories.

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4.2.1.1 DESIGNING RHEOLOGICAL BEHAVIOR

4.2.1.2 NATURAL AND SYNTHETIC POLYMERS: RHEOLOGICAL PROPERTIES AND APPLICATIONS

Natural and synthetic polymers are used in a multitude of formulations to design rheological properties for various applications. This chapter discusses rheological additives for both water-based systems and anhydrous systems. Rheological additives for water-based systems include natural gums and natural gum derivatives, cellulosics, starch derivatives, clays, polyethylene glycols, fatty alcohols, and polymers. Anhydrous systems are more often modified by the use of silicas, polyethylenes, fatty acids, castor oil derivatives, aluminum/magnesium hydroxide stearate, stearic acid derivatives, or organoclay pre-dispersions. Some of these additives are used for both aqueous and nonaqueous formulations.

Most rheological additives contribute more than basic flow and viscosity properties. Many also provide suspension properties, stabilize emulsions, and improve stability to shear, heat, pH, or stability to freeze/thaw cycles. They can also promote penetration, lubricity, and slip and modify the ultimate skinfeel of the finished product. Overall, the addition of rheology modifiers contributes to emulsion stability and improves the shelf life of the product. The rheological additives that can be utilized to achieve these properties in various applications can be split into two categories: additives that can be used to modify water and additives that can be used to modify liquids other than water. Though this basic classification system can be useful, it should be remembered that many cosmetic systems contain both water and oil phases and can therefore use rheological additives from either group, depending on the need.

There are many rheological additives a formulator may select to obtain the desired properties of the final formulation. Often several rheological additives would perform well in a particular formulation and the formulator must make some choices. Considerations regarding the product application, labeling, consumer targets, and pricing come into play. However, more important considerations are the requirements of processing and the stresses the formulation will encounter. Therefore, the pH of the formulation, the processing temperature, shear circumstances, the packaging conditions, and the consumer's handling of the product are extremely important. The formulator must also consider the desired appearance and properties of the final formulation.

4.2.1.3 RHEOLOGICAL ADDITIVES USED TO OBTAIN SPECIFIC PROPERTIES

Using a rheological additive to create higher viscosity can greatly contribute to the emulsion stability of the finished formulation and therefore produce a longer product shelf life. These thickeners may be naturally or synthetically derived, organic or inorganic polymers. The effects of product viscosity changing due to changes in pH, temperature, or shear can be stabilized by using the right rheological additive. Thickeners also help to suspend active ingredients or pigments in formulations, such as using carbomers in lotions or organoclays in nail polish. Higher viscosity in formulas, especially skin and hair care products, is often associated with luxuriousness and therefore improved consumer acceptance.

Surfactant systems, such as shampoos, body washes, and shower gels can be very difficult to thicken and stabilize. Typical rheological additives used for these surfactant systems include carbomer, gums, gum derivatives, starch derivatives, aluminum/magnesium hydroxide stearate, hydroxypropylmethylcellulose, or simply salt.

Thixotropic rheology modifiers create viscosity in the formulation; however, when the formulation is applied to the skin or hair, it becomes thin so it can be evenly spread. In other words, these additives can cause the product's viscosity to decrease when the shear rate is increased. They do not immediately return to their original viscosity when the shear is removed, but the original viscosity will be recovered if given enough time. Therefore, formulators must consider the level of shear to which the formula will be subjected in the laboratory or in the manufacturing environment.

If the rheological additive in the formula is shear-sensitive, such as with large polymers or silicas, then the overall product rheology can be affected. The product structure may break down and the final product will no longer have the intended rheology. On the other hand, rheological modifiers like clays and organoclays and

some gums, like carrageenan, thrive on shear and will not break down under these conditions. The viscosity may even build higher with these shear-loving additives.

It is important to be aware of time and temperature when working with thixotropic rheology modifiers. The cooling rate of the product can affect viscosity and structure formation. If one tries to measure the viscosity of a thixotropic material without control of the length of time before testing, one may get results that are not reproducible since the time on the viscosity recovery curve is not controlled. The formulator must also keep these sensitivities in mind when testing and reporting accelerated stability results for products with thixotropic ingredients.

Pseudoplastic rheology modifiers are shear thinning when the shear rate is increased; however, they return to their original viscosity when the shear is removed. Good examples of pseudoplastic rheology modifiers are gums, like xanthan or guar gum. Carbomers, cellulosics, clays, and silicas also exhibit excellent pseudoplastic properties. The rheological additive is often chosen to achieve the desired product attribute when used by the end consumer. For example, a hand lotion may be formulated to rub in quickly, whereas a massage lotion or barrier cream will be formulated to have more play time and rub in slowly.

Rheological additives can increase the resistance to flow or yield value. It is important to have even distribution of the active ingredients, colors, and other additives in the formulation emulsion or suspension. Increasing the yield value can contribute to this stability. Flow properties can make or break consumer acceptance of products like lotions or shampoos. If the desired amount of product is not easily pumped or squeezed out of the package, the product itself may be wonderful, but the consumer may fail to purchase it again. Products like nail polish require good flow properties from their rheological additives to evenly distribute the wet polish on the nail; however, the brush marks must then disappear on the dry polished nail.

Emulsion technology is another key area for the use of rheological additives. Stabilizing emulsions against phase separation can be accomplished with two different rheological approaches: adding a rheological additive to the external phase or adding it to the internal phase. The first approach is the one most frequently used. By choosing an additive that either increases the viscosity of the external phase or introduces a yield value to that phase, the formulator can slow down the flow of the internal phase through the external one. Formulators can also adjust the rheology of the emulsion by incorporating a rheological additive into the internal phase. The stabilizing effect here is caused by making the internal phase more "deformation resistant." Using an oil-in-water emulsion as an example, a viscosity-increasing additive in the oil phase will make the oil droplets more resistant to deformation as they flow past each other. The harder they are to deform, the harder it will be for the oil phase to flow through the aqueous phase, thereby increasing the time required for creaming, phase separation, or coalescence.

Finished product stability is often a result of ensuring emulsion stability of the formula. Rheological modifiers are key ingredients in providing emulsion stability to formulations such as lotions, sunscreens, or cleansing products. As described above, these ingredients can be added to the internal or external phase, but are most often added to the external phase. They prevent phase separation often by building the viscosity of the product and slowing down the potential flow of one phase through the other.

There are many rheological additives that can be used to stabilize emulsions and often more than one is used in a formula. These additives not only thicken and suspend to stabilize the emulsions, but go beyond that to actually develop a polymer network that keeps the oil droplets from coalescing. Typical rheological modifiers used to stabilize formulas include natural gums and natural gum derivatives, cellulosic derivatives, starch derivatives, clays, fatty alcohols, synthetic polymers, or silicas. For example, synthetic polymers are often used for emulsion stability in creams and lotions or personal cleansing products. In aqueous systems, starch derivatives inhibit syneresis, which is the separation of water from the formulation. Controlling this moisture migration extends the shelf life of the product by maintaining a stable emulsion.

Insoluble additives are increasingly added to personal care formulations. These additives are often very fine in particle size and have a tendency to settle out of the formulation over time. Rheological modifiers are used to suspend these particles and create a stable product. Suspending agents are rheological additives since they change the rheology of the fluid, making it more difficult for the solid particles to flow through. Many suspending agents form a loosely bound structure within the liquid, and the solids become trapped and are supported in the formula. If the weight of the solid exceeds the strength of the supporting structure, the solid will settle. Therefore, fine particles suspend more easily than very large particles. Antiperspirants, sunscreens, liquid makeups, mascara, and nail polishes all depend on suspending agents to help distribution of active ingredients and pigments. Lipsticks also benefit from suspending agents. When a lipstick is manufactured, it is held in a molten state until it is poured into molds. When melted, it is critical that the pigments stay in uniform suspension; otherwise, some lipsticks of the batch may contain more pigment or color than others. After injecting the hot lipstick mass into a mold, it is also important that the pigment does not settle into the tip of the stick, or the lipstick's appearance will suffer. Examples of rheological additives that act as suspending agents include natural gums and natural gum derivatives, cellulosics, synthetic polymers, carbomer, starch derivatives, clays, silicas, polyethylenes, castor oil derivatives, and organoclays.

Rheological additives that are good film formers can enhance and stabilize foam in formulations such as shampoos, shaving creams, or body washes. A

product that produces more foam or lather does not necessarily mean the product is working better; however, many consumers believe this to be true. Consumers want good foam properties and for most, it is an indication that the product is cleaning the skin or hair better. While surfactants create the foam, rheological additives aid in the foam's properties and stability. Natural gums and natural gum derivatives, cellulosic derivatives, starch derivatives, fatty alcohols, and polyquaterniums help provide lather and stabilize foam, which improves consumer acceptance of the commercial product.

The film-forming properties in products like styling formulas, sunscreens, and shaving products are often attributed to the rheological modifiers used in the formula. For example, in sunscreens these additives help to distribute the active ingredients to protect the skin from the sun's rays and may even improve water resistance.

The lubricity of a formula can easily be modified by the addition of rheological polymers. When a cream or lotion is rubbed onto the skin, the amount of friction created can greatly influence the consumer's sensory perception of the product. Lotions, shaving products, shampoos, soaps, body washes, lipsticks, and sunscreens all benefit by improved lubricity. Natural gums and gum derivatives, starch derivatives, polyethylene glycols, castor oil derivatives, and stearic acid derivatives all impart lubricity to creams and lotions, cleansing products, and more.

Both nonionic and cationic rheological modifiers are added to formulations to improve the conditioning properties of the product. In shampoos, conditioners, and styling gels, cationic polymers help to smooth and control the hair. Polyquaterniums, cellulosics, starch derivatives, polyethylene glycols, and carbomers are good examples of rheology modifiers that provide conditioning properties.

Before a formulator selects the rheology modifiers to incorporate into the formula, they must determine the desired product appearance of the finished lotion, gel, suspension, or solid. Typical questions might be: Is the target a clear or opaque product? Would gloss inhibit or benefit the consumer perception of the product? Once these decisions have been made, an appropriate rheological additive can be selected. For example, if a clear formula is desired, a synthetic polymer, carbomer, or polyethylene could be tested. On the other hand, if the desired outcome is an opaque formula, clays or organoclays may be selected. Starch derivatives or gums could impart glossiness to the formula, if that is the objective.

Another choice a formulator must make is how the product will be administered from the container. Will it be pumped, poured, squeezed, etc? As discussed, the flow properties of a formulation are greatly affected by the choice in rheological additives incorporated into the formula. If a short texture (not stringy or slimy) is desired, a starch derivative in a lotion or silica in toothpaste is a good choice.

Another example is using polyethylene glycols, which come in a wide range of molecular weights, to adjust the desired formulation structure by increasing or decreasing molecular weight. Gums, gum derivatives, and highly substituted cellulosics can provide a long texture to formulas, if that is the aspiration.

Short-chain, low-molecular-weight polymers with low viscosity (thickening) properties are excellent rheological modifiers to improve the luxury and body of the formulas without drastically increasing the viscosity of the overall system. An example is using maltodextrin in a massage lotion where the formulator wants to increase the play time or time to rub the lotion into the skin.

4.2.1.4 RHEOLOGICAL ADDITIVES FOR AQUEOUS SYSTEMS

The category of rheological additives for water-based systems includes a number of natural and synthetic ingredients including gums and gum derivatives, cellulosic and starch derivatives, clays, polyethylene glycols, fatty alcohols, and polymers.

While there is an increasing consumer interest for natural formulations and the use of natural ingredients, the definition of "natural" is often debated. Also, the desire to formulate green formulations that are safe and mild, yet still effective, is a challenge to formulators. Historically, natural gums were often used for rheology modification; however, they have fallen out of favor with formulators due to their innate variability and availability. Because they are agricultural products, these natural polymers can vary depending on the year, climate, yield, etc. They also introduce the potential for microbiological contamination to the formulation. On the positive side, natural gums can provide a stable viscosity at a wide pH range and greatly improve product stability. They can also provide the formulation with salt tolerance. They can exhibit pseudoplastic or thixotropic rheological properties and are added into the water phase of an emulsion. Some gums can be pituitous (stringy or slimy), which can cause problems for rheological flow from containers, especially those with small pumping orifices where they can pull back into the container upon pumping. Examples of natural gums include carrageenan, xanthan, guar, tragacanth, locust bean, karaya, and alginates. Synergistic effects can be achieved by the combination of some of the natural gums with clay products, such as the use of xanthan or guar gum with aluminum magnesium silicate.

Hydroxypropyl guar is a good example of a nonionic, natural gum derivative with pseudoplastic properties. It develops high viscosity in a short amount of time, so it is a very versatile thickener. This rheological modifier is often used in lotions, shaving products, and shampoos not only for viscosity, but for its ability to impart lubricity. These derivatives are also compatible with alcohols, electrolytes, and surfactants, which make them even more functional in personal care formulations.

Cellulose is another naturally occurring polysaccharide, and through chemical modifications it accounts for a family of rheological additives that includes cellulose gum, hydroxyethylcellulose, methylcellulose, and hydroxypropylcellulose. Due to the modifications, some of the derivatives display excellent salt tolerance, surfactant compatibility, or film-forming properties, which make them particularly useful in certain shaving products, shampoos, and other hair care products. They can impart suspension properties to mascara, liquid makeup, and other pigmented products.

While cellulose gum provides thixotropic rheology, hydroxypropylcellulose, and hydroxyethylcellulose are good examples of pseudoplastic rheology modifiers that are thickeners and emulsion stabilizers. These rheology modifiers provide a stable viscosity in hair conditioners where many thickeners are not compatible with the cationic polymers. In addition, their film-forming properties enhance hair care products such as styling gels. Cellulosic derivatives may be hydrophobically modified to increase the surface activity of the rheological modifier.

When added to the aqueous phase of the formulation, starch-based derivatives help build viscosity, stabilize emulsions, suspend other ingredients, and improve film-forming properties of the formulation. With certain modifications, starch derivatives can prevent syneresis or the movement of water out of the emulsion. This extends the product shelf life and improves consumer acceptance. Starch derivatives can be stable to a wide range of pH, shear, and temperature. They also provide emulsion stability to freezing and thawing conditions. These polymers provide short-textured, thixotropic viscosity to a wide variety of formulations including creams, lotions, body washes, shaving creams, shampoos, and other aqueous emulsions. Because they are good film formers, starch derivatives improve foam properties in personal wash products, shampoos, and shaving products. They can also form freestanding films to use as a delivery vehicle for other ingredients. Some starch derivatives are emulsifiers and can be used to encapsulate active ingredients or fragrances, while others are designed to improve conditioning or flow properties.

Maltodextrins are low-molecular-weight glucose polymers derived from starch. They are used to improve the body or luxuriousness of creams and lotions, shaving products, and cleansing products. Since they are low molecular weight, they improve play time of a formula without greatly increasing the viscosity. They are very water soluble and often used in soap and cleansers, bath products, and pouched powder products to increase dispersibility of the formulations. They also impart humectant properties.

Clays can exhibit pseudoplastic rheological properties in water-based systems, but can also be highly thixotropic depending on the type of clay and formulation used. They are used primarily for viscosity enhancement, flow control, suspension,

and emulsion stability. There are different types of clays, and the different natural formations of these clays along with the different chemistries account for the various properties they provide. Clays may include bentonites, hectorites, synthetic hectorites, and magnesium aluminum silicates. Bentonites are based on aluminum silicates, while hectorites are based on magnesium silicates. Clays are very often used in combination with other thickeners, especially organic ingredients like cellulosics or gums. Clays tend to form opaque or tinted dispersions that must be considered when developing formulations. Hectorite is normally lighter in color than bentonites. These rheological modifiers are generally used in shampoos, lotions, and liquid makeups.

Polyethylene glycols (PEGs) are available in a range of molecular-weight polymers of ethylene oxide. These materials are soluble not only in aqueous systems but also in nonaqueous systems such as alcohols and glycols. They are widely compatible and unaffected by electrolytes. They are used as thickeners, humectants, and lubricants in creams and lotions, and as co-gelling aids for antiperspirant sticks. They exhibit high viscosity to form a matrix that suspends fine particles of active ingredients or pigments; however, when pressure or shear is applied they can easily break down to release the particles. The higher the molecular weight, the more effective the polymer is in providing viscosity. Polyethylene glycols below a molecular weight of 700 are liquid at room temperature. The PEGs can be used to create a particular melting point for personal care sticks and pastes. They can also help solubilize other ingredients like bath oils in body wash products.

Fatty alcohols, such as cetearyl alcohol, a blend of cetyl and stearyl alcohol, are widely used as rheological additives in lotions, creams, and hair care products. They not only create viscosity, but can also act as an emulsifier, emollient, opacifier, and foam booster. These ingredients are used in both aqueous and nonaqueous formulations and help to stabilize both water-in-oil emulsions and oil-in-water emulsions. They can be used to change rheology dependent on processing time and temperature.

Synthetic polymers contribute high viscosity and are used to thicken, suspend, and stabilize formulations. These polymers are used extensively in cosmetics formulations and are a polymer of choice for clear formulas. The main base used for most synthetic polymers is acrylic acid, and the polymer is described as a homopolymer (made entirely of one kind of polymer), copolymer (combinations of more than one type of polymer), or crosspolymer (copolymers with a cross-linking agent). These materials are supplied in a tightly coiled acid form and become effective only when uncoiled by neutralization with a base. These polymers create viscosity by swelling; they do not dissolve in water. Their effectiveness comes from both long-chain entanglement and hydrogen bonding; they are typically used at very low concentrations ranging from 0.1% to 0.5%. Most synthetic polymers

are limited by salt sensitivity, pH sensitivity, and the inability to withstand high levels of shear. Many synthetic polymers are also sensitive to UV light, and the formulation viscosity can be lost if exposed for periods of time. This is a concern for sun-care lotions and similar products.

Acrylic acid polymers, or **carbomers** are added to the aqueous phase and build pseudoplastic viscosity, create clear gels, and stabilize emulsions. They are often the main rheological additive in creams and lotions, body washes, shampoos, and hair-styling products for providing viscosity and film forming. Carbomers are used in lotions not only to create viscosity in the finished product, but also to allow the product to break down when applied to the skin due to their sensitivity to salt, thus allowing the lotion to rub in quickly. Synthetic cationic polymers are compatible with both cationic and nonionic surfactants and are used to thicken, suspend, and condition formulations such as hair care products.

Polyquaternium products are typically cationic polymers where the base polymer may be a cellulosic, synthetic, or other polymer. Because of the positive charge, these polyquaterniums are good conditioners and are widely used in hair care products. They are film formers, so they work well in hair-styling and conditioning products that contain cationic additives. They can bond with the hair or skin and form a protective film to prevent reaction to harsh surfactants or chemical treatments. They can improve slip and prevent moisture loss, thereby providing humectant properties. Some polyquaterniums are nonionic and also provide film-forming and stabilizing properties to cleansing, conditioning, and styling products.

In surfactant systems such as body washes, shampoos, or hand soaps, a common viscosity modifier is simply salt, sodium chloride. Especially when used with anionic surfactants, increasing the salt content of the formulation will increase the viscosity; however, a point is reached where adding additional salt will then decrease the viscosity. Formulators will typically create a salt curve measuring the viscosity of the product as the salt level is increased. The viscosity of the formula will increase to a peak and then as more salt is added, the viscosity will quickly decrease. The concentration of salt selected for the formula is typically the concentration just before the peak of the curve.

4.2.1.5 RHEOLOGICAL ADDITIVES FOR NONAQUEOUS SYSTEMS

Rheological additives for nonaqueous systems include silicas, polyethylenes, fatty acids, castor oil derivatives, aluminum magnesium hydroxide stearate, stearic acid derivatives, and organoclay pre-dispersions. The functional properties and potential applications for these additives cover a wide range of topics and products.

Silicas, or **silicon dioxides**, are used for building viscosity, for creating thixotropy, and for providing suspension properties. They build their thixotropic

structures in oil systems through a network of long chains and hydrogen bonds. These very fine particulates can be either porous or not, depending on their manufacturing process. The liquid process (hydrated silicas) yields a very porous material that can absorb oils, while the vapor or "fumed" process yields a higher surface area and a significantly reduced bulk density. Precipitated silica, a form of hydrated silica, is manufactured in both porous and nonporous forms, giving a product range of oil-absorption and thickening properties. Hydrated silicas and fumed silicas can provide the formulation with pseudoplastic or thixotropic rheological properties. Silicas are used in powder and liquid formulations for hair and skin care. They build structure and viscosity in toothpaste formulas. In powder formulations, they act as anti-caking agents or carriers as well as improving flow properties. In liquid formulations, they contribute to emulsion and viscosity stability, moisture resistance, and provide suspending properties. Care must be taken to avoid high shear processing after the addition of silica, since it can destroy the hydrogen-bonded network. Silicas can also be used to modify aqueous formulations.

Polyethylenes and their variants are thixotropic rheological modifiers used primarily for their thickening and suspending properties in anhydrous systems such as lipsticks, antiperspirant sticks, and mascaras. They improve water resistance, form films, improve heat stability, and can form clear gels when quickly cooled, allowing the formation of a fine thixotropic crystal structure. To achieve good properties, polyethylenes need to be heated to 80°C or higher, depending on the grade of polyethylene used. Polyethylenes contribute to emulsion stability and film-forming properties, which makes them suitable for creams and lotions, sunscreens, and hair care products.

Fatty acids are typically added to the oil phase of an emulsion to provide viscosity and stability to the formulation. In cleansing products like shampoos and body washes, they act as foam stabilizers and suspending agents to entrap the dirt and wash it away. Fatty acids and fatty-acid derivatives can be derived from plant or animal sources. Soybean, coconut, and palm oils are often used to manufacture fatty acids and fatty-acid derivatives. These additives greatly improve lubricity of the formulation.

Castor oil derivatives are used to increase viscosity of anhydrous formulations like ointments, lotions, foundations, lipsticks, and mascaras. They can also contribute to the moisturizing effects of a formulation on the skin. A good example of a castor oil derivative is trihydroxystearin, a thixotropic rheology additive that provides a high degree of viscosity, stabilizes emulsions, improves water repellency, and helps with suspension of active ingredients or pigments. It is used with organoclays to control the flow and recovery time in mascaras. When using castor oil derivatives, heat and shear, under carefully controlled process conditions, are necessary for full rheological development.

Aluminum magnesium hydroxide stearate is a complex of aluminum magnesium hydroxide and stearic acid, which can form gels in a number of cosmetic oils. These gels tend to be colorless and stable over a broad range of temperature. This ingredient provides thixotropic viscosity and emulsion stability to formulations like creams and lotions, ointments, antiperspirants, eye makeup, and lipsticks. This rheological modifier can suspend pigments and active ingredients and can increase the temperature stability of the formula.

Stearic acid derivatives are often used in cleansing formulations for viscosity and foam stabilization. They contribute a moisturizing effect to many formulas. Besides the thickening effects, these additives can contribute to the emulsion stability and emollient properties, improving the skinfeel of creams and lotions. Stearic acid esters impart a pearlescent appearance to shampoos and body washes.

Highly thixotropic rheology modifiers, organoclays are reaction products of hydrophilic clays with long-chain quaternary compounds, for example, quaternium-18 hectorite. The quaternary ammonium compound provides compatibility with nonaqueous liquids, allowing the primary clay rheology to be expressed in nonaqueous systems such as oils, esters, and silicones through a hydrogen-bonding structure between platelets. The organoclays are the rheological additives of choice in nail polish, makeups, hair gels, antiperspirant roll-ons, aerosols, and waterproof mascaras, due to their thickening and suspending abilities. To be fully dispersed, organoclay powders need chemical activation and very high shear; therefore, they are often available as pre-dispersions, where the organoclays are in fully activated and sheared forms in a number of cosmetic oils. Organoclay gels are unaffected by high-temperature or high-shear processing, though laminar flow can reduce the viscosity of the overall system. These rheological additives are added to the oil phase of the formulation and are often the first choice to thicken oil-based systems.

Some rheology modifiers typically used in nonaqueous systems may also be used in aqueous systems depending on the formulation. For example, synthetic polymers and polyethylene glycols can be used to modify either type of system.

New, very specialized rheology modifiers are being developed with specific goals in mind. Many are vegetable derived to target the growing natural market. Some of these polymers provide viscosity, water repellency, and improved product appearance as well as stability. It is in the formulator's best interest to educate themselves on new ingredients.

The formulator has many choices in determining the rheological additive that is right for their particular formulation. The decision must be based on the type of rheology required in the finished product. However, the formulator must be cognizant of compatibility with other formulation ingredients, the pH of the formula, the processing conditions, the need for clarity, and the desired consumer reception.

As in many cases, the final answer can be reached by several different paths. The formulator must consider the rheological additives available and weigh the advantages and drawbacks of each one. By continuing to learn and experiment with new rheological additives, new and unique formulas can be developed.

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GLOSSARY

Acrylic acid polymers: High-molecular-weight rheology modifiers produced from acrylic acid and used as thickeners, emulsion stabilizers, and to create clear gels.

Aluminum magnesium hydroxide stearate: A complex of aluminum magnesium hydroxide and stearic acid used to form colorless gels in oil.

Carbomer: Polymers of acrylic acid used as thickeners, emulsion stabilizers and to create clear gels.

Castor oil derivatives: Rheology modifiers used to increase viscosity of anhydrous formulations.

Cellulose-Based Polymers: Naturally occurring polysaccharides usually chemically modified to create versatile rheological modifiers.

Clays: Naturally occurring minerals used for increasing viscosity, improving suspension properties, and controlling flow.

Conditioning: Improving surface properties by moisturizing, changing surface chemistry, or adding lubricity.

Emulsion: A mixture of oil and water where one phase is suspended as tiny droplets in the other phase to create a stable dispersion.

Fatty acids: Rheological additives based on natural acids, which create viscosity, lubricity, and improve stability of formulations.

Fatty alcohols: High-molecular-weight alcohols used as thickeners, opacifiers, and foam boosters.

Film formers: Ingredients capable of forming a film or continuous sheet on a surface or as a bubble.

Flow: The deformation or movement of a fluid.

Hydroxypropyl guar: Nonionic, natural gum derivative used to create viscosity, impart lubricity, and improve stability in formulations.

Lubricity: The reduction in friction when two surfaces are rubbed together.

Maltodextrin: Low-molecular-weight saccharide polymers derived from agricultural starch products and used to impart solids, lubricity, and suspension to formulations.

Natural gums: Polysaccharide polymers based on agricultural products and used as thickeners and rheology modifiers.

Organoclays: Reaction products of hydrophilic clays with long-chain quaternary compounds used as rheology modifiers in nonaqueous systems.

Polyethylene glycols: Polymers of ethylene oxide available in a wide range of molecular weights and used as rheology modifiers in formulations.

Polyethylenes: Rheological modifiers used as thickeners and suspending agents in anhydrous systems.

Polyquaternium polymers: Cationic polymers, based on cellulosic, synthetic, or other polymers, creating conditioning and film-forming properties.

Pseudoplastic: Shear thinning with immediate recovery when the shear is removed.

Rheological additives: Ingredients that modify flow characteristics of a fluid and add other functional properties to a formulation.

Salt: Common salt is sodium chloride, which is used for increasing viscosity in surfactant systems.

Sodium chloride: Common salt used to increase viscosity in surfactant systems.

Silica: A chemical compound, also known as silicon dioxide, used for building viscosity, structure, and suspension properties in formulations.

Silicon dioxide: A chemical compound, also known as silica, used for building viscosity, structure, and suspension properties in formulations.

Starch derivatives: Naturally derived polymers based on agricultural products usually chemically modified to create versatile rheological modifiers.

Stearic acid derivatives: Rheology modifiers used for viscosity, foam stabilization, and emollient properties.

Syneresis: The separation of water from a gel.

Synthetic polymers: Polymers, not derived from natural ingredients, used as thickeners and suspending agents in formulations.

Suspending agents: Ingredients that support the dispersion of solid particles in a liquid, making it difficult for the particles to flow through the liquid and settle out.

Thickener: Ingredient with the ability to create viscosity in a formulation.

Thixotropic: Shear thinning with a delayed recovery when the shear is removed.

Viscosity: A measure of the resistance of a fluid to deformation or flow. Thickness.

Yield value: The stress required to initiate flow or movement of a fluid.

RHEOLOGY MODIFIERS AND CONSUMER PERCEPTION

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ABSTRACT

All consumer product formulations have an inherent rheological character that is dictated by the raw materials used and how they are combined in the formulation. The rheological character is a description of the flow behavior over a range of shear rates and with the passage of time while being sheared. There are many parameters that contribute to the overall rheological character of a formulation. Viscosity is the most common rheological parameter encountered in formulation. Said simply, viscosity is the measure of flow of the fluid, over a range of shear rates from very low (like sitting in a container, or being poured) to very high (like being applied to the skin upon rubbing). Sometimes the viscosity is constant over the range of shear rates and with the passage of time. At other times, the viscosity may increase or decrease with shear and with the passage of time. Yield stress is a critical rheological parameter for imparting stability to emulsion formulations and to optical effects formulations, such as cleansers or hydroalcoholic formulations that have some type of optical effect, such as beads or air bubbles. Viscoelasticity is a third rheological parameter. Viscoelasticity is the balance of the viscous and elastic components of a material. It impacts the formulations visual aesthetic when it is at rest and the pick-up and cushion of formulations during rub-in. The array

of formulation attributes that are affected by its rheological character makes the design of the rheological behavior of a formulated product a crucial step in order to make a product behave in the desired way in the consumer's hands and during use.

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4.2.2.1 INTRODUCTION

In this chapter we describe some of the key rheological modifier polymers, their structure in general, and the variables of structure that can be built in in order to provide a range of behavior in cosmetic and personal care formulations.

In many formulations, the rheological properties provided by the primary functional materials can provide the desired physical behavior and flow properties for the formulation. For example, an emulsion may be stable and sufficiently thickened only by the inclusion of emulsifiers and oils, and this resulting formulation will have its own unique rheological profile. Often, however, the primary functional materials of a formulation, such as emulsifiers and oils, are not sufficient to impart long-term stability, thickening, and a desirable rheology. In this case, polymeric rheology modifiers are typically employed. Polymeric rheology modifiers have the ability to impact multiple aspects of formulations, including formulation stability and the product's full sensory profile. The typical rheological parameters that impact personal care formulations are viscosity, yield stress, and viscoelasticity. It is critical to choose a polymeric rheology modifier that can help to deliver the desired product aesthetics to your formulation. The most commonly used synthetic polymeric rheology modifiers are modified sulfonic acid–based polymers, polyacrylic acid–based polymers, and alkali swellable emulsion–type polymers.

4.2.2.2 RHEOLOGICAL PARAMETERS

a. Viscosity

A primary function of polymeric rheology modifiers is to provide increased viscosity of the solvent, such as water. Viscosity is a measure of the resistance to flow when a shear stress is applied. For ideal viscous fluids, the value of the ratio of the shear stress, τ, to the corresponding shear rate, $\dot{\gamma}$, is a material constant called the apparent viscosity, η. These kinds of fluids are often referred to as Newtonian fluids. One example of such a fluid is polydimethyl siloxane. Many types of fluids in personal care exhibit a *pseudoplastic* or shear-thinning behavior with the applied force. In these fluids, the value of the ratio of the shear stress to shear rate decreases with the shear rate. Equations 1 and 2 and Figure 5 illustrate these behaviors:

$$\eta = \frac{\delta}{\gamma}\text{; h is constant, Ideal of Newtonian Fluid} \quad (1)$$

$$\eta(\dot{\gamma}) = \frac{\delta}{\gamma}\text{; } \eta \text{ varies with the shear rate, e.g., shear-thinning fluids} \quad (2)$$

With rare exceptions, Newtonian and pseudoplastic fluids are the two most common fluid types encountered in personal care formulations at room temperature and under typical conditions of usage. Note, however, that a large variety of rheological behavior, such as plastic, pseudoplastic, dilatant, rheopectic, thixotropic, is found in many other industrial fluids and formulations and has been extensively documented in the literature (1).

Graphical examples are best suited for illustrating rheological behavior, although there are numerous mathematical models that are used to describe such behavior (2) (3). Two types of graphs are commonly used. One is a plot of apparent viscosity versus shear rate as shown in Figure 1:

Figure 1. Typical graphical representation of viscosity versus shear rate for aqueous personal care formulated products.

The other way to graphically describe the rheological properties of a liquid formulation is to plot the shear stress versus shear rate data as shown in Figure 2. In this way data obtained at various temperatures and at varying concentrations of the rheology modifier and other formulation ingredients can be obtained and studied to fully characterize the rheological properties.

Figure 2. Typical graphical representation of shear stress versus shear rate for aqueous personal care formulated products.

A number of aqueous formulations in personal and home care obey the rheological flow behavior described in Figures 4 and 5. These include shampoos, bath gels, body washes, liquid soaps, hair gels, toothpastes, skin creams, lotions, pomades, etc. Many of these formulations are emulsions or contain dispersed solid matter such as beads, mica, pearlizing crystals, seeds, etc. However, from a

rheological viewpoint, the difference among these products is not essential; it is more of a quantitative than qualitative nature.

b. Viscosity Measurement

The viscosity of aqueous formulations in personal care is typically measured using various types of rotational and oscillatory rheometers. Rotational rheometers typically measure the torque on a probe such as a rotating plate, cylinder, or a spindle of certain specific geometry, in contact or immersed in the test fluid. The measured torque is correlated to viscosity by the instrument's processor. Typical measuring systems of the rotational type include a) concentric cylinders, b) cone-and-plate, c) parallel-plate and d) relative measuring systems. Brookfield rotational viscometers are relative measuring systems (4). They are extensively used to measure the viscosity of aqueous compositions of various kinds. They are inexpensive and simple to use; however, they only measure relative viscosity values or instrument-dependent values since the viscosity is measured with a specific spindle with no information of shear rate, which is required according to Newton's Law in equation 1. Oscillatory rheometers are instruments in which the probe instead of rotating is oscillating according to a periodic oscillatory motion, such as for instance a sinusoidal motion of either constant amplitude or frequency. The rheological information obtained includes a number of parameters that assess the viscous and elastic components of the formulation in terms of the elastic or storage modulus (G'), the viscous or loss modulus (G") and the loss factor (tan δ). One of the main advantages of this system is that the strain or deformation imposed on the fluid is very small. Regardless of the instrument used, temperature control is essential to perform accurate measurements, particularly in the case of aqueous formulations whose viscosity can significantly vary with a change of only ±1°C.

c. Viscosity of polymeric rheology modifiers

In order to understand the different thickening mechanisms of polymeric rheology modifiers it is necessary to review the basics of viscosity in relation to polymer solutions. When a fluid is at rest, solvent molecules move rapidly under Brownian motion. When a fluid is flowing, solvent molecules move rapidly in the direction of flow. A low viscosity fluid is obtained when the solvent molecules are undisturbed and able to flow rapidly. When solvent molecules are disturbed and need to flow around or through an obstacle, they move along a longer path or more slowly, both of which decrease the rate of flow of the fluid. The resulting macroscopic behavior, in both cases, will be that of a high-viscosity fluid.

Polymers in solvent exhibit a hydrodynamic volume. The hydrodynamic volume is the effective volume of space that the polymer fills. The chemical composition of a polymer will dictate the nature and strength of the interactions between that

polymer and its solvent. In a good solvent, the polymer has strong interactions with the solvent and exists in a more expanded conformation. This expanded conformation takes up more effective space and therefore the hydrodynamic volume of the polymer is greater. Solvent molecules flowing around or through a polymer with a greater hydrodynamic volume will move more slowly and will result in a high-viscosity fluid. As the hydrodynamic volume of a polymer in solution increases, so does the viscosity. By contrast, in a poor solvent, the interaction of the polymer with the solvent is unfavorable, so the polymer interacts preferentially with itself. This results in a more collapsed conformation. This collapsed conformation takes up less effective space and therefore the hydrodynamic volume of the polymer is smaller, resulting in a lower viscosity. As a general rule, the greater the hydrodynamic volume of the polymer, the higher the viscosity of the fluid. Polymeric rheology modifiers impart thickening properties based on this principle of hydrodynamic volume.

d. Yield Stress

Another primary function of polymeric rheology modifiers in formulations is to provide a yield stress or yield point. A fluid with a yield point begins to flow only when the external forces acting on it are larger than a certain shear stress commonly known as yield stress, τ_o. Equation 3 is a mathematical model of one such fluid from the many that have been proposed in the literature (5), where $f(\eta, \gamma, \tau)$ may be a mathematical function of the viscosity, η, shear rate, γ, and shear stress, τ.

$$\tau = \tau_o + f(\eta, \gamma, \tau) \tag{3}$$

Yield stress is a critical parameter to providing stability in emulsions and suspensions. In practice, the yield stress of personal care formulations is used to estimate the suspending power of the rheology modifier and to predict the shelf life of a product. Yield stress can be measured or estimated in many ways. The procedure of choice to estimate yield stress must be validated for each formulation by conducting shelf life stability tests. In many instances the yield stress of a formulation can be estimated by linear extrapolation of the shear stress data to zero shear rate (6). For this, it is important to measure shear stress at the lowest shear rates possible under very well-controlled experimental conditions. The yield stress, τ_o, shown in Figure 5, was measured in a rotational viscometer and required shearing the fluid at very low shearing rates through the use of a very sensitive rheometer, under well-controlled experimental conditions.

Synthetic polymeric rheology modifiers may be linear or cross-linked polymers. Cross-linking of polymer molecules provides a network structure that imparts a particular yield stress to an aqueous formulation. The degree of cross-linking impacts the yield stress such that typically polymers with a higher degree of cross-linking exhibit higher yield stress.

e. Viscoelasticity

A third critical rheological parameter in the design of personal care formulations is the viscoelasticity. Viscoelasticity is the balance between the viscous and the elastic components of materials. Elastic materials deform when subjected to an applied force, and the deformation reverses completely and spontaneously when the applied force is removed. Viscous materials deform when subjected to an applied force, and the deformation stops when the applied force is removed. Most practical materials exhibit both viscous and elastic behavior and are referred to as viscoelastic. The balance of the viscous and the elastic components dictates the behavior of the material when a forced is applied (7).

Viscoelasticity can be measured with a rotational viscometer or a rheometer using an oscillatory technique. The relationship between the viscous component and the elastic component is described by the ratio, tan δ, of the storage or elastic modulus (G') and loss or viscous modulus (G"), Equation 4.

$$tan\ \delta = \frac{G''}{G'} \tag{4}$$

When *tan* δ < 1, the elastic modulus is dominant. When *tan* δ > 1, the viscous modulus is dominant. A model strain sweep of a viscoelastic material is shown in Figure 3.

Figure 3. Model strain sweep of a viscoelastic material. G' is the storage modulus and G" is the loss modulus.

4.2.2.3 SYNTHETIC POLYMERIC RHEOLOGY MODIFIERS

There are a variety of polymeric rheology modifiers available to the personal care industry, including both synthetic and natural polymers. Natural polymeric rheology modifiers derived from polysaccharides are discussed in more detail by

Laba (8). Synthetic rheology modifiers are produced by polymerization of various types of monomers. Among the synthetic polymers, those prepared by free radical–initiated polymerization of vinyl, acrylate, or methacrylate and acrylamide or methacrylamide monomers constitute an important class of commercially available materials (9, 10). Depending on the monomers used, these polymers may be anionically or cationically charged; the anionic polymers being the most common synthetic rheology modifiers. The anionic charge in these polymers is typically provided by carboxylic and sulfonic moieties existing along the backbone of the polymers. Furthermore, and for convenience, these polymers are classified based on the form they are supplied as powder, liquids, emulsions (oil in water), and invert (water in oil) emulsions. In this chapter we will review the most commonly used synthetic polymeric rheology modifiers in personal care formulation: modified sulfonic acid–based polymers, polyacrylic acid–based polymers, or alkali-swellable emulsion type polymers.

a. Modified Sulfonic Acid (AMPS®) Polymers

Polymeric rheology modifiers based on modified sulfonic acid chemistry are often referred to as AMPS® polymers. The chemical structure of AMPS® is acrylamido-2,2-dimethyl propane sulfonic acid, and the AMPS® monomer is present in all AMPS® polymers designed for rheology modification. Different co-monomer structures are combined with AMPS® to provide a range of polymeric rheology modifiers. All of these polymers are chemically cross-linked, which provides, in part, their thickening ability. The range of different co-monomers leads to polymers that have different effects on many attributes provided by the polymer in formulations. These include, for example, electrolyte compatibility, thickening effects, and skinfeel sensorics.

Ammonium acryloyldimethyltaurate/VP copolymer (Aristoflex® AVC)[19], Figure 4, is one of the most commonly used AMPS®-based polymeric rheology modifiers. The co-monomer in Aristoflex® AVC is vinylpyrrolidone (VP).

Figure 4. Ammonium acryloyldimethyltaurate/VP (Aristoflex® AVC) copolymer chemical structure.

Ammonium acryloyldimethyltaurate/beheneth-25 methacrylate crosspolymer (Aristoflex® HMB), Figure 5, is a hydrophobically modified polymeric rheology modifier, due to the beheneth-25 methacrylate co-monomer. The addition of

hydrophobic groups to the AMPS® polymer chain provides a rheology modifier with an increased affinity for hydrophobic formulation components, such as oils and silicones. These polymers act as thickeners due to the cross-linking present in all Aristoflex polymers as well as efficient polymeric emulsifiers that can stabilize high oil loads without the need for traditional emulsifiers.

Figure 5. Ammonium acryloyldimethyltaurate/beheneth-25 methacrylate crosspolymer (Aristoflex® HMB) chemical structure.

There are distinct rheological differences between Aristoflex AVC and Aristoflex HMB due to the differences between the vinylpyrrolidone and beheneth-25 methacrylate co-monomers used in polymerization. Within the class of AMPS® polymers, a wide range of final chemistries is available based on selected co-monomers for use with the AMPS® monomer. Variations in the co-monomer structure and the cross-linking of the polymer chains significantly affect the thickening and yield properties of AMPS®-based polymers.

b. AMPS® Polymers Rheological Properties

AMPS®-based polymers have a permanent negative charge due to the sulfonic acid moieties in the AMPS® co-monomer, independent of pH. These polymers have a high degree of cross-linking so that although the charged sulfonic acid moieties repel each other, a permanent network structure exists. The negatively charged sulfonic acid moieties interact strongly with polar solvents, such as water, and the solvent swells the network structure to produce a larger hydrodynamic volume and thus thickening of the solvent-based formulation. This interaction of solvent and polymer is presented schematically in Figure 6.

The co-monomer structure affects the strength of the polymer-solvent interactions as well as the degree of swelling of the network structure. Aristoflex polymers with higher charge density or more polar moieties will swell more quickly and reach a greater overall viscosity. As with all polymeric rheology modifiers,

an increased polymer molecular weight results in increased thickening at lower polymer concentrations. The viscosity profile varies depending on these properties as shown in Figure 7.

Figure 6. Swelling of network structure of AMPS®-based rheology modifiers.

Figure 7. Viscosity as a function of polymer concentration for AMPS®-based polymeric rheology modifiers Aristoflex® AVC and Aristoflex® Velvet, AMPS®-based terpolymer. Brookfield Viscometer Model RVDV-1, 20°C, 20rpm.

Aristoflex polymers with high degrees of cross-linking have a more elastic network, resulting in higher yield values and more elastic character. The *tan* δ as a function of polymer concentration for Aristoflex® HMB is shown in Figure 8 (11).

Figure 8. Elastic modulus, G', as a function of frequency for 0.5%, 0.7%, and 1.0% Aristoflex® HMB in DI water. Values of *tan* δ are at $1s^{-1}$.

The yield point of Aristoflex® HMB in DI water is 0.3% (11). At all Aristoflex HMB concentrations above the yield stress, *tan* δ < 1 indicates a dominant elastic component. An increase in the concentration of Aristoflex HMB provided an increase in the elastic component of the gel.

c. Polyacrylic Acid Polymers

An important class of anionic acrylate-based rheology modifiers is based on high-molecular-weight cross-linked polyacrylic acid technology. These polymers are commonly known by their INCI names carbomer and acrylates/C10-30 alkyl acrylate crosspolymer. They are used extensively in a variety of aqueous formulations including personal care, home care, pharmaceutical, and other industrial applications. Some examples are given on Table 1.

Table 1: Examples of Formulated Products with Carbomer and Acrylates/ C10-30 Alkyl Acrylate Crosspolymer

Body Care: Bath Gel, Body Wash, and Creams	Sun/After Sun: Tanning Lotions and Soothing Creams	Eye Color Cosmetics: Eye Shadow, Mascara
Face/Neck Care: Creams and Soaps	Foot Care Creams	Hair Conditioner
Shampoo	Hair Treatments: Hot Oils, Cold Waves, Straighteners	Lip Color Cosmetics: Lip Color, Lip Pencil, Rouge, Creams
Hand/Nail Care	Shower Products	Eye Color Cosmetics: Eye Lash, Eye Shadow, Eye Liner
Hair Styling: Gels and Pomades	Women's Fragrances	Shaving Preparations: Foam and Gel
Eye Care Creams	Face Color Cosmetics, Makeup	Women's and Men's Fragrances
Liquid Soap and Detergents	Foundations, Fluid Illuminators, Glitter	Bath Additives
Sun Care: Sunscreen Lotions, Creams and Spray	Face Color Cosmetics - Primers	Depilatory Products
Hair Colorants and Hair Dyes	Body Color Cosmetics	Eye Cleansers

The chemical structure of these polymers is shown in Figure 9.

A B

Figure 9. A) Chemical structure of carbomer and **B**) Acrylates/C10-30 alkyl acrylate crosspolymer, R = C_{10}-C_{30} Alkyl.

d. Polyacrylic Acid Polymers Rheological Properties

The viscosifying properties of polyacrylic acid polymers are strongly dependent on molecular weight, degree of cross-linking, the level of hydrophobic alkyl groups in their backbone, the pH and concentration in the formulation. Additionally, the charge character of acrylates-based polymers is typically imparted by the carboxylic acid functionality and is therefore dependent upon pH of the formulation. At low pH, below about pH 3.5, the carboxylic acid groups on the polymer are protonated and thus have no effective charge. This results in decreased polymer-solvent interactions, which provide a collapsed polymer conformation and low hydrodynamic volume. Thus, at pH less than about 3.5, acrylates-based polymers provide relatively low viscosity increase to water-based formulations. However, as the pH is increased above pH 3.5, neutralization occurs and the carboxylic acid group is deprotonated and exhibits a negative charge. Deprotonated carboxylic acid functionalities interact strongly with polar solvents, such as water. In addition, neighboring carboxylic acid moieties on the same polymer molecule repel each other due to charge interactions. This results in an increased hydrodynamic diameter and significant thickening of the formulation as illustrated in Figure 10.

Figure 10. Neutralization with a base creates negative charges along the backbone of the polymer. These repulsive forces uncoil the polymer into an extended, highly swollen structure.

In practice, and as result of this process, the viscosity of aqueous solutions of polyacrylic acid polymers exhibits a significant increase at pH of 3.5 to 5.0, reaching a maximum plateau at pH 5.5 to 10 and then decreasing at pH above 10 as shown in Figure 11 (12).

Figure 11. Neutralization scheme for polyacrylic acid–based rheology modifiers. Carbopol® Ultrez 10 and Carbopol® ETD 2050 polymers are cross-linked carbomers and Carbopol EDT 2020 is a cross-linked, hydrophobically modified carbomer.[20] All polymers at 0.5 wt%, neutralized with NaOH. Viscosity measured at 25°C with Brookfield viscometer Model RVT with spindle rotating at 20 rpm.

The degree of cross-linking of polymeric rheology modifiers is a critical parameter affecting the viscosification properties. Figures 10 and 11 show the viscosity values for aqueous solutions of carbomer polymers synthesized with relatively low degrees of cross-linking, Figure 12, and higher degrees of cross-linking, Figure 13, at various concentrations of the polymer. These data show that in general, the higher the degree of cross-linking of the polymer, the greater the viscosity it can provide to a formulation.

Figure 12. Relative viscosity of aqueous solutions of carbomer polymers with relatively low degree of cross-linking. pH ~ 7.5 adjusted with NaOH; viscosity measured at 25°C in a Brookfield RVT viscometer with spindle rotating at 20 rpm.

Figure 13. Relative viscosity of aqueous solutions of carbomer polymers with relatively high degree of cross-linking. pH ~ 7.5 adjusted with NaOH; viscosity measured at 25°C in a Brookfield RVT rheometer with spindle rotating at 20 rpm.

The degree of polymer cross-linking also affects the yield stress values. Figure 14 shows the Brookfield Yield Stress Values (6) for aqueous solutions of carbomer polymers synthesized with various degrees of cross-linking at various concentrations of the polymer. The equivalent degree of cross-linking for this set of carbomers increases in the following order:

CBP934 > CBP980 > CBP940 > Ultrez 10 > Ultrez 21 > ETD 2020> ETD 2050 > CBP941[21]

Typically, yield stress values increase with an increased degree of cross-linking of the polymer molecules. However, parameters such as polymer molecular weight also affect the yield stress. For example, CBP934 has the highest degree of cross-linking of the series evaluated, but the polymer molecular weight is low. The yield stress is less than that of polymers with lesser degrees of cross-linking due to the influence of the molecular weight.

Figure 14. Brookfield yield stress values for aqueous solutions of carbomer polymers with various degrees of cross-linking as a function of polymer concentration at pH 7.0. Yield stress values were measured using the Brookfield method at 25°C. All polymer solutions were neutralized with NaOH.

e. Alkali Swellable Emulsion Polymers - ASE/HASE Polymers

Alkali Swellable Emulsions (ASE) and Hydrophobically Modified Alkali Swellable Emulsions (HASE) polymers constitute a group of rheology modifiers widely used in the formulation of personal and home care products. These are also carboxy-functional polymers prepared by the free radical polymerization of

ethylenically unsaturated monomers. They are polymerized in aqueous emulsion (latex) of water-insoluble monomers that are emulsified in water by the use of suitable surfactants or suspending agents. A detailed account of the synthesis of these materials and properties as rheology modifiers was published by Jenkins (13, 14) and Shay (15). Liquid emulsion rheology modifiers are supplied as low-viscosity fluids that can be easily added and dispersed in aqueous formulations without the need of special mixing equipment or extended mixing times. Once incorporated into the formulation they are neutralized with base to allow them to swell and thicken the product. These polymers have been used extensively in a number of personal care products such as hair styling gels and pomades, shower products, shampoo, face/neck care, body care creams, liquid body soaps, hair colorants, hair treatments, shaving gels, liquid hand soap, etc.

As shown in Figure 15, the backbone of both alkali-swellable emulsion polymers (ASE) and hydrophobically modified alkali-swellable emulsion polymers (HASE) consist primarily of methacrylic acid and an assortment of acrylate or methacrylate alkyl ester monomers. In some cases relatively small amounts of other carboxylated monomers such as acrylic acid, fumaric, and itaconic acids are used. Additionally, HASE polymers typically contain 0–3 mol % of hydrophobic groups attached to the polymer backbone by means of a medium-length polyethylene oxide, hydrophilic segment, or spacer.

ASE

HASE

Figure 15. Chemical structure of alkali-swellable emulsion polymers where - m, n, and p represent the moles of monomeric (e.g., acrylic or methacrylic acid, alkyl (R) acrylate or methacrylate esters) units in the backbone of the polymer and q is the moles of the ethylene oxide spacer connecting R″ to the backbone. R″ may be a cetyl, stearyl, or behenyl fatty acid alkyl group, i.e., the associative group of the polymer. Y may be H of CH3.

f. Rheological Properties of ASE/HASE Polymers

Viscosification using alkali-swellable emulsion polymers (ASE) and hydrophobically modified alkali-swellable emulsion polymers (HASE) is dependent upon pH of the formulation. In aqueous solution and in their unneutralized state the polymer chains are hydrophobic; hence they exist in the form of coiled emulsion droplets. Upon neutralization of the carboxylic groups in the backbone of the polymers with a base such as sodium hydroxide, the emulsion droplets swell and the polymer coils expand, resulting in significant viscosity increase. This process is schematically illustrated in Figure 16.

Figure 16. Schematic representation of the polymer swelling process of ASE or HASE rheology modifier as a function of neutralization with base.

The viscosifying properties of ASE or HASE polymer rheology modifiers are strongly dependent on a number of factors including molecular weight, degree of cross-linking, the number of associative groups in the backbone, the pH and temperature of the solution, the concentration of thickener in the formulation, interaction with surfactants, oils, electrolyte, other ingredients in the formulation, etc.

The molecular structure of the polymers has a significant effect on the performance of the polymers as rheology modifiers. While the composition of the polymer backbone in ASE and HASE polymers may be for the most part equivalent, the addition of small quantities of an associative group in HASE-type polymers has a significant effect on the rheological properties of the polymer. Figure 17 compares the viscosity of an ASE polymer (Carbopol® Aqua SF-1, SF1 polymer; INCI name Acrylates Copolymer) with a HASE polymer (NovethixTM L-10 Copolymer, NL10; INCI name Acrylates/Beheneth-25 Methacrylate Copolymer)[22] under equivalent test conditions. The backbone composition of both rheology

modifiers is approximately equivalent. NL10, a HASE-type polymer, also includes small amounts of an associative thickener monomer; see Figure 17. As result, it can be easily seen that L10 exhibits large viscosity gains as the concentration of the polymer increases. Conversely the viscosity of SF1 exhibits a linear dependency with its concentration. The hyperbolic viscosity gains in NL10 are due to structuring via the associative groups, which tend to form stable physical intermolecular bridges.

Figure 17. Viscosity dependency of ASE (Carbopol® Aqua SF-1 polymer) and HASE (Novethix™ L-10 Copolymer) polymer mucilage in water vs. concentration. RVT Brookfield viscometer rotating at 20 rpm, 20°C, pH 7.0 to 7.5 adjusted with NaOH.

4.2.2.4 RHEOLOGICAL PROPERTIES AND THE CONSUMER EXPERIENCE

The rheological profile of personal care formulations should be a primary consideration in developing consumer-preferred products. The interactions of polymers in simple solvents as described previously, such as water, are well understood. However, in personal care formulations the interaction of polymeric rheology modifiers

with other ingredients adds a level of complexity and impacts their rheological behavior. Some considerations in these practical applications include interaction with charged species, such as emulsifiers, surfactants, or electrolytes, and combination with other functional ingredients, such as preservatives. It is also advantageous to accommodate specially required rheological profiles that connote claimed or desired consumer benefits.

Polymeric rheology modifiers have the ability to impact multiple flow behavior aspects of personal care products. Examples of these include: formulation stability, the appearance of the product, the application of the product, and the sensory feel of the product during and after use. Formulation stability is of primary importance to all formulators, and the rheology of the product contributes significantly to the stability, especially the yield stress imparted by the rheology modifier. Consumers experience a product in multiple stages during use, and all of these experiences lead them to their final decision on the product benefits. The sensory profile of a product is a tool that formulators use to help deliver the claimed benefits to the consumer, and the rheological character of a formulation influences many stages of the overall sensory experience. The appearance of the product at rest in the package is the first visual cue to the consumer of the benefit of the product. The viscosity and the viscoelasticity of the product are important during this initial experience. The application of a product to skin is an experience that can be controlled by the rheology of the product, in particular the shear behavior. The feel of the product on skin during use and after use are also impacted by the polymeric rheology modifier, particularly in the elasticity and shear behavior and also by the chemistry of the polymer molecule. Depending on the desired benefits and claims of the product, polymeric rheology modifiers can be chosen to help connote to the consumer the desired product benefits.

In the field of personal care there are many different types of formulations. Each of these has basic rheological needs, as outlined in Table 2.

Table 2: Basic rheological needs of personal care formulations

Formulation Type	Components					Rheological Needs
	Water	Solvent	Surfactant / Emulsifier	Oil	Poly-mer	
Gels	X	(X)			X	Viscosification; shear thinning
Optical effects cleansers	X		X		X	Low yield stress; pourable

Conventional O/W emulsions	X		X	X	X	Yield stress; shear thinning; viscoelasticity
Crème gels	X			X	X	Yield stress; shear thinning; viscoelasticity

The components of each type of formulation can offer unique challenges to developing a desirable rheological profile. The same polymer molecule can deliver a different rheological profile depending on the other components present in the formulation. In the following sections we will address these different formulation types in more detail.

4.2.2.5 POLYMERIC RHEOLOGY MODIFIERS IN EMULSION FORMULATIONS

Rheology modifiers are commonly employed in conventional emulsions and in crème gels. Conventional emulsions are formulated with amphiphilic emulsifiers to reduce interfacial tension between the oil droplets and water external phase (O/W emulsions) or water droplets and oil external phase (W/O emulsions). In conventional emulsions, polymeric rheology modifiers impart yield stress for stability, viscoelasticity for visual and feel aesthetics, and shear-thinning properties for application aesthetics. Crème gels are emulsions that do not contain traditional emulsifiers. The polymeric rheology modifier stabilizes the internal phase droplets in the external phase. It also imparts all of the yield stress, viscoelasticity, and shear-thinning properties as in a conventional emulsion.

a. Yield stress for emulsion stability

Polymeric rheology modifiers serve a primary function in emulsion formulations of providing enhanced stability in systems that are inherently thermodynamically unstable. Rheology modifiers provide suspension of emulsion droplets, which decreases the rate of instability due to coalescence or creaming. Suspension properties are imparted by yield stress and an increased yield stress will provide increased stability. The concentration of polymer employed to impart stability must be balanced to provide an adequate yield stress while maintaining a flowable liquid that can be utilized by a consumer.

Loeffler and Miller studied the yield stress as a function of Aristoflex® AVC concentration in both a conventional emulsion formulation and a crème gel formulation, Figure 18 (16).

Figure 18. Yield stress of Aristoflex® AVC as a function of concentration in crème gel and traditional emulsion formulations.

An initial yield stress was observed at 0.3% Aristoflex® AVC in the crème gel formulation. An initial yield stress was observed at 0.375% Aristoflex® AVC in the emulsion formulation. At all Aristoflex® AVC concentrations, the yield stress of the crème gel was significantly higher than that of the conventional emulsion. The interaction of the oil droplets with the polymeric rheology modifiers is different in the crème gel and the conventional emulsion due to the presence of traditional emulsifiers in the latter. Miller and Loeffler studied the yield stress as a function of Aristoflex® HMB concentration as shown in Figure 19 (17).

Figure 19. Yield stress of Aristoflex® HMB as a function of concentration in crème gel and traditional emulsion formulations, and aqueous gels.

A similar trend was observed, where the initial yield stress was observed at a lower Aristoflex® HMB concentration for the crème gel compared to the conventional emulsion, 0.275% and 0.3%, respectively. The yield stress of the crème gel was significantly greater than that of the conventional emulsion at all Aristoflex® HMB concentrations. It was also observed that the yield stress in both a crème gel and a conventional emulsion was significantly greater than in an aqueous gel. Thus, the presence of oils in the formulation increased the overall yield stress.

b. Relationship between rheological properties and the sensory experience

It is important to establish a correlation among the rheological properties of the emulsion with subjective properties that a skin care cream user would experience during rubbing of the emulsion over her skin. There are four primary sensoric properties that can be clearly related to the rheological parameters of an emulsion formulation:

- Speed of breakdown during rub-in
- Spreadability during rub-in
- Cushion
- Skin afterfeel once rub-in is complete

c. Speed of Breakdown

Speed of breakdown during rub-in is governed by the initial interactions between the polymer in the emulsion formulation and skin. The chemistry of synthetic rheology modifiers dictates their interaction with skin and moieties contained within skin, such as NaCl. Both AMPS®-based and polyacrylic acid rheology modifiers rely on their charge character and cross-linked network structure to provide thickening to a formulation. Upon exposure to NaCl, the repulsions between neighboring anionic charges are effectively screened, resulting in a viscosity decrease. Also, NaCl concentration gradients between the skin and the formulation cause a decrease in swelling of highly cross-linked networks in these polymers. Osmosis drives water diffusion out of the swollen polymer network and into the high-salt region of the skin, which results in a collapse of the polymer network. During rub-in, this decrease in viscosity is perceived by consumers as the breakdown of the formulation. A schematic representation of this collapse mechanism is shown in Figure 20 for AMPS®-based rheology modifiers. Variations in co-monomer structure, degree of cross-linking, and other parameters can influence the speed of breakdown during rub-in on skin.

Water hydrates and swells anionic polymer

Osmetic water diffusion due to salt on skin surface; polymer collapses

Figure 20. Collapse of network structure of AMPS®-based rheology modifiers upon contact with skin.

d. Spreadability

Spreadability during rub-in is the ease with which the product is spread over the skin and can be directly related to the shear response of the formulation. The response of an emulsion to shear can connote different product benefits to the consumer, though as a general rule, shear-thinning formulations are ideal for rubbing over the skin. The shear response for a crème gel, a conventional emulsion, and an aqueous gel containing 0.6% Aristoflex® HMB was evaluated by Miller and Loeffler, Figure 21 (17).

Figure 21. Shear-thinning behavior of 0.6% in crème gel and traditional emulsion formulations, and aqueous gels.

All formulation types exhibited shear-thinning behavior. The rate of shear thinning was similar for the crème gel and the conventional emulsion. The aqueous gel rate of shear thinning was similar up to 10 s^{-1}. The viscosity decrease as a function of shear rate was approximately linear for both the crème gel and the conventional emulsion. The crème gel viscosity was greater initially and this increase was maintained over all shear rates.

A conventional emulsion was evaluated for shear response as a function of rheology modifier chemistry. The composition of the emulsion is shown on Table 3. It is important to note that the amount rheology modifier that was added to the emulsion was adjusted to obtain viscosity between 28,000 and 33,000 mPa·s, measured at 25°C using an RVT Brookfield rheometer with spindle rotating at 20rpm. This narrow range of viscosity was selected as representative of the viscosity of a good skin cream.

The flow curves in Figure 22 show that there are practically no differences in the rheograms of the different emulsions throughout the entire shear-rate range. These data indicate that all of the emulsions containing Carbopol® polymers are shear thinning, which makes them ideal for rubbing over the skin.

Figure 22. Steady state flow curves for the emulsion in Table 3 thickened with different Carbopol® polymers. No electrolyte added.

Since the surface of the human skin contains electrolytes such as NaCl, determining the rheological behavior in the presence of NaCl is important. Figure 23 shows the flow curves for the same emulsions to which 0.05 wt% of NaCl was added. The addition of small amounts of electrolyte offers differentiated flow patterns.

Figure 23. Steady state flow curves for the emulsion in Table 3 thickened with different Carbopol® polymers and containing 0.05 wt% of NaCl.

Emulsions formulated with Ultrez 10, 20, and 21 have high electrolyte tolerance and exhibit relatively low viscosity drop in the presence of salt. As will be explained below, these polymers give emulsions having varying degrees of cushion and pick-up and in general they tend to feel less watery upon rubbing over the skin. On the other hand, emulsions formulated with CBP940 or CBP980 polymers are more sensitive to salt; the viscosity of the emulsion falls to relatively lower values in the presence of electrolyte. These emulsions tend to break down faster and feel light and more watery.

Table 3: Water in Oil Screening Emulsion

Part	Trade Name	INCI	% weight					
A	Carbopol® Ultrez 10 polymer	Carbomer	0.40					
	Carbopol® Ultrez 20 polymer	Acrylates C10-C30 Alkyl Acrylate Crosspolymer		0.75				
	Carbopol® Ultrez 21 polymer	Acrylates C10-C30 Alkyl Acrylate Crosspolymer			0.38			
	Carbopol® 940 polymer	Carbomer				0.30		
	Carbopol® 980 polymer	Carbomer					0.40	
	Carbopol® 934 polymer	Carbomer						0.50
	Deionized Water		Q.S. to 100					
B	C1215 Alkyl Benzoate	C12-15 Alkyl Benzoate	10.00	10.00	10.00	10.00	10.00	10.00
	Cremophor® A6**	Ceteareth-6 and Stearyl Alcohol	1.80	1.80	1.80	1.80	1.80	1.80
	Cremophor®A25**	Ceteareth-25	1.20	1.20	1.20	1.20	1.20	1.20
C	TEA (99%)	Triethanolamine	QS to pH = 6.0 – 6.5					
D	Glydant® Plus Liquid***	DMDM Hydantoin (&) Iodopropynyl Butylcarbamate	0.40	0.40	0.40	0.40	0.40	0.40

** Cremophor is a registered trademark of BASF; *** Glydant is a registered trademark of Lonza.

e. Pick-Up and Cushion

Viscoelasticity is an important rheological parameter to evaluate when considering the "at rest" aesthetics, pick-up, and cushion of emulsions. Viscoelasticity relates to the "stability of shape" of the formula as well as the cushion during application. Stability of shape dictates how the formulation will appear at rest in the package. The viscous component of the formulation, G", relates to the flowability of the formulation. The elastic component of the formulation, G', relates to the stability of shape of the formulation. The relationship between the viscous component and the elastic component is represented by tan δ as discussed previously in Equation 4. Formulations with tan δ > 1 have a larger viscous component and are more flowable. Formulations with tan δ < 1 have a larger elastic component and have more stability of shape. The viscoelasticity of Aristoflex® AVC was evaluated by Loeffler and Miller (Figure 24) (16).

Figure 24. Frequency dependence of storage and loss moduli for emulsion with 1% Aristoflex® AVC.

The Aristoflex® AVC provides high elasticity in the formulation. This will result in a formulation that appears stiff and solid. The pick-up and cushion of an emulsion formulation are also related to the elastic component. High elastic component imparts poor pick-up, and it may be desirable to include formulation

components that lower the elastic component or increase the viscous component in order to improve the pick-up aesthetic of the formulation. Formulations with greater elasticity will have more cushion and may give the perception of a richer formulation. Formulations with a greater viscous component will have low cushion and may give the perception of lightness or freshness. A balance of the viscous and elastic components is critical to connote the desired benefits of a formulation to the consumer.

As with the shear-thinning behavior, the viscoelasticity of an emulsion containing a polymeric rheology modifier can be affected by the presence of NaCl. Dynamic or oscillatory rheological studies indicate that conventional emulsions present various degrees of elasticity and yield stress depending on the chemistry of the rheology modifier. Figure 25 shows the elastic modulus, G', for the emulsions on Table 3 with and without electrolyte added to the emulsion for different carbomer polymers.

Figure 25. Effect of electrolyte on the Elastic Modulus, G', and Yield Stress rheological properties of the skin care emulsion in Table 3, prepared with various carbomer polymers.

The elasticity of the emulsions decreases considerably in the presence of electrolyte. For instance, G' for the emulsion with Carbopol® 940 polymer decreases from about 600 Pa to about 150 Pa upon addition of 0.05% NaCl. This seems to indicate that the emulsion in contact with skin would turn rapidly much less elastic and possibly spread out much more easily. Conversely the elasticity of the emulsion prepared with Ultrez 20 polymer remains practically unaffected by the electrolyte. Interestingly, while the elastic modulus for most polymers is lower in the presence of electrolyte, the plateau region does not seem to be significantly affected and shows Yield Stress (inflexion region) within the same range.

f. Skin Afterfeel

As discussed in reference to the breakdown of polymeric rheology modifiers upon contact with skin, the chemistry of these polymers affects their interaction with skin, both on initial contact and after application. The skin surface has a chemical makeup that interacts with formulation ingredients through hydrogen bonding and hydrophobic associations. These skin–ingredient interactions and associations impart a skinfeel to each formulation. Thus the chemistry of the rheology modifier has an impact on the skinfeel during use and the skin afterfeel once the product is rubbed in. Molecules with large hydrophobic groups interact with the skin to give a substantive sensation. Consumers generally acknowledge these materials to have high moisturization or conditioning benefits. Synthetic polymeric rheology modifiers can be designed so that the comonomers contribute to the salt tolerance and the chemical interactions with skin in order to provide different sensory profiles to the consumer that connote a range of benefits from the formulation. For example, Aristoflex® AVC has a low salt tolerance and minimal chemical interactions with the skin due to the vinylpyrrolidone co-monomer. The resulting sensory profile is a rapid breakdown upon application to skin and a neutral feeling on the skin during and after application. Aristoflex® HMB has a higher salt tolerance. The hydrophobic beheneth-25 pendant chain interacts with the skin through hydrogen bonding, and the long carbon chain provides a substantive feeling to the skin. The resulting sensory profile is a slower breakdown and a moisturized feeling on the skin during and after application.

g. Correlation of Rheological Measurements and Consumer Perception in Emulsion Formulations

When developing new formulations to delight the consumer's senses, the combination of technical measurement science and consumer sensory characterization is critical. The rheological technical measurements described throughout this discussion can be used in conjunction with consumer sensory testing.

Quantitative Descriptive Analysis, QDA, for sensory characterization of consumer products (18) was used for the subjective evaluation of emulsion attributes, during and after rubbing the emulsion over the skin, for five subjective parameters defined as:

- Cushion (thickness): is the amount of material being felt between the fingertips and the skin while making the three rotations with the respective finger. 1 is designated as the thinnest, 6 as the thickest.
- Quickness of break: defined as how fast the emulsion structure breaks down. This is typically characterized by a cooling or wet sensation. 1 is

designated as the slowest to break (more rubs), 6 as the fastest to break (less rubs).

- Wetness: the amount of perceived wetness during the three rotations (when structure is most likely starting to break or has been broken) with the fingers. 1 is designated as the least wet, 6 as the most wet.
- Spreadability: the ease with which the product is spread over the skin. 1 is designated as the most difficult to spread, 6 as the easiest to spread.
- Afterfeel intensity (residue): is the perceived amount of material left covering the skin. 1 is designated as the least residue, 6 as the most residue left on skin.

The results of the sensory characterization study are shown in Figure 26.

Figure 26. Quantitative Descriptive Analysis, QDA, for sensory characterization of the skin care emulsions in Table 3, as a function of the type of carbomer polymer.

The effect of differing carbomer polymer chemistries on the overall sensory attributes of the emulsion is significant. For instance, the data indicate that the

emulsion prepared with Carbopol® Ultrez 20 polymer gives the most cushion, it feels less wet and it does not break down readily, it offers more resistance to spreading, and it is perceived to leave residue after application. On the other hand, the emulsion prepared with Ultrez 10 is perceived as wet, it spreads and breaks easily, and leaves less residue. In sum, the sensorial characteristics of the polymers can be compared and ranked as follows:

1) Cushion: Ultrez 20> Ultrez 21=CBP934=CBP980>CBP940> Ultrez 10
2) Quickness of break: Ultrez 10>CBP940> CBP934>CBP980= Ultrez 21> Ultrez 20
3) Wetness: Ultrez 10>CBP934= Ultrez 21>CBP980>CBP940>Ultrez 20
4) Spreadability: Ultrez 10>CBP940>CBP980=CBP934= Ultrez 21> Ultrez 20
5) Afterfeel intensity: Ultrez 20> Ultrez 21>CBP980=CBP940>CBP934> Ultrez 10

The sensory attributes of the emulsions correlated with many of their rheological properties. For instance, in general the perception of wetness, quickness of break, and spreadability seem to be attributes of the emulsions prepared with carbomer polymers with the high sensitivity to electrolyte such as Ultrez 10, CBP940, CBP980, and CBP934. The emulsions formed with these polymers under the presence of NaCl have relatively low viscosity and low elastic modulus. On the other hand, the emulsions formed with polymers such as Ultrez 20 and 21 gave more cushion, were more difficult to spread, and were perceived as less wet. These polymers gave emulsions with high modulus and their viscosity was less sensitive to electrolytes.

4.2.2.6 POLYMERIC RHEOLOGY MODIFIERS IN HYDROALCOHOLIC FORMULATIONS

Many consumer products utilize a hydroalcoholic solvent system. Such products include alcohol hand sanitizers, sunscreen gels, and hair-styling products. The interactions of polymeric rheology modifiers and the solvent system are critical for providing the rheological needs of such systems: viscosification and shear-thinning flow behavior.

The viscosity of a hydrophobically modified AMPS®-based polymer, Aristoflex® HMB, in various organic solvent/water systems was evaluated my Morschhauser et al. (Figure 27). The viscosity as a function of organic solvent concentration was studied for three organic solvents: 1,2 propylene glycol, ethanol, and acetone (11).

Figure 27. Viscosity of 1% Aristoflex® HMB solution in water/solvent mixtures. Brookfield viscometer, 20°C.

The viscosity of 1% Aristoflex® HMB in deionized water was approximately 50,000 mPa·s. The addition of 1,2 propylene glycol did not significantly affect the viscosity up to a 70:30 ratio of 1,2 propylene glycol:water. The combination of ethanol and water showed an initial decrease in viscosity at a 20:80 ratio of ethanol:water. The viscosity decrease with addition of ethanol was approximately linear up to 60% ethanol where a more significant viscosity decrease was observed. Acetone had the most significant impact on viscosity with an initial decrease in viscosity with addition of 10% acetone and a continued decrease in viscosity with an increase in acetone concentration. A significant decrease in viscosity from about 15,000 mPa·s to about 2,000 mPa·s was observed between 60% and 70% acetone, respectively. These differences in organic solvent effect on viscosity can be directly attributed to the interaction of each solvent system with the Aristoflex® HMB polymer.

Of the above water/solvent systems, the ethanol/water solvent system is most commonly used in personal care formulations. The effect of polymer concentration on the viscosity of these systems was evaluated in 62% ethanol/water gels (Figure 28).

Figure 28. Viscosity of Aristoflex® HMB solution in 62% ethanol/water mixture.

At Aristoflex® HMB concentrations below about 1.25%, the viscosity of the gel is less than about 30,000 cP. Above about 1.25% polymer, the viscosity of the gel increases significantly, reaching about 650,000 cP at 2.0% Aristoflex® HMB. This is typical behavior of polymers in solvent. Below a critical polymer concentration, known as the critical overlap concentration (c*), polymer molecules do not interact with one another in solution. As polymer concentration increases, a concentration is reached where polymer molecules begin to overlap and then entangle, which exponentially increases the viscosity. The concentration where polymers begin to overlap is c*. The value of c* for Aristoflex® HMB in 62% ethanol/water is approximately 1.25% polymer.

Generation of a graph of viscosity as a function of polymer concentration, in the chosen solvent system, can aid in determination of the appropriate polymer concentration to provide the desired product viscosity. It can also aid the formulator in understanding the sensitivity of the formulation to small deviations in polymer concentration that may be encountered during manufacturing. It is critical if attempting this technique, that the formulator consider other components of the formulation that may affect the viscosity and polymer-solvent interactions. The viscosity as a function of Aristoflex® AVC polymer concentration in a full formulation, containing 62% ethanol/water plus silicones and emollients, is shown in Figure 29.

Figure 29. Viscosity of Aristoflex® AVC containing formulation in 62% ethanol/water mixture. Formulation also contains silicones and other emollients.

The viscosity increases exponentially after a critical polymer concentration of about 0.8% Aristoflex® AVC. The viscosity increase above the c*, 0.8% Aristoflex® AVC, is more gradual in the full formulation compared to a simple gel due to the complex environment with the inclusion of silicones and emollients.

In the case of thickening of hydroalcoholic solutions with carbomer polymers, the critical factor is to be able to select the correct base neutralizer for the amount of alcohol that is to be gelled. Since Carbopol® polymers contain unneutralized carboxylic acid groups in their backbone, these could be neutralized with practically any base to affect the solubility or degree of swelling of the polymer in hydroalcoholic solutions. If the wrong neutralizer is used, the salt of the Carbopol® polymer will precipitate out because it is no longer soluble in either the water-ethanol or water-isopropanol blend. Table 4 gives recommended neutralizers for various alcohol levels.

Table 4: Recommended Neutralizers for Hydroalcoholic Systems

Alcohol wt%	Neutralizer
20%	Sodium Hydroxide
30%	Potassium Hydroxide
60%	Triethanolamine
60%	Tromethamine
80%	Aminomethyl Propanol
90%	Tetrahydro-xypropyl Ethylenediamine

Alcohol wt%	Neutralizer
90%	Diisopropanolamine
90%	Triisopropanolamine
>90%	PEG-15 Cocamine

This indicates that for hand sanitizers containing 60% to 90% parts of ethanol or isopropanol, highly viscous and clear compositions can be easily obtained by using 1 wt% of a carbomer polymer using triisopropanolamine as the neutralizer to achieve pH 7.5.

4.2.2.7 POLYMERIC RHEOLOGY MODIFIERS IN OPTICAL EFFECTS CLEANSING FORMULATIONS

The rheological needs of surfactant-based systems are unique compared with organic solvent systems or emulsion systems. Consumer preference for a cleansing product is typically for Newtonian flow behavior so that the product pours easily from the bottle and is evenly distributed on the skin or hair surface before dilution and lathering can begin. Newtonian flow is achieved in surfactant systems by micellar thickening mechanisms, such as high surfactant concentration or salt thickening. Viscosifying polymers, such as PEG-150 distearate, can also provide Newtonian rheology in surfactant systems. These mechanisms for thickening and providing Newtonian flow cannot be used in optical effects cleansers where a yield stress is required to suspend particles, beads, or air bubbles. In these systems it is critical to provide a yield stress for suspension stability while maintaining a low viscosity so that the product is pourable. This is achieved using polymeric rheology modifiers whose function in the formulation is to provide this critical yield stress for suspension.

Miller and Loeffler studied the suspension capability and the viscosity of a model surfactant system comparing a polymeric rheology modifier (Aristoflex® HMB) and sodium chloride, Figure 30 (17).

Both the Aristoflex® HMB and NaCl increased the viscosity of the surfactant system. Aristoflex® HMB exhibited a c* of about 1.2% AMPS®-Beheneth-25, above which the viscosity was in a typical cleansing product viscosity range. This same viscosity range was achieved with approximately 4–5% NaCl. Particles with an average diameter of 850 μm and density of 0.03 g/cm^3 were suspended in each surfactant formulation. The stability of the particles was evaluated visually and is labeled as either “creaming” (unstable) or “stable” in Figure 19. It is evident from Figure 19 that viscosity alone cannot provide stable suspension of these particles. The Aristoflex® HMB imparted a low yield stress in addition to viscosity, which provides suspension stability, Figure 31 (17).

Figure 30. Viscosity and stability to creaming of Aristoflex® HMB and NaCl containing shower gel formulations.

The critical polymer concentration, c_τ, required for suspension stability was determined to be approximately 0.3% Aristoflex® HMB, using a visual stability method. Therefore, the yield stress will be high enough to suspend the particles at polymer concentrations above 0.3% Aristoflex® HMB. However, 1.0–1.5% Aristoflex® HMB was required to provide a consumer-desired viscosity (Figure 19). Stable suspension of particles throughout the formulation is necessary to connote the consumer benefit and to make a visually appealing product.

ASE and HASE-type polymers can also be designed to provide extended shelf life to optical effects cleansing formulations. Carbopol® Aqua SF-1 has a relatively high yield stress under most conditions of formulation. To illustrate the suspending power of the polymer in a surfactant composition such as a facial scrub, a cocktail of water-insoluble particles was added to the formulation to observe particle sedimentation or creaming. Table 5 shows the compositions of the mixture of particles, which include a range of particles with various diameters and specific gravity that are commonly used in the formulation of personal care products.

Figure 31. Yield stress and stability to creaming of Aristoflex® HMB in shower gel formulation.

Table 5: Composition of the mixture of particles used to test the stabilizing effect of ASE and HASE polymers

Type	**INCI Name**	**Specific Gravity**	**Particle Size µm**
Glitters	Synthetic Fluorphlogopite and Titanium Dioxide and Iron Oxides	2.9–3.2	20–95
	Synthetic Fluorphlogopite and Iron Oxides	2.9–3.2	95–730
Polyethylene	Polyethylene	0.92	0–315
	Polyethylene	0.92	400–800
Unispheres®[23]	Mannitol and Cellulose and Hydroxypropyl Methylcellulose and CI 77289 and Tocopheryl Acetate	1.1–1.2	1000–1500
	Mannitol and Cellulose and Hydroxypropyl Methylcellulose and CI 77492 and Tocopheryl Acetate	1.1–1.2	500–900
Jojoba Beads	Jojoba Esters and CI73360 and Talc	0.9	250–600
Floraspheres®[24]	Jojoba Esters	0.9	1200–1700
Natural scrubs	NA	>1	NA

The mixture of particles was added at about 1% wt. to a model facial wash surfactant composition with a target viscosity (Brookfield RVT @ 20 rpm, 25°C) of 8,200 cP and pH 5.4.

To assess the stabilizing ability of the polymer, samples of the model facial wash surfactant composition were kept at 45°C for one week. The suspending ability of the polymer was assessed visually by observing the migration or agglomeration of the suspended particles in clear glass containers. The following scores were used to assess the shelf life of the formulation with respect to stabilizing the suspended particles.

Scores for suspension:

4 = good, suspended

3 = fair, slight sedimentation

2 = poor, half sedimentation

1 = very poor, not suspended at all

The results of this test are shown in Figure 32 and Figure 33 where various ratios of copolymers SF1 and NL10 were added to the formulation as rheology modifiers and suspending agents. It can be observed that good suspension is obtained when SF1 is the only suspending agent (ratio = 100/0) added to the composition. At ratios greater than 80/20 of SF1 to NL10, the suspending ability of the system is still good. However, as the concentration of NL10 increases, the suspending power of the system becomes rapidly deficient in spite of the significant gains in Yield Value and viscosity. These data underscore again that measuring the yield stress of a formulation requires careful consideration and interpretation. Furthermore, the methods to measure yield stress require proper validation by conducting shelf-life studies on the formulation in question.

Figure 32. Suspending ability of polymers in a Facial Wash Formulation containing a total amount of 1.8 wt% polymer at various ratios of Carbopol® Aqua SF-1 / Novethix™ L-10 Copolymer. BYV = (η @ 0.5 rpm - η @ 1.0 rpm) / 100, pH = 6.6. All measurements on samples aged for one week at 45°C.

Figure 33. Viscosity and Brookfield Yield Value, BYV, of the Facial Wash Formulation containing a total amount of 1.8 wt% polymer at various ratios of Carbopol® Aqua SF-1 / Novethix™ L-10 copolymer. Data obtained with RVT Brookfield Viscometer rotating at 20 rpm, 20°C, pH 6.6 adjusted with NaOH. All measurements on one week at 45°C aged samples.

CONCLUSION

Rheological properties of all formulation types affect the consumer's perception of the product. The stability of the product is critical to formulators to successfully bring a product to their consumer. The viscosity, shear behavior, yield stress, and viscoelasticity are critical rheological parameters that impact the product's visual aesthetics and in use-sensory properties. Understanding the effect of your rheology modifier on all aspects of the product's function can improve the design of your product and aid in delivering consumer-preferred formulations that connote the desired or claimed product benefits.

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SILICONES IN PERSONAL CARE PRODUCTS: POLYDIMETHYL SILOXANES, ORGANOSILICONE POLYMERS, & COPOLYMERS

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ABSTRACT

Silicone polymers have been known since 1880, however they did not become a major ingredient in the personal care industry until the 1990s. The designation "silicone polymers," as will be seen in this chapter, covers a variety of silicon-based materials that are characterized by molecules or polymers that contain the Si-O-Si bonds. For convenience, and ease of introducing these materials to you the reader, we have initially simplified our speaking about these materials to "silicone polymers." However, one of the goals of this chapter is to distinguish that "silicone polymers" actually represent a whole range of materials ranging from

simple silicon-based molecules (e.g., silanes) to "straight" polydimethyl siloxane polymers (of varying molecular weight to linear and cyclic variations of these; to "organosilicone" copolymers of different chemistries (like "T" configurations or "ABA" type polymers; to cross linked variations of silicon-based materials which vary in the degree of cross-linking to generate yet another class of gelled "silicone polymers" of varying degrees of rheological behavior and applications in the personal care and cosmetic industries. Further, as we delve deeper into this enormous and widely useful class of materials in the cosmetic and personal care industry, one of our intentions is to make sure that the "old" use of the term "silicone copolyol" (from its initial INCI definition), disappears forever since there is no one such material as "silicone copolyol!"

We also address the apparent trend away from "silicones" in light of their reputation as useful, but not "natural"—and how the technology is evolving to deal with this issue without losing the unique properties the materials are famous for. Further, the chapter addresses the potential toxicity question of very low molecular weight cyclic silicones, which provide a unique, noncooling effect on evaporation and alternatives to these cyclic silicones known as cylcomethicone (D4 and D5).

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PREFACE

Silicones have become ubiquitous in our industry and, in fact, there are few product categories that do not have "silicone" present. This fact has been made abundantly clear by the rapid growth in the number of patents related to silicone ingredients and their compositions. We also note their use in formulations for numerous cosmetic and personal care categories and the many synergistic effects obtained with other components.

There are a few key, unusual functional attributes that make silicone a "must have" in a personal care formulation. These include: better surface tension reduction than that obtained with organic surfactants and, at much lower concentrations; as well as the capability of the forming associative films upon dry-down. Further, in view of the presence of the silicone moiety, these materials have expanded emulsification properties beyond those of organic surfactants. They also have foaming ability in both aqueous and nonaqueous environments the capability to disperse pigments, and an ability to generate a desirable, unusual sensory/aesthetic silky feel.

As we have stated above, silicones provide an outstanding feel and unparalleled sensory performance that a formulation would be lacking without the presence of silicone. This chapter addresses the selection process, which is drawn from a host of molecular choices that vary in their structure and stereochemistry.

4.2.3.11 INTRODUCTION

Silicones can drastically change the performance of a cosmetic formulation by the modification of two salient properties: (1) insolubility in both hydrocarbon oils and water, as well as (2) surface and interfacial properties when hydrophilic and hydrophobic moieties are present. While these two properties seem simple enough, a good understanding of the two, and the value they bring to formulations, is key to the proper selection of organosilicone polymers in order to obtain specific desirable properties. In short, silicone polymers should be used in a formulation only when they provide benefits not provided by other classes of polymers.

Today, the global consumer is faced with many often-contradictory desires when selecting a cosmetic or personal care product. The cosmetic industry is driven by the consumer in the sense that if a cosmetic formulation does not feel good on topical application, they will not buy it no matter how well it performs. As we enter the "Green Chemistry" age, consumer demands have evolved and changed towards use of natural products. The desire for formulations that are "green" has become a major focus when formulating a cosmetic formulation. While the definition of green chemistry has been debated and will be addressed later in this chapter, one thing is clear: Consumers are looking for products that are "green," and contain a greater percentage of sustainable raw materials. This push towards green products has some limitations; none bigger than the fact that while the consumer wants a green product, the demand for cost-effective performance still remains. This dichotomy, and schism, makes the proper selection of silicone-based raw materials even more critical. It is commonly accepted that silicone is not green. This leads to the concept that silicone compounds should be incorporated into cosmetic formulations in minimal concentrations. Essentially, formulations utilize silicone to drive performance upwards and not as a main ingredient. This means that judicial selection of the most efficient silicone will be used in formulations, and silicone- based products will be used only when other less costly options are unavailable. Determining the efficiency of silicones in formulations is an absolute requirement, and this chapter provides insights on methodologies to fine-tune formulations and achieve the optimal balance between perception, cost, and performance.

4.2.3.12 SOLUBILITY

It is important to understand that the term "silicone polymer" refers not to one material but to many classes of products. The simplest class is a polymer that contains only dimethylpolysiloxane, and this term generally refers to the term "silicone fluids." Other classes of silicone polymers contain organic groups besides, or in place of the methyl groups and are referred to as organo-functional silicone polymers. One of the unique properties of silicone fluids is the fact that they are insoluble in

both water and oil (Figure 2.0.1). Since oil and water are two of the most common ingredients in cosmetic formulations, how they interact with the silicone polymers is a significant key to formulation performance.

Figure 2.0.1: Solubility

This difference in solubility from both water and hydrocarbon oil has long been an invitation to the chemistry community to rethink the terms "hydrophobic" and "hydrophilic." In the cosmetic and personal care world, in which there are primarily only two immiscible phases, oil and water, the terms hydrophilic and hydrophobic are sufficient. In fact the whole concept of hydrophilic/lipophilic balance (HLB) by Griffin (discussed at http://en.wikipedia.org/wiki/Hydrophilic-lipophilic_balance) has been eminently useful to describe the behavior of "organic" surfactants.

When the silicone moiety is introduced, new terms become necessary. This is due to the fact that a hydrophobic material can be either oil-loving (oleophilic) or silicone-loving (siliphilic).[i] The term "group opposites" has been applied to this concept.[ii] Table 2.0.1 shows the classifications.

Table 2.0.1

Solubility Characteristic Terms	
Hydrophilic (water loving)	Hydrophobic (water hating)
Oleophilic (oil loving)	Oleophobic (oil hating)
Siliphilic (silicone loving)	Siliphobic (silicone hating)

There are several new terms introduced in Table 2.0.1. With these distinctions, materials can now be broken down into more specific categories. A hydrophobic (water-hating) product can be either oleophilic or siliphilic. Oleophobic (oil -hating) materials may be either hydrophilic or siliphilic. Siliphobic (silicone-hating) materials may be either oleophilic or hydrophilic. If a hydrophobic or siliphobic fiber is treated with "silicone" or with a hydrocarbon, the fiber will become oleophobic at the surface. Thus, for application in waterproofing fibers, selection of the proper solubility characteristics is critical.

4.2.3.13 SURFACE TENSION

The other unique and salient characteristic of silicone compounds, either silicone fluids or silicone polymers containing other functional groups (i.e., organo-functional) is the fact that silicone-containing polymers have much lower liquid/air surface tension as compared to hydrocarbon oils and water. Surface tension has been defined as: "the energy required to break through the surface of a substance."[iii] In a bulk solution, the molecules at the air/solution interface behave differently than a molecule in the bulk solution. This leads to superior surface-active properties. An interesting example of this behavior is that of water. Water, at the air/water interface has a high surface tension (72 dynes/cm), leading to a high boiling point (100°C) and the ability to suspend a paper clip gently placed upon its surface! This high boiling point is surprising because of the low molecular weight of water (18 g/mol). The major reason why the surface tension of water is so high is because of hydrogen bonding. When compared to hydrocarbon oils and water, their surface tension is significantly lower than that for silicone. Table 3.0.1 shows typical surface tension values.

Table 3.0.1[iv]

Surface Tension	
Material	**Surface Tension (dynes/cm)**
Mercury	472.0
Water	72.6
Squalane	46.2
Soap Solution (1%)	38.8
Mineral Oil	33.1
Dimethicone (i.e., polydimethyl siloxane)(20cP)	26.6

Surface Tension	
Material	**Surface Tension (dynes/cm)**
Acetone	23.7
Hexamethyldisiloxane	22.7
Ethanol	22.2
Cyclomethicone (cyclic silicone with 4 repeating units of O-Si-O(D4)	20.6
Diethyl ether	17.0

The low surface tension demonstrated by polydimethlysiloxane relates directly to its structure, how the molecules orient at the liquid/air surface, and the intermolecular forces. Silicone fluid is a silicone polymer consisting of repeating units of Si-O [–(O-Si$(CH_3)_2)_n$-]; its structure is shown below (Figure 3.0.1).

Figure 3.0.1: Structure of Silicone Fluid (Polydimethylsiloxane)

Silicone fluid (polydimethylsiloxane) does *not* have strong intermolecular forces, and the only intermolecular forces it possesses are dispersion forces that refer to their superior ability to spread out on surfaces. Furthermore, the silicone polymer has very flexible Si-O bonds in the polymer backbone, and is highly branched due to the methyl groups. This branching affects the way the polymer chains interact with one another. The presence of methyl groups on the polymer prevents chains from packing tightly, thus decreasing surface tension. Figure 3.0.2[ii] shows a computer-generated structure for a silicone fluid. The polymer is known to coil up on itself, much like DNA, and this may be visible, somewhat, in Figure 3.0.2 below.

Figure 3.0.2:[ii] Silicone Surfactants (Organo-Functional Silicones)

Surfactants are a class of compounds commonly used in cosmetic formulations. An extensive discussion of surfactants is presented elsewhere in this text. Surfactants have been defined as: substances that change the properties of a surface.[3] When a surfactant is added to a solution in which it has some solubility, it migrates to the air/liquid interface. This migration causes a disorder in the surface wherein the hydrophobic portion is ejected from the liquid (water), thus decreasing surface tension since the interface now appears hydrophobic. Below is a graphic of how surfactants lower the surface tension of a material.[5] The graphic covers a range of surfactant concentration from below the critical micelle concentration (i.e., not enough to entirely cover the air/liquid surface) to higher levels where the surface is filled and excess surfactant is pushed under the surface, migrate to each other and form "micelles," which are clumps of surfactants with their outside consisting of the surfactants' hydrophilic portion and the inside of the micelle consisting of their hydrophobic portions.

Altering the surface tension of materials provides many of the desirable properties that silicone copolymers bring to cosmetic products as outlined in Table 3.0.2.

Table 3.0.2

Desirable Properties of Silicone Polymers
1. Skinfeel
2. Spread
3. Film Formation
4. Dry feel

Further discussion of critical micelle concentration (CMC) and how silicone affects the surface tension of a bulk solution will appear later in this chapter.

4.2.3.14 SILICONE NOMENCLATURE

Silicone fluids are polymers made up of building blocks or monomers. The word "polymer" can be broken down into "poly" meaning many and "-mer" meaning unit. Typically silicone polymers are is made up of repeating silicone oxygen bond units. These "repeat" units are called monomers and they have to be at least di-functional. When these monomer units react with themselves they can either form a linear polymer chain (silicone fluid) where the more of them that react, the higher the molecular weight (into the millions for silicone rubber), or rings that are coined (cyclics). Cyclics are essentially polymer chains that form when one end group reacts with the other, forming a ring. There are several common building blocks, so named by how many silicon-oxygen bonds they contain. Figure 6.0.1 below contains a list of the most common building blocks used in silicone polymers.[6] The figure also shows a shorthand designation for each structure that is quite useful in describing these complex materials: M, D, T, and Q.

As seen in the table, each building block is named by how many silicon-oxygen bonds it has; "M" units or mono-substituted groups are "chain terminators." They only have one reactive site, so they have to be on the end of the polymer chain, or as a pendant group of the silicone polymer chain. Pendant groups are groups that "hang" off of the main polymer backbone or polymer chain. M units cannot be in the silicone polymer backbone because they only possess one reactive group. Unlike the M unit, the D unit is di-substituted and will be found in the polymer backbone. The T and Q units are used as cross-linkers, linking two or more polymer chains together. Typically, chemists refer to silicone polymers as silicone fluids and identify their structures by their letters and viscosities. An example of this is $MD_{50}M$. The structure of this polymer is shown in Figure 6.0.2.

Building Blocks		
Name	Structure	Numer of Si-O bonds
M Mono-		1
D Di-		2
T Tri-		3
Q Quat-		4

Figure 6.0.1

Structure of $MD_{50}M$

Figure 6.0.2

$MD_{50}M$ is a silicone polymer made up of 50 D units, end-capped with M units. It is synthesized by the reaction of MM with cyclic D_4 or D_5. A detailed discussion of cyclo-silicones and fluids will appear later in this chapter.

It must be clearly understood that the chlorosilane materials made in the Rochow process result in the building blocks that are found in the polymers and for which the nomenclature is derived.

All of the units mentioned so far are useful for synthesizing silicone fluids or nonfunctionalized (e.g., with organic groups, for example) silicone fluids. To functionalize silicone polymers, new building blocks with reactive sites are needed. Figure 6.0.3 outlines some of the common reactive building block sites. These are stereochemical positions created by the basic starting structure where other reactive materials can be attached. One example of this would be a short hydrocarbon chair, or a short chain of polyethylene glycol or, getting more complex, low-molecular-weight copolymers (either random or block) of ethylene oxide and propylene oxide.

Reactive Building Blocks			
Name	Structure	# of Si-O bonds	# of Reactive Sites
M* Mono-		1	1
D* Di-		2	1
T* Tri-		3	1

Figure 6.0.3

It is important to note that there is no Q^* reaction site shown. The Q building blocks have four silicone-oxygen bonds and therefore they cannot have a reactive site bonded to them. However, even these can be reacted with M end blockers to make MQ resins, which also are another type of silicone gels that have found usefulness in cosmetics.

Silicone polymers can contain from one to several reactive sites. They are typically constructed by reacting either M^*M^* with a cyclic, a reactive cyclic with MM, or M^*M^* with a reactive cyclic. Reactive cyclics are typically denoted as D_{5*}.

They follow the same nomenclature as above. If we take the polymer from above (MD_{50}) and add reactive sites, its name gets changed to $MD^{*}_{50}M$. The structure is shown in Figure 6.0.4.

Figure 6.0.4

These reactive sites can be placed at three parts of the polymer backbone. They are named after what the polymer resembles following the reaction. Figure 6.0.5 shows illustration of the three common structural types.

Name	Illustration
1. Comb	
2. Terminal	
3. Star	

Figure 6.0.5

Theses three categories are so named by the theoretical shapes of the polymer backbones. The first category is called comb. This is because when the chemist draws out the structure it appears like a hair comb. All of the reactive sites are on one side (pendant) and along the backbone of the polymer or comb. It should be taken into consideration that the polymer backbone has free rotation and the reactive groups can be in many different arrangements when the polymer chain is in motion. The second category is so named terminal. This category is self-explanatory by the fact that the only reactive groups on the polymer are at the chain ends. The final category is the star category. This category is so named because it could be envisioned that this polymer chain would have reactive groups on all sides of it and at the ends. Such molecules grow outward from a center in a multiplicity of "arms."

It is important to note that the functional group shown above is the Si-H bond. This is not the only reactive site used in the synthesis of functional silicones. There is a long list of functional groups that can be incorporated to fully utilize silicone chemistry.

a. Summary of Silicone Polymer Structure Types

The silicone backbone that is prepared by the technology developed by Rochow makes up what is called the *construction* of the silicone polymer. It is the number and location of the so-called Ds, Ms, Ts, and so on that determines one major part of the function of the polymer. The other parameter that helps define the structure-function behavior of these materials is called *functionalization* and is determined by what the M*s D* and the like *are reacted with* to make organo-functional silicones. There are indeed a great number of products that can be made using this technology.

4.2.3.15 VOLATILE SILICONES

Volatile silicones comprise a class of compounds that have a low heat of vaporization; that is, they evaporate from skin easily, providing a cooling effect. Evaporation of a material from the skin causes a cooling effect. Cyclomethicones conform to the structure shown in Table 7.0.1.

Table 7.0.1

Cyclomethicone			
Structure	**N**	**Common Name**	**Name**
	3	D3	Cyclotrisiloxane
	4	D4	Cyclotetrasiloxane
	5	D5	Cyclopentasiloxane
	6	D6	Cyclohexasiloxane

The INCI name "cyclomethicone" refers to a family of cyclic dimethyl siloxanes that includes cyclotetrasiloxane (D_4), cyclopentasiloxane (D_5), and cyclohexasiloxane (D_6), a family that has come under increased environmental/regulatory scrutiny in recent years. Cyclomethicone compounds are commonly used in cosmetic products to provide a solvent that feels dry on the skin. Key areas where this family of materials is used include antiperspirants, color cosmetics, and as a base solvent to blend with fragrance oils and perfume oils. Cyclomethicone is a clear, odorless silicone that leaves a silky-smooth feel when applied to the skin. Cyclomethicone compounds possess a cyclic structure rather than the chain structures of linear dimethyl silicones (dimethicone). D_4 evaporates from the skin and is volatile, resulting in a lower heat of vaporization, but it also has a very low surface tension. In other words, the volatility of D_4 is only one of the reasons it has a dry skin feel, but its low surface tension resulting in spreadability on the skin cannot be ignored. However, the physical chemistry that results in the dry feel of cyclomethicone evaporating is a complex one. Volatility is but one aspect of the complex phenomenon that contributes to a dry feel in a solvent used in cosmetics. In view of the safety concerns associated with the low-molecular-weight cyclomethicones, replacements have been sought. These must also provide similar viscosity and surface tension reduction (which affects spreadability), and must be nonflammable and cost effective (a formidable combination!). Clearly, a D_5 replacement that is flammable, defatting, and expensive is unacceptable. Table 4.2 shows the Heat of Vaporization of some compounds. Table 7.0.2 shows that it takes significantly less energy to evaporate D_4 or D_5 than either water or ethanol.

Table 7.0.2[7]

Heat of Vaporization	
Materials	**Heat of Vaporization (cal/g)**
Water	539
Ethanol	210
D_4	31
D_5	31

The cyclic silicones in Table 7.0.2 have a dry feel when applied to the skin, for two main reasons. First, they have the ability lower surface tension of the formulation, containing oil and thereby leading to a drier-feeling product, since they are surface active in oils. Second, since the cyclics are so volatile, they evaporate rapidly when they are exposed to the temperature of the skin. There are a number of cosmetic applications in which a dry skinfeel is important. These include antiperspirants, skin serums, and sun-care products. Table 7.0.3 shows some other characteristics of cyclic silicones.

Table 7.0.3

Cyclic Silicones					
INCI Name	**Viscosity (cS)**	**Molecular Weight (g/mol)**	**Solubility Parameter**	**Refractive Index**	**Flash Point (°C)**
Cyclotetrasiloxane	2.5	297	7.4	1.394	55
Cyclopentasiloxane	4.2	371	7.4	1.397	76
Cyclohexasiloxane	6.8	445	7.4	1.399	93

Recently, the use of D_4 in personal care applications has been abandoned because of safety concerns. The most common replacement has been D_5, and to a lesser extent, D_6. While drawn from the same family, these materials have different physical properties and do not result in the same dry feeling as D_4.

Cyclomethicone is used in a variety of products. From a commercial view, most importantly they have been used in antiperspirant formulations because they:

(1) Impart a soft-silky feel to the skin
(2) Provide excellent spreading
(3) Leave no oily residue or buildup
(4) Detackify formulations
(5) Are nongreasy
(6) Are compatible with a wide range of cosmetic ingredients
(7) Lower surface tension and
(8) Provide transient emolliency on the skin.

Cyclomethicones are used in many other applications including:

Table 7.0.4

Cyclomethicones in Cosmetic Application
1. AP / DO
2. Hair Sprays
3. Cleansing Creams
4. Skin Creams, Lotions
5. Stick Products
6. Bath Oils
7. Suntan
8. Shaving Products
9. Makeup
10. Nail Polish

a. Cyclomethicone Replacements

As we have said, over the years, the cosmetic industry has been actively seeking cyclomethicone replacements. D_4 is no longer used in our industry and despite some indications that the underlying conclusions that it is problematic in cosmetic products is faulty, the consumer simply will not accept these materials in formulation. The sharp consumer sensitivity to cosmetic formulation additives will also have an effect on D_5. While it is guilt by association, it is my feeling that long-term D_5 will also disappear. Products that contain large quantities of D_5 will be first to feel the wrath of consumers. In fact, in the future the use of silicone in formulation will be limited to those polymers that (a) provide a function that is not attainable from other types of materials and (b) are effective at very low concentrations. Put metaphorically, if a cosmetic product is a gourmet meal, the silicone additives will be the spice to the meal and not the meat and potatoes.

One approach to cyclomethicone replacement is to use low-molecular linear dimethicone. The most common linear polydimethylsiloxane (dimethicone) used to replace the cyclics range from 0.65 cSt to 3 cSt fluid. Despite the added cost, of these materials over that of cyclomethicone, they have enjoyed some success because of their similar properties. (See Table 7.1.1.)

Table 7.1.1

D5 Replacements				
INCI Name	**Hexamethyl Disiloxane**	**Dimethicone**	**Dimethicone**	**Dimethicone**
Viscosity (cSt)	0.65	1.00	1.50	2.00
Molecular Weight	162	236	311	385
Specific Gravity	0.76	0.82	0.85	0.87
Refractive Index	1.375	1.382	1.387	1.389
Solubility Parameter	6.7	6.9	7.0	7.0
Flash Point (°C)	–3	34	56	87

Another approach to replacing cyclomethicone is to use a silicone that is a methicone and not a dimethicone. Dimethicone compounds contain a dimethylsiloxane group; methicone compounds contain only mono methyl silioxanes wherein the other methyl group is replaced with a non-methyl organo-functionality. These polymers are not made from D_4. When a properly selected methicone is added to triglycerides or other esters, such a material: (1) will lower the surface tension at low concentration, making esters and triglycerides feel more like silicone; and (2) can be used in an ester or triglyceride at less than 5 percent, providing a cost-effective D_5 like feeling.

Methicone

R is $-CH_2CH_3$

The desirable dry effect achieved on the skin by the efficient reduction of surface tension of a natural oil, or low-viscosity ester, by means of using low concentrations of ethyl methicone offers a valuable formulation tool for the formulator. Replacing D_5 with combinations of natural oils and a small amount of alkyl silicone results in a replacement that is greener than D_5 (due to the fact that natural oils are present). Table 7.1.2 shows an example of how D_5 can be replaced by another silicone in an antiperspirant formulation.[6]

Table 7.1.2

Antiperspirant Formulations[8]			
Material	**Formulation**		
	A	**B**	**C**
Part A			
Aluminum/Zirconium Tetrachlorohydrex-gly	24.000	24.000	24.000
PEG/PPG 18/6 Dimethicone	2.500	2.500	2.500
Part B			
Cyclopentasiloxane	30.000	0.000	0.000
Ethyl Methicone	0.000	30.000	1.500
Glycine SoJa (Soybean Oil)	0.000	0.000	28.500

Antiperspirant Formulations[8]			
Material	**Formulation**		
	A	**B**	**C**
Part C			
Isohexadecane	9.000	9.000	9.000
PPG-14 Butyl Ether	9.000	9.000	9.000
Hydrogenated Castor Oil	2.500	2.500	2.500
PEG-8-Distearate	1.000	1.000	1.000
Stearyl Alcohol	18.000	18.000	18.000
Part D			
Talc	3.000	3.000	3.000
Silica	0.500	0.500	0.500
Part E			
Fragrance (Parfum)	0.500	0.500	0.500
Total	100.000	100.000	100.00

Procedure:

1. In a side vessel, combine Phase A ingredients. Impeller mix to uniformity.
2. In main vessel, heat Phase B ingredients to 70°C under agitation. Continue mixing and add Phase A. Bring to 75°C.
3. Combine Phase C in side vessel, heat to 85°C under impeller agitation, and mix to uniformity.
4. Add Phase C to AB under homogenization. Maintain batch at 80°C.
5. Pre-combine Phase D. Add to batch under homogenization. Begin cooling.
6. Add Phase E at 70°C under homogenization. Continue cooling.
7. Pour batch into sticks at 65°C.
8. Heat-treat surfaces.

As seen in Table 7.1.3, the addition of the mixture of 1.5% ethyl methicone and 28.5% soybean oil (Formula C) functions in a very similar way to Formula A and Formula B. The inclusion of low-cost oils or esters as the majority of the D_5 replacement offers a cost-effective way to replace D_5. Soybean oil can be replaced by other triglycerides or by dry esters including capric / caprylic triglyceride.

Ethyl methicone significantly lowers the surface tension when added into an oil or ester. The lowering of surface tension of the triglyceride or ester provides outstanding feel and improves the spreadability of a formulation. Table 4.6 shows the general information of ethyl methicone.[8]

Table 7.1.3

Ethyl Methicone[8]	
Description	A polymer under REACH with a CAS number of 63148-54-9
Typical Properties	**Appearance:** Clear Liquid
	Active Content (%): 100
	Color, Gardner: 3 Max
Uses and Applications	Provides excellent dry feel and lubricity in personal care formulation. Replacement for D_4 and D_5 in applications including but not limited to antiperspirant, hair glossers, resins, moisturizers, lotions, and pigmented products. Product can also be blended with low-viscosity esters and triglycerides, mimicking a D_5-like skin feel.

b. Summary of Successful Replacements for Cyclomethicones

Volatile silicone compounds are used primarily for their dry feel and ability to deliver in a more cosmetically acceptable manner to the hair and skin. While effective and a time-honored approach, replacing cyclics in formulation has recently become a hot topic. The replacement of volatile silicones in cosmetic and personal care formulations can be very difficult, but there are several ways of approaching this problem. Replacing the volatile silicones primarily with organo-modified silicones seems to be the most viable approach at the present time. Consumer perceptions will all drive the speed at which replacements are made, but cost effectiveness, flammability, and content of sustainable raw materials will also drive what gets chosen.

4.2.3.16 SILICONE FLUIDS

Silicone fluids are a well-known and well-established class of compounds used in personal care formulations. They encompass a variety of compounds that range in viscosity from 1 to 1,000,000 cSt. Silicone fluids have an MD_nM structure as illustrated in Figure 8.0.1.

Silicone Fluid Dimethicone

Figure 8.0.1

They contain no organo-functionality other than the methyl groups, and consequently are classified as homopolymers.

Since silicone fluids have the same structure and are homopolymers, the major difference among them is their molecular weight. As the degree of polymerization (DP) is increased, so is the molecular weight and viscosity. This leads to silicone fluids being classified and sold by their viscosity. Such polydimethyl siloxanes are Newtonian (constant viscosity over a broad range of shear rates) to the point that they are used as viscosity standards. Viscosity in turn is determined by the "n" value in the formula. Obtaining the proper viscosity in a cosmetic formulation is very important to the formulating chemist.

There are two major methods for obtaining a silicone fluid with the proper viscosity. The first is to polymerize the silicone to a specific molecular weight, and the second is to blend a higher-molecular-weight silicone with a lower-viscosity one. This approach results in a bimodal distribution and generates silicone fluids that have very different properties than a mono-modal (one-silicone polymer) product made to the same viscosity. Figure 8.0.2 shows the viscosity/molecular weight relationship.[vii]

Silicone Fluids			
Viscosity at 25° C (Centistokes)	**Approximate Molecular Weight**	**Approximate "n" Value**	**Average Construction**
5	800	9	$MD_{9}M$
50	3,780	53	$MD_{53}M$
100	6,000	85	$MD_{85}M$
200	9,430	127	$MD_{127}M$
350	13,650	185	$MD_{185}M$
500	17,350	230	$MD_{230}M$
1,000	28,000	375	$MD_{375}M$
10,000	67,700	910	$MD_{910}M$
60,000	116,500	1,570	$MD_{1570}M$
00,000	139,050	1,875	$MD_{1875}M$

Figure 8.0.2

Silicone fluids are commonly used in personal care applications at low concentrations (typically < 3 % wt). Figure 8.0.3 shows some common formulations and typical use levels. It is important to note that every formulation is different, and these numbers can vary depending on the specific formulation.

Applications of Silicone Fluids[1]		
Product Type	**Desired Effect**	**Use Level (% wt)**
Skin Lotion	Desoaping	0.1
	Rub-out	0.1–0.5
	Protection	1.0–30.0
	Feel	0.5–2.0
Skin Cleaner	Lubricity	0.1–0.5
	Wetting	0.1
Antiperspirant	Anti-whitening	0.5–2.0
	Detackification	0.5–2.0
Preshave Lotion	Lubricity	0.5–2.0
Aftershave Lotion	Feel	0.5–2.0
Makeup	Water Resistance	1.0–5.0
Shaving Cream	Reduce Razor Drag	0.5–2.0

Figure 8.0.3

One common problem with formulating with silicone fluids is their compatibility with the formulation. Along with the viscosity difference between low-molecular-weight silicones and high-molecular-weight ones is that the solubility of the silicone changes drastically with molecular weight. A perfect example of this is the silicone fluids having a viscosity of up to 200 cSt. These are soluble in anhydrous ethanol, while those with a viscosity of 350 cSt and above are insoluble. A list of the viscosity of common materials is found in Figure 8.0.4.

Viscosity Comparison of Silicone Fluids[1]	
Viscosity (cSt)	**Reference Object**
1–5	Water
10	Kerosene
20	Transformer Oil
50	Sae-5 Oil
100	Sae-10 Motor Oil
350	Sae-30 Motor Oil
500	Sae-30 Motor Oil
1,000	Light Syrup
2,500	Pancake Syrup
10,000	Honey
25,000	Chocolate Syrup

Viscosity Comparison of Silicone Fluids[1]	
Viscosity (cSt)	**Reference Object**
50,000	Ketchup
60,000	Thick Molasses
100,000	Hot Tar
250,000	Peanut Butter
1,000,000	Paste/Caulk

Figure 8.0.4

a. Low-Viscosity Silicones

This class of compounds consists of silicone fluids from 5 cSt up to, but not including 50 cSt. Commonly, the viscosity includes 5, 10, and 20 cSt. They are primarily used as an ingredient in a number of personal care products due to their high spreadability, low surface tension, and subtle high skin lubricity. These fluids are clear, tasteless, odorless, and provide a nongreasy feel on the skin. They are used in a wide variety of antiperspirants, skin creams, skin lotions, suntan lotions, bath oils, hair care products, etc.

These polymers have become very attractive to replace D_5 in formulations. The aesthetics of these materials mimic the attractive properties that D_5 possesses. These properties arise from their low surface tension and their ease of spreading, giving a dry feel and lack of volatility.

b. Standard-Viscosity Fluids

Standard-viscosity fluids are all covered by the CAS# 63148-62-9. They range in viscosity from 50 to 1,000 cSt. The typical grades include 50 cSt; 100 cSt; 200 cSt; 350 cSt; 500 cSt; and 1,000 cSt.

c. High-Viscosity Fluids

High-viscosity fluids are all covered by the CAS# 63148-62-9. They range in viscosity from 10,000 to 60,000 cSt.

d. Ultra-High-Viscosity Fluids (Gums)

As their names suggest, high-viscosity fluids are called gums because of the physical properties they possess. As the molecular weight is increased, these materials become more gum-like and behave like silly putty. They have a viscosity ranging from 100,000 to 1,000,000 cSt. The CAS number for these materials is CAS 63148-62-9.

Ultra-high-viscosity silicone fluids, while very sticky in bulk, when blended with cyclomethicone and/or low-viscosity fluids provide a soft velvety feel when

applied to hair and skin. The low-viscosity fluid acts as a plasticizer in that it allows for an overall reduction of the viscosity. A brief description of the viscosity-reduction phenomenon is that the low-viscosity fluid acts like a solvent and allows the longer-molecular-weight polymer chain to move more freely. This blend of high-molecular-weight and low-molecular-weight polymers produces a product with a cosmetically elegant feel initially, but a rich velvety feel upon spreading. Generally the blend consists predominantly of low-viscosity silicone (generally 50 cSt) and is primarily about 50 to 80% by weight in the blend. These blended products improve the feel of lotion products, and provide a hydrophobic, protective, but breathable barrier to the skin that imparts a softness and emolliency to the product.

Despite the fact that ultra-high-viscosity silicone fluids are linear in structure, they have such a high degree of polymerization ("n") value that they flow very slowly. This is very typical of a high-molecular-weight silicone polymer. Typically, as molecular weight increases past a critical value called the molecular weight of entanglement, the polymer chains entangle. This entanglement restricts movement and changes the polymers' physical properties. Their linearity of structure and lack of organic functionality are salient characteristics of this class of compounds. Figure 8.4.1 shows the viscosity of an ultra-high-viscosity fluid (60,000 cSt).

Figure 8.4.1 60,000 cSt silicone fluid[viii]

e. Summary of Silicone Polymer Behavior

Silicone fluids, sometimes called silicone oils, are a class of linear polymers that range in viscosity from very low to extremely high values. While there are benefits to both extremes, there are some solubility formulating issues and differences that arise when comparing the high and low. Typically, solubility and viscosity are the primary concerns to formulating chemists. There are two common approaches

to matching viscosity and solubility: 1) direct synthesis of a molecular-weight polymer with those properties, and 2) blending a low-molecular-weight polymer into a high-molecular-weight silicone fluid. In products that are not the result of blending, the viscosity is directly related to the molecular weight, which in turn is related to the number of D units present between the two D units. As the number of D units increases, so does the viscosity and molecular weight. Blends of high-viscosity fluids with either low-molecular-weight fluids or cyclomethicone result in an efficient way to deliver the high-molecular-weight fluid to the hair or skin. The use of silicone fluids in many formulations is primarily limited by solubility.

4.2.3.17 RESINS AND ELASTOMERS

Elastomers and resins have become very interesting materials based on their physical properties. These materials are very similar and there is no set definition of what a resin is and what an elastomer is. Before we describe the difference between elastomers and resins, it is important to have a brief discussion of cross-linking and stress-induced flow behavior. Cross-linking, as the name implies, is a method that links two or more polymer chains together. Figure 9.0.1 shows an illustration of a non-cross-linked polymer and cross-linked polymer chains.

Figure 9.0.1

While the term "stress-induced flow" seems a bit complex, it is a simple concept. "Stress-induced" simply means the application of a force over a given area, which forces the polymer to move. This can be as simple as pushing your finger into a polymer. As your finger comes into contact with the polymer, it must absorb the energy of the stress (force/unit area) being applied by the tip of your finger. This is accomplished by the polymer chains slipping past one another under the applied stress. As the force of your finger is increased, once the initial barrier to movement is exceeded, the polymer chains will start to flow past one another. If

the polymer chains are cross-linked, or chemically bound together, they can only move so far away from one another. This leads to some very interesting and unique properties.

Silicone elastomers and resins contain cross-linking moieties that link two or more polymer chains together. This linking makes it impossible for the polymer chains to pass by each other. Elastomers are lightly cross-linked materials. An excellent example of this is a rubber band or the rubber used in automotive tires. When a stress is applied to an elastomer, the material will stretch. When the stress is removed, the cross-links will allow for the polymer chains to spring back into their original position. Everyone who has thrown a bouncy ball into a wall is familiar with elastomers. "Elastomers are a group of polymers that can easily undergo very large reversible elongation (up to 1,000%) at relatively low stress levels." The term "elastomeric" applies to the physical polymer properties and not its chemistry. As the degree of cross-linking is increased, the material loses its mobility and becomes very rigid. Instead of flexing like less cross-linked elastomers do, the highly cross-linked material becomes brittle and will actually snap instead of moving with the applied stress. This phenomenon leads to an important question, "When do elastomers become resins?" While the definition of the two is somewhat blurred, what is clear is the difference in physical properties. The cross-linking can be introduced in many different ways, but it is the cross-link density (the number of cross-linking groups per non-cross-linking group) that determines the eventual properties. Figure 9.0.2 show a photograph of a silicone resin and silicone elastomer.

Silicone Resin and Elastomer

Figure 9.0.2

More detailed discussion of this subject can be found in an excellent reference by Paul C. Painter and Michael M. Coleman.

Figure 9.0.3 shows some common properties of silicone gums, elastomers, and resins.

Structural Considerations[1]	
Silicone Gum	Linear Product
	No Cross-Linking
	Free Rotation Around the Si-O-Si Bond
	Oily or Sticky Feel
	No Film Formation
	Function as Plasticizers
Silicone Elastomer	Lightly Cross-Linked
	Rotation Around Si-O-Si Bond
	Provides Rubbery Films
	Provides Waterproofing
Silicone Resin	Heavily Cross-Linked
	No Rotation Around the Si-O-Si Bond
	Non-Rubbery Film
	Will Peel Off and Crumble

Figure 9.0.3

a. Resin Types

There are several types of silicone resins and elastomers. Each type is based on what groups are involved in the cross-linking process. For silicone fluids, cross-linking is limited to T and Q units. The most common types are the so-called Q resins.

b. MQ Resins

MQ resins are an extremely interesting and unique class of silicone resins. These materials possess many unique properties that are unlike silicone fluids and organic resins. They are synthesized by the reaction of monofunctional silane (M) and tetrafunctional silane (Q). The molecule has only “M” and “Q” groups, hence the name “MQ resin.” Such compounds are commonly drying to the skin and often are formulated with silicone fluids as plasticizers. The cross-linking of this type of

resin is due to the nature of the Q silane. As discussed in a previous section, the Q unit has four oxygen atoms. These four reactive sites produce a cross-linked network. The structure of these materials can be quite complex and each structure will have different properties. Huang et al. used ^{29}Si NMR to show the effect of the acid catalyst on the MQ structure.[ix] The structure of this type of product is shown in Figure 9.2.1.[x]

Graphic Representation of A MQ Resin[11]

Figure 9.2.1[11]

As depicted in Figure 9.2.1, the MQ resin forms a "cage-like" structure that forms highly organized materials. This leads to their very interesting properties both in and out of solution. The "cage-like" structure is produced only when the ratio of M to Q is low enough. As shown in Figure 9.2.2, MQ resins with a high M to Q ratio do not form a cage structure and are liquids at room temperature.

As seen in Figure 9.2.2, the cage structure does not begin to form until the ratio of M to Q reaches 2 to 1. This cage structure changes the properties of the resin dramatically.

The physical properties of these resins can be tuned by controlling the M to Q ratio. A resin with a high M to Q ratio will have liquid properties. The opposite is also true since a resin with a very low M to Q ratio will be a resin. These resins are typically soluble in hydrocarbons and can modify cosmetic products at very low concentrations.

M/Q Ratio	Structure
4.0	
2.0	

1.2

Figure 9.2.2

c. MDQ Resin

This type of product not only has "M" and "Q" groups, but it also has D groups, hence the name "MDQ resin." This type of material has a lower cross-link density than the MQ resin type and can be engineered to be either a resin (plastic-like) or an elastomeric (rubbery) depending upon the Q to D ratio.

d. Silicone Crosspolymers

Silicone crosspolymers are silicone polymers that are cross-linked with another silicone polymer; they can either be resins or elastomers. Figure 9.4.1 shows a schematic representation of a dimethicone/vinyl dimethicone crosspolymer.

Illustration of a Silicone Crosspolymer

Figure 9.4.1

As seen from Figure 9.4.1, each silicone polymer backbone must possess a reactive group on the polymer backbone. This means the polymer must possess a reactive site such as M^*, D^* . . . etc. In this case the cross-linker is vinyl dimethicone.

These resins/elastomers have different properties including: solubility, aesthetics, and structure. The major difference between MQ resins and crosspolymers is the structure of the polymer backbone and the structure of the finished system. Crosspolymers contain dimethyl groups on the silicone backbone. As seen above, the MQ resins form a cubic structure with each of the Q units making up the corner of the cube. The vinyl dimethicone crosspolymer is cross-linked by a much more flexible silicone polymer. These silicone polymers are composed of $Si(CH_3)_3$-O-$Si(CH_3)_2$-O- bonds, where the MQ is comprised of SiO_2 bonds very similar to inorganic silicone quarts. Table 9.4.2 shows the building blocks of the cross-linked system and the possible structures they form after cross-linking.

Table 9.4.2

Material	Structure Single Unit	Building Block	Structure
MQ			
Crosspolymer			

As seen from the illustration, the structures of these two systems are quite different, and this difference in structure leads to very different performance and application in the cosmetic and personal care field.

4.2.3.18 DIMETHICONE COPOLYOL (PEG/PPG DIMETHICONE)

At this juncture, we note that we have addressed "simple" silicones (i.e., those with no functionality such as the incorporation of water-loving or oil-loving groups attached to the silicone "backbone." As mentioned previously, silicone fluids have limited application due to their solubility properties relative to hydrocarbon oils and water. In this section we address how silicone fluids can be modified to increase their polarity and provide surfactant-type behavior at fluid interfaces. It is important to note that these modifications are done by chemical reaction between a reactive silicone and a vinyl-containing compound.

The most common modification that can be conducted to increase polarity and water solubility is to incorporate poly(ethylene glycol) (PEG), poly(propylene glycol) (PPG), or copolymers of both (either random or block type) onto the silicone polymer backbone in the so-called pendant T-configuration. After the addition of PEG or PPG, at varying positions along the silicone backbone, and in varying amounts relative to the backbone molecular weight, the silicone is called dimethicone copolyol. Dimethicone copolyols are silicone copolymers that contain at least two different polarities covalently bonded onto the same polymer backbone; in this case they have both water-soluble and silicone-soluble groups. Polymers that

possess at least two different polarities on the same polymer backbone are referred to as amphilic polymers.

When added into water at low concentrations, dimethicone copolyols (of which there are literally thousands of varieties) migrate to the air/water interface. As more dimethicone copolyol is added into the water, the interface becomes saturated and a critical point is achieved. When additional dimethicone copolyol is added, it cannot migrate to the interface, so they start to form micelles. This critical point where the interface is saturated and spherical micelles begin to form is called the critical micelle concentration (CMC). Critical micelle concentration values are determined by monitoring surface tension change with concentration. Figure 10.0.1 shows an illustration of what happens upon addition of a silicone copolyol surfactant into bulk aqueous solution.[14]

Figure 10.0.1

As seen in Figure 10.0.1, the surface tension begins to decrease in relationship with the amount of surfactant added. When the interface becomes saturated with the surfactant, the surface tension levels off and stops decreasing.

The key to surfactant efficacy in decreasing surface tension is the ability of the surface-active silicone copolymer to migrate to the interface and take up the maximum free volume on that interface. This maximization of free volume at the interface and its affinity for the interface make dimethicone copolyol extremely effective at lowering surface tension. The surface-active material distorts and packs into the lowest entropy configuration with the water-hating portions extending out of the liquid below. This leads to the dimethicone copolyol being able to be used at very low concentrations and the ability to drastically change the properties of a solution.

Dimethicone copolyols can provide many unique and desirable properties.
- Lower Surface Tension
- Provide Emulsification
- Provide Wetting
- Provide / Alter Foam
- Conditioning
- Alter Skinfeel

Dimethicone copolyols can exist in many different forms. A very common structure is shown in Figure 10.0.2.

PEG / PPG Dimethicone Structure

Figure 10.0.2

As seen in Figure 10.0.2, there are several variables that can be modified to change the performance of dimethicone copolyol surfactants. One of the most important is the ratio of a (silicone-soluble portion) to b (water-soluble portion), as well as the molecular weight of the a and b segments, which are both controllable and affect surfactant behavior. The higher the ratio of a to b, the less water soluble the organosilicone surfactant. Also, the overall molecular weight of the dimethicone copolyol can drastically change properties.

a. Wetting Properties as a Function of Molecular Weight[7]

The term "wetting" refers to how a material spreads and coats a surface. For example, if a water droplet is placed on a lotus leaf, the water will minimize the interaction with the leaf and produce a high contact angle. If a good wetting agent is added—either to the surface of the leaf or in the water solution, that same drop will spread out, coating the surface of the leaf. In turn making the leaf "wet," this is where the term wetting comes from. The spreading and wetting phenomenon is not only related to the air/liquid surface tension generated by the organosilicone surfactant but is also a function of the interfacial tension between the two

liquids that are typically present in an emulsion, along with the silicone surfactant. Figure 10.1.2[15] shows an illustration of Young's Equation and how the contact angle is defined.

Figure 10.1.2

As seen in Figure 10.1.2, the contact angle is the angle the water makes with the surface. In this illustration, a low contact angle ($\theta < 90°$) means the surface is hydrophilic. Large contact angles ($\theta > 90°$) lead to a surface that is hydrophobic.

Another measure of wetting is the time required for a standard skein of fiber, or of hair, to drop in an aqueous solution. This type of wetting time is called Draves Wetting (ASTM D2281). The lower-molecular-weight dimethicone copolyols have faster wetting times than their high-molecular-weight counterparts. The lower-molecular-weight polymers allow for more efficient packing efficiency. The materials with lower molecular weight are extremely effective at the higher concentration of 1.0 % w. Their wetting speeds are almost instantaneous—meaning the wetting is controlled by the rapid diffusion of the dimethicone copolyol to the air/water interface. An interesting finding is that wetting speed is slowly reduced as the molecular weight is increased. The slope of it increases once the molecular weight reaches a value of over 2,000. This implies that a rapid-wetting conditioner can be prepared by proper selection of organosilicone molecular weight. As the molecular weight of a polymer is increased, its ability to function in different capacities changes. This is due to hydrogen bonding and achieving lowest free energy. Figure 10.1.3 shows this.

Function of Dimethicone Copolyols vs. Molecular Weight	
Molecular Weight (Da)	**Function**
500	Wetting
2,500	Emulsification
10,000	Conditioning
50,000	Waterproofing

Figure 10.1.3

If the ratio of a to b is held constant, the molecular weight of the polymer controls its wetting properties. As shown in Figure 7.1.2, as molecular weight increases, the wetting ability decreases. Figure 10.1.4 shows the graph of molecular weight vs. wetting time.[18]

Draves Wetting[7]

Figure 10.1.4

b. Eye Irritation as a Function of Molecular Weight[7]

Not only do wetting properties depend upon molecular weight, but the molecular weight is also a controlling factor in eye irritation. Lower-molecular-weight polymers penetrate into the eye, causing more irritation to the eyes. As molecular weight increases, irritation decreases. However, there is a very definite molecular weight effect over which irritation becomes little or no problem. Figure 10.2.1.

Draize Primary Ocular Irritation Scale	
Moderately Irritating	25.1–50.0
Mildly Irritating	15.1–25.0
Minimally Irritating	2.6–15.0
Practically Non-Irritating	0.6–2.5
Non-Irritating	0.0–0.5

Figure 10.2.1

The data in Figure 10.2.1 was generated on a series of PEG 8 dimethicone copolymers with the same a to b ratio, by increasing the total number of a and b units. The eye irritation was run at 20% active and was evaluated over one and seven days.

Figure 10.2.2

c. Formulation Ingredient Interactions[1]

One of the most important factors facing the formulator of cosmetic products is how additives to formulations will interact with each other. This complication can include hydrophobic / hydrophilic interactions between ingredients, hydrogen

bonding between ingredients, insolubility or limited solubility of ingredients, and micellar interactions, just to name a few. This explains why there is a great deal of difficulty for the formulator to adapt raw material supplier data on the function of organosilicone products in a pure aqueous environment with data obtained in more complex systems. Figure 10.3.1 shows the effect of adding PEG-8 dimethicone to an aqueous solution of deionized water. The graph is quite ordinary and looks like what one might expect for a CMC. Notice, however, that the surface tension is significantly lower than for fatty surfactants (i.e., below 30 dynes/cm).

Figure 10.3.1

When the same PEG-8 dimethicone copolyol is added to a 1% solution of sodium lauryl sulfate, a completely different result is obtained. Figure 10.3.1 does not look like a CMC (critical micelle concentration) graph. Instead, it shows an interaction effect between the two surfactants as they compete for the surface at the air/water interface. While not a CMC graph in the classical sense, this graph and its shape are very enlightening as to the effectiveness of the silicone surfactant when added to the organic surfactant. Since there is no clear break point as is seen with the CMC graph, we created a new point so the effectiveness of the addition of a silicone surfactant to another surfactant vis-à-vis surface tension reduction can be determined. That term is designated the RF_{50} and it is described in Figure 10.3.3.

RF_{50} Definition[xiii]
RF_{50} = the concentration of silicone surfactant added to reduce the surface tension to 25 dynes/cm.

Figure 10.3.3

Figure 10.3.4 shows the results of addition of two different PEG-8 dimethicone molecules being added to a 1% solution of two different sulfated fatty surfactants.

Silicone Surfactant	Molecular Weight	Co-Surfactant	RF_{50} (%)
A-208	616.3	SLS	1.2
A-208	616.3	SLES-2	1.2
C-208	1,556.5	SLS	1.5
C-208	1,556.5	SLES-2	3.5

Figure 10.3.4

It is interesting to see from Figure 10.3.4 that when SLS is used as a co-surfactant, for the higher MW dimethicone copolyol the result has an RF_{50} of 1.5%, while the lower-molecular-weight dimethicone copolyol has an RF_{50} of 1.2%. This shows that the low-molecular-weight organosilicone copolymer is more efficient at lowering the surface tension when a co-surfactant is involved.

Upon comparing the RF_{50} in the samples containing SLES-2, the results are more dramatic. The RF_{50} for the low-molecular-weight dimethicone copolyol is 1.2, while the higher-molecular-weight copolyol is 3.5%. This is much more significant and is driven by the hydrogen bonding between the two moles of polyoxyalkylene on the SLES, making getting to the surface of the solution much harder for the larger C-208 molecule. The molecular weight of the silicone polymer has an impact upon the efficiency of the molecule to migrate to the interface and lower surface tension. The larger the molecule, the less efficient the molecule is in lowering surface tension.

How can these observations and behavior be applied when creating a cosmetic formulation?

1. It will be less efficient to use a higher-molecular-weight organosilicone copolyol to lower surface tension effectively in sodium laureth 2 sulfate systems (simply referred to as SLES-based systems). It would be more efficient to use a lower-molecular-weight dimethicone copolyol.
2. The evaluation of surface tension reduction in a formulation needs to be determined to maximize the effectiveness of the silicone in formulations. There is simply no other alternative.

Delivery of the silicone to the surface (interface) is a prerequisite to obtaining a consumer-perceivable result in formulations. The consumer can easily feel a difference in a formulation where there is a surface tension difference. As shown above, when organosilicone surfactants are added to others there is a foreseeable impact on efficacy. This efficacy can be measured by RF_{50}.

Another mostly neglected structural variable that can dramatically change RF_{50} is the solubility of the silicone being added. Insoluble materials cause formulation problems, and too-soluble materials result in the expensive silicone ingredient going down the drain. Products that form microemulsions (i.e., are thick and blue in color when added to water) are most efficient.

Consider the structure in Figure 10.3.5.

PEG-8 Dimethicone

Figure 10.3.5

As the ratio of a to b changes, the water solubility changes. This is because the "a" subscript (i.e., the number of repeating units of that species) is related to a group that lowers water solubility, while the "b" group represents the amount of the species that improves aqueous solubility. A polymer with an a:b ratio of 100:1 will be insoluble in water while one with an a:b ratio of 1:100 will be very water soluble. Somewhere in between these extremes, there will be a polymer that forms a microemulsion. It will be that polymer in the series that will offer the best effectiveness in solution. Using a wide range of a to b ratios, the optimal polymer can be determined that provides a desired microemulsion. Knowing the a:b ratio (D:D* ratio) provides a means to calculate the ratio at which a microemulsion can be formed. From the formula, shown in Figure 7.2.6, we can calculate the critical ratio needed to make a microemulsion.

Solubility in 10% H_2O			
D	**D***	**Calculated**	**Observed**
5	1.0	Insoluble	Insoluble
5	2.0	Soluble	Soluble
10	2.0	Insoluble	Insoluble
10	2.5	Micro	Micro
10	3.0	Soluble	Soluble
20	3.5	Insoluble	Insoluble
20	4.0	Micro	Micro
20	4.5	Soluble	Soluble
24	4.0	Insoluble	Insoluble
24	4.5	Micro	Micro
24	5.0	Soluble	Soluble
40	6.0	Insoluble	Insoluble
40	7.0	Micro	Micro
40	8.0	Soluble	Soluble

Figure 10.3.6

$\Omega = (0.17)x + 1$

Where Ω is the minimum number of D* units and x is the number of D units

It is always recommended that microemulsion-forming polymers can be used in cosmetic formulations, since these will be most effective at getting to the surface.

d. Water Tolerance[xiv]

In the previous section water-soluble silicones and silicone surfactants were discussed. In this section we introduce yet another important organosilicone-based phenomenon to formulating chemists. To begin our discussion we will have to first consider what happens when water is added to an anhydrous formulation. Most commonly, formulations will split or separate when water is added. Typically, in a nonsilicone, standard organic surfactant system this is because the addition of water changes the HLB of the system thereby decreasing the solubility of one of the components in the formulation. A useful new concept has emerged and it is called water tolerance. Basically, water tolerance is a measure of how much water can be added to a formulation before the formulation splits.

Dimethicone copolyols are interesting polymeric surfactants in that they can be utilized to increase the water tolerance of a formulation. To test how the dimethicone copolyol affects the formulation, the water tolerance of the formulation has to be determined. The determination of water tolerance is based upon the fact that dimethicone copolyols are soluble in anhydrous isopropanol, independently of their solubility in water. Water can be added to the isopropanol solution until a haze develops. This haze signifies the water tolerance of the solution, and the concentration of water tolerated by the formulation is called the "water tolerance." A common procedure for determining the water tolerance is described in Figure 10.4.1.

Water Tolerance Test
1. Dissolve 2.0 g of dimethicone copolyol in 100.0 g of isopropanol.
2. Slowly titrate in deionized water into the isopropanol solution.
3. Record the number of grams of water needed to produce the first haze.

Figure 10.4.1

Figure 10.4.1 shows the water tolerance of specific silicone compounds. As the D:D* ratio (a:b ratio), i.e., the number of D units (hydrophobic) compared to the number of water soluble groups (D*), increases, the materials become more water insoluble and the water tolerance drops. What is very interesting and warrants consideration is the fact that one mole of propylene oxide will increase water tolerance as much as four moles of ethylene oxide does.

Figure 10.4.2

e. Antiperspirant Release[17]

Along with the interesting wetting and water-tolerance phenomena we have presented in this chapter, dimethicone copolyols are of interest in a wide variety of applications. One of the more significant product categories is the use of dimethicone copolyol in antiperspirant applications. The copolyol contains PEO or PPO groups that are capable of coordination with different materials. This interaction between polyoxyethylene groups in dimethicone copoylols, while providing dryness in feel, inhibits the efficient release of the active from antiperspirant sticks. Several formulations were prepared and evaluated. The formulations are shown in Figure 10.5.1.

Antiperspirant Formulation		
Material	**Formulation**	
	A	**B**
Reach AZP-908	24.0	24.0
PEG-12 Dimethicone	-	2.5
PEG/PPG 8/8 Dimethicone	2.5	-
Cyclomethicone	30.0	30.0
Polydecane	9.0	9.0
PPG 14-butyl ether	9.0	9.0
Hydrogenated Castor Oil	2.5	2.5
PEG-8-Distearate	1.0	1.0
Stearyl Alcohol	18.0	18.0
Talc	3.0	3.0
Cab-o-sil-MS	0.5	0.5
Fragrance	0.5	0.5

Figure 10.5.1

The formulations shown in Figure 10.5.1 were evaluated and tested. The procedure and results are shown below.

A.P. Release Methodology

Reagents:
1. Silver Nitrate (0.05%N)
2. 10% nitric Acid

Apparatus:
1. Constant temperature water bath.
2. Lab stirrer with RPM reading
3. 250 ml beaker
4. Analytical Balance
5. Weighing dish
6. Spatula
7. Filter paper (Whatman # 4)
8. Funnel
9. Automatic Chloride titrator (Brinkman)
10. Magnetic Stirrer
11. Thermometer

Procedure:
1. Weigh 199 grams of DI water into a 250 ml beaker and heat to 37ºC
2. Place the beaker in a constant temperature water bath and mix well at 250 RPM with an overhead mixer.
3. Weigh 1.1 gram of antiperspirant stick by shaving wih a spatula from the surface of the stick
4. M ix for 30 and proceed to step 6.
5. Repeat steps 1-3 three more times only this time for 60, 120 and 240 minutes
6. Filter through filter paper and collect liquid for chloride titration.
7. Add 15 mo of 10% nitric acid and mix well.
8. Analyze for chloride ion using the auto titrator
9. Calculate % chloride

$$\frac{\text{\% Chloride titrated}}{\text{Theoretical \% chloride}} \times 100 = \text{\% Active}$$

The release of chloride ion is an indication of the effectiveness of the antiperspirant. Figure 10.5.2 shows the results.

Release Data				
Formulation	**30 min**	**60 min**	**120 min**	**240 min**
A PEG-8/PPG-8 Dimethicone	96.2 %	96.5 %	98.8 %	-
B PEG-12 Dimethicone	43.0 %	61.3 %	80.5 %	90.6 %

Figure 10.5.2

As seen in Figure 10.5.2, the introduction of poly (propylene oxide) groups into the polymer chain results in more efficient release.

f. Summary

As a class of materials, dimethicone copolyols offer the formulator the advantage of using aqueous solutions and introducing water into formulations that are typically anhydrous. However, if the dimethicone copolyol used is too water soluble in the formulation, it will be of little value to that formulation. If the expensive silicone compound stays in the water phase and ends up down the drain, it is ineffective. Alternatively, if the product is too insoluble, clear systems will not be obtained. The particular product needs to be matched to the formulation (not just to water) to ensure it ends up at the interface where silicone can perform its "magic."

4.2.3.19 ALKYL DIMETHICONE

Alkyl silicones are a class of amphilic silicones that contain within one molecule both a silicone-soluble group and an oil-soluble group. These materials are of interest to the personal care formulator for a number of reasons. They are very useful polymers because they are soluble in nonpolar solvents, they migrate to the surface between the oil and the substrate, they lower surface tension of oils, and they improve the spreadability of formulations on skin and hair.

It is well known that a fatty surfactant like sodium lauryl sulfate added to water will decrease the surface tension from 72 dynes/cm to as low as 30 dynes/cm. The same phenomenon occurs when an alkyl silicone is added into oils; with one notable exception, the oil will have an initial surface tension of 32 dynes/cm. Figure 11.0.1 shows the effect.

Figure 11.0.1[16]

As seen from Figure 11.0.1, the surface tension of soybean oil was decreased drastically from about 32 to about 22 dynes/cm. This surface tension reduction causes an increase in spreadability. This increase in spreadability makes the oil feel dryer (more silicone-like) and provides a more elegant feel on the skin. The reduction of surface tension in such oils is dependent upon the ability of the alkyl silicone to reach the liquid surface and orient itself properly. An effective silicone product must possess a balance between solubility and function. The alkyl silicone must be sufficiently soluble to provide clarity, yet sufficiently insoluble to migrate to the surface. The most efficient polymer for reducing the surface tension is the one that can provide the lowest surface tension at the lowest concentration.

a. Alkyl effects

Along with the ability to lower surface tension and increase the solubility of the silicone in oil, alkyl dimethicone copolymers can also change the physical properties of the materials that they are added to. By changing the alkyl group attached to the silicone backbone, a wide variety of products can be obtained. Attaching alkyl groups containing 16 or less carbons atoms in their alkyl chain will result in a liquid product at ambient temperature. Alkyl groups with 18 or more carbon atoms in their alkyl chain are solid at ambient temperatures. Adding alkyl dimethicone, with long carbon-pendant groups (≥ 18 carbons) to a liquid product will cause the material's melting point to increase. The opposite is true for a product that has a high melting point. In other words, adding alkyl dimethicone, with a small carbon-pendant groups (≤ 16 carbons), can drastically lower the melting point of the product and add liquidity.

b. Silicone-to-alkyl ratio effects

Alkyl dimethicone polymers not only have the ability to form solid and liquid products, they also have the ability to change the physical properties of the solid. By tuning, or changing, the silicone to alkyl ratio (changing the "a" to "b" ratio in the structure), the physical properties of the solid can range from a very brittle opaque solid (high alkyl) to a translucent wax (high silicone). Increasing the percentage of silicone will result in a softer wax with minimal effect upon melting point. This ability to control the tensile strength applies not only to the polymer in bulk but also when it is added into a product's formulation. The addition of a silicone with the correct silicone-to-alkyl ratio provides a formulation chemist the ability to "stiffen" or "soften" a solid.

c. Example: Cetyl Dimethicone

The structure of cetyl dimethicone is shown in Figure 11.3.1.

Cetyl Dimethicone

Properties	
Melt Point:	37°C
Solubility:	Esters, Triglycerides, & Other Oils

Figure 11.3.1

When cetyl dimethicone is added to oil-based formulations, cetyl dimethicone improves spread on the skin of that oil by lowering surface tension. Figure 11.3.2 shows the CMC curve of cetyl dimethicone in mineral and soybean oil.[16]

Figure 11.3.2

This results in the oil phase feeling more siliphilic (silicone-like), and adds a silicone- like feel. Cetyl dimethicone is added to sunscreen formulations since it increases spreadability on the skin surface and also tends to increase SPF values, because more uniform films of sunscreen actives are deposited.

d. Behenyl Dimethicone

The structure is shown in Figure 11.4.1.

Behenyl Dimethicone	
Properties	
Melt Point:	37°C
Solubility:	Esters, Triglycerides, & Other Oils

Figure 11.4.1

Alkyl silicones having high content of solid alkyl groups (50% or more of the polymer) will provide a translucent occlusive coating to the hair and skin. As the amount of silicone increases, the material becomes less soluble in the oil and the resulting wax is no longer occlusive. Figure 11.4.2[xv] shows this phenomenon.

10 % Behenyl Dimethicone in Olive Oil				
Olive Oil	**A**	**B**	**C**	**D**
-	20% Silicone 80% Alkyl	40% Silicone 60% Alkyl	60% Silicone 40% Alkyl	80% Silicone 20% Alkyl

Figure 11.4.2[18]

As seen in Figure 11.4.2, as the amount of silicone in the polymer is increased, the solubility in olive oil decreases. This leads to an opaque gel that melts upon the application of heat.

Another interesting phenomenon when it comes to alkyl silicones is the critical gelation concentration. As seen in our last example, 10% wt of behenyl dimethicone added to olive oil produced a solid product. If a lower concentration of alkyl silicone is added to an oil, will the oil be a liquid or a solid? To investigate this phenomenon, we studied the concentration at which a particular oil or silicone can be gelled and provide a nonflowing wax. The point where the oil or silicone stops

flowing is called the gel concentration. Alkyl dimethicone was added to 50-viscosity silicone fluid at varying concentrations. The resulting materials were evaluated for flow behavior. The results of this test are shown in Figure 11.4.3.[16]

Alkyl Silicone in 50-Viscosity Silicone Fluid

0%	10%	20%	30%	40%
Liquid	Liquid	Solid	Solid	Solid

Figure 11.4.3[16]

As seen in Figure 11.4.3, the critical gellation concentration (CGC) was between 10 and 20 wt %. It is important to note that the CGC will be different for every solvent and should be evaluated before the formulation is put together.

4.2.3.20 MULTI-DOMAIN ALKYL SILICONES

The previous section of this chapter has focused on alkyl silicone polymers. In this section, we will discuss a new class of alkyl silicone where there are at least two different alkyl groups covalently bonded to the same silicone backbone. This class of alkyl silicones contains at least two alkyl groups, one of which is solid at room temperature (C16 and above) and at least one of which is liquid at room temperature (C8 to C16). These alkyl silicones fall into a category called multi-domain silicones. A generic structure is shown in Figure 12.0.1.

Multi-Domain Alkyl Silicone

Figure 12.0.1

These multi-domain silicones have very interesting phase behavior. The different pendant groups can aggregate together and form pockets of solid and liquid in a highly organized structure. Figure 12.0.2 shows a representation of these highly organized structures.

Figure 12.0.2

In order to further study these multi-domain polymers, photomicroscopy was employed. These photomicrographs show the differences in the structure of the wax. Two different materials were evaluated. The first is the multi-domain product; the second is a blend having the same percentages of alkyl groups but on different polymers. This comparison will show if multi-domains act differently than two alkyl silicones blended together. The results are shown in Figure 12.0.3[16] and Figure 12.0.4.

Phase Contrast Microscopic Images	
Multi-Domain Polymer	Polymer Blend

Figure 12.0.3[16]

Polarized Light Microscopic Images	
Multi-Domain Polymer	Polymer Blend

Figure 12.0.4

Optical Images	
Multi-Domain Polymer	Polymer Blend

Figure 12.0.5

Physical Properties	Multi-Domain	Polymer Blend
Melting Point	34–38°C	56°C
Visual Appearance	Translucent Gel	Opaque Gel
Shear-Induced Flow	Yes	No

As seen from the polarized micrographs, multi-domain silicones are drastically different from a polymer blend of the same composition. In the multi-domain silicone, both alkyl chains are covalently bonded to the same silicone backbone. This restricts the degree of freedom of each alkyl chain

and drastically affects the properties of the solid. As two long alkyl chains (in the multi-domain silicone) come into contact with one another, they "stick together" and form a solid domain. As the long alkyl chains get incorporated into these solid domains, the small alkyl chains get excluded and start to form small liquid domains. Since both domains are attached to the same polymer backbone it is impossible for them to completely separate. This causes each solid and liquid domain to be confined into small regions and causes each solid domain to be surrounded by a liquid domain. The small domain size leads to many of the different properties of the solid, from scattering light (large domains scatter more light and are opaque to smaller domains that don't scatter much light and are translucent) to low melting point. The fact that the solid domain is surrounded by the liquid domain allows for the solid to flow under pressure. When a stress is applied to the solid, the solid domains can slide past one another more easily. This allows the solid to flow without breaking. In the polymer blend, the domains are not limited in size. Each domain can be very large size and this accounts for greater light-scattering ability (opaque solid) and the solid also becomes brittle. Since these multi-domains are highly organized compared to the blend approach, they can provide novel and specific properties needed for formulations. A comparison of a co-reacted product having liquid and solid domain (multi-domain) and a blend of alkyl silicones having the same ratio of alkyl groups is seen below.

a. Syneresis Improvement with Multi-Domain Silicones

Syneresis is a condition that exists in products that have raw materials with minimal solubility in each other. Over time, these formulations separate and generate an oil phase, which migrates to the surface. This phenomenon is called "sweating" or more accurately, syneresis. It is usually undesirable in cosmetic products and must be corrected to ensure stability.

Syneresis in pigment products such as lipsticks is difficult to correct since the presence of the pigment masks the underlying problem. If the same formulation—devoid of pigment—is melted, it will be observed that even when melted, the formulation will not be clear. Additionally, there will be a relatively wide melt range (4 or more degrees C). Both situations are indicative of incompatibility. Addition of the proper alkyl silicones to the formulation devoid of pigment will reveal that both problems are addressed. The melt point tightens to a much narrower range of 1–2 degrees and the clarity will improve. This will resolve or minimize the syneresis. The addition of multi-domain alkyl silicones will not only help resolve syneresis, but will provide a difference in cushion of the product and payoff of the color.

b. Summary

Alkyl silicones offer a cosmetic formulator a number of opportunities to alter the feel, consistency, and rheology of oil-based formulations. Alkyl silicones can also be used for the desirable attributes they provide in oil phases in the oils themselves, serums, emulsions, and any other form in which the oil is present. Adding structure to the oil phase will stabilize emulsions. Furthermore, alkyl silicones will provide (a) lower surface tension, (b) gelation, and (c) tactile changes.

4.2.3.21 ALKYL DIMETHICONE COPOLYOL

Alkyl dimethicone copolyols are the product of the co-hydrosilylation reaction of one or more alkyl group and one or more polyoxyalkylene group. This results in a silicone polymer backbone that has both a hydrophobic group and a hydrophilic group. Cosmetic products are named using what is called INCI **(International Nomenclature of Cosmetic Ingredients**) rules, which name these materials alkyl PEG / PPG dimethicone. The incorporation of both an oil-soluble and a water-soluble group onto the organosilicone backbone results in a series of compounds best known as emulsifiers. A typical compound has the structure shown in Table 13.0.1.

Table 13.0.1

Structure of Alkyl Dimethicone Copolyol

The INCI name for this material is lauryl PEG 8 dimethicone. Variation on the values of a and b has resulted in the development of a new series of emulsifiers that together make up an emulsifier kit that can be used to evaluate emulsifiers in

both regular (oil-in-water) and inverse (water-in-oil) emulsions. The composition is shown in Table 13.0.2.[7]

Table 13.0.2

Composition of Silicone Emulsifiers				
Material	**EO (%)**	**Alkyl (%)**	**3D HLB**	
			x	**y**
Lauryl PEG-8 Dimethicone A	**48**	**6**	**9.6**	**1.2**
Lauryl PEG-8 Dimethicone B	**39**	**13**	**7.8**	**2.6**
Lauryl PEG-8 Dimethicone C	**28**	**22**	**5.6**	**4.4**
Lauryl PEG-8 Dimethicone D	**16**	**32**	**3.2**	**6.4**

The ability to assess emulsion stability and make changes to improve it is a key concern to the formulator. Often the stability cannot be ascertained for weeks or longer using storage at low, ambient, and elevated temperature. The contraction of the development cycle experienced by the formulation chemist makes this approach problematic at best. A newer screening method has been developed using the range of emulsifiers listed above, which confers upon the formulator the ability to quickly ascertain basic information on emulsion stability in between three and 24 hours. This approach not only speeds up the formulation process but also allows for evaluation of both the inverse and regular emulsions in a shorter time period. The process begins with the evaluation of how the emulsifiers interact with the oil phase. Using the formulation 13.0.3, equal amounts of oil and water are evaluated, allowing for the creation of both a regular and invert emulsion using the four test emulsifiers. This basic work will allow for the first rapid evaluation.

Formulation 13.0.4[1]

Test Emulsion Formulation[1]	
Material	**Concentration (wt %)**
Water	47.0
Oil	47.0
Emulsifier	5.0
Salt	1.0

Procedure:

1. Place emulsifier or emulsifier blend into the oil phase.
2. Mix well, noting clarity.
3. Add salt to water phase.
4. Heat both phases to 50°C
5. Add water phase to oil phase and use mixer mix for 120 seconds.
6. Note appearance.

The above process is repeated with emulsifier blends depending upon the results of the first emulsion. The formulation is set up to allow for the evaluation of the ability of the emulsifiers to make regular (o/w) and inverse emulsions (w/o). The use of a common kitchen mixer further simplifies the process, as it will allow for rapid determination of emulsion stability. The technique is not a replacement for regular emulsification equipment in production but rather, is simple a tool for testing emulsion stability. Table 13.0.4 is used to expedite the evaluation of emulsions.

Sheer Mixer

Sheer apparatus for lab evaluation

Figure 13.0.5

Table 13.0.6

Lauryl PEG 8 Dimethicone in o/w Emulsion			
Time = 0			
J-208-212	**J-208-412**	**J-208-612**	**J-208-812**
Time = 3.0 h			
J-208-212	**J-208-412**	**J-208-612**	**J-208-812**
Time = 24.0 h			
J-208-412	**J-208-612**	**J-208-812**	

A clear separation can be seen in the J-208-212 sample in three hours. This trend continues and is much more dramatic in 24 hours. As seen above, some emulsions split after three hours and aren't compatible with this type of emulsion. This test produces very interesting results. More importantly, this test shows how slight modifications to the silicone polymer can produce an inverse emulsion, as in the case of the J-208-412 and standard emulsions, J-208-812. This shows the versatility of the silicone polymers; it is possible to rapidly modify the polymer to produce a good emulsifier for both inverse and regular emulsions.

4.2.3.22 GREENING WITH SILICONE

There has been a great deal of interest in "green chemistry." While the definition of "green" can be open to interpretation, the desirability to use sustainable raw materials in our products is clearly a goal.

But in addition to being made from sustainable raw materials, formulations need to meet their expectations. The goal therefore is obtaining formulations with the maximum content of sustainable raw materials, with highest performance, that are cost effective and aesthetically appealing—Ah, this is the holy grail. Clearly, a balanced approach is required and totally green is a destination; this is a journey not a one-step process.

The majority of consumers will always consider cost, performance, and impact on human health as the critical primary considerations and, in many instances, the deciding factor in consumer acceptance. However, this alone is not enough.

While the concept of green products is straightforward, the ability for the formulator and the consumer to quantify the greenness of a given shampoo or other consumer product is elusive. Given a proper understanding, the consumer and formulator can make better-informed and better-educated decisions about the best combination of green properties and formulation attributes for their products. In other words, the needs of the consumer, the needs of the environment, and conformation with regulatory guidelines can be intelligently determined. All too often, the determination of the greenness of a raw material or formulation is more an emotional than a scientific decision and requires either an all-or-nothing approach to environmental stewardship. Simply put, materials are either green or they are not. The consumer's demand for many formulation benefits cannot be achieved with all green ingredients; therefore, "non-green" products are required. The consumer then needs a systematic approach to develop a measurable metric for the level of greenness in a formulation and trade off some greenness for performance. This quest has resulted in the development of "Green Star Rating" defined in U.S. Patent Application **20090259409** system, or simply "GSR."

The Green Star Rating is calculated by taking the Green Star Value and multiplying it by the amount of actives in the product (% solids). To obtain the Green Star Rating for a formulation, simply add up the contributions by each of the raw materials. Once this number is known, the effect of replacing one ingredient in a formulation with a "greener" compound can be ascertained. Specifically, if a raw material used in a formulation at 20% by weight with a Green Star Rating of 1 is replaced with a product with a Green Star Rating of 7, the impact on the formulation is (7–1) times 0.20 or 1.2. This means that many more renewable resources are being used in the formulation and its consumption of this product will have a less negative impact on the environment. This approach allows the formulator to make greener products, with a higher Green Star Rating, and allows the consumer to choose these greener products.

The Green Star Rating is determined using the following steps:

(1) Determining the empirical formula for chemical compounds used to make formulated products;
(2) Determining which portions of the molecule are green;
(3) Determining the percentage by weight of the green portion of the molecule;
(4) Determining the green star value and optionally;
(5) Optimizing the formulation by selecting components with the greatest green star value.

Hair Glosser

Formulation 14.0.1

Material	**Concentration (% wt)**
Cyclomethicone and Dimethiconol	95.0
Argon Oil	5.0
Green Star Rating:	**0.5**

Formulation 14.0.2

Material	**Concentration (% wt)**
Olive Oil	80.0
Octyldodecyl Citrate Crosspolymer	15.0
Argon Oil	5.0
Green Star Rating:	**10.0**

Formulation 14.0.3

Material	Concentration (% wt)
Olive Oil	80.0
Octyldodecyl Citrate Crosspolymer	14.0
Argon Oil	5.0
Behenyl dimethicone / Vinyl Dimethicone Crosspolymer	1.0
Green Star Rating:	**9.9**

The above formulations were evaluated on hair tresses for softness, gloss, and spreadability on a scale of 1–5 (5 is best).

Table 14.0.4

Property	Formula 10.1	Formula 10.2	Formula 10.3
Softness	3	4	5
Gloss	3	4	4
Spreadability	4	3	5
Total	10	11	14
GSR	**0.5**	**10.0**	**9.9**

The analysis above would need to be evaluated to pick the product that is overall best for the market or markets. If the objective is an all-green product, then Formulation 10.2 would be the winner. If the market calls for strictly on performance and not on green, best overall product is desired Formulation 10.3.

a. Summary

While the term "green chemistry" might not be the best to describe this new approach to chemistry, it is a step in the right correct direction. What is clear from this is that a formulation with 99.99% renewable resources is better than one with 99.99% nonrenewable materials. The formulation of products for the personal care market requires consideration of many factors, including, but not limited to: maximum green content, highest performance, optimal cost effectiveness, and most esthetically appealing product. With that in mind, the incorporation of small amounts of an effective organosilicone into a cosmetic or personal care product to give that product optimal performance may be the future of silicone chemistry.

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SILICONE ELASTOMER APPLICATIONS

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ABSTRACT

Silicone elastomers are a broad class of cross-linked dimethicone polymers that form three-dimensional polymeric structures and provide a beneficial texture and appearance to cosmetics. They are generally formulated into products to enhance consumer appeal by yielding a preferred sensory profile, while functioning for example as anti-aging ingredients such as that of a line filler and soft-focus particle. They also can act as sebum absorbers to reduce excess oil on the surface of the skin and act as mattifying agents, which is just another way to say they make the skin less shiny. Further, these cross-linked dimethicone polymers are capable of delivering actives by acting as controlled-release polymers and can also reduce the oily texture of high-oil-content formulas. Several formula examples are provided to show how silicone elastomers may be co-formulated with common ingredients such as sun protection actives and pigments in order to provide elegant daily wear cosmetics or lotions.

Having described in great detail the varying structural types of silicone-based elastomers and gels in a previous section of this book; we come now to a description of their usefulness in cosmetic and personal care products.

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4.2.3.21 SILICONE ELASTOMERS FOR IMPROVED CONSUMER ACCEPTANCE OF A PRODUCT: ACHIEVING THE "WOW" EFFECT

There is always opportunity to offer products that make people say "wow" (sensory elegance) while enhancing the delivery of actives that promote skin health, beauty, and the perception of ageless elegance. As described previously, silicone elastomers are a broad class of cross-linked dimethicone polymers that form a three-dimensional structure and can provide a sought-after texture and appearance to cosmetics. The key to achieving the optimal structure is to create an interstitial pore volume, defined by the cross-link density of the silicones. This elastic volume can be swollen (filled) with a solvent or even a small active material for obtaining enhanced delivery attributes. Depending on the chemistry and process to make them, silicone elastomers may be called silicone crosspolymers, dimethicone crosspolymers, dimethicone/vinyl dimethicone crosspolymers, or Polysilicone-11 (CTFA designation). Altogether, this class of cosmetic ingredients provides a wide range of elegant textures and promotes compatibility with other commodity ingredients. They are generally formulated into products to enhance consumer appeal by yielding a preferred sensory profile, while functioning as a line filler, sebum absorber, soft-focus particle, and mattifying agent. Higher-end cosmetic creams and lotions may contain a significant percentage of silicone elastomer, as this type of formulation helps create a positive consumer feedback loop that reinforces the performance and value associated with a brand. Silicone elastomers (in all their wide varieties) are available as loose powders, as swollen mixtures in cosmetic solvents, and as dispersions in water. The basic polymers can be further compounded into anhydrous and aqueous "formula bases" to help remove the need for later homogenization and/or to help take the guesswork out of the correct emulsifier blend required for stabilizing silicones in a formula.

The name "elastomer" is associated with this class of materials because the primary polymer shares many of the elastic qualities of rubbers, including medical-grade silicone rubber. One distinction we would point out is that the final size of the cosmetic-grade silicone elastomer is on the micron scale, typically on the order of 5–50 microns. This sizing property provides a small enough material to be

generally invisible on skin, but large enough (nano-free) to never be able to penetrate the dermis. This type of material is known for being ultra-mild, breathable, and nonocclusive. It can also be used to deliver soluble actives when the elastomer interstitial pore size is matched to the size of the active ingredient of choice.

A linear polymer like dimethicone is a versatile material that helps reduce drag, provide some silicone texture, and form a breathable protective barrier film on the skin. Unlike silicone elastomers, dimethicone is not promoted as a mattifying ingredient since it really does not absorb sebum and is actually glossy in appearance.

The main difference between the linear dimethicone and a cross-linked dimethicone elastomer is that when solvents are introduced into the product, linear polymers become soluble, whereas elastomers swell or expand, and do not lose their semi-spherical shape. This difference in behavior allows solvents, sebum, and actives to be transported in and out of the interstitial pore area. When volatile solvents are used in the formula, they disappear from the material upon dry-down and leave an invisible elastomeric sponge behind. This sponge is capable of soaking up all of the excess sebum produced by the skin to provide a desirable, continuous matte appearance throughout the day.

As described previously, the chemistry of forming an elastomer or crosspolymer can be extremely varied, but most commercially available elastomeric silicones share at least one common attribute in that an existing dimethicone polymer containing a reactive hydrogen "methicone" site is cross-linked with a terminal divinyl dimethicone or an organic diene to form rungs like a ladder across two or more polymers. The net result is a three-dimensional structure that is able to absorb or release solvents and actives as generalized in Figure 1.

Figure 1: Generalized dimethicone crosspolymer prepared with vinyldimethicone cross-links.

When optimized in production, this reaction is processed into a soft material that presents a unique "silky" texture that only silicone elastomers are able to provide. Each manufacturer has proprietary methods and recipes to create their exact type of product required. There is a wide choice available in terms of cross-linkers and divinyl compounds that commercially yield a nearly limitless variety of elastomers with different cosmetic attributes.

Less common varieties of silicone crosspolymers can also be formed by cross-linking pendant sulfur groups on dimethicone in the presence of catalysts like titanium dioxide, zinc oxide, or hydrogen peroxide, or by cross-linking polymethylsiloxanes (dimethicones) by a tin catalyst. Higher alkyl groups can be substituted for methyl groups in a wide variety of ways to promote compatibility with hydrocarbon solvents and alkyl esters. Likewise, ethoxy and/or propoxy (EO/PO) copolyol functional groups can be added for self-emulsifying properties.

4.2.3.22 COSMETIC ATTRIBUTES – TEXTURE AND OIL CONTROL (MATTIFYING)

Even small amounts of oil (sebum) on your skin can produce a shiny or uneven appearance. An overproduction of sebum results in oily skin or oily zones (combination skin) where the oil is more localized. The "T-zone," which is the facial area across the forehead and down the nose and chin, is a problem area that typically benefits from the use of oil-control products. Oil-control products are designed to counteract sebum production by soaking up oil and maintaining a soft, matte appearance.[1] An absorbent is a class of ingredients that can pick up and hold liquids. Small hard talc, minerals, and other polymers can act to absorb sebum on the surface. Among these ingredients, silicone polymers are exceedingly mild and safe on skin, they have no color or odor, they are highly oxidatively stable (even when heated), and they do not promote free radical formation under UV exposure. Moreover, consumers are able to discern the improvements in sensory properties and oil control provided by formulas containing silicone elastomers with mattifying properties, and this attribute set is directly linked to their popularity in cosmetics.

Sebum absorption, polymer swelling, and the pore volume (interstitial area) of an elastomer depend on the number (cross-link density) and polymer length of the dimethicone and divinyl dimethicone chains. A high cross-link density produces a hardened product that has less freedom to expand, even in the presence of a good solvent. Lightly cross-linked polymers create a flexible product with more solvent-absorbing capacity, but offers less refined texture. The perfect combination of polymer chain length and cross-link density provides ideal expansion, oil absorption, and a smooth texture on skin. If the combination is close, but not perfect, pilling may occur during rubbing as well as dry-out to give a visible powdery texture that is undesirable. A silicone elastomer with the INCI name Polysilicone-11 is a

highly refined combination of the above attributes that is offered in a wide variety of solvent combinations for tailoring the application.

Typical solvents combined with silicone elastomers are cyclomethicones (which have continued to receive safe-as-used opinions from recent Canadian CIR and EU-based SCCNNF reviews[2]), dimethicones, alkanes (dodecane and coconut alkanes), and other compatible emollients. Cyclopentasiloxane is the most widely used solvent, as it helps to loosen sebum and ultimately volatilizes off, thus exchanging cyclopentasiloxane (D5) for sebum in the elastomer space.[1] This solvent exchange leads to extended mattifying effects. Likewise, volatile and semi-volatile dimethicones can be used in place of cyclomethicones (along with actives, colorants, and sunscreens) to be used in elegant daily wear formulations as shown in the examples below.

4.2.3.23 EXAMPLE COSMETIC FORMULAS WITH SILICONE ELASTOMERS

	FORMULA	UV SHIELD/REPAIR BB CREAM, SPF~15	
Phase	**Ingredient**	**INCI**	**%**
A	GRANSIL DMG-3	Dimethicone & Polysilicone-11	10.000
	DIMETHICONE, 5CST	Dimethicone	8.000
	GRANSURF 67	PEG-10 Dimethicone	2.000
	GRANSURF 50C-HM	Dimethicone & PEG/PPG-18/18 Dimethicone	2.500
	GRANSIL 530	Dimethicone	1.000
	UV CUT TIO2-55-CG	Titanium Dioxide & Caprylic/Capric Triglyceride & Stearic Acid & Alumina & Polyhydroxystearic Acid	15.000
	PROTACHEM CTG	Caprylic/Capric Triglyceride	3.000
	EUXYL PE 9010	Phenoxyethanol & Ethylhexylglycerin	1.000
	BENTONE 38V	Disteardimonium Hectorite	1.000
B	SUNCROMA YELLOW IRON OXIDE	Iron Oxide	0.726
	SUNCROMA RED IRON OXIDE	Iron Oxide	0.218

	SUNCROMA BLACK IRON OXIDE	Iron Oxide	0.072
	RBTD-MS3	Titanium Dioxide (CI 77891) & Methicone	3.632
	SERICITE DNN	Mica	3.632
	DIMETHICONE, 5CST	Dimethicone	4.140
	GRANSURF 67	PEG-10 Dimethicone	1.380
C	DEIONIZED WATER	Water	30.700
	GLYCERINE	Glycerin	4.000
	BUTYLENE GLYCOL	Butylene Glycol	2.000
	GRANACTIVE 1518	Water & Polysorbate 20 & Pentapeptide-21 & Morus Alba Root Extract & Resveratrol & Scutellaria Lateriflora Extract & Hydrangea Arborescens Root Extract & Phenoxyethanol & Capryl Glycol & Potassium Sorbate & Hexylene Glycol	5.000
	SODIUM CHLORIDE	Sodium Chloride	1.000
		Total:	100.000

Procedure:

1. Combine Phase A in the main kettle and homogenize at 1000 to 1500 rpm until uniform.

2. Combine Phase B and run ball mill until the drawdown passes 10 micron on Hegman Gauge.

3. Combine Phase C in the support kettle and mix until the solids are dissolved.

4. Add Phase B into the main kettle while homogenizing and continue mixing for at least 20 minutes.

5. Add Phase C very slowly to the main kettle while homogenizing. At the end, increase to speed to 1500 to 2500 to finish the batch.

The wide variety of solvent options available in combination with silicone elastomers ultimately allows consumers to pick the cosmetic products best suited for their skin type. One typically applies these products before putting on the rest of your makeup, but many of them can be reapplied over makeup throughout the

day without having any detrimental effects. As we described earlier, talc and silicone powders are popular products for oil absorption and slip. The example formula below shows how a combination of silicone elastomer (Polysilicone-11) and silicone powder (polymethylsilsesquioxane) can be emulsified into a very smooth-feeling serum. This formula offers the flexibility of further designing in additional choices of oil-soluble and/or water-soluble actives (where applicable) without losing the premium texture profile.

	FORMULA	**SMOOTH W/S SERUM**	
Phase	**Ingredient**	**INCI**	**%**
A	GRANSIL DM-5	Dimethicone & Polysilicone-11	20.000
	DIMETHICONE, 5CST	Dimethicone	20.000
	JEECHEM ISP	Isopropyl Palmitate	5.000
	GRANSIL PSQ	Polymethylsilsesquioxane	5.000
	GRANSURF 67	PEG-10 Dimethicone	1.500
	GRANSURF 50C-HM	Dimethicone & PEG/PPG-18/18 Dimethicone	0.500
	PROTASORB O-80	Polysorbate 80	0.700
	VITAMIN E ACETATE	Tocopheryl Acetate	0.200
	EUXYL PE 9010	Phenoxyethanol & Ethylhexylglycerin	0.600
B	DEIONIZED WATER	Water	35.500
	BUTYLENE GLYCOL	Butylene Glycol	10.000
	CARBOPOL ULTREZ 10 POLYMER	Carbomer	0.600
	SODIUM HYDROXIDE, 25% AQ SOLUTION	Water & Sodium Hydroxide	0.400
		Total:	100.000

Procedure:

1. Combine Phase A in the main kettle and mix until uniform.
2. Combine Phase B in the support kettle and mix until no lumps.
3. Add Phase B into A while mixing and mix until uniform.

4.2.3.24 SUMMARY

Silicone elastomers are three-dimensional polymeric structures that can be combined in a delivery solvent of choice to provide premium texture- and oil-control attributes that are recognized by consumers in top-selling cosmetics around the globe. They act as line fillers, soft-focus particles, sebum absorbers, mattifying agents, texture modifiers, and controlled-release polymers. The can be formulated in most common types of cosmetic creams and lotions, including pigmented cosmetics, and originally impacted the industry in oil-control foundations and other products where enhanced mattifying and sensory properties are highly desired. In many respects, silicone elastomers are able to further enhance a basic formula such that it is more able to meet a higher level of consumer acceptance in order to reinforce or couple together brand performance and brand loyalty.

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ABSTRACT

It is well known that whitening products represent a very large category for skin care in Asia , especially in Japan where beauty products feature the function of Bihaku (beautiful white). Eastern women tend to have yellowish skin, so whitening in Asia is equivalent to anti-wrinkles for Caucasians. A light complexion was considered as a sign of elegance among nobles, educated people, and members of the higher classes in China since ancient times. As far back as the ancient Qin Dynasty (221–205 BCE), there have been records on Chinese medicines that have beauty functions. For example, there were records about using Bai Zhi (*Angelica dahurica*) in facial cream during this period. In the later Tang Dynasty skin care grew in popularity.

Like the women in ancient China, women in ancient Japan were also concerned about maintaining a white skin. No wonder that the faces of Japanese women are white in the drawings and paintings. More recently, in the last half-century, there appeared the first whitening powders in Japan and 20 years ago such products became a real boom. Along with this evolution came some regulation such as Quasi-Drug in Japan but also more sophisticated mechanism-based products beyond the classic anti-tyrosinase activity. This chapter looks at the evolution of the approach to whitening in the last 50 years and then explains the mechanism of action of the different active families and specific ingredients within those families. It also covers lightening of dark spots and moves from the basic molecules through more and more complex molecular ingredients and finally to modalities and possibilities based upon molecular biological approaches.

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4.2.4.1 HISTORIC EVOLUTION OF WHITENING PRODUCTS IN JAPAN

In this chapter we present a unique view of the evolution of the category of skin whitening from the point of view of company and product introductions during each decade beginning with the 1960s.

In the 1960s, the first whitening powders appeared in Japan, for example, Soie de Reine Beauty C powder by Kanebo in 1966. The product contained an oil-soluble form of vitamin C and the goal was to provide a safe alternative to the bleaching action of hydrogen peroxide.

In the 1970s, the first whitening product series appeared with the introduction of Real Beauty (Kanebo) and Natural Shine (Albion) in 1971, and Freshure (Shiseido) in 1976.

In the 1980s, new lightening ingredients were developed and used in Lumiera (Pola) in 1980; Faircrea (Kanebo) in 1984 and in 1985 in UV White (Shiseido) and Sekkisei (Kose). This is also the time where the product form changed from a powder to an essence introduced by Estée Lauder (Swiss Whitening) in 1986, Kose (Sure White) in 1988—the year when Sansho Seiyaku obtained regulatory approval by the Japanese Ministry of Health, Labour, and Welfare (MHLW) to use kojic acid as quasi-drug (a short list of regulated actives used by skin care companies to use the claim "whitening")—and Kanebo in 1989 (Kanebo Blanchir). An essence corresponds to a serum in occidental countries: a water-based thin emulsion.

In the 1990s the skin-whitening category began to boom and extend its reach to the mass market as younger customers were targeted. Shiseido launched Whitess Essence (with arbutin), Kanebo launched Freshel White C (with "penetrating" vitamin C), and Kao launched Shokubutsu (with vitamin C as well). Closer to the

year 2000 the lines were again altered to new forms of products such as wipe-off lotion-type and soap-type products for cleansing, which performed by removing old keratinocytes.

After creating new type of products in the beginning of the 21st century, the trend has been to create more exclusive quasi-drug (QD) actives for lightening skin. This approach was accelerated in 2001 due to the BSE (Bovine Spongiform Encephalopathy) issue. The largest companies: Kose, Lever, Pola formulated out the bovine placenta due to this "mad cow disease." Shortly after Kojic acid was introduced as a QD in 1988. During that period this ingredient fell into disrepute due to alleged safety issues concerning possible carcinogenic effects (not confirmed since then). In view of this setback, most companies were condemned or limited to use vitamin C derivatives, with tyrosinase inhibition as the targeted activity. At present, the cosmetic industry is looking for exclusive ingredients from green and sustainable sources (preferably plants) that have a novel mechanism for skin whitening. Thus, there is a significant opportunity for ingredient seekers and formulators of novel, safe skin-whitening products that tie into the exploding demand by consumers for green and sustainable resources.

4.2.4.2 WHAT IS A WHITENING QUASI-DRUG?

It is not possible to talk about skin whitening without explaining what is the meaning of a whitening quasi-drug in Japan. A quasi-drug (QD) refers to the food, cosmetic, and drug standard regulated by the Japanese Ministry of Health, Labor, and Welfare (MHLW) under the Pharmaceutical Affairs Law. JSQI (Japanese Standard of Quasi-Drug Ingredients) and QD JSQI are the official standards set by the Japanese government for ingredients to be incorporated into final QD products, and labeled QD to advertise that the product is a government-approved medicated quasi-drug. This QD status has become a powerful marketing tool for many Japanese and foreign firms in the Japanese market. Obtaining QD status requires time and monetary investment, thorough documentation, and rigorous testing of safety and efficacy, all of which is regulated and approved by the MHLW. The efficacy of a QD is typically milder than that of a pharmaceutical drug, but has been proven effective in a specific claim category as either an excipient (additive) or an active at a specific dosage.

For skin whitening, this category in Japan is created for functional actives that will prevent hyperpigmentation or improve hyperpigmentary disorders. Most of the time the whitening products target an enzyme called tyrosinase. Tyrosinase is an enzyme needed for the production of melanin.

The following is a list of whitening QD in Japan in order of approval (from earliest to most recent) and it includes a description of their mode of action:

Bovine placenta:

This is a QD active that has been very popular and long used along with the use of vitamin C and its derivatives. The main source of placental extract was bovine, but it could also come from porcine origin, which has been recently used to replace the bovine due to the BSE issue. BSE was thought to be transmitted to human beings. The form of this disease in humans is the Creutzfeldt Jakob Disease, which killed numerous humans in the UK. Hatae et al. (1) and Ito (2) proposed, in the *Fragrance Journal of Japan*, the mode of action of placental extract as being an increased cell turnover to remove the melanin from the upper layers of the skin, or the role of high concentrations of minerals and amino acids, to reduce melanin synthesis.

Vitamin C (Ascorbic acid) derivatives:

Vitamin C and its derivatives are by far the most popular skin-whitening ingredients in use in Japan. Ascorbic acid and its derivatives are antioxidants, and as such they can block, for example, the conversion of DOPA to DOPAquinone; and from DOPAquinone, the melanin synthesis pathways can be blocked since the reaction pathway diverges to produce either eumelanin or pheomelanin (3). Most vitamin C derivatives approved as QD can be used as such by all customers. Some companies have had to obtain regulatory approval for the benefit of others! Takeda obtained the QD status for magnesium-ascorbic acid 2-phosphate in 1988 (use level must be 3%), and Shiseido obtained the QD status for L-ascorbic 2-glucoside in 1994 (use level must be 2%). Much later on, Nikko chemical, a supplier, obtained the QD status for use of tetra-isopalmitoyl ascorbic acid in 2007 (use level must be 3%). This oil-soluble form of vitamin C is easier to use and prevents UV-induced skin pigmentation through its antioxidative properties (4). The first company to introduce a QD product featuring this form was Green Coop in the QD line *Shion.*

Ascorbic acid

Tetra-isopalmitoyl ascorbic acid

Other forms of vitamin C: vitamin C-ethyl, L-ascorbic acid2-phosphate, etc, . . . are on the market but not necessary as QD.

Kojic acid:

The hydroxylation of L-tyrosine to L-DOPA is catalyzed by the action of the copper-containing glycoprotein tyrosinase enzymes located in the melanocyte's melanosome. Molecules chelating copper atoms can reduce the activity of the tyrosinase, and as a result, the melanin synthesis. This is how kojic acid functions as a skin whitener (5), and Shansho Seiyaku Co., Ltd obtained the whitening QD status in 1988.

Kojic acid

Kojic is a QD at the use level of 1%; Mishima et al. evaluated the molecule in clinical tests to treat freckles, age spots, and post-inflammatory hyperpigmentation (6). Due to some concern about the potential carcinogenic effect, the MHLW (Ministry of Heath, Labour, and Welfare) started in March 2003 to discourage companies to use Kojic acid. Although they somewhat came back on their decision, most companies remain hesitant to resume its use and prefer to consider alternatives.

Arbutin:

Arbutin was submitted by Shiseido to the MHLW and successfully obtained QD status in 1989 at the use level of 3%. Arbutin is found in the leaves of cowberry and is a derivative of hydroquinone.

Arbutin Cowberry

According to K. Maeda and M. Fukuda, arbutin is a naturally occurring beta-D-glucopyranoside of hydroquinone and is effective in the topical treatment of various cutaneous hyperpigmentations characterized by hyperactive melanocyte function. They report that arbutin inhibits the tyrosinase activity in cultured human melanocytes at non-cytotoxic concentrations. Melanin production is inhibited significantly by arbutin, as determined by measuring eumelanin radicals with an electron spin resonance spectrometer. The study of the kinetics and mechanism for inhibition of tyrosinase confirms the reversibility of arbutin as a competitive inhibitor of this enzyme. The utilization of L-tyrosine or L-dopa as the substrate suggests a mechanism involving competition with arbutin for the L-tyrosine binding site at the active site of tyrosinase. These results suggest that the depigmenting mechanism of arbutin in humans involves inhibition of melanosomal tyrosinase activity, rather than suppression of the expression and synthesis of tyrosinase.

Ellagic acid:

Lion applied for ellagic acid, a polyphenol, for whitening QD application at 0.5%. Approval was granted in 1996.

Ellagic acid

The discovery of ellagic acid was made by the chemist M. Chevreul in oak galls and more precisely studied by M. Braconnot in 1831. Ellagic acid is found in oak species like *Quercus alba* and *Quercus robur*. The green alga *Myriophyllum spicatum* produces ellagic acid; so too does the medicinal mushroom *Phellinus linteus*. However, the highest levels of ellagic acid are found in blackberries, cranberries, pecans, pomegranates, raspberries, strawberries, walnuts, wolfberries, and grapes. Considering the growing popularity of these fruits and nuts as sources for green and sustainable cosmetic actives, ellagic acid may have considerable room for growth in the skin-whitening area.

The mode of action is again (like kojic acid) copper chelation.

Chamomilla extract:

Kao, in 1998, obtained the QD status at 0.5% for this anti-inflammatory. This is quite unusual, as there is only one plant extract approved as QD so far and the mechanism does not involve tyrosinase. In the proposed mechanism of action for

chamomilla extract, cells communicate with each other and, once activated by UVB, the keratinocytes produce alpha-MSH and endotheline, which will bind to specific receptors in the *stratum corneum* and activate the melanocytes. Chamomille extract acts as an antagonist of this receptor and therefore reduces the "call" for melanin production.

Rucinol (4-n-Butylresorcinol):

Pola obtained a QD status for Rucinol at 0.3% in 1998. 4-n-butylresorcinol, is a resorcinol derivative that has an inhibitory effect on both tyrosinase and tyrosinase-related protein-1 and -2, and is featured in BA Grand Luxe or Whitissimo White Shot Spots, clear products of Pola as examples.

4-n-butylresorcinol

Linoleic acid:

Linoleic acid

Linoleic acid (LA) is an unsaturated omega-6 fatty acid called 18:2(n-6). Chemically, linoleic acid is a carboxylic acid with an 18-carbon chain and two "cis" double bonds; the first double bond is located at the sixth carbon from the omega end. This fatty acid can be obtained from hydrolysis of vegetals (such as safflower, evening primrose, or kukui seed oils). Linoleic acid (omega-6) is located in the cell membranes and can therefore decrease the tyrosinase level and accelerate tyrosinase degradation. This results in less melanin being produced, and an accompanying whitening effect on the skin.

Sunstar obtained the QD status for Linoleic acid at 0.1% in 2001.

Tranexamic acid:

Tranexamic acid

Tranexamic acid is a drug used to treat melasma. It is proven to inhibit UV-induced hyperpigmentation and melanocyte activity (lightening skin). This is especially true in people with melisma, or dark spots, where the pigment-suppressing abilities of tranxemic acid works best on localized areas of irregular melanin distribution. Tranexamic acid prevents the binding of plasminogen (originating from endothelial cells), to keratinocytes, which is thought to be a possible mechanism for melasma treatment.

This material is featured in Shiseido's Haku Melanofocus, and Shiseido is the company who obtained QD status for tranexamic acid in 2002.

4-Methoxy potassium salicylate (4 MSK):

The following year, in 2003, Shiseido obtained one more exclusive QD approval with 4 MSK; the mode of action is tyrosinase inhibition, and this whitening ingredient is featured in Clé de Peau la Crème manufactured by Shiseido.

4- MSK

Adenosine monophosphate disodium salt:

This active is QD when used at 3% and the status was obtained by Otsuka Pharmaceuticals Co. in 2004. This AMP salt acts as a fuel to the cells. They therefore produce more energy and, as a result, the epidermal turnover is increased and the cells containing melanin will exfoliate faster. This reduces the melanin content in the skin.

Adenosine monophosphate disodium salt

Dipropyl-biphenyl-2,2'-diol (Magnolignan):

This compound belongs to the family of polyphenols; it looks like the magnolol of magnolia. This active was approved for QD at 0.5% and was submitted for QD approval by Kanebo in 2005.

Magnolignan retards or slows down the maturation of tyrosinase.

4-HPB: 4-(4-Hydroxyphenyl)-2-butanol:

This active is a competitive inhibitor of tyrosinase. It can be found in white birch and Nikko's maple extract. The material is also known as rhododendrol.

4-(4-Hydroxyphenyl)-2-butanol

Kanebo, again, obtained approval for this molecule as QD in 2007.

Tranexamic acid cetyl ester hydrochloride:

Partnering with Nikko chemical, Chanel was the first non-Japanese company to gain approval for a new whitening molecule in 2009.

The mode of action is similar to the tranexamic acid used by Shiseido.

This ingredient is the most recent approval in Japan.

From a review of the information presented previously it is obvious there has been an accretion of approvals for ingredients that lighten skin. The year 2007 was special, as two molecules got approved! It is anticipated that the approved list will grow longer in the coming years, as this category is very important in Japan and Asia in general.

4.2.4.3 WHAT ARE THE QUASI-DRUG ADDITIVES?

Whitening is not just quasi-drug, there are also QD additives that are important to create a bouquet, which means a multifaceted approach to treat the question of whitening at different levels and a multifunctional approach in treating skin pigmentation. This is illustrated in the following schematic, which also summarizes

the various mechanisms of action and discusses the different types of strategy to reduce melanin synthesis, distribution, etc.

a. - In the melanocytes

Tyrosinase

Tyrosinase has been an early target for the whitening products. In molecular biology, tyrosinase is mainly involved in two distinct reactions of melanin synthesis; firstly, the hydroxylation of a monophenol and secondly, the conversion of an o-diphenol to the corresponding o-quinone. o-Quinone undergoes several reactions to eventually form melanin. Tyrosinase is a copper-containing enzyme present in the skin that catalyzes the production of melanin from tyrosine by oxidation.

As oxidation is a key to effective whitening, strong antioxidants are candidates to inhibit tyrosinase activity, and this is one of the functions of ascorbic acid derivatives. However, plant extracts are also used for their antioxidant properties, especially licorice root extracts as they contain glabridin, a strong antioxidant molecule:

Many cosmetic products in Asia contain licorice extracts for whitening.

Another molecule that has antioxidant properties is an amino acid called ergothioneine:

Ergothioneine

This molecule is a copper chelator and tyrosinase is copper-dependant. As a result, ergothioneine is an interesting molecule for achieving skin whitening, along with ellagic acid and kojic acid.

Arbutin and 4 MSK are examples of actives with competitive inhibition to tyrosinase.

Yet another strategy is to inhibit tyrosinase maturation or tyrosinase synthesis; in addition to magnolignan one can consider a zinc glycine complex:

This molecule was tested at 1% in an *in vivo* study and showed very good whitening effects in two weeks. Not only does this complex reduce the synthesis of tyrosinase but it also induces the synthesis of an antioxidant protein, metallothionein, a rich peptide with copper-chelating properties.

The most recent approach is to consider an action on MITF (microphthalmia-associated transcription factor) and to down-regulate this gene so the tyrosinase is not activated.

MITF is the transcription factor for tyrosinase (TYR) and tyrosinase-related protein 1 (TYRP1), which plays a key role in melanocyte development. MTIF binds to a symmetrical DNA sequence (E-boxes) (5'-CACGTG-3') found in the tyrosinase promoter.

MITF down-regulation can be achieved with a water extract of an alga called *Dictyopteris membranacae*.

melanosomes

This extract also down-regulates OA1, the gene regulating the number and size of melanosomes as well as MART1, the gene responsible for the maturation of

melanosomes and MLPH, the gene responsible for the migration of melanosome. Tested at 3% *in vivo* on Asian skin, the reduction of skin pigmentation was as high as 36% in three months.

b. - In the keratinocyte

Here the strategy is to limit the communication between the keratinocytes and the melanocytes. UV stimulates keratinocytes (and can break their DNA); therefore keratinocytes look for protection, they "ask" the melanocyte to produce melanin to be used as a "cap" for the next time keratinocytes are exposed to UV. The melanin will be carried in the melanosome to the "fingers" of the melanocytes, the dendrites, and given back to the keratinocytes.

The way keratinocytes signal their need for melanin is through alpha MSH messenger and endothelin messenger. These messengers find receptors on the membrane of the melanocyte and trigger the making of melanin. Undecylenoyl phenylalanine is a molecule that will act as alpha-MSH antagonist—no stimulation of the melanocyte eventually translates into less melanin production and a lightening action in the same way, but this time by preventing the endothelin adhesion. Ascophyllum nodosum extract is an algae extract that can block endothelin adhesion to the membrane of the melanocyte. *In vivo* study has confirmed the whitening effect of this active.

There is also another new target: POMC for achieving skin lightening. POMC is activated by the cytokines released by the keratinocytes under UV. Once POMC is activated, alpha MSH and endothelin are released. The down-regulation of POMC will stop the communication between keratinocyte and melanocyte.

c. - In the nerves—a new approach to the reduction of dark spots

As if all these interesting biochemical approaches are not enough to stimulate new ideas for skin whitening products, Codif international discovered that nerves also stimulate the melanocytes and increase the dendricity via a peptide called Substance P. Substance P is well known to be present in areas that are exhibiting pain. In this approach to skin whitening, the concept is called neurowhitening.

An extract of *Pancratum maritimum* (white sea lily or sea daffodil) can down-regulate POMC and reduce the stimulation from keratinocyte but moreover it can decrease the number of Substance P receptors. In other words, this extract prevents the stimulation of melanocytes from the nerves. Substance P receptors, once activated, increase the number of dendrites in the melanocytes. The dendrites are like these arms that the melanocytes use to distribute the melanin to the surrounding keratinocytes; it is reported that one melanocyte can reach out up to fifty keratinocytes:

Skin structure: far-reaching dendrites of melanocytes

Limiting the number of dendrites is especially efficient on age spots when used at 1.5% in three months.

Skin pigmentation is a complex issue and multi-tiered problem. There are various options upstream via limitation of melanocyte activation. There is yet another road to explore: the melanin is produced, wrapped up in melanosome, and sent to the dendrite—ready to be delivered. Here an enzyme is critical: PAR-2. If PAR-2 is blocked, the melanin will not be transferred.

The goal here is to seal off the melanin transfer.

Artemisia capillaris works very well as it is a PAR-2 inhibitor, and as a result it reduces melanin transfer from melanocytes to keratinocytes. Genomic tests were performed to confirm that Artemisia inhibits PAR-2.

Artemisia is a good addition to a multifunctional bouquet of actives that are needed to achieve a whitening effect in a cosmetic formula.

Tests have been performed at different levels, first of all in cell culture and pseudo melanin. Where Artemisia is used to reduce melanin transfer into the keratinocytes, there is less fluorescence. As seen hereafter:

Control (no Artemisia)

With 0.67% Artemisia (melanin inhibition is visible)

Further tests were run on skin models with UV applied. Melanin is still produced, but where Artemisia is used the melanin does not migrate to the surface, as seen hereafter:

Control (no Artemisia—melanin migration) 500 µg/ml Artemisia (limited migration; melanin stays at the bottom)

The same test was performed on the skin, and there was no tanning response when Artemisia was used at 2%, three times a day for three weeks. Tests were performed by Maruzen Pharmaceuticals in Japan.

d. - For the corneocytes

The last cells to target are at the very surface of the skin: the corneocytes. The goal here is to accelerate the epidermal turnover and with the desquamation the melanin will go away. To do so, varying chemical exfoliators are usually used: lactic acid, retinol derivative, and retinol-like glucosamine derivatives.

Another elegant approach to skin whitening is to increase the expression of the protein of desquamation, a group of serine proteases called kallikrein, which are capable of cleaving peptide bonds in proteins. The names of these human desquamation enzymes are KLK5 (*stratum corneum* tryptic enzyme) or KLK7 (*stratum corneum* chymotryptic enzyme), and certain marine exopolysaccharides are increasing these enzyme expressions.

Chemical exfoliation or enzymatic desquamation could also be combined with an active working at the melanocyte and/or keratinocyte level to enhance skin-whitening activity.

CONCLUSION

Whitening or skin lightening is a very important category in skin care and is taken very seriously in Asia, as this is one of the most important signs of aging there. For almost 50 years now the category of Quasi-Drug has been used to allow the safest and most efficient active, but for many years the choice was very limited to placenta, ascorbic acid derivatives, and kojic acid. In the last ten years there have been are six new molecules approved for QD application, but each is exclusive and limited by the company that applied for the ingredient patent. As the

process takes minimum of five years and can cost up to 5 million dollars, the bar to achieving success is very high. In the coming years new molecules are likely to be approved—of different categories: peptides, polysaccharides—and with new targets. It will remain a vibrant category.

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MARINE INGREDIENTS FOR SKIN CARE: AN OCEAN OF RESOURCES

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ABSTRACT

What are algae? Something greenish-brownish, slimy, smelly, and attached to the rocks when the tide is low . . . slippery if you walk on it and after all, not that clean. This was yesterday's perception.

We are far removed from this perception nowadays when we think about the ingredient space occupied by the Marine Ingredients for Cosmetics category. Today, in cosmetics and personal care ingredients, one can chose from active fractions of large algae, polysaccharides from planktonic origin, or actives from plants from the coasts of many landmasses around the world. There are numerous ingredients available from marine origin, and they support many of the desirable claims consumers want to see when they buy their cosmetic and personal care products. In this chapter, we present an overview of marine ingredients and point out their value to the cosmetic and personal care formulator.

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4.2.5.1 MARINE SOURCES OF ALGAE

a. What is an alga?

IN THE BEGINNING . . . it is believed that there were only bacteria on earth. Some of them evolved and had chlorophylian pigments; thus, the first algae were born. They could use the energy of the sun via photosynthesis to produce oxygen (O_2). They were major participants in the production of oxygen surrounding our planet—without algae, life would not have evolved as we know it today. Prehistoric evidence of the presence of the earliest algae is found in the fossils of stromatolites (Figure 1) [1]. These coral-like structures were created by the precipitation of sediments of cyanophycae—also known as the blue algae named *Phormidium*.

There are more than 25,000 kind of algae—there is more difference between a brown alga and a green alga than between the same green alga and a tree in the forest. Blue algae are close in structure to red algae (*Rhodophycae*), which are close to that of brown algae (*Pheophycae*) [2].

Algae are usually in water (but not always). They are different from other plants, as they do not bloom, they have no roots, no stem, and no leaves. All algae have in common a green pigment that provides the chlorophyll and therefore their ability for photosynthesis: (Figure 2) [3] [4].

Figure 1: Stromatolites

Figure 2

The two major categories of algae are: unicellular and microscopic. The latter type comprises an important part of the plankton and macroalgae. Examples of microalgae are: *Anacystis*, *Phormidium* (Figure 3), *Spirulina* . . . and their size can be as small as fraction of a millimeter.

Figure 3: Phormidium

Example of macroalgae are: *Laminaria*, *Palmaria*, *Fucus*, *Chondrus* (Figure 4) . . . and these types can be as long as 40 meters.

Figure 4: Chondrus

Blue algae molecules are used for cosmetic applications: enzymes, such as photolyase, and various types of exopolysaccharide. Macroalgae are rich in polysaccharides, which can be hydrolyzed into bioactive oligosaccharides. This important process will be discussed further later in this chapter.

b. Plants from the shore

The climate condition and soil composition by the shore are not optimal for plants to grow in view of the variation of climatic conditions and the composition of the soil. The plants growing in these areas develop a resistance to high salinity in the face of their repetitive exposure to the tides, and they have developed a resistance to dryness as well [5] [6]. Their adaptive response of the algae to the harsh environment led to production of defensive capabilities and protective secondary metabolites that may be beneficial to skin care applications and offer a significant opportunity for further exploration beyond the encouraging results and products available at this writing.

Figure 5: Salicornia

Examples of coastal plant: Salicornia (Figure 5) and Rock Samphire (Figure 6).

Figure 6: Rock Samphire

c. Other resources from marine origins

Minerals are in fashion in cosmetics, and the water of the ocean is a balanced cocktail of trace elements and minerals (more than 63 elements of the periodic chart have been identified). These minerals can be supplied as salts, or in the form of water including Hawaiian deep seawater, and various waters from the lagoons of Polynesia and the Dead Sea of Israel.

Figure 7: Lithothamnium

Microdermabrasion is also an important field where sea minerals provide an alternative to chemical polyethylene beads. Such materials are opportunities for formulators to investigate the white sands of Bora Bora, the black volcanic sands of Tahiti . . . or back to the algae powders of yellow or blue lithothamnium.

Lithothamnium (Figure 7) can be crushed to different sizes for milder face care or harsher foot care.

4.2.5.2 EXAMPLES OF THE USE OF MACROALGAE IN COSMETICS

a. Moisturization

Macroalgae produce polysaccharides [7]; these large polymers of sugar are in the "skin" of the algae. There is an amazingly striking similarity observed when comparing a cross-section of an alga (Figure 8) with the histology of human skin (Figure 9).-

The polysaccharide polymers present in the algae can retain a good level of moisturization—a result of repetitive exposure to low tides and the accompanying low-moisture environment.

Figure 8: alga skin

Figure 9: human skin

It is interesting to combine the contained polysaccharide polymers from microalgae with amino acids from chlorella and sea minerals from plankton, versus a placebo. With daily application, there is a cumulative, beneficial effect in enhanced moisturization (Figure 10). The combination of amino acids from microalgae with sea minerals strengthens the NMF level to produce an immediate hydration effect. It is observed that with high-molecular-weight marine polysaccharides a second skin forms on the epidermal surface to reduce water loss.

Figure 10: hydration effect of a combination of microalga AA, sea salts, and macroalga polysaccharide

b. Slimming

Marine ingredients can be used for sculpting the body. cAMP is key for activation of the lipase, which will initiate lipolysis. However, various factors limit the expression of cAMP. These include the presence of phosphodiesterase neuropeptide Y binding on its receptor and adrenaline binding to its alpha 3 receptor. Extracts of *Laminaria digitata* can inhibit all these negative factors and favor lipolysis. Figure 11 illustrates this fat-burning effect: pictures 11a and 11b show adipocytes with their fatty content in red. Treatment with *Laminaria digitata* extracts (11b) results in a reduction in the size of adipocyte and elimination of fatty acids. Another brown algae called *Sphacelaria scoparia* acts directly on the key adipocyte differentiation factors. *Sphacelaria* inhibits pre-adipocytes from maturing into adipocytes and reduces the activity of enzymes involved in fat accumulation: they are GLUT 4, the glucose transporter; FAT, the fatty-acid transporters. The pre-adipocyte, a type of mesenchydermal cell, is not allowed to turn into adipocyte and possesses fibroblast-like activity. The algae stimulates collagen I and IV fiber synthesis in adipocytes as well as collagen I synthesis in fibroblasts. This process

results in the reduction in the thickness of adipose tissue and, as a consequence, one can achieve skin remodeling and firming.

Figure 11a: untreated adipocytes

Figure 11b: adipocytes treated with *Laminaria digitata* extract

c. Fountain of youth

Laminaria digitata, an alga contains sugars with specific biological activity. As the fibroblasts age, they change shape, become larger and produce less collagen. Older fibroblasts have markers of senescence such as beta galactosidase as well as chaperone molecules APJ. The application of *Laminaria* extract gives fibroblasts their "young appearance," the senescence factor disappears but the chaperone molecule remains. Collagen synthesis of mature skin then returns to the same condition as found in young skin. Skin is tightened and wrinkles are filled in.

Figure 13: anti-wrinkle effect of a Laminaria digitata extract, before (left) and after (right) 28 days of daily application.

Tested *in vivo*, wrinkles are reduced (Figure 13) and the volunteers appear younger in appearance. So the rejuvenation effect at the cell level translates into rejuvenation of people's skin as well.

d. Oligosaccharides

Polysaccharides of marine origin can be severed, on a molecular level, into smaller oligomers called oligosaccharides. There are polysaccharides found in the

membrane of *Laminaria* with a repetitive structure of mannuronic acid (M) and guluronic acid (G) (Figure 14).

Figure 14: structure of the oligosaccharide obtained by depolymerization of the polysaccharide from membrane of *Laminaria*.

The team of Professor Kawada [8] discovered that the oligosaccharide made repeating units of M and G act as acofactor for EGF (Epidermal Growth Factor) and these can stimulate keratinocyte growth. Testing the Laminaria Oligo Alginate *ex vivo*, using skin-biopsy methods, has further confirmed that Professor Kawada's hypothesis was correct (Figure 15). The recovery from wounds, using skin biopsy techniques as the model, is faster with the treatment of oligosaccharides.

Figure 15: Cut area is indicated by the black arrows. On the left: wound-healing process in a cut human epidermis; on the right: wound-healing process in a cut human epidermis treated with the oligoalginate.

The addition of zinc to this oligosaccharide gave additional benefits such as acne control as well as wound-healing activities (Figure 16).

Figure 16: Observation of acneic skin before (on the left) and after (on the right) 1 month of daily treatment with the oligoalginate zinc chelated.

4.2.5.3 EXAMPLE OF THE USE OF MICROALGAE IN COSMETICS

a. Exopolysaccharides

Exopolysaccharides, also known as EPS, are high-molecular-weight polymers mainly composed of polymeric sugars [9]. They are produced and secreted by microorganisms/microalgae directly in their environment to provide protection, nutrition, or adhesion to objects and rocks. Each microorganism produces its own specific exopolysaccharides with a unique sequence of sugars.

The marine exopolysaccharides (EPS) have no land-based equivalent and represent a new and original growing source of interesting functional ingredients for the cosmetic field. They can be used to improve aesthetics and provide a slippery feel like polyorganosiloxanes. These materials can be used to deliver immediate benefits and are used as line-filler polymers, mattifying agents, sebum absorbers, exfoliators, boosters of immunity, etc.

For example, EPS that mainly consist of galacturonic acid (Figure 17) is capable of absorbing sebum (Figure 18) and providing both immediate and long-term effects in skin mattifying (Figure 19).

Figure 17: structure of the mattifying EPS

Figure 18: demonstration of the affinity of the mattifying EPS with sebum. Left: before gentle shaking; Right: after

Figure 19: mattifying effect of the EPS on the forehead, 1 hour and 8 hours after a single application.

On the other hand, EPS composed of galactose, galacturonic acid, glucose, glucuronic acid, and mannose (Figure 20) can form a second skin at the surface of the epidermis. These are useful to fill in fine lines (Figure 21) and decrease the depth of wrinkles (Figure 22).

Figure 20: structure of the filling EPS

Figure 21: Observation of skin surface using electron microscope, before (left) and after (right) the application of the filling EPS

Figure 22: visualization of the wrinkle-filling effect of the EPS, 15 minutes and 3 hours after a single application

EPS composed of repeating units of galactose and N-acetyl-glucosamine (Figure 23), are known to stimulate keratinocyte differentiation (Figure 24) and improve the natural exfoliation of the *stratum corneum*.

Figure 23: structure of the exfoliating EPS

INVOUCRINE (INV) nvolved in forming the cornified envelope

TRANSGLUUTAMINASE 1 (TGM1) esures assembly of proteins that make up the cornified envelope

SMALL PROLINE RICH PROTIN (SPRP) precursor proteins in the formation of the cornified envelope

LATE CORNIFIED ENVELOP (LCE) precursor proteins in the formaion of the cornified envelope

CORNEODESMOSINE (CDSN) pivotal role in cohesion of the stratum corneum

NICE 1 involved in termnal differentiaion of keratinocytes

Figure 24: stimulating effect of the exfoliating EPS on genes involved in keratinocyte differentiation

b. Photolyase

UV radiation causes dimerization of adjacent pyrimidine bases in DNA. This action is classified as DNA damage and it can lead to the cell death. Photolyase (EC 4.1.99.3) is an enzyme (Figure 25) that is present in many species such as *Anacystis nidulans*, a blue-green algae. Photolyases are DNA repair enzymes that repair damage caused by exposure to ultraviolet light.

The repair is activated by visible light and known as photoreactivation.

Figure 25: photolyase

In order to preserve the enzyme's repair activity, the best way to use this ingredient is by encapsulation in liposomes, which protect the enzymes' capabilities

and deliver them effectively. The ability to reduce DNA damage on skin is shown in Figure 26.

Figure 26: picture at the top showing a lot of fluorescence due to the important quantity of dimers. The picture at the bottom is one hour after the application of 1% of the liposome containing the photolyase. There are 45% less dimers (10).

c. Thioreduxine/thioreduxine reductase

At the origin of life there was less ozone and a more aggressive effect of UV light on the DNA of blue algae.

Some of these algae invented the "instant repair" taking the energy of light and using their photolyase to do an instant photorepair; while others did not have such an enzyme. The stromatolites mentioned earlier are made by the blue algae *Phormidium* and have existed for at least a billion years. The way they survived

is with their glucoside-biopterin (Figure 27), a cofactor that activates the enzyme thioredoxin—a small redox protein acting as powerful antioxidant and preventing the formation of dimers under UV attack (Figure 28).

Figure 27: Biopterin

Figure 28: *Phormidium* extract activates thioredoxine and less dimers are created (less fluorescence).

There is great value for the cosmetic industry in the category of sunscreens by combining the extract of both blue algae in formulations to achieve the maximum protection of the DNA present and instant repair activity.

4.2.5.4 EXAMPLE OF THE USE OF COASTAL PLANT IN COSMETICS

a. Retinol-like

The Rock Samphire (Figure 6) *Crithmum maritimum* is the sole species of the genus *Crithmum*. It is an edible wild plant found on southern and western coasts of Britain and Ireland, on Mediterranean and western coasts of Europe including the Canary Islands, North Africa, and the Black Sea.

More recently Rock Samphire has been evaluated for its applications in skin care: the samphire oil extract offers the benefits of retinoids but without the unwanted irritation and photosensitivity. The other "retinoid like" activities seen include reduction of sebum secretion, reduction of lines and wrinkles due to premature aging.

This active extract can up-regulate CRABp 2 (c Retinoic Acid Binding protein) receptor like retinol and down-regulate the genes responsible for cell cohesion (Figure 28). As the bridges between the cells are weakened, exfoliation of the skin will increase and leave the skin smooth and uniform.

Figure 29. Effect of *Samphira* on adhesion protein expression

A treatment with *Samphira* modulates expression of differentiation genes compared to vehicle-treated epidermis: (1) increase of cellular retinoic acid-binding protein (CRABPs II); (2) decrease of cytokeratine CK1 and CK6.

Samphira decreases the expression of genes such as desmocollin 3 and 4, desmoplakins I, II, & III—which are part of the desmosome structure. The application of retinoid derivatives modifies the expression of these genes (Humphries et al. 1998). In this way, corneocytes peel off more easily because their inter-corneocyte adhesion is reduced, thus leading to the reduction in the size of the cornified layer.

b. The discovery of AQP 8

Salicornia (Figure 5) is a halophyte (salt-tolerant) plant that grows in salt marshes, on beaches, and among mangroves. These are occasionally sold in grocery stores or appear on restaurant menus as "sea beans," but their resistance to dryness has

led to research in its moisturizing properties. It was a real discovery by Codif International to find that the oil extract of Salicornia stimulates AQP8 (Aquaporine 8, aka ammoniaporine) and AQP3 expression (Figure 29). Salicornia helps to rehydrate skin from many different angles. These include, for example: increasing production of urea, filllagrin, natural moisturizing factor (NMF), and lipids. Until this major finding, AQP8 had not yet been discovered in the skin.

As a result of using Salicornia treatment, skin hydration is improved and transepidermal water and urea are reduced.

Figure 29: increase of aquaporines by the Salicornia oil

CONCLUSION

This chapter provides an overview (albeit, brief) of marine ingredients that are used to reduce wrinkles, pore size, sebum flow, and dryness. They can also be used for tanning or whitening (see chapter 3 A.16) as well as for slimming or plumping. While the early "simple algae extracts" had a "typical" odor, advances in the technology have expanded our awareness of the import of the depth of technology and usefulness of more sophisticated algae and their extracts. There are more and more products featuring exopolysaccharides, enzymes, oligosaccharides, etc. arising from marine origin. The trend is expected to continue, as these ingredients are natural, sustainable when farmed appropriately, and well accepted by the public.

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TOPICAL REDUCTION OF VISIBLE SKIN DETERIORATION DUE TO CELLULITE

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ABSTRACT

Perhaps no single cosmetic problem receives as much bad publicity as the subject of cellulite. To a great measure, this negative publicity is due to the many so-called experts in the field of cellulite. Scientific journals are replete within accurate descriptions of this condition. Greater success in dealing with this issue is hampered by the lack of basic understanding of this disorderthat produces emotional discomfort for a high percentage of women regarding their perception of beauty.

One would think a disorder that affects females almost exclusively would provide a clue to its cause. We call it a disorder because it is not truly a disease and in the true sense of the word, it is a natural consequence of a physiological process.

This chapter describes a possible explanation for the etiology of cellulite as well as suggestions for ingredients that can be useful in topical cosmetic applications. As far as the commonly known name, "cellulite," this is sufficient for our purposes since the more technical name, "gynoid lipodystrophy," does not shed any light on understanding the disorder, from a cosmetic point of view.

There are several reviews on the theories and treatment of cellulite, and for a more in-depth discussion, we refer the reader to reference 1.[1] Keep in mind that much that has been written about cellulite in the past ten years is not based on accurate facts and we must dig a bit deeper to gain an understanding of what the phenomenon is, what causes it, and how to deal with it from a cosmetic point of view.

The term "adipose tissue" has taken on an entirely new meaning in the last ten years. We know now that this is not just a storage tissue, or a protective cushion. Rather, such tissue is actually a highly dynamic metabolic organ that has multiple

functions. In this chapter we limit our discussion to cellulite and the adipose tissue. The reader, however, is referred to an interesting article on subcutaneous adipose tissue (2).

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4.2.6.1 A POSSIBLE ETIOLOGY FOR CELLULITE

First, we need to look at the anatomy of cellulite. True cellulite occurs only on the buttocks and thighs, to a distance about four inches above the knees. Obviously there will be some variations between individuals, but for the most part cellulite does not occur on the abdomen or the arms or the breasts. Why? The adipose tissue inthe buttocks and thighs is reserved for nutritional purposes in pregnancy during lean times. The adipose tissue in the upper thighs and buttocks contains twice as many alpha-2 receptors as it does beta receptors. The alpha-2 receptor inhibits lipolytic, or fat-burning, action so that this fatty tissue is not easily mobilized.[2] *This is why dieting and exercise are not very effective in the treatment of cellulite.*

The second important area we must look at is the anatomical structure of the fat. The fatty tissue of men and women is structured in a series of connective tissue bands known as trabeculae. These tough collagen bands keep the fat in a somewhat ordered array and essentially two layers. See Figure 1 and Figure 1a below.

There is a deep layer of fat that rests immediately upon the muscles. This in turn is separated by a superficial layer of fat immediately under the dermal layer of the skin,which is a thin fascia, and is called the superficial fascia.

Within the literature there are several errors that identify four to five layers of fat, along with a second major error that identifies male fatty distribution as being in a crisscross of compartment-like containers. This error has spread continuously throughout the cellulite literature, for it is quoted in just about every article one reads. No one to the author's knowledge has ever duplicated the findings of this prior work by Nuremberger and Muller![i] These investigators used two-dimensional histological preparations of skin. It is quite difficult to visualize a three- dimensional structure with this type of preparation. They also did not have the advantage of three-dimensional ultrasound studies.

Current studies employing 3-D ultrasound do not show evidence of crisscross collagen structure, or differences between male and female structure, other than the quantity and thickness of the collagen trabeculations (see Figure 2).The only distinguishing characteristicbetween male and female subcutaneous fat is the following:female fat is thicker, as measured from the bottom of the dermis to the muscle layer then in the male. The fat globules are generally larger than in the male adipose tissue. One can see this in Figure 1. Notice the size of the lobules and the thicknessof the fatty layers.

Figure 1. Human Anatomy of Thigh Skin: Male vs. Female

Figure 1a. Male vs. Female Adipose Tissue. Inverted Contrast.

In cellulite, the primary structural abnormality lies in the disruption of the described collagen connective tissue bands. The collagen is known as Type I collagen, a tough ubiquitous structural component. In cellulite these bands are dissolved, either completely or partially, resulting in a disorganized and chaotic adipose tissue layers. This can be seen again in Figure 1 but can be more readily demonstrated by a 3-D ultrasound as seen in Figure 2.

Figure 2. A 3-D Ultrasound of a 5-cm square section of cellulite thigh skin from a 28-year-old female. Taken with 7.5 mHz transducer.The black area represents the adipose tissue. Fat does not reflect ultrasound.

The immediate question arises:What could possibly cause such destruction in an otherwise well- organized tissue? The answer is very surprising. It took one author, P.T. Pugliese, M.D.,tenyears to appreciate the fact that cellulite is not a fat disorder; rather,it is a consequence of another innocuous physiological process, collagenase destruction of endometrium. Dr. Pugliese searched for a source of

collagenase in the body that would have the power to dissolve type I collagen and which appears almost immediately after puberty.[3] The menstrual cycle appeared to be the most likely candidate. To appreciate the role of the menstrual cycle and the associated hormones and enzymes in the genesis of cellulite we must review the physiology of the menstrual cycle.

4.2.6.2 THE MENSTRUAL CYCLE

Briefly, the menstrual cycle produces a succulent hospitable environment for a pregnancy. In the absence of pregnancy, endometrium of the uterus (inner lining) must be discarded and renewed on a monthly basis. The renewable environment is known as the endometrium, and it greatly enlarges during the early phase of the menstrual cycle under the influence of estrogen. The major structural components are a collagen type I matrix and an adequate supply of small blood vessels. This is shown in Figure 3. While the hormone relationship is fairly well understood, the role of the destructive enzymes and associated cytokines is less well understood. The following explanation represents our current understanding of the biological compounds involved in the menstrual cycle.

The Hypothalamic-Pituitary-Ovarian System

Figure 3

4.2.6.3 THE MATRIX METALLOPROTEINASES

In the absence of a pregnancy, estrogen and progesterone fall to very low levels. This initiates a process that removes the endometrium by a collagenolytic process called shedding. Specific enzymes known as matrix metalloproteinases cleave the collagen fibers and break them down into peptides and amino acid, which are recycled in the metabolic pool. These enzymes originate from fibroblasts and perhaps other cells of the endometrium stromal cells in particular. These special enzymes are called matrix metalloproteinases, or MMPs.

Regarding our present focus, we will only cover the basics of MMPs to allow the reader to appreciation of the real nature of cellulite. There are four major classes of MMPs along with natural biological inhibitors, known as TIMPs, or tissue inhibitors of matrix metalloproteinases. The four classes of MMPs are: the collagenases, the gelatinases, stromelysins, and membrane-type enzymes (MT-MMPs). All of these enzymes contain zinc metal at the active site, are synthetized in an inactive form known as a preproenzyme and secreted into the extracellular matrix (ECM). They are activated in the ECM; they recognize and cleave the ECM components, and are inhibited by both serum inhibitors and extracellular specific MMP inhibitors.

The **collagenases** are grouped as MMP-1, MMP-8, and MMP-13. These MMPs can cleave many types of collagen including Types I, II, III, V, and XI, which are fibrillar.[4] When these collagen types are cleaved they become more soluble and form gelatin (the origin of Jello).

The **gelatinases**, MMP-2 and MMP-9, can now take over and attack type IV collagen (the basement membrane of the epidermis), fibronectin, and laminin.

The **MT-MMPs** appear to have a key role in this regulation as well as in controlling other MMPs.

TIMPs are tissue inhibitors of MMPs. There are four types TIMP I, TIMP II, TIMP III, and TIMP IV. We shall not discuss these compounds in detail.

4.2.6.4 THE MENSTRUAL CYCLE, MMPS, AND OVULATION

Keep in mind as you read this section that menses occur each month, and the rise and fall of these enzymes is critical to both ovulation and menstruation. Cellulite is a spinoff process of these enzymes, as are many of the other connective tissue disorders.(It is possible that as these proteolytic processes occur they may produce small peptides with antigenic properties that eventually result in antibody product against tissue components).[5] Ovulation is a major process necessary for an eventual pregnancy. In Figure 4 the steps of the process are outlined. Menses occur about two weeks after ovulation.

Figure 4

1. LH initiates a biochemical pathway that stimulates the production of progesterone, cytokines, and prostaglandins.
2. These biochemical agents act on granulosa cells and thea cells to produce MMPs and TIMPs.
3. The MMPs destroy the follicle, permitting the egg (ovum) to be released.
4. The TIMPs may orchestrate the formation of the corpus luteum by controlling cellular proliferation and steroidogenesis.

4.2.6.5 THE MMPS AND THE MENSTRUAL CYCLE

Following the late secretory phase in the menstrual cycle, there is a fall in the plasma levels of estrogen and progesterone. This is apparentlya critical step in the cycle that triggers actual menstruation. Since the endometrium is a thick mass of collagen and blood vessels it needs to be shed in a matter of days to allow for the new growth of endometrium. Vigorous and swift lysis of the interstitial matrix by proteinases[6] is required. Whilewe do not know all of the hormonal intricacies, we do know that the ovarian steroids, estrogen and progesterone, initiate the stimulation of endometrial fibroblasts to secrete MMP-1. After initiation by sex hormones,MMP secretion is under control of interleukin IL-1alpha.[7]

4.2.6.6 THE KEY STEP RELATING MENSES TO THE GENESIS OF CELLULITE

MMP-1 is triggered to be formed in, and secreted by, the fibroblasts—not only in the uterus but also in other fibroblasts of the body. This statement is based on the fact that women get MMP-associated diseases such as temporomandibular joint disease[8] and anterior cruciate ligament disease.[9] In the thighs and legs the main target is the collagen bands, that is, the trabeculae that support and separate the fatty tissue into finite compartments. These bands are broken down, causing the free fatty tissue to move upwards and laterally to fill the available space. Where there are no bands, the fat will herniate through the dermis and produce a hill (no bands), and valleys will be produced where the bands are still attached. *This is the basis of the undulating appearance of cellulite.* Fat is stored not only as reserve food, but also to protect certain areas of the body, and in the case of the female it provides sexual attraction. What can be done? Alas, the current treatments are based on traditional and erroneous concepts, some which are actually harmful in that they exacerbate the condition. Here's the inside picture of cellulite:

1. Fatty tissue is no longer confined to anatomical compartments.
2. Fat is expanded in a nonphysiological manner,with new fat cells filling newly created spaces.
3. As new MMP insults exacerbate this damaged tissue, repair becomes impossible.

What can we do to correct this situation? Considering that estrogen and related cytokines are the initiating force, we need first of all to stop the repetitive damage. Next we must institute an effective repair system that will restore the collagen to its normal physiological structure. Finally we must restore the fat to its normal configuration, which in effect will eliminate the signs of cellulite. It must be borne in mind that cellulite is the result of a continuous physiological process and though it is treatable it is not curable. Since body fat is the second largest producer of estrogen even at menopause, there will be endogenous estrogen that will continue this destructive process.

4.2.6.7 TOPICAL THERAPY FOR REVERSING THE APPEARANCE OF CELLULITE

Anti-estrogens—the key component

It is surprising that are oriental women have a very low incidence of cellulite. Perhaps it is genetically based, but it may also be related to the soy products they consume in their diet. Soy contains a very potent phytoestrogen known as genistein. Due to its structure similarity to 17β-estradiol (estrogen), genistein,

a phytoestrogen, can compete with it and bind to estrogen receptors. However, genistein shows much higher affinity toward estrogen receptor β than toward estrogen receptor α.[10] Isoflavones such as genistein and daidzein are found in a number of plants including lupin, fava beans, soybeans, and kudzu.

Figure 5. Genistein

Genistein has estrogenic activity 100–1000 times less than estradiol and is known to be a cell proliferator. While it has exhibited anticancer properties it has also been shown to promote certain estrogen-sensitive cancers. It would appear however that since a rather large part of the Asian diet consists of sources of genistein, the incidence of estrogen-sensitive cancers does not seem to be beyond the normal worldwide incidence.

As a blocking agent 0.5–1% in a topical product should be adequate to inhibit estradiol.

a. Chysin—an aromatase inhibitor

Estrogens are made from testosterone precursors by an enzyme known as aromatase. Blocking the action of aromatase will decrease the amount of estrogen in the body and increase the amount of testosterone. It is used primarily by bodybuilders, but has also found use in treating dark circles under the eyes.

b. Chrysin is a naturally occurring flavone, a type of flavonoid. It is found in the passion flowers *Passiflora caerulea* and *Passiflora incarnata*. It is also found in chamomile, in the mushroom *Pleurotus ostreatus*, and in honeycomb. At high concentrations, chrysin is reported to be an aromatase inhibitor *in vitro*. However, studies performed *in vivo* show that orally administered chrysin does not have clinical activity as an aromatase inhibitor. However, chrysin is effective topically and appears to have many positive physiological effects on skin, from decreasing pigment to preventing cancer.[11,12,13]

Figure 6. Chrysin

Chrysinis not very water soluble, but is soluble in a number of organic materials including alcohol. For starting cosmetic formulations, the authors suggest a 1% concentration. So many other ingredients can affect the solubility and penetration that the final concentration in the formula will need to be optimized by an empirical process.

c. DIM, or Diindolymethane

Diindolymethane is gaining popularity as an anti-aging product. It is the active ingredient in cruciferous vegetables that accounts for much of their activity, including their being anti-estrogenic. A good starting concentration for this ingredient for an anti cellulite topical preparation is approximately 0.5%. Considering its action is different than that of the flavonoids, DIM could possibly enhance the action of the other anti-estrogens.

4.2.6.8 AGENTS THAT BLOCK MMPS

After inhibition of estrogen, the next most important ingredient in an anti cellulite product would be one that would inhibit the action of the MMPs on degradation of collagen. In our laboratory grapeseed extract and pine bark extract would inhibit collagenase type I up to 70 percent at a concentration of 1.4%. At this level we have obtained the maximum inhibition, as there is no difference observed over that level. At levels as low as 0.5% some inhibition has been obtained, but this does not occur at levels above 30%. It is suggested that several anti-collagenase ingredients be tried in the formula to maximize the effect. Examples of such ingredients include: EDTA, EGTA, cysteine, and histidine.

4.2.6.9 MOBILIZING ADIPOSE TISSUE

It is a characteristic of adipose tissue to fill available space, since a well-known biological principle is that nature abhors a vacuum. One of the perpetuating mechanisms in cellulite is the continual replacement of fat that has been mobilized or displaced upward. In order to have the trabeculae replaced in an orderly fashion it is advantageous to mobilize the existing fat while at the same time the body is restructuring the connective tissue foundation for the replacement of new adipose tissue. What follows are several suggestions for ingredients that can help to perform this task.

The most potent mobilizers are the natural catecholamines of the adrenaline series. Exercise of course not only helps to mobilize fat, but at the same time will produce an abundance of catecholamines. There are of course additional agents, such as forskolin from the plant *Coleus forskolii*, growth hormone, thyroid hormone, leptin, and testosterone.

4.2.6.10 BLOCKING PHOSPHODIESTERASE

Phosphodiesterase is an enzyme that destroyed camp, which is needed to phosphorylate lipase. Lipase of course is the enzyme responsible for the hydrolysis of triglycerides to free the fatty acids from the glycerol molecule. Glycerol is metabolized as a carbohydrate entity while free fatty acids are then shuttled into the mitochondria for oxidation through the Krebs cycle and electron transport system. It is well known that xanthines will block phosphodiesterase. The addition of theophylline or caffeine would help to mobilize fat by allowing an increased production of cAMP.

4.2.6.11 SHUTTLING FAT INTO MITOCHONDRIA

Mitochondria are the energy-producing organelles in the cell. They mobilize and metabolize in the cytoplasm of the adipocyte intofree fatty acids. Thereafter, the free fatty acids shuttle into the mitochondria and are reduced to carbon dioxide, water, and energy via the Krebs cycle and the electron transport system. Carnitine is used as a carrier to shuttle the free fatty acids into the mitochondria.Carnitine transports long-chain acyl groups from fatty acids into the mitochondrial matrix, where through β-oxidation they are metabolized to acetyl CoA before entering the citric acid cycle. Fatty acids must be activated before binding to the carnitine molecule to form acylcarnitine by being attached with a thioester bond to coenzyme A (CoA). The acyl group on CoA can now be transferred to carnitine and the resulting acylcarnitine transported into the mitochondrial matrix.[14]

Metabolism of Triglycerides

Figure 7

4.2.6.12 REBUILDING COLLAGEN

With the damage to the connective tissue stopped, and the fat being mobilized, we are in a position to begin to reconstruct collagen. A first suggestion is to have the subject wear a pair of mild-compression pantyhose. By mild compression, this means that pressure should not exceed ten to fifteen millimeters in the toe and should gradually decrease pressure at the knee and mid thigh, so that groin pressure will be near zero. Any restrictive underclothing such as bikini underwear will impede the venous return to the heart and result in increased pressure in the interstitial tissues. By compressing the veins, the diameter of the vessel is reduced, which decreases the pressure in the vein by increasing the velocity of the blood flow (Bernoulli's law). This results in the interstitial fluid moving into the veins and the lymphatic vessels, thereby reducing the interstitial pressure. The net result is greater metabolic activity in the fatty tissues. This increased pressure has a dramatic influence on the dynamics of fluid transfer. One only needs to observe the edema in a woman's lower legs and feet after standing at work all day to see this process in action. Just sitting at a desk will cause venous blood to pool in the lower legs, since the only adequate method of returning venous blood from the leg is by walking. Generally the pressure in the feet of a standing individual is 90 to120 millimeters of mercury. The addition of the pantyhose will decrease the diameter of the veins, thereby increasing the velocity of the blood flow and decreasing the interstitial pressure, by virtue of Bernoulli's law.

4.2.6.13 COLLAGEN STIMULATORS

Perhaps no single natural product is better able to stimulate fibroblasts to produce collagen than asiatic acid, derived from *Centella asiatica*.[15,16] Vitamin C, or ascorbic acid, is able to stimulate the production of collagen to threefold and is an important addition to anti cellulite preparations.[17] Vitamin C of course is critical to cross-linking of collagen. In the treatment of cellulite, the critical phase is to rebuild the collagen, in a normal pattern. To achieve this, it is necessary that the subject be ambulatory and have a regular exercise program that principally involves walking. Since adipose tissue in the cellulite area, that is, the buttocks and thighs, is not easily mobilized because of the presence of a large number of alpha to inhibitory receptors, it will take some time to mobilize the fat and rebuild the connective tissue. Studies for efficacy therefore should be a minimum of 90–180 days.

4.2.6.14 CLINICAL STUDIES

Most clinical studies use thigh diameter as an index of efficacy. However, the skin surface topography, and the shape and position of the buttock are a far better parameter to measure. Figure 8 shows photographs of the female posterior of a subjects. Subject A is a photograph of a post-surgery patient who had a "butt lift"

and additional plastic surgery to produce more rounded buttocks. Subject B is the classical "rectangular cellulite buttock."

Since the buttocks usually represent the largest pendulant mass in the body, this soft overlying tissue, composed of skin and fat, would be subject to gravitational forces that will tend to not only pull it down,but force it together in the center. This is unlike the gravitational pull on the breasts which, due to the curvature of the chest, are forced down and out.

Subject of plastic surgery showing circular-type buttocks. This is a rectangular buttock somewhat exaggerated in size.

Figure 8. Shapes of buttocks

Additional Ultrasound Figures

Enhanced photo accentuates the surface irregularities. This is a far better parameter to measure than circumference of the thighs. Note how the buttocks are flat. This is very characteristic of advanced cellulite.

A 3-D system is employed in this ultrasound photograph to measure the volume of the herniated glob of fat. Each segment views the fat globules from a different side. The lower-left corner of the illustration is actually a top view of the globular. The white and blue illustration on the right illustrates the 3-D composite picture of the globule with an actual measured volume, 0.92 ml.

This photograph represents a 3-D ultrasound depicting slices made at 90 degrees to each other at the top of the photo. The lower-left corner shows a view from the top of the section; the right lower side is a 3-D representation showing from what part of the specimen the slice was taken. This is represented by the blue section.

CONCLUSION

Cellulite is a serious cosmetic disorder resulting from a normal physiological process. At present there is no known cure, but there is a rational approach to dealing with this bane of women's beauty. The etiology of cellulite is hormonal. It is a consequence of estrogenic activity on fibroblasts, resulting in destruction of collagen tissues that support the storage of fat in the thighs and buttocks. By blocking the estrogen receptors on the fibroblast, the estradiol effect is attenuated so that less collagenase is produced. By mobilizing the displaced adipose tissue, the fibroblast within the structure of the connective tissue of the dermis is able to restructure the lipid compartments. Effective approaches to dealing with reducing cellulite centers on blocking estrogen and inhibiting collagenase as well as mobilizing displaced fat and restructuring the collagen compartments within the fatty tissue. Much more needs to be known about cellulite, but unless the hormonal genesis forms the basis of our understanding, current approaches may not be adequate.

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INGREDIENTS III
ANTI-AGING

TOPICAL RETINOIDS

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ABSTRACT:

Topical retinoids are derivatives of vitamin A used widely in the field of dermatology. They are indicated for treating a variety of conditions including acne, psoriasis, pigmentary disorders, and photo-aging. Retinoids are available over the counter in cosmeceutical products or by prescription. The most common side effect is irritation, including redness, scaling, and dryness.

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4.3.1.1 INTRODUCTION

Retinoids are derived from vitamin A and may be synthetic or natural. They are commonly used in dermatology to treat a wide array of skin conditions. This class of medication can be administered orally or topically; however, the latter is more common and will thus be the focus of this chapter.

The first topical retinoid was developed over 40 years ago in the form of all-*trans* retinoic acid (ATRA), which is otherwise known as tretinoin. It was brought to the market in the 1970s as a treatment for acne under the trade name Retin-A. It was not until 1986 that the beneficial effects of tretinoin on photo-aging were first published.[1] This breakthrough research spurred the development of numerous prescription and over-the-counter products designed to treat wrinkles and other signs of aging.

4.3.1.2 PHARMACOLOGY

Vitamin A exists in three forms: an aldehyde (retinal), an alcohol (retinol), and a carboxylic acid (retinoic acid), which is the active form in the skin. All-trans retinol is the form of vitamin A delivered to the skin by way of the bloodstream after the metabolism of dietary retinyl esters. Within the cell, all-trans retinol is either oxidized to form retinoic acid or stored as retinyl esters, depending on whether retinoic acid is needed by the cell. This may explain why topical retinoic acid is more irritating than topical retinol; it can't be stored and thus may overwhelm cellular processes and lead to adverse effects.

Retinoic acid and synthetic retinoids bind to specific receptors in the nucleus. There are two groups of receptors: RARs and RXRs. Each type is further subdivided into three subtypes: alpha, beta, and gamma. RAR-gamma is the most important mediator of retinoid activity in the skin. The drug-receptor complex then functions by influencing the transcription of DNA. Structural differences between retinoids determine their binding affinity to transport proteins and nuclear receptors, which ultimately affect their physiologic actions.

4.3.1.3 INDICATIONS:

a. Acne

The pathogenesis of acne is thought to involve four key elements: plugged follicles, inflammation, *Proprionibacterium acnes*, and excess sebum. Retinoids decrease the retention of debris in hair follicles, thereby preventing the formation of the microcomedo, which is thought to be the initial lesion of acne. In addition, anti-inflammatory effects occur through the inhibition of toll-like receptor 2, which down-regulates inflammatory mediators such as NF-κB, IL-8, and IL-20. Topical retinoids, however, do not directly inhibit excess sebum or *P. acnes* counts.

Because topical retinoids are effective in treating both inflammatory and non-inflammatory acne, they are considered a cornerstone of any effective acne treatment regimen. FDA-approved retinoids for the treatment of acne include tretinoin, adapalene, and tazarotene.

b. Melasma and Post-Inflammatory Hyperpigmentation

Retinoids are a valuable adjunctive treatment for disorders of hyperpigmentation often used in conjunction with skin-bleaching agents like hydroquinone. Retinoids function by blocking transcription of tyrosinase, inducing desquamation, dispersing pigment granules, and enhancing epidermal cell turnover.

Combination therapies, most frequently consisting of hydroquinone, tretinoin, and a topical steroid are thought to be superior to monotherapy in the overall management of melasma. Tretinoin allows for the epidermal penetration of hydroquinone and the incorporation of a topical steroid helps to lessen the irritation of retinoids and hydroquinone.[2]

c. Photo-Aging

Topical retinoids have been shown to ameliorate the deleterious effects of chronic sun exposure on the skin. Indeed, topical retinoids have been proven to reduce epidermal thinning and dysplasia, increase new collagen formation in the dermis, increase new vessel formation, and exfoliate retained debris in the hair follicles.[1]

As such, tretinoin is FDA-approved to treat signs of aging such as fine facial wrinkles, mottled facial hyperpigmentation, and tactile facial roughness.

The main over-the-counter vitamin A derivative is retinol. Long considered to be the original cosmeceutical, it is commonly formulated with moisturizers and antioxidants to be used as a nightly cream or serum. The concentration of retinol is usually 1–2%, but benefits can be seen with as little as 0.1%.[3] In general, retinol is less irritating but also less effective than prescription retinoids. Some examples of popular retinol-containing cosmeceuticals and pharmaceutical retinoids are given in Table 1.

4.3.1.4 ADVERSE EFFECTS

The major side effect of topical retinoids is local irritation, which may include redness, scaling, dryness, itchiness, stinging, and burning. Consumers may also experience increased sensitivity to ultraviolet radiation; therefore a sunscreen is recommended when using topical retinoids. Poor tolerance to these adverse effects is a common reason for noncompliance or discontinuation of the product. While using topical retinoids, it is important to avoid using products that contain salicylic acid or alcohol to minimize irritation.

Table 1: Examples of OTC and pharmaceutical retinoid products

OTC Retinol Products	Pharmaceutical Retinoids
RoC Retinol Correxion Deep Wrinkle Night Cream	Tretinoin (Retin-A, Atralin, Renova, Refissa, Avita)
Neutrogena Rapid Wrinkle Repair	Adapalene (Differin)
Skinceuticals Retinol 1 Maximum Strength Refining Cream	Tazarotene (Tazorac)
SkinMedica Tri-Retinol Complex	

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PEPTIDES FOR ANTI-AGING SKIN CARE

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ABSTRACT:

The first peptides for cosmetic use were developed in the early 1990s. Since that time, peptides have become an increasingly popular "cosmeceutical" ingredient in anti-aging products. These interesting and useful molecules are comprised of chains of at least two amino acids linked by a "peptide bond." The peptide bond is a chemical bond characterized by a sharing of electrons between atoms that are formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule. This relationship constitutes the primary linkage of all protein structures. There is a specific association between the amino acid sequence in the peptide chain and its resulting bioactivity. Peptides are typically active at very low concentrations. The major challenge for the use of these molecules in the cosmetic and personal care area is to develop a peptide and formulate it into a vehicle that can be delivered to the target site in skin in order for the peptide to be effective.

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4.3.2.1 PEPTIDES FOR ANTI-AGING SKIN CARE

Introduction

The term "cosmeceutical" was introduced over forty years ago by the famous dermatologist Albert Kligman, M.D. The word is ambiguous from a regulatory point of view since, in the United States, there is no such defined category for skin care products that is regulated by the Food and Drug Administration. However, recognizing the legal schism that exists between "drug" and "cosmetic," the industry jumped at a chance to have a word that described the many new, effective ingredients it was developing that fell into the schism. Interestingly, at one point, Dr. Kligman told an audience he was sorry he had proposed the term, but, nevertheless, the industry needed a term to describe such materials.

It is generally accepted that cosmeceutical ingredients are safe, will not harm the individual and may actually be beneficial in skin care products. The market for "high performance" skin care products remains strong as an aging population seeks products to help restore its youthful appearance. The ingredients formulated in cosmeceuticals are important, as they provide the basis for efficacy and other marketing claims for the formulation. Peptides provide product differentiation and enhanced-performance claims, including "naturally derived" and other holistic "well-being" concepts. Such claims align with the popular concept of healthy aging (1). They are of high interest for use in cosmeceutical products, as they function to modulate cell proliferation, cell migration, inflammation, melanogenesis, protein synthesis, and cellular regulation. These materials are generally not considered immunogenic and they break down into natural amino acids conferring a high degree of safety for use in cosmetic products.

4.3.2.2 WHAT IS A PEPTIDE?

As stated above, a peptide is a chemical entity consisting of at least two amino acids that are joined together by a peptide bond. Alternatively, the peptide bond is called an amide bond, and is formed when the carboxyl group of one amino acid

chemically links to the amino group of the next amino acid, releasing a molecule of water (Figure 1). Surprisingly, the International Union of Pure and Applied Chemists (IUPAC) provides no specific definition of a peptide. Short amino acid chains are considered peptides; long amino acid chains are termed proteins. Peptides consisting of two amino acids are termed a dipeptide, three amino acids are called a tripeptide, and so on. Peptides consisting of less than 20 amino acids are referred to as an oligopeptide. A polypeptide may consist of 50 to 100 amino acids. Amino acid chains above this range are usually considered proteins. Peptides may be found in a variety of configurations. For example, they occur as naturally occurring peptide sequences: peptides generated by the chemical or biochemical breakdown of proteins and peptides synthesized from naturally occurring or synthetically derived amino acids. In addition to length, the most important characteristic of a peptide is the amino acid sequence. The sequence order is crucial with respect to the biological properties of the peptide. These molecules perform a variety of biological functions; they may travel around the body until they interact with a target receptor either on the cell surface or within the nucleus of the cell. The interaction triggers additional activity resulting in a biological response.

Figure 1: Chemical structure of a peptide bond. The star indicates location of the peptide bond site.

4.3.2.3 SKIN STRUCTURE AND PEPTIDE CATEGORIES

As discussed in detail in other parts of this text, skin is composed of three differentiated layers. The epidermis, dermis and hypodermis. The epidermis and dermis are separated by a basement membrane rich in extracellular matrix (ECM) proteins. ECM proteins include collagens, laminin, fibronectin, elastins, and proteoglycans (2). The ECM anchors cells and organs together and also serves as a mediator of receptor-induced interactions between cells. The dermis provides a supporting

matrix including collagen and elastin. The largest class of fibrous ECM molecules consists of the collagens, which include 16 different types of collagen. Collagen in the dermal matrix is composed of type I (80–85%) and type III (8–11%) collagen. Collagens are primarily responsible for the tensile strength of skin (3). Damage to the collagen network causes healing to take place. Cellular behavior in the ECM is related to signaling by matrix components to cells through cell membrane receptors. Common receptors are known as integrins, and are found on the cell surface. Some of the signaling molecules are produced by proteolytic degradation in the ECM that release soluble peptides termed matrikines (4).

Signal Peptides-Matrikines

Matrikines result from the degradation of extracellular matrix proteins. They function as cell messengers involved in wound-healing and tissue repair. Matrikines are peptide fragments released by the process of enzymatic protein breakdown during tissue renewal (5). Two classes of matrikines have been characterized: natural matrikines that signal directly from the extracellular matrix area, and cryptic matrikines that require separation of peptide bonds for cell signaling (6). Matrikines are found in collagen, elastin, decorin, laminins, fibronectin, and tenascins. Palmitoyl pentapeptide-3 introduced by Sederma is an example of a matrikine peptide. The trade name for this peptide is Matrixyl®. According to the patent filed in 1999 and subsequent publications, 50 parts per million (ppm) of this peptide have been shown to produce a significant benefit to lines and wrinkles around the eyes when compared to the vehicle alone in a randomized study (7, 8). Thus, this technology falls into the characterization of peptides as interesting and useful skin anti-aging ingredients.

Antioxidant Peptides

Reactive oxygen species (ROS) can damage cells by oxidizing membrane phospholipids, proteins, and nucleic acids.

The damaging effects of ROS are normally kept under control by endogenous antioxidant systems including glutathione, ascorbic acid, and enzymes such as superoxide dismutase (SOD), glutathione peroxidase, and catalase. When these systems are excessively stressed by ROS, resulting oxidative reactions damage exposed cells (9). Large doses of antioxidants have been found to be effective in many animal models of diseases associated with oxidative stressors. Despite these observations, clinical trials with antioxidants in humans, such as vitamin E or recombinant human SOD, have generally failed to demonstrate significant benefits, leading to a proposed concept termed the “antioxidant paradox” (9). A possible

explanation for this paradox is that many of the antioxidants can also have pro-oxidant activity in addition to antioxidant activity. Such "bipolar" activity occurs especially in the presence of transitional metals such as copper (Cu (II). Another possibility for this paradox is that some of the antioxidants do not reach the relevant sites where free radicals are generated. Large proteins such as SOD do not penetrate cell membranes and are therefore ineffective against intracellular ROS.

Neuropeptides

There are functional and anatomical connections between the brain and skin. In the skin, "itch" (pruritus), excessive sweating (hyperhidrosis), and "flushing" (facial erythema), as well as many dermatoses such as atopic dermatitis, psoriasis, seborrhoeic eczema, prurigo nodularis, lichen planus, chronic urticaria, and alopecia areata, can be triggered or aggravated by stress (10). Neuropeptides released from cutaneous tissue and immune cells in response to stress stimuli are important for regulation of the cutaneous immune response and repair of skin. Since skin and brain are derived from the same type of tissue, this may help explain why peptides in skin act as neurotransmitters and other peptides are involved in itch, pain, pigmentary changes, and various forms of cutaneous inflammation (11).

Carrier Peptides

Carrier peptides function as facilitators of transport mainly for trace elements, including copper and manganese. In skin, this functionality is significant for wound-healing and regulation of various enzymatic processes. The tripeptide H-Gly-His-Lys-OH (GHK) was originally identified in human plasma and has a high affinity for copper^{2+} (Cu^{2+}). It acts as a signaling peptide and a carrier molecule for copper, which is a cofactor for several enzymes involved in collagen and elastin formation. The copper peptide was shown to stimulate wound-healing and to reduce fine lines and wrinkles (12, 13). By means of this mechanism, we again see the usefulness of peptides as "anti-aging" ingredients for skin.

Enzyme Inhibitor Peptides

Enzyme inhibitor peptides directly or indirectly inhibit enzymes. Several di- and tripeptides are known to be angiotensin-converting (ACE) inhibitors. ACE inhibitors are used to reduce blood pressure when taken orally. ACE inhibitors also prevent angiotensin I conversion to angiotensin II, a potent vasoconstrictor. Further, they also prevent the enzyme from inactivating the vasodilator bradykinin (7). Sederma has marketed a dipeptide, known as dipepetide-2, under the trade name Peptide VW commercialized for anti-aging skin care use (7).

4.3.2.4 BIOACTIVE PEPTIDES MARKETED FOR SKIN CARE PRODUCTS

A major challenge for cosmetic chemists working with peptides is to formulate the most appropriate peptide in a suitable vehicle to deliver the peptide to the appropriate target in skin at the appropriate time. This is a significant challenge in view of the fact that peptides are subject to: degradation by proteases in skin, undesired side effects that occur via interaction with different receptors in skin, and limited permeability into skin. To deal with these issues, chemically modified peptides are designed with enhanced bioavailability and stability, and this is a useful strategy employed by peptide manufacturers.

The most direct approach to achieving the goal of delivering the peptide intact is to modify side chains of single amino acids. The introduction of side-chain functional groups that do not occur naturally in peptides restricts their conformational flexibility and enhances metabolic stability. Another approach is to modify the peptide chain itself (14). A third approach is to construct the peptide with non-natural amino acids, which can enhance the stability of the peptide and provide further biological benefits (14).

Small peptides contain two to five amino acids, medium-sized peptides consist of four to 50 amino acids, and long peptides contain 40 to 80 amino acids. Small proteins contain 70 to 120 amino acids. The solubility of a peptide in an aqueous solution is largely dependent on the amino acid composition. Peptides with a large proportion of basic and acidic amino acids are likely to be readily soluble in aqueous buffers at physiological common pH of approximately 7.4.NT Peptides are modified as determined by their intended use. For example adding a fatty acid, a palmitoyl group to a water-soluble peptide can enable it to penetrate the lipophilic stratum corneum of skin. A large number of polar amino acids will improve solubility in aqueous systems as well (15).

Improved delivery of the peptide to skin can also be achieved by means of the use of fatty-acid derivatives to increase the lipophilic property of the peptide. An example of this approach is palmitoyl pentapeptide-3 (pal-KTTKS), commercialized under the trade name Matrixyl® by Sederma. Matrixyl is a five amino-acid chain consisting of lysine-threonine-threonine-lysine-serine. Palmitoyl is a fatty-acid derivative intended to increase penetration into skin. Matrixyl is claimed to have an anti-aging effect in that it has been observed to reduce fine lines and wrinkles. GHK copper-binding peptide, originally isolated from human plasma, was discovered during the 1970s (16). This peptide is useful for enhancing wound-healing via stimulation of components in the extracellular matrix (7). Table 1 lists examples of peptides marketed as cosmeceuticals in skin care products.

Table 1: Common examples of peptides used in cosmetic products, mechanism of activity, source, peptide type, and application in skin care (7, 16).

Peptide/Trade Name	**Source**	**Peptide Type/Mechanism of Action**	**Alternate Names**	**Cosmeceutical Application**
Palmitoyl Oligopeptide/Biopeptide-CL	collagen	Signal Peptide/Stimulates collagen and glycosaminoglycans synthesis	Pal-GHK	Anti-aging, anti-wrinkle, skin moisturization
Palmitoyl tripeptide/ Syn®-col	Thrombospondin I	Signal peptide/ collagen synthesis via TGF-ß		Improves stretch marks, anti wrinkle, skin moisturization, improve skin tone.
Acetyl hexapeptide-3/ Argireline®	SNAP-25	Neurotransmitter inhibitor/Botox-like via SNARE inhibition		Anti-wrinkle, skin moisturizer, improve skin tone and firmness
Peptide/Trade Name	Source	Peptide Type/Mechanism of Action	Alternate Names	Cosmeceutical Application
Palmitoylpentapeptide-3/Matryxl®	Synthetic, procollagen I fragment	Signal peptide/stimulates collagen I, III, IV, fibronectin, elastin, and glucosamoglycan production.	Pal-KTTKS, palmitoyl oligopeptide	Anti-aging, anti-wrinkle
Copper tripeptide complex/Lamin®	Synthetic, human serum	Signal and carrier peptide/Promotes large collagen aggregate degradation and more regular collagen synthesis, elastin, proteoglycans, glycosaminoglycans		Anti-inflammatory, anti-wrinkle, after skin resurfacing, skin moisturizer, hair growth stimulation
Dipeptide diaminobutyroyl benzylamide diacetate/Syn®-ake	Synthetic, Waglerin 1	Neurotransmitter inhibitor/Botox-like via acetylcholine receptor, mimics effect of Waglerin 1 found in the venom of the Temple Viper	Tripeptide-3	Anti-wrinkle. Botox-like effect

An example of a modified "biomimetic" peptide is a five amino-acid peptide with a cyclic structure marketed by Merck KGaA, Darmstadt, Germany under the trade name RonaCare® Cyclopeptide-5, INCI name Cyclotetrapeptide-24 Amino-cyclohexane Carboxylate. The peptide consists of five amino acids. Of these, four amino acids; L-arginine, glycine, L-aspartic acid, and D-phenylalanine are found in nature, while the fifth amino acid, aminocyclohexane carboxylic acid (ACHA), is synthetic (Figure 2 and 3). The peptide is claimed to improve the appearance of wrinkles and fine lines.

Figure 2: A cyclic peptide consisting of the amino acids arginine ®, glycine (G), aspartate (D), and aminocyclohexane carboxylic acid (ACHA). The amino acid sequence R G D is known in the literature as an amino acid sequence for stimulating wound healing.

Figure 3: Chemical structure of aminocyclohexane carboxylic acid.

Arginine is an essential amino acid associated with wound-healing. Various publications report that peptides consisiting of arginine, glycine, and aspartic acid (RGD) facilitate wound-healing by binding to specific receptors that signal for collagen renewal. Penylalanine helps to stabilize the protein from breakdown caused by proteases found in skin. The non-natural peptide AHAC helps to keep the peptide stable and provide a cyclic structure that optimizes selectivity for the targeted integrin receptor in skin (17).

CONCLUSION

There is a growing demand for anti-aging ingredients as the world's aging population increases. Various publications have reviewed peptides commonly used in cosmeceuticals (7, 8, 16). Peptides have great potential for use in anti-aging products, as they have a multitude of properties suitable to address common complaints associated with aging skin. A major challenge to formulating with peptides is the ability to enable peptides to penetrate skin. Permeability is associated with acid dissociation constant, molecular size, binding affinity, solubility, and partition coefficient (16). Other important factors are the compatibility and stability of the peptide in the formulation (7). From the perspective of cosmetic "ingredient-seekers" and formulators, the family of peptides, from the simplest to the more complex chemical modifications, is and will continue to be a useful foundation for the application of creativity in designing new and more efficacious anti-aging ingredients.

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HYDROPHOBIC (NONPOLAR) AMINO ACIDS

Alanine (Ala, A)

Isoleucine (Ile, I)

Leucine (Leu, L)

Methionine (Met, M)

Phenylalanine (Phe, F)

Proline (Pro, P)

Tryptophan (Trp, W)

Valine (Val, V)

HYDROPHILIC (POLAR) UNCHARGED AMINO ACIDS

Asparagine (Asn, N)

Cysteine (Cys, C)

Glutamine (Gln, Q)

Glycine (Gly, G)

Serine (Ser, S)

Threonine (Thr, T)

Tyrosine (Tyr, Y)

MICRORNAS IN SKIN PHYSIOLOGY

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ABSTRACT

This chapter provides a short introduction to microRNA biology, summarizing and discussing existing evidence for the role of microRNAs in the skin, particularly in epidermal differentiation and renewal, pigmentation, skin protection and dermal senescence. Some noncoding DNA regions of human genome are transcribed into noncoding RNAs (ncRNAs). Among these ncRNAs, microRNAs represent a major component of RNA interference-mediated gene silencing. MicroRNAs bind to complementary sequences on target messenger RNA (mRNA) transcripts, generally resulting in translational repression or target degradation, leading to subsequent gene silencing. Cellular protein expression and a large number of biological processes are influenced by microRNA regulation of gene expression. Indeed, the critical phenotype of mice lacking key enzymes of the microRNA biogenesis pathway in the skin confirmed the essential function of microRNAs in their tissue. Moreover, a growing body of evidence supports the concept that microRNAs have an essential role in orchestrating the morphogenesis and homeostasis of epidermis and skin appendages. Profiling studies have identified numerous differentially regulated microRNAs associated with physiological and pathological processes. Among them, miR-203 is specifically expressed in keratinocytes and plays a crucial role in the epidermal differentiation processes.

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4.3.3.1 RNA INTERFERENCE AND MICRORNAS—TIMELINE OF THE DISCOVERIES

The completion of the Human Genome Project in 2003 has confirmed that a large amount of DNA sequences (98% of the genome) are not protein-coding regions. Moreover, these noncoding regions are not simply "junk DNA" (Ohno 1972) but have a major regulatory role in gene expression. About 43% of noncoding DNA is transcribed mainly as introns and noncoding RNAs (ncRNAs). Among ncRNAs, short interfering RNAs (siRNAs), microRNAs (miRs), Piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), and long ncRNAs (lncRNAs) are involved in RNA interference (RNAi). RNA interference (RNAi) is the inhibition of gene

expression by small double-stranded RNA molecules. MicroRNAs were discovered in the early 1990s in *C. elegans*. Since then, microRNAs led to extensive research, discoveries, exponential bibliographical publications, and studies with a growing number of microRNAs reported in dedicated databases such as MirBASE (www.mirbase.org) with 24521 entries (MirBASE v10, June 2013) and still counting.

a. Discovery of microRNAs

The discovery of microRNAs is very recent when placed in the timeline of the discoveries in genetics, covering these last two centuries (Figure 1, based on Darnell 2011). Briefly, the discoveries of the different amino acids extended from 1819 to 1935, and the study of the proteins started with the invention of the word "protein" by Jöns Jacob Berzelius in 1838 and culminated with the understanding of their structure and sequence (Frederick Sanger and Hans Tuppi 1951). Meanwhile, DNA was identified by Friedrich Miescher as "nuclein" in 1869, and the famous double-helical structure was elucidated by James Watson and Francis Crick in 1953, based in part on the work of Rosalind Elsie Franklin. RNA, although known for a long time, was first demonstrated to have a messenger role (François Jacob and Jacques Monod), then an adaptor role as transfer RNA, allowing gene expression. The central dogma of molecular biology, expressed by Francis Crick in 1958, explains the flow of information from DNA to protein; RNA being the intermediate. Finally, the uncovering of the genetic code explaining the correspondence between DNA coding sequence and the corresponding protein sequence is due to Marshall Nirenberg, in 1966. Only recently, however, has the regulatory role of noncoding RNA been evidenced, with the discovery of RNA interference and microRNAs.

Figure 1: Main discoveries in genetics.

b. The concept of RNA interference

Epigenetics was, until the end of the 1980s, a topic related only to chemical modifications of DNA or proteins, allowing transcriptional repression or activation. However, in the early 1990s, several studies reported *RNA-mediated inhibition of protein expression*, also called *gene-silencing*, occurring at the post-transcriptional level; which is related to epigenetics regulation of gene expression. First observed in plants by Richard Jorgensen and colleagues (Napoli et al. 1990), gene silencing was also described in the mold *Neurospora crassa* (Romano and Macino 1992), and named "quelling." These seminal observations introduced the concept of post-transcriptional gene silencing (PTGS) or RNA silencing (Figure 2).

Figure 2: Timeline of the discoveries in microRNA research.

In 1998, Fire and Mello observed in the worm *Caenorhabditis elegans* that double-stranded RNA (dsRNA) was capable of triggering sequence-specific inhibition of protein expression, a phenomenon that they called "RNA interference" (Fire et al. 1998). This term encompasses the post-transcriptional inhibition of gene expression by two types of small RNA molecules: *small interfering RNAs* (siRNAs) and microRNAs. Both siRNAs and microRNAs are small double-stranded RNAs. They differ in that siRNAs arise from long exogenous RNA precursors such as viruses, transposons ,or plasmids taken up by cells (they can also be applied on cells as synthetic siRNAs), while microRNAs are transcribed from the cellular genomic DNA and further processed.

MicroRNAs are small double-stranded noncoding RNAs (20–25 nucleotides in length). The first microRNA, Lin-4, was discovered in the worm *Caenorhabditis elegans* in 1993 by Victor Ambros and colleagues (Lee et al. 1993) and the first human one, *Lethal-7* or *Let-7*, was discovered in 2000 by Gary Ruvkun and

collaborators (Reinhart et al. 2012; Pasquinelli et al. 2000) (Ref Figure 2). The subsequent development of miR cloning alowed the identification of microRNA homologs in many vertebrate species, demonstrating the evolutionarily conservation of the major microRNAs across many species.

In only thirteen years, known human microRNA precursors reported in MirBASE, reached the number of 1872 (corresponding to 2578 mature microRNAs) (MirBASE v10, June 2013), giving evidence of the high interest of the scientific community for this new type of cellular components. The future elucidation of the entire human repertoire of microRNAs will be an important step in fully understanding their wide range of functions.

The discovery of RNA-mediated post-transcriptional regulatory mechanisms and the contribution to biology and medicine are so important that in 2006, the Nobel Prize in Physiology or Medicine was awarded to Andrew Z. Fire and Craig C. Mello for the discovery of RNA interference (Ref Figure 2), opening the way to breakthrough technologies to study the effects of the loss of function of specific genes in mammalian cells (Almeida et al. 2011) and to the use of these technologies in clinical therapeutics (Sindhu et al. 2012).

4.3.3.2 MICRORNAS – NOMENCLATURE, STRUCTURE, FUNCTION, MECHANISM OF ACTION

MiRNAs follow a particular nomenclature, allowing their identification in databases such as MirBASE, and insuring homogeneous principles of naming for the scientific community (Kozomara and Griffiths-Jones, 2014). The following are the main nomenclature rules for microRNAs. Hence, a sequential numerical identifier is assigned to each newly discovered microRNA: miR-29, miR-30, miR-31, etc. Usually, the mature microRNA sequences are designated “miR,” whereas the precursors and genes are labeled “mir.” The complete name of a microRNA should use a three- to four-letter code to indicate the species. *Homo sapiens* species, for example, will be indicated by the letters “hsa” (e.g., hsa-miR-29). In mouse, the prefix will be “mmu” (*Mus musculus*), whereas for drosophila, it will be “dme” (*Drosophila melanogaster*). In this review, as we will mainly refer to human microRNAs, unless stated differently, and we will use the microRNA name without indicating the letter code for species. Orthologous microRNAs in different species will bear the same numbering: for example, hsa-miR-29 in human and mmu-miR-29 in mouse. Lettered suffixes indicate related (paralogous) mature sequences, only differing at one or two positions—for example hsa-miR-29a and hsa-miR-29b, which are expressed from precursors hsa-mir-29a and hsa-mir-29b, respectively. Distinct genomic loci and precursor sequences that express identical mature sequences have numbered suffixes, e.g., hsa-mir-7-1, hsa-mir-7-2, and hsa-mir-7-3. Finally, the mature sequences are assigned names of the form miR-155

(the predominant product) and miR-155* (from the opposite arm of the precursor). A microRNA precursor can give two different mature sequences named -5p (from the 5' arm) and -3p (from the 3' arm).

MicroRNAs mediate their regulatory action through binding to the 3' untranslated region (3'UTR) of target gene mRNAs, usually resulting in translational repression and gene silencing (Bartel et al. 2004 and 2009; Feramisco et al. 2010). MicroRNAs are present in the entire genome, both as *intergenic microRNAs* and as *intronic microRNAs* (found in intronic regions of protein-coding transcripts).

RNA interference mediated by microRNAs, is a complex multi-step mechanism including transcription, nuclear processing, nuclear export, and cytoplasmic processing. MicroRNA biogenesis starts from the microRNA gene in the nucleus: intergenic microRNAs are transcribed by polymerase II (Cai et al. 2004; Lee et al. 2004) or polymerase III (Borchert et al. 2006) and give rise to a primary microRNA (pri-miR), whereas intronic microRNAs are co-expressed in the intron region of a protein-coding transcript, through a polymerase II-dependent promoter, and the resulting microRNA precursor (pre-miR) enters the microRNA pathway (Figure 3).

Figure 3: MicroRNAs: nuclear biogenesis, processing, and functions.

Pri-microRNAs are several kilobases long, capped, polyadenylated primary precursors, with hairpin-like structures, possibly polycistronic (i.e., bearing more than one microRNA). The stem-loop structure is cleaved by nuclear RNAse III Drosha, with the help of the double-stranded RNA-binding domain protein DGCR8, to release a microRNA precursor (pre-microRNA) (Cai et al. 2004; Lee et al. 2003; Lee et al. 2011). The Drosha-DGCR8 complex is called the "microprocessor" and allows nuclear maturation of primary microRNAs. All the previous steps occurring in the nucleus, the export of pre-microRNAs to the cytoplasm is mediated via the Exportin-5 protein (XPO5), and its cofactor Ran-GTP (Bohnsack et al. 2004; Lund et al. 2004). In addition to XPO5, Exportin-1 protein (also termed XPO1) can also participate in the export of microRNA precursors through the nuclear membrane, into the cytoplasm (Castanotto et al. 2009). Once in the cytoplasm, pre-microRNAs are further processed by the action of RNAse III DICER1 and TRBP cofactor, which remove the remaining hairpin structures to give birth to a microRNA duplex of 20 to 25 nucleotides in length. Finally, to be capable of modulating gene expression, one of the two strands of the microRNA, the mature microRNA, is loaded into the *RNA-Induced Silencing Complex* (RISC), which is composed of DICER1, TRBP, and the protein AGO2 (*Argonaute 2*) (Wilson and Doudna 2013). The integrated microRNA guides RISC to its specific mRNA targets either causing their degradation or directly inhibiting their translation into proteins (Feramisco et al. 2010).

Actually, depending on the degree of complementarity between the microRNA and the target mRNA, two different mechanisms of RISC-mediated gene regulation can occur (Doench et al. 2003; for review Stefani and Slack 2008). When the mature microRNA exhibits total complementarity with the target mRNA, the AGO2 component of RISC is capable of degrading mRNA, whereas if base pairing is incomplete, the silencing is achieved by preventing translation. Hence, microRNAs are capable of decreasing translation of human genes through two different silencing mechanisms. Moreover, each microRNA might target several messenger RNAs (Lim et al. 2005), and a single messenger RNA can also be targeted by multiple microRNAs (Brennecke et al. 2005). This increases the level of complexity in microRNA functions.

4.3.3.3 MICRORNAS REGULATE VARIOUS ASPECTS OF HUMAN PHYSIOLOGY AND EPIGENETICS

It has been estimated that human microRNA repertoire could reach or exceed 2000 genes, and that microRNAs may target 30% to 60% of the transcripts in humans (Friedman et al. 2009). Since microRNAs were recognized as a distinct class of biologic regulators with conserved functions, research has revealed their multiple roles in post-transcriptional regulation of gene expression, through transcript

degradation and translational suppression. Different sets of expressed microRNAs are found in different cell types and tissues (Lagos-Quintana et al. 2002). For example, among all microRNAs showing a cell-specific location/expression, miR-203 expression is restricted to keratinocytes (Sonkoly et al. 2007). By affecting gene regulation, microRNAs are likely to be involved in the main biological and physiological processes such as development and organogenesis (Stefani and Slack, 2008; Zhao et al. 2005), differentiation (Chen et al. 2004) proliferation and apoptosis (O'Donnell et al. 2005), growth control, stem cell activity, homeostasis, metabolism, signal transduction, and many others.

All the known mechanisms capable of altering the normal expression of a gene can affect the expression of a microRNA, including mutations and deletions, amplifications, chromosomal translocations, or transcriptional regulation dysfunction, leading to aberrant and dysregulated microRNA expression. Aberrant expression of microRNAs has been observed in numerous disease states, and microRNA-based therapies are under investigations (Tran et al. 2008; Li et al. 2009b; Fasanaro et al. 2010; Zhang et al. 2013).

4.3.3.4 MICRORNAS AND SKIN PHYSIOLOGY

a. Introduction on skin

The skin is the largest organ of the body, representing 15% of total body weight in the adult. Human skin shows a highly complex organization and involves the communication of multiple cell types. Skin is composed of three layers: the epidermis, anchored to a basement membrane (the dermo-epidermal junction), delimiting the underlying dermis and finally, subcutaneous hypodermis containing adipocytes. Skin is interspersed with various structures, such as hair follicles, sebaceous glands and sweat glands, blood vessels, lymphatics, and nerves.

Epidermis, the outermost layer of the skin, is a keratinized stratified squamous epithelium providing chemical, physical, and mechanical protection and serving as a first barrier to microbial infection. During the human embryonic development, epidermis is first constituted by a single-cell layer of ectodermal origin, the periderm, that will undergo a stratification process allowing the formation of the different layers of differentiated cells required to fulfill its barrier function (basal, spinous, and granular layers) (Figure 4). The main types of cells that make up the epidermis are keratinocytes, melanocytes, dendritic cells (including Langerhans cells), T-cells, and Merkel cells. The cells of the proliferative basal layer will alternatively differentiate into hair follicles.

Figure 4: Skin architecture and main processes regulated by microRNAs.

The mesenchymally derived dermis, mainly composed of matrix components (collagens, elastin, fibrillar proteins), also contains also cells, among which are fibroblasts, and immune cells such as mast cells, macrophages, dendritic cells, innate lymphoid cells, and T-cells (Heath and Carbone 2013). The integrity of the dermo-epidermal junction (DEJ) area, located between the epidermis and the dermis, is essential to ensure skin cohesion. Capillary blood and lymphatic vessels are spread throughout the dermis, and nerves access the dermis and the epidermis.

b. MicroRNAs and cutaneous biology

In the skin, microRNA-mediated gene regulation is involved in the main biological processes. To our knowledge, microRNAs were observed and described in skin for the first time in 2005 (Krützfeldt et al. 2005). The evidence of microRNA implication in skin has been demonstrated by the knockdown of DICER1 gene in mouse embryonic skin progenitors, which led to the epidermis-specific removal of microRNA activity and to a disturbed epidermal morphogenesis (Yi et al. 2006).

Many studies revealed that microRNAs are involved in skin homeostasis and cellular processes including development, stem-cell differentiation, signal transduction, metabolism, genomic stability, apoptosis, hair follicle development, skin and hair pigmentation, wound-healing and inflammation (Shilo et al. 2007;

Bostjancic and Glavac 2008; Sand et al. 2009; Feramisco et al. 2010; Hildebrand et al. 2010). MicroRNAs are also dysregulated in melanoma and nonmelanoma skin cancer (Kunz 2013; Sand et al. 2012)

Sets of microRNAs were identified as being important in skin physiology and function:

Holst and collaborators conducted a microRNA expression profiling study, in skin biopsies from eight healthy volunteers, and pointed out the 31 most abundantly expressed microRNA species in human skin (Holst et al. 2010). These microRNAs were selected because they had an expression level exceeding 100-fold over the background. MiR-574 appeared to be the most expressed, with almost a 450-fold expression compared to background. The microRNAs identified in this study are as follow (in increasing abundance): miR-203, miR-200c, miR-296-3p, miR-765, miR-539, miR-34a, miR-323-5p, miR-638, miR-608, miR-122, miR-145, miR-23b, miR-768-5p, miR-23a, miR-768-3p, miR-32, miR-206, miR-210, miR-205, miR-297, miR-672, miR-125b, miR-923, miR-574-5p, miR-595, miR-654-5p, let-7f, let-7d, let-7a, let-7c, and let-7b (Holst et al. 2010).

As a review, Aberdam and collaborators set-up a list of 34 microRNAs expressed in the epidermis: miR-15b, miR-16, miR-17, miR-18, miR-19b, miR-20, miR-21, miR-24, miR-27a, miR-27b, miR-30b, miR-34a, miR-92, miR-93, miR-99b, miR-106b, miR-125a, miR-125b, miR-127, miR-130a, miR-133b, miR-141, miR-191, miR-200a, miR-200b, miR-200c, miR-203, miR-205, miR-429, Let-7b, Let-7c, Let-7f, Let-7g, Let-7i (Aberdam et al. 2008).

Studies of the miRNAome in various pathologies such as psoriasis (Sonkoly et al. 2007, Aberdam et al. 2008, for review), systemic scleroderma (Li et al. 2010), atopic dermatitis (Aberdam et al. 2008, for review), keloids (Li et al. 2013) pointed out the deregulation of the expression of various microRNA sets.

All these studies showed the growing importance of microRNAs at different cell type levels in normal and abnormal skin physiology. The role of particular microRNAs of physiological relevance will be discussed in the following paragraphs.

c. Epidermal renewal and skin barrier

Skin integrity requires an efficient epidermis renewal to maintain the skin barrier function throughout life. The constant renewal of the epidermis is supported by progenitor cells mainly located in the basal cell layer, able to proliferate and to asymmetrically divide to engage suprabasal differentiation (Muffer et al. 2008; Pincelli and Marconi, 2010). These progenitors, also called *"keratinocyte stem cells"* (KSCs) or *"somatic stem cells of the epidermis"* (SSCEs) reside in a special microenvironment called *niche*, in the basal epidermis, and constitute less than 1% of the basal population. A delicate equilibrium between stemness and

differentiation controls epidermal renewal. Keratinocyte differentiation is a highly coordinated multi-step process regulated by autocrine and paracrine intercellular signaling mechanisms (such as growth factors . . .), and other external stimuli, leading to the expression/repression of genes and microRNAs. SSCEs are also observed in other compartments such as hair follicles and sebaceous glands, corresponding to cellular reservoirs (Beck and Blanpain 2012).

In the epidermis, several microRNAs regulate keratinocyte differentiation, but the most important player in this process is miR-203 (Yi et al. 2008).

d. MiR-203 is a master regulator of epidermal differentiation:

MiR-203 has been identified as one of the highly expressed microRNA in healthy human skin (Holst et al. 2010). Sonkoly and coworkers showed that miR-203 formed a gradient with low expression in the basal cell layer and high expression in the more differentiated suprabasal layers, and that it was expressed more than 100-fold higher in skin compared to other organs in human, and that miR-203 was specific to keratinocytes (Sonkoly et al. 2007). MiR-203 pattern of expression is in accordance with an implication in suprabasal keratinocyte differentiation (Sonkoly et al. 2010).

Interestingly, miR-203 was shown up-regulated in psoriasis, a pathology in which keratinocytes show abnormal states of proliferation and differentiation (Bostjancic et al. 2008), suggesting that the fine homeostasis of this microRNA is necessary to maintain a healthy skin. MiR-203 has also been recently identified as specifically overexpressed during the earliest process, leading to the specification of embryonic stem cells into keratinocytes (Nissan et al. 2011).

MiR-203 is involved in the transition of KSCs to “transit-amplifying” keratinocytes (Aberdam et al. 2008; Beck and Blanpain, 2012). In the adult epidermis, miR-203 is detectable only in the upper layers (Yi et al. 2008). In addition, it has been shown that miR-203 overexpression–induced keratinocyte differentiation and miR-203 inhibition with a specific anti-miR (anti-miR-203) resulted in decreased expression of involucrin (a protein involved in the cornified cell envelope, in the *stratum corneum*) after calcium treatment (Sonkoly et al. 2010). MiR-203 permits the switch between basal keratinocyte proliferation and suprabasal differentiation, by repressing stemness, through the post-transcriptional repression of target genes expressed in basal KSCs (Yi et al. 2008; Sonkoly et al. 2010).

e. P63, SOCS3, Zfp281, JUN, and ABL1 are the major mir-203 targets in the epidermis:

Several studies have identified miR-203 target genes in skin. The major target described in KSCs is the transcription factor DNp63, the principal p63 isoform, represents a master regulator of the stratification process and is responsible for

maintaining the proliferative potential of the epithelial stem cells. By controlling the expression of DNp63, miR-203 is involved in the transition of "KSCs" to transit-amplifying keratinocytes, behaving as the molecular switch for epithelial stratification (Lena et al. 2008; Aberdam et al. 2008).

SOCS3, a negative regulator of the JAK/signal transducer and activator of transcription (STAT) pathway is another potential target, but this is subject to controversy, as contradictory studies exist: while Lena and coworkers showed that despite bio-informatic alignment of miR-203 with SOCS3 3'UTR, the levels of SOCS3 mRNAs were not decreased with the increase of miR-203 levels, when keratinocytes were stimulated to differentiate *in vitro* (Lena et al. 2008), another study demonstrated the degradation of SOCS3 transcript in a luciferase reporter assay (Wei et al. 2010).

Evidences indicate that miR-203 can be considered as a tumor suppressor, targeting well-known proto-oncogenes such as c-jun (commonly deregulated in a wide range of cancers, including skin tumors), and c-abl (Abelson murine leukaemia viral oncogene homolog 1, or ABL1). Similarly to other miR-203 targets in the skin, c-jun is preferentially expressed in the basal proliferative layer of the epidermis. The suppression of miR-203 in basal cell carcinoma tumors was associated with a marked increase of c-jun expression (Sonkoly et al. 2012). MiR-203 was also shown to control the expression levels of ABL1, a classic oncogene involved in hematopoietic malignancies, where miR-203 is epigenetically silenced by hypermethylation (Bueno et al. 2008). An additional potential miR-203 target expressed in the basal-layer keratinocytes, is represented by the zinc-finger protein 281 (ZNF281) that may contribute to the basal-suprabasal cell transition (Yi et al. 2006; Aberdam et al. 2008).

Finally, some miR-203 minor target genes such as the *endothelin A receptor* (EDNRA), and the *protein phosphatase eyes absent homolog 4* (EYA4), have also been reported in the basal layer of the epidermis (Hildebrand et al. 2010).

f. Other microRNAs important in epidermal renewal

To analyze changes in microRNA expression during keratinocyte differentiation, the expression of 377 microRNAs has been evaluated after calcium-induced differentiation of primary human keratinocytes *in vitro*, and compared to microRNA expression patterns observed in epidermal stem cells, transient amplifying or terminally differentiated keratinocytes isolated from human skin (Hildebrand et al. 2010). In these experiments, 13 up-regulated and 1 down-regulated (miR-376a) microRNAs were observed after three days of calcium treatment. After seven days of cultivation in the presence of calcium, differential expression reached the numbers of 55 up-regulated and 8 down-regulated microRNAs. The comparison of these expression patterns with the ones obtained

with the transient amplifying or terminally differentiated keratinocytes from skin, allowed the identification of a set of microRNAs that may cooperate during skin differentiation (Hildebrand et al. 2010). Indeed, apart from miR-203, nine additional microRNAs were associated with human keratinocyte differentiation *in vitro* and *in vivo*: 8 microRNAs up-regulated during differentiation (miR-23b, miR-26a, miR-27b, miR-95, miR-200a, miR-210, miR-224 and miR-328) and one microRNA down-regulated (miR-376a).

A recent study highlighted the role of miR-574-3p and miR-720 in the regulation of p63 expression, *via* the protein iASSP (Beck and Blanpain 2012, for review). p63 and iASPP are both expressed in the basal epidermal keratinocytes, and iASPP is involved in the regulation of several members of the desmosomal proteins, tight junction and gap junction components, and b1 integrin. The expression of the protein iASSP, under the control of p63, represses miR-574-3p and miR-720, diminishing their impact on the degradation of p63 transcripts, thus forming a positive autoregulatory feedback loop.

MiR-24 is expressed in the suprabasal layers of human epidermis and induces differentiation *in vivo* by controlling actin adhesion dynamics and cytoskeletal modification during keratinocyte differentiation (Amelio et al. 2012).

Another study reported that miR-200 and miR-205, both highly expressed in normal skin, targeted ZEB1 and ZEB2, the transcriptional repressors of E-cadherin, playing an essential role in maintaining epithelial stability (Banerjee et al. 2013).

Finally, the miR-193b/355a cluster has been very recently shown to have anti-proliferative and anti-migratory properties in human keratinocytes, consistent with potential tumor suppressor functions in the epidermis (Gastaldi et al. 2013).

g. Skin pigmentation

Skin pigmentation plays a critical role in skin protection at the cellular/DNA level, particularly at the level of the basal keratinocytes allowing epidermal renewal. Melanocytes synthesize melanin in special organelles called melanosomes, which upon maturation are transferred to keratinocytes, and form a supranuclear melanin cap, thus protecting genomic DNA from harmful effects of solar UV irradiation. Several biological processes are implicated in the control of skin pigmentation: melanocyte signaling pathways involved in the transcriptional regulation of melanogenic enzymes, tyrosinase and related enzymes, melanosomal packaging and transfer, autophagic melanin scavenging in keratinocytes, and inflammation (Cichorek et al. 2013). Each of these processes is potentially regulated by post-transcriptional silencing by microRNAs; however, further research will be needed to elucidate the whole mechanism.

There is a great interest in the scientific and medical community to study microRNAs involved in the progression of melanoma, a most dangerous form of skin cancer affecting melanocytes. Kozubek and colleagues determined the microRNA transcriptomic signature distinguishing specimens of melanoma from normal human skin and set up a list of 40 microRNAs. Top-ten microRNAs deregulated in human melanoma was as follows: miR-205, miR-211, miR-15b, miR-26a, miR-451a, miR-203, miR-23b, miR-26b, miR-877, and let-7i-5p (Kozubek et al. 2013). One remarkable member of this list is miR-203 (down-regulated in melanoma), the well-known regulator of keratinocyte differentiation, that evidently also plays a role in melanocyte pigmentation. Hence, the decrease of miR-203 was described to play a pivotal role in melanoma, through reducing melanosome transport and promoting melanogenesis by targeting kif5b, the kinesin superfamily protein 5b, and through negative regulation of the CREB1/MITF/Rab27a pathway (Noguchi et al. 2013).

Albeit the majority of the studies on microRNAs and pigmentation report the modulation of microRNA expression in melanoma cells, several studies on normal human melanocytes NHEM pointed out the potential role of specific microRNAs in human skin pigmentation:

Wu and colleagues described in 2008 the effect of miR-434 targeting tyrosinase in human melanocytes (Wu et al. 2008). A study in Alpaca melanocytes showed the implication of miR-25 as a regulator of pigmentation, targeting MITF expression (Zhu et al. 2010). The increased expression of miR-145, by targeting genes implicated in melanogenesis (Sox9, Mitf, Tyr, Trp1) and genes important for melanosome transport (Myo5a, Rab27a, and Fscn1), induced depigmentation (Dynoodt et al. 2013). In the same study, several microRNAs were identified after forskolin and UV treatment of mouse melanocytes: some of them were up-regulated (miR-130b, miR-182, and miR-9) or down-regulated (miR-125b, miR-139-5p, miR-145, miR-155, miR-193, miR-206, miR-218, miR-221, miR-222, miR-28, miR-335, miR-365, and miR-455), suggesting their implication in the regulation of skin pigmentation (Dynoodt et al. 2013).

In a recent publication, miR-125b was identified as a potent regulator of melanogenesis, as its expression level was inversely related to pigment levels (Kim et al. 2014). Interestingly, the authors demonstrated a modulation of miR-125b expression by methylation of the *MIR125B-1* gene promoter.

Surprisingly, microRNAs originating from other cell types seem to control melanocyte pigmentation: indeed, Kim and colleagues recently demonstrated that miR-675 was secreted in exosomes by keratinocytes, and that it could regulate melanocyte pigmentation through a mechanism involving MITF (Kim et al. 2013). This observation enlarges our vision of the mechanisms of action of microRNAs, and brings to our attention the potential role of extracellular microRNA-containing

exosome as cell-cell mediators (Hu et al. 2012). Exosomes containing mRNAs and microRNAs are also important in pathological context, as their content is modified in cancers such as melanoma, and their characterization may lead to the identification of useful diagnostic biomarkers (Xiao et al. 2012).

h. Dermal physiology

Several studies have investigated microRNA expression in dermal fibroblasts and showed their involvement in the compositional remodeling of the dermis by the regulation of fibroblast senescence and extracellular matrix (ECM) protein synthesis.

Gu and Iyer evidenced the implication of 33 microRNAs in the reentry of fibroblasts into the cell cycle and proliferation, through the regulation of three signaling pathways: the PI3K pathway, the JAK/STAT pathway, and the MAP kinase pathway. Several microRNAs belonging to the let-7 family (let-7b c, d, e, f1, f2, g, i) as well as several other microRNAs (miR-15a, miR-15b, miR-21, miR-22, miR-23a, miR-23b, miR-31, miR-34a, miR-99a, miR-100, miR-107, miR-125a, miR-125b, miR-130a, miR-143, miR-145, miR-199, miR-214, miR-221, miR-222, miR-320, miR-368, miR-376a, miR-424) were hence identified (Gu and Iyer, 2006). Additional microRNAs have been identified for their role in replicative aging and senescence in fibroblasts: down-regulated miR-17, miR-19b, miR-20a and miR-106a (Hackl et al. 2010); down-regulated miR-21, miR-143, miR-145, miR-155, and up-regulated miR-10b, miR-23a, miR-26a, miR-34a, miR-146a, miR-199, miR-542 (Bonifacio and Jarstfer 2010); up-regulated miR-152 and miR-181a, targeting respectively integrin α5 and collagen XVI (Mancini et al. 2012); down-regulated miR-7, miR-18, miR-20, miR-29a, and up-regulated let-7b, miR-26, miR-30, miR-125a, miR-181, miR-199, implicated in fibroblasts proliferation and regulation of ECM synthesis (Suh et al. 2012); miR-29 and miR-30, targeting b-myb and the pRb pathway implicated in the control of cellular proliferation (Martinez et al. 2011; Martinez and DiMaio 2011). Finally, members of miR-29 family (miR-29a, b, and c) were found up-regulated during induced and replicative senescence in fibroblasts (Feliciano et al. 2011; Uglade et al. 2011), particularly miR-29b, which is a potent post-transcriptional repressor of collagen type I (Cheng et al. 2010).

Deregulation of physiological context in the dermis leads to conditions that helped identify microRNAs regulating fibroblast functions. Scleroderma, or systemic sclerosis, is a localized or systemic autoimmune disease affecting the skin, with excessive collagen deposition, fibrosis, and vascular alterations. However, little is known about the exact mechanism mediating the excessive collagen expression. Several microRNAs have been associated with this disorder. For example, the decrease of microRNA let-7a observed in patients contributes to the

excessive expression of type I collagen (Makino et al. 2013). Levels of miR-92a in dermal fibroblasts and in the sera of patients with systemic scleroderma was found increased. In fibroblast. The increase of this microRNA targeting MMP1, a major protease degrading collagens in the dermis, may participate to the accumulation of collagens observed in this disease (Sing et al. 2012). Similarly, dermal fibroblasts and the sera of patients showed a decreased miR-150 level in patients suffering from scleroderma. As a result, integrin b3 and collagen I expressions were increased (Honda et al. 2013). In another study, miR-7 down-regulation was also evidenced in patients with localized sleroderma (Etoh et al. 2013). Similarly, variations in the seric levels of miR-206 and miR-21 was correlated with scleroderma, and was proposed as a diagnostic association (Koba et al. 2013). A microarray chip analysis, comparing the miRNAome of fibroblasts from patients with systemic scleroderma to healthy individuals, identified six miRNAs: miR-206, miR-133a, miR-125b, miR-140-5p (up-regulated), and miR-23b and let-7g (down-regulated) (Li H. et al. 2013).

The study of another skin condition, keloids, allowed the identification of microRNA modulating the expression of collagens. Keloids are an overgrowth of scar tissue due to abnormal wound-healing process after skin injury, characterized by fibroblast proliferation and overproduction of extracellular matrix such as collagens. The study of microRNAs differentially expressed between keloid tissue and corresponding normal skin tissue pointed-out 32 microRNAs (Liu et al. 2012). Among them, up-regulated miR-21, miR-382, and miR-4269, and down-regulated miR-200c, miR-203, and miR-205 were validated by quantitative real-time PCR. A more recent study on normal and keloid skin fibroblasts revealed the differential expression of nine microRNAs, six of which were significantly up-regulated in keloid fibroblasts, including miR-152, miR-23b-3p, miR-31-5p, miR-320c, miR-30a-5p, and hsv1-miR-H7; and three were significantly down-regulated: miR-4328, miR-145-5p, and miR-143-3p (Li et al. 2013). MiR-196a was also shown to be involved in the expression of collagens during keloids formation (Kashiyama et al. 2012).

In conclusion, the dermis plays an important role in the aging process, resulting in altered appearance of the skin, and the emergence of wrinkles. The capacity of dermal fibroblasts to secrete good quality and sufficient quantity of extracellular matrix proteins through time is important to face the senescence process.

i. MicroRNAs and the hypodermal adipocytes

The process of adipogenesis corresponds to the differentiation of mesenchymal stem cells (MSC) into pre-adipocytes and then the maturation of these pre-adipocytes into functional adipocytes. Two principal types of fat are present in mammals: the white adipose tissue, which is predominant in adult human, and the

brown adipose tissue, present in significant amounts in newborns. Hypodermal adipocytes are part of the white adipose tissue and are involved in skin energy balance: they allow the storage of energy as fat in structures called lipid droplets. Lipid reserves occupy most of the cell space and can be released according to energy need in the tissue. Extracellular environment, hormones, and autocrine/paracrine signaling factors regulate adipocyte differentiation and maturation. The identification of microRNAs implicated in adipocyte metabolism is of high interest, in order to better understand hypodermal physiology and represent potential therapeutic targets against lipid storage disorders and obesity (McGregor and Choi 2011).

Several microRNAs have been identified as modulators of adipocyte physiology and obesity in mice. Indeed, the possibility to obtain genetically modified mice (expression knock-out of a given gene), specific mouse strains (such as *"ob/ob"* or obese mice), or adipocyte cell lines (such as 3T3-L1 pre-adipocyte cell line) designates these animals as a model of choice. A recent article reviews these microRNAs: Let-7, miR-15a, miR-17/92, miR-21, miR-24, miR-27, miR-31, miR-103, miR-107, miR-125b, miR-138, miR-143, miR-150, miR-200, miR-210, miR-221, miR-222, miR-326, miR-355, miR-378, miR-448, miR-519d (McGregor and Choi 2011).

Few studies are available on microRNAs involved in adipocytes growth and differentiation in humans. Essentially, the down-regulation of miR-155, miR-221 and miR-222 together seems to be required for adipocyte differentiation from human MSCs. These microRNAs act on two known key regulators of adipogenesis: the peroxisome proliferator-activated receptor gamma (PPARγ) and the CCAAT/enhancer binding protein beta (C/EBPβ) (Skårn et al. 2010). Another publication shows that miR-22 is able to influence negatively the differentiation of MSC in adipocytes: miR-22 expression inhibits MSC adipogenic differentiation and favors osteogenic differentiation by repressing the histone deacetylase 6 (HDAC6) (Huang et al. 2012). As for MiR-30, it was shown to induce the differentiation of MSC into adipocytes, by repressing osteogenic differentiation through targeting RUNX2 (Zaragosi et al. 2011). Another microRNA described in the inhibition of MSC differentiation in adipocytes is miR-138. Its overexpression revealed a reduced lipid droplet accumulation in cells, and a reduced expression of key adipogenic transcription factors such as C/EBPa and PPARg (Yang et al. 2011). MiR-21 seems to participate in adipocyte differentiation via the modulation of TGFb signaling (Kim et al. 2009). The inhibition of miR-26b is enough to reduce differentiation and formation of lipid droplets (Song et al. 2014). Another interesting microRNA is miR-27b, described to be able to suppress adipogenesis in multipotent adipose-derived stem cells by targeting PPARg (Karbiener et al. 2009). MiR-130 has also been reported to modulate PPARg and influence negatively adipogenesis (Lee et al. 2011). Recently, the knockdown of miR-143, a microRNA

already known to regulate adipocyte maturation by targeting mitogen-activated protein kinase 7 (ERK5) (Esau et al. 2004), did not reveal major changes, suggesting that miR-143 is only a fine tuner of the adipogenic differentiation and not a master regulator (He et al. 2013).

Specific microRNAs have also been identified in the brown adipose tissue. As an example, the miR-193b-365 cluster is a key regulator of brown fat development. These particular microRNAs are also involved in myogenesis repression for routing the differentiation to brown adipose tissue (Sun et al. 2011).

In conclusion, specific microRNAs can modulate and control adipogenesis (white or brown), and the deregulation of their expression may participate in fat accumulation disorders. Indeed, miR-519d has been associated with human obesity, implicated in regulating the expression of PPAR family members during adipogenesis (McGregor and Choi 2011).

j. Hair follicle morphogenesis

During the embryonic development, morphogenesis of hair follicles is initiated through interactions between the periderm cells and the underlying dermis, leading to localized invagination of epithelial cells to form hair follicles. The mature hair follicle is constituted of various layers of epithelial keratinocyte cells surrounding the hair shaft, and contains also specialized dermal fibroblast cells in the dermal papilla, and melanocytes, responsible for hair color. The outermost keratinocyte cell layer of the hair follicle, the outer root sheath, is contiguous with the basal layer of the epidermis. Hair follicles undergo cycles of growth (anagen phase), regression (catagen phase), and rest (telogen phase) (Alonso and Fuchs 2006). MicroRNAs play essential roles in hair follicle organogenesis, as the epidermal-specific Dicer deletion in mice resulted in hypoproliferative follicles, with no hair shaft (Andl et al. 2006).

Only a few studies report the role of microRNAs in human hair follicle morphogenesis and cycling, as most of them were performed on mice and other animals. However, a list of 31 microRNAs expressed in the hair follicle was compiled by Aberdam and collaborators: miR-15a, miR-15b, miR-16, miR-17, miR-18, miR-20, miR-21, miR-24, miR-27b, miR-92, miR-99b, miR-106b, miR-125b, miR-126, miR-127, miR-130a, miR-143, miR-152, miR-196, miR-199a, miR-199b, miR-206, miR-214, miR-351, Let-7a, Let-7b, Let-7c, Let-7d, Let-7f, Let-7g, Let-7i (Aberdam et al. 2008). The underlined microRNAs were also expressed in the epidermis. One microRNA of these lists, miR-196 (miR-196a-2) was recently shown to target tyrosinase expression, and a particular miR-196a-2 mutation (rs11614913 C allele) may account for a decreased risk of vitiligo (Huang et al. 2013).

Interestingly, miR-221, miR-125b, miR-106b, and miR-410 were found up-regulated in androgenetic alopecia (or male pattern baldness, MPB), suggesting their possible implication in the pathogenesis of MPB (Goodarzi et al. 2010).

4.3.3.5 INTEREST OF MICRORNAS IN THE EVALUATION *IN VITRO* OF ANTI-AGING DERMO-COSMETIC INGREDIENTS

a. Skin aging

Two distinct types of aging are affecting the skin, both contributing to structural and functional decline. Aging caused by our genetic inheritance and the changes in gene expression as time goes by, is called chronological or intrinsic aging, and can be mimicked *in vitro* by a high number of cell subcultures, known as "replicative senescence." The other type of aging is referred to as extrinsic aging, and is due to environmental factors such as sun exposure, and particularly repeated exposure to ultraviolet light (UV), overemphasizing the effects of intrinsic aging. It is noteworthy that environmental factors influencing skin aging are determining factors in the condition of mature skin, and play a central role in skin appearance impairment by inducing cellular senescence.

Whereas chronological aging is characterized by an atrophic pale skin (sun-protected) with fine wrinkles and laxity-inducing exaggerated facial expression lines, extrinsic aging leads to a rather hypertrophic skin, as the result of anti-inflammatory, anti-oxidant, and DNA-protectant responses to the damaging effects of UV irradiation. Moreover, photo-aged skin comes along with many manifestations ("age spots") corresponding to irregular pigmentation (e.g., freckles and solar lentigines); xerosis and leathery skin; senile purpura, cherry angiomas, and telangiectasias due to blood vessel abnormalities; comedones and premalignant skin tumors such as seborrhoeic keratoses; and a more pronounced wrinkling, compared to sun-protected aged skin.

b. MicroRNAs and cellular senescence

Cellular senescence is an "irreversible" state of cell cycle arrest that is induced during cellular aging and is involved in tissue maintenance (Bilsban et al. 2013). In the skin, senescence is associated with phenotypic changes in cutaneous structure and cells, and the appearance of markers of aging (senescence-associated beta-galactosidase, etc.). Many genes have been reported to be involved in the regulation of senescence: tumor suppressor genes (e.g., p53), senescence genes (e.g. p16, p21) and senescence suppressor genes (e.g., telomerase), oncogenes (e.g., MYC), stemness genes (e.g., SOX2), genes related with epigenetics (e.g., SIRT1, SATB1), inflammation-related genes, DNA damage signaling, cytoskeletal remodeling (e.g., TGF, WNT), and various transcription factors (Lafferty-Whyte et al.

2009; Olivieri et al. 2013). All these genes participate in a network of interactions regulating senescence, ending up with the highly regulated p53–p21 and p16–pRB pathways (Xu and Tahara 2013).

One strong piece of evidence that microRNAs can significantly modulate the senescence process *in vivo* is the conditional DICER ablation in adult mouse tissues, resulting in a premature senescence phenotype (Mudhasani et al. 2008). In regard to senescence regulation and induction, specific microRNAs have emerged as key modulators of the p53–p21 and p16–pRB pathways, and are related to inflammation, cellular senescence, and age-related diseases, cancer included. Hence, these microRNAs can be considered as *"inflammation-associated"* (inflamma-miRs), *"senescence-associated"* (SA-miRs), or *"cancer-associated"* (onco-miRs) (Bilsland et al. 2013).

Several publications reviewed the known SA-miRs (Lafferty-Whyte et al. 2009; Olivieri et al. 2013). Thus, Lafferty-Whyte and colleagues listed the main SA-miRs: miR-20a, miR-29c, miR-34a, miR-34b, miR-34c, miR-217, miR-369, miR-371, miR-372, miR-373, miR-499, let-7f (Lafferty-Whyte et al. 2009). Olivieri and colleagues recently updated this list, pointing out 27 human SA-miRs and 21 human inflamma-miRs, and observed 12 microRNAs common to the two lists. Interestingly, the majority of the microRNAs considered both as SA-miRs and inflamma-miRs (miR-9, miR-19a, miR-19b, miR-20a, miR-21, miR-29a, miR-126, miR-146a, miR-146b, miR-155, miR-181a, and let-7) are also deregulated in human cancers (Olivieri et al. 2013). Moreover, some of these microRNAs, and others, are associated with the most common age-related diseases (atherosclerosis, ischemic heart disease, diabetes mellitus type 2, kidney disease, cataracts, sarcopenia, osteoporosis . . .) (Schraml and Grillari 2012), confirming the importance of microRNA homeostasis in the aging process.

Interestingly, in very old people, a signature of up-regulated small noncoding RNAs (including microRNAs) was recently reported in circulating mononuclear cells from centenarians, compared to octogenarians and young people (Serna et al. 2012). The identified SA-miRs were miR-21, miR-130a, miR-494, miR-1975, and miR-1979. SCARNA17 (also called U91), a small nuclear RNA possibly involved in telomerase regulation, was also up-regulated in centenarians. The identification of this RNA signature may help us understand the molecular mechanisms and gene expression regulation endowing centenarians with extreme longevity (Serna et al. 2012).

Intrinsic senescence: The deregulation of particular microRNAs has been shown to play a role in intrinsic senescence. For example, SA-miR-22 was shown up-regulated in senescent fibroblasts, and suspected to be able to repress cancer progression by inducing cellular senescence, as it is known to be down-regulated in various cancer cell lines (Xu et al. 2011). In contrast, miR-24 was down-regulated

in senescent fibroblasts, whereas its target, p16 (pRB pathway), was up-regulated (Lal et al. 2008). MiR-34a is one of the microRNAs to have attracted a lot of attention, as it is considered a tumor suppressor regulating apoptosis in cancerous cells (Yamakuchi et al. 2008). MiR-34a up-regulation is observed in both permanent growth arrest states of replicative senescence, and in hydrogen peroxide–induced premature senescence of replicating lung fibroblasts (Maes et al. 2009). Interestingly, the epigenetics-related enzyme SIRT1, believed to regulate cell growth and apoptosis by deacetylating molecular targets that include p53, is a target of miR34a. Finally, miR-34a may also regulate the expression of transcription factor E2F3, which regulates cell-cycle progression (Yamakuchi et al. 2008). In epidermal keratinocytes, replicative senescence is associated with diffuse chromatin remodeling and epidermal terminal differentiation, and microRNAs are modulated during this process. As an example, miR-191 was identified as one of the most up-regulated miRNAs in senescent keratinocytes, targeting SATB1 (Special AT-rich Binding protein 1) and CDK6 (Cyclin Dependent Kinase 6) mRNAs (Lena et al. 2012). The identification of SATB1, a protein known as an epigenetic "higher order chromatin organizer" (Mir et al. 2012) implicated in the early development of epidermis (Fessing et al. 2011), suggests that up-regulation of miR-191 may lead to senescence-associated epigenetic modifications, inducing a new cellular gene expression profile, also inducing senescence (Lena et al. 2012). Another study reported that several SA-miRs (i.e., miR-138, miR-181a, miR-181b, and miR-130b) were up-regulated in human primary keratinocytes after replicative senescence, and participated in the regulation of ΔNp63 and SIRT1 (Rivetti di Val Cervo et al. 2012).

Extrinsic senescence: Other microRNAs were identified for their implication in extrinsic senescence. The deregulation of miR-15a, miR-20a, miR-20b, miR-93, and miR-101 has been shown to play a role in extrinsic senescence through targeting the p53–p21 and p16–pRB pathways (Greussing et al. 2013). A study pointed out the differential expression of miR-146a in UVA-irradiated fibroblasts, suggesting its implication in the regulation of aging-related genes (Li et al. 2013). MiR-34c, already known to be involved in intrinsic senescence, was also shown up-regulated in UV-induced senescence in cultured fibroblasts (Zhou et al. 2013).

The study of protein components involved in human diseases associated with phenotypic senescence led to the discovery of several microRNAs regulating their expression level. Nuclear lamins are a good example of such an approach. Lamin B1 (LMNB1) together with lamin A (LMNA) are major constituents of the nuclear envelope of cells. Lamin A mutations have been observed in a premature aging syndrome called *progeria*, whereas mutations in lamin B1 cause adult autosomal dominant leukodystrophy (ADLD). Decline of LMNB1 in senescent human dermal fibroblasts and keratinocytes was recently observed by Dreesen and

colleagues, showing that miR-23a could mediate the decrease of LMNB1 mRNA. This phenomenon is also observed in chronologically aged human skin tissue (Dreesen et al. 2013).

Cellular senescence has also been reported to be associated with the release of exosomes, which derive from lysosomes, a mechanism regulated by p53 protein in senescent cells. These senescence-associated exosomes can transfer microRNA and protein cargos between cells, mediating cell-cell communication during cellular senescence (Lehmann et al. 2008). Exosome-mediated microRNA transfer is an important mechanism of intercellular communication (Valadi et al. 2007) and potentially constitute a novel mechanism for long-range signaling during senescence. Hence, the release of exosomes from senescent cells may induce inflammation and microenvironment alteration in age-related diseases. Furthermore, the observation of intercellular signaling organelles open the door to investigate altered circulating microRNA levels as biomarkers of senescence (Lehmann et al. 2008; Bilsban et al. 2013; Xu and Tahara, 2013).

c. Tissue-engineering and microRNA studies

For the past few years, the use of bioengineered skin equivalents has encountered a strong development in pharmaceutical and dermo-cosmetical companies, allowing the access to reproducible and coherent testing models, as substitutes for cultured organotypic tissues and human skin. 3D reconstructed skin is a real technological advance allowing to obtain tissues close to *in vivo*, produced in a mastered environment with chemically defined medium, and has also medical applications in tissue-engineered skin bioconstructs for autografts, after severe burns or wounds (Shevchenko et al. 2010). Tissue reconstruction starts with primary cells extracted from human skin (keratinocytes, melanocytes, fibroblasts, etc.), obtained from patients undergoing surgery. Cells are then cultured in a specific medium and amplified in monolayer conditions to select mainly proliferative cells, that will be conserved frozen in liquid nitrogen, and will constitute a cell bank. The second step is the 3D reconstruction of a well-differentiated tissue, starting from the frozen cells. Epidermis can be reconstructed as a fully differentiated epithelium, with additional autologous cell types (e.g., melanocytes, to obtain a pigmented tissue) and also grown on a dermal-equivalent tissue, elaborated with autologous fibroblasts grown in a collagen scaffold (this model is sometimes referred to as "full thickness"), that will secrete proteins of the dermo-epidermal junction at the interface, with the aim to mimic the histological structure of normal skin, with both the epidermal and dermal layers.

Engineered epidermis and reconstructed skin constitute great models to study the role of microRNAs in skin, particularly keratinocyte proliferation and differentiation processes, or the relationships between keratinocytes and melanocytes

during melanogenesis and skin pigmentation. Models including a dermal equivalent compartment allow assessing the implication of microRNAs in fibroblast physiology, secretion of the dermal extracellular matrix, and communication between dermis and epidermis. Skin reconstruction also gives the opportunity to study microRNA expression in the context of the donor (influence of age, gender, ethnicity, skin phototype, epigenetic environmental factors . . .) and during the chronological differentiation of the skin epithelium. The use of anti-microRNAs or pre-microRNAs in these reconstructed models opens the way to study the effect of specific microRNAs on gene expression, protein localization, affected signaling pathways and biological processes, and the subsequent morphological and functional consequences.

CONCLUSION

Since the discovery of microRNAs in humans and the evidence of their expression in the skin, a growing number of researches proved that microRNAs are important regulators of the main biological processes, such as skin development, epidermal morphology, proliferation and differentiation of keratinocytes, hair morphogenesis, melanin production and skin or hair pigmentation, dermal ECM synthesis and adipocyte physiology. Microarray, RNA sequencing of microRNA, qPCR, and qPCR array are the main experimental approaches that have been developed to perform expression profiling of microRNAs, and to identify which are expressed in particular cell types, and their level of expression, under several conditions. It will be important in the future to better understand the mechanisms controlling microRNA expression, and the role of epigenetics, particularly DNA methylation, histone modifications, and chromatin organization. It will also be crucial to determine which microRNAs are important to ensure the physiological cohesion and the homeostasis of the skin, considered as a whole organ, and their role in other skin cell types such as Merkel cells, afferent neurons, immune cells, etc. Moreover, the deregulation of microRNA expression, either up- or down-regulated, observed in many skin pathologies and cutaneous cancers, shows that the impairment of microRNA expression may be implicated in the pathogenesis of these affections. In this case, microRNA signatures could serve as novel diagnostic markers of such pathological conditions. The role of extracellular, possibly circulating microRNA-containing exosomes has to be further investigated in an intercellular and inter-tissular context, and exosome content characterization may also serve for pathological profiling.

Elucidating the role of microRNAs and the processes they regulate in the skin, in healthy and in pathological conditions, will constitute important challenges for future research and therapeutic strategies.

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GLOSSARY:

3'UTR: 3' untranslated region of a messenger RNA, located downstream of the stop codon.

AGO2: Argonaute 2 is encoded by AGO2 gene and is implicated in short interfering RNA mediated gene silencing.

BAT: Brown adipose tissue: Tissue specialized to burn lipids for heat generation and energy expenditure as a defense against cold and obesity.

B-MYB: *V-Myb Avian Myeloblastosis Viral Oncogene Homolog-Like 2.* B-myb is a member of the *myb* family of transcription factor, encoded by MYBL2 gene, and is a nuclear protein involved in cell cycle progression.

C/EBP β: CCAAT/enhancer binding protein beta, encoded by CEBPB gene. The protein can bind the promoter and upstream element and stimulate the expression of the collagen type I gene, and is also implicated in immune and inflammatory responses.

DICER1: A Ribonuclease III family enzyme. Dicer was given its name by Emily Bernstein, a graduate student at Cold Spring Harbor Laboratory, who first demonstrated the dsRNA "dicing" activity of this particular enzyme.

DGCR8: *DiGeorge syndrome critical region 8***.** This gene encodes a subunit of the microprocessor complex that mediates the biogenesis of microRNAs.

DROSHA: A Ribonuclease III family enzyme implicated in microRNA processing. The "microprocessor" is a complex comprising the RNase III enzyme Drosha and the double-stranded RNA-binding protein DGCR8, catalyzing the nuclear step of microRNA biogenesis.

ECM: Extracellular matrix. ECM is the extracellular scaffold present in all kinds of tissues, involved in tissue morphogenesis, differentiation, and homeostasis.

ERK5: *Extracellular signal-regulated kinase 5.* ERK5 is a member of the MAP (mitogen-activated protein) kinase family. Synonym: *Mitogen-Activated Protein Kinase 7* (MAPK7).

FSCN1: *Fascin Homolog 1, Actin-Bundling Protein.* This gene encodes a member of the fascin family of actin-binding proteins.

iASSP: *Apoptosis stimulating proteins of p53 (ASSP) inhibitor.* Also known as *Protein Phosphatase 1, Regulatory Subunit 13 Like* (PPP1R13B-Like).

miR: MicroRNA. A category of noncoding ribonucleic acids (RNAs).

MITF: *Microphthalmia-associated transcription factor.* The corresponding protein regulates the expression of melanogenesis enzyme genes.

mRNA: Messenger ribonucleic acid.

MYO5A: Myosin VA is an actin-based motor protein involved in cytoplasmic vesicle transport and mRNA translocation.

NHEM: Normal human epidermal melanocyte.

NHF: Normal human fibroblast.

NHK: Normal human keratinocyte.

Orthologous genes: Relates to genes for which the homology is the result of speciation. Orthologous genes are hence encountered in different species.

Paralogous genes: Relates to genes for which the homology is the result of gene duplication.

PKC: Protein-kinase C is a family of serine- and threonine-specific protein kinases involved in diverse cellular signaling pathways, that can be activated by calcium and diacylglycerol, and phosphorylate in a wide variety of protein targets.

PPARg: Peroxisome proliferator-activated receptor gamma nuclear receptors. PPARs form heterodimers with retinoid X receptors (RXRs), thus regulating the transcription of various genes.

pRb pathway: Retinoblastoma (Rb) pathway, involved in the control of cell proliferation and apoptosis.

RAB27A: Gene encoding a ras-related protein, belonging to the rab family of small GTPases.

RAN-GTP: *RAS-related nuclear protein*, binding a GTP molecule**.**

RISC: RNA-induced silencing complex, also known as *RISC loading complex* (RLC), or *microRNA loading complex* (miRLC). RISC is composed of DICER1, AGO2, and TRBP.

RNase III: Ribonuclease III enzyme. DROSHA and DICER1 are RNAse III enzymes.

RQ: relative quantification. RQ allows expressing of changes in gene expression, in a given condition, relative to a reference condition (e.g., untreated control).

RUNX2: Runt-related transcription factor 2. This gene encodes a member of the RUNX family of transcription factors, essential for osteoblastic differentiation and skeletal morphogenesis.

SCARNA17: Small Cajal Body-Specific RNA 17 (synonym: U91). SCARNA17 is a small nucleolar RNA (snoRNA) associated with small nucleolar ribonucleoproteins.

SIRT1: *Sirtuin 1 (Silent information regulator 1).* The encoded protein is a member of the sirtuin family, homologs to the yeast Sir2 protein.

SOCS3: *Suppressor of cytokine signaling 3.* This gene encodes a member of the *STAT-induced STAT inhibitor* (SSI), also known as *suppressor of cytokine signaling* (SOCS) family.

SOX2, SOX9: Transcription factors involved in embryonic development, stem-cell maintenance and in the determination of cell fate.

SSCE: Somatic stem cell of the epidermis.

TGFb: Transforming growth factor beta is a family of cytokines, including TGFb1 and 2, regulating proliferation, differentiation, adhesion, and migration in many cell types.

TRBP: *Transactivating response RNA binding protein*, also known as TARBP2, is a member of the RNA-induced silencing complex (RISC), with DICER1 and AGO2.

Tyr, Tyrp1, Tyrp2: Tyrosinase and Tyrosinase-Related Protein 1 and 2 are melanosomal enzymes involved in the melanin biosynthetic pathway.

UTR: Untranslated region.

WAT: White adipose tissue. Serves primarily to store extra energy as triglycerides, lipid droplets.

WNT: *Wingless-Type MMTV Integration Site Family.* Family of signaling proteins implicated in developmental processes.

XPO1: *Exportin 1*, or *chromosome region maintenance 1* (CRM1). The protein encoded by this gene mediates leucine-rich nuclear export signal (NES)-dependent protein transport.

XPO5: *Exportin 5.* This gene encodes a member of the karyopherin family that is required for the transport of small RNAs from the nucleus to the cytoplasm, in association with ran-GTP.

ZEB1, ZEB2: *Zinc finger E-box binding homeobox 1, 2.* The protein zeb1 is a transcription factor, whereas zeb2 is a DNA-binding transcriptional repressor.

AMINO ACIDS

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ABSTRACT

Everyone ages.

Everyone wants to stop aging.

But is that really what we want? In this chapter, the author asserts that *we want to accumulate as many healthy years as possible but still maintain the appearance of youth and vigor.* Medical science has extended our life span and useful years dramatically by addressing disease and overall health issues. Cosmetic and Personal Care science can help mitigate the appearance of aging and increase one's overall attractiveness.

There are twenty "standard" proteinogenic amino acids that combine in unique ways to make peptides, proteins, enzymes, muscle, hair, skin, and organs. These twenty amino acids are also converted in the body through various metabolic pathways to produce useful substances. Amino acids topically applied exhibit useful properties such as acting as antioxidants, humectants, and free radical scavengers. They have also been shown to increase collagen production. Amino acids can also be derivatized "ex-somes" to produce cosmetically useful materials such as emollients, humectants, surfactants, moisturizers, and protectants that are beneficial in cosmetic formulation to address the appearance of aging. In this chapter we discuss the various amino acids, their derivatives, and the benefits to skin and hair formulation from a formulator's perspective.

It is our intention that this detailed chapter covering the structure and behavior of amino acids and their derivatives, on both skin and hair, will provide formulators and marketers with new pathways to create desired consumer products and to explore this interesting field further.

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4.3.4.1 OVERVIEW OF AMINO ACIDS

a. Production

Amino acids are the building blocks of life. An amino acid is an organic compound that contains both an amino (NH-) functionality and a carboxylic acid (COOH) functionality. There are twenty standard amino acids that combine in specific ways to produce peptides, polypeptides, proteins, enzymes, muscles, and skin. These twenty amino acids are divided into nonessential amino acids and essential amino acids. The nonessential amino acids are those that we produce within our bodies in sufficient amounts for life and health. These are glycine, L-cysteine, L-alanine,

L-tyrosine, L-arginine, L-proline, L-aspartic acid, L-serine, L-glutamic acid, L-aspargine, and L-glutamine. The essential amino acids are just as important for life and health as the nonessential amino acids, but we do not make them or at least do not make sufficient amounts of them in our bodies to support life and health. Accordingly, we must obtain these amino acids from the food chain or more recently, from supplementation. The essential amino acids are L-mehtionine, L-tryptophan, L-threonine, L-phenylalanine, L-valine, L-lysine, L-leucine, L-histidine, L-isoleucine. Beyond these twenty biological important amino acids there are more than 500 other amino acids that are not useful biologically. There are also a myriad of derivatives that are designed for specific useful functions such as cleansing, moisturizing, lubrication, etc.

The Fermentation Method

"In 1908, Professor Kinue Ikeda discovered glutamic acid as a flavor component of seaweed and soy hydolysates. After neutralization with caustic soda, he isolated Monosodium Glutamate. This was not only the birth of the first industrial scale amino acid production from natural raw material hydrolysis but also the fundamental process for the isolation of other amino acids." [1]Most of the amino acids that are used in cosmetic and personal care products now are produced by a fermentation process using natural materials. In the fermentation method of amino acid production, microorganisms convert nutrients to various components necessary to themselves. Raw materials such as sugars and syrups are added to the microorganism culture and the proliferating microorganisms produce amino acids. Enzymes play a key role to degrade and synthesize substances. Consecutive reactions by 10 to 30 kinds of enzymes are involved in the fermentation process that produces the various high-purity amino acids.

It is necessary to screen the microorganisms to find the one that produces the greatest amount of the specific amino acid sought. Since one gram of natural soil contains on the order of 100 million microorganisms,[2] it is possible to pick out the useful one. There are a number of methods to select the microorganisms including mixing soil and water in a blender and then centrifuging the mixer. The microorganisms will be suspended in the supernatant.[3] They can then be cultured using the appropriate medium. In general, microorganisms produce the 20 amino acids in amounts necessary for themselves through a mechanism that regulates the quantities and qualities of the enzymes to yield amino acids in the amounts needed. "It is necessary to release this regulatory mechanism in order to manufacture the target amino acid in large amounts."[4]

The yield of an amino acid depends on the quantity and quality of the enzymes. Yield increases if the enzymes involved in the production of the target amino acid are present in large quantities, while it decreases if those enzymes are present in small amounts. "Suppose that a microorganism has a metabolic pathway

A->(a)->B->(b)->C->(c)->D (a, b, and c are enzymes). In order to produce only amino acid C in large amounts, one has to enhance the actions of only enzymes a and b and to get rid of the action of enzyme c. Strains are improved using various techniques to make this process possible."[5]

Other methods of producing amino acids are by enzymatic reaction and extraction:

- **In the enzymatic reaction method** an amino acid precursor is converted to the target amino acid using one or two enzymes. This allows the conversion to a specific amino acid without microbial growth eliminating the slower fermentation process from glucose.
- The **extraction method** entails natural proteins that are degraded to the various amino acids. The amount of each amino acid present in the raw material protein restricts the yield.

Overall, the fermentation method has the advantage of mass production at low cost.

From these twenty amino acids further derivitizations can be done to produce useful materials. The amino acids can be acylated and neutralized to form surfactants, acylated and esterified to produce emollients, cyclizated (via dehydration) to produce humectants, acylated to form functional products and neutralized to produce soap, etc. These and other derivitizations lead to many useful products for personal care applications, and more than about 500 "amino acids" recognized today.

b. Properties

There are twenty amino acids that are used by the body to produce proteins, etc. An amino acid is an organic compound that contains both a carboxylic acid group (COOH) and an amino group (NH2+) on the same molecule. An alpha amino acid is one in which the amino group is attached to the alpha carbon (that which is immediately adjacent to the carboxylate group). Further differentiation among the various amino acids is found in the side chains (R).

Fig. 1

This can be demonstrated in the following table:

Structures of Amino Acids (Table 1)

NONPOLAR, HYDROPHOBIC	R GROUPS	POLAR, UNCHARGED
Ajanine Aja A MW = 89		Glycine Gly G MW = 75
Valine Val V MW = 117		Serine Ser S MW = 105
Leucine Leu L MW = 131		Threonine Thr T MW = 119
Isoleucine Ile I MW = 131		Cysteine Cys C MW = 121
Phenylalanine Phe F MW = 131		Tyrosine Tyr Y MW = 181
Tryptophan Trp W MW = 204		Asparagine Asn N MW = 132
Methionine Met M MW = 149		Glutamine Gln Q MW = 146
Proline Pro P MW = 115		POLAR BASIC Lysine Lys K MW = 146
POLAR ACIDIC Aspartic acid Asp D MW = 133		Arginine Arg R MW = 174
Glutamine acid Glu E MW = 147		Histidine His H MW = 155

Solubility curves of amino acids that are relatively soluble in water

Figure 2

The solubility of L-Alpha Amino Acids is shown in the following charts:

The "breaks" in the curves of the behavior of some of the amino acids shown are the temperatures at which one mole of bound water is given up by that amino acid.

Solubility curves of amino acids that are relatively insoluble in water

Figure 3

It has been found that topically applied amino acids absorb into the epidermis and into the hair shaft. The first was done by a skin-stripping study and the latter through radio-tagged amino acids and amino acid analyzer.[8]

The amino acid analyzer measures the amino acids that are added to the hair shaft as well as those that are washed out. The radio tagging only measures amino acids that are added to the hair shaft. By using a combination of these analyses it is possible to determine how much amino acid penetrates the hair.

Figure 4

Figure 5

Function in Body Table 2: Amino acids as the building blocks of the body have many positive effects.

Valine Leucine Isoleucine	All of these three amino acids are called branched-chain amino acids (BCAAs). They perform the important functions of increasing proteins and serving as an energy source during exercise.
Alanine	It is an important amino acid as an energy source for the liver.
Arginine	It is an amino acid needed to maintain normal functions of blood vessels and other organs.
Glutamine	It is an amino acid needed to maintain normal functions of the gastrointestinal tract and muscles.
Lysine	It is a representative essential amino acid and tends to be insufficient when we are on a bread- or rice-centered diet.
Aspartic acid	It is contained in asparagus in large amounts. It is a fast-acting energy source.
Glutamate	It is contained in wheat and soybean in large amounts. It is a fast-acting energy source.
Proline	It is the main component of "collagen," which constitutes the skin and other tissues. It serves as a fast-acting energy source.
Cysteine	Cysteine can often be deficient in infants.
Threonine	It is an essential amino acid that is used to form active sites of enzymes.
Methionine	It is an essential amino acid that is used to produce various substances needed in the body.
Histidine	It is an essential amino acid that is used to produce histamine and others.
Phenylalanine	It is an essential amino acid that is used to produce various useful amines.
Tyrosine	It is used to produce various useful amines and is sometimes called aromatic amino acid together with phenylalanine and tryptophan.
Tryptophan	It is an essential amino acid that is used to produce various useful amines.
Asparagine	It is an amino acid that is located close to the TCA cycle (place of energy generation) together with aspartic acid.
Glycine	It is used to produce glutathione and porphyrin, a component of hemoglobin.
Serine	It is used to produce phospholipids and glyceric acid.

1.1 Derivatives of Amino Acids for Personal Care formulation:

Amino acids can be derivatized into many useful materials for formulation in the cosmetic and personal care field. The following chart shows just a few of the possibilities. Glutamic acid can be acylated with a fatty acid and neutralized to produce an anionic surfactant acyl glutamate. Glutamic acid can also be acylated and esterified to produce emollients, and it can be cyclized by dehydration to form a humectant. Aspartic acid can be polymerized and neutralized to produce polyaspartate, a film former and humectant. Latter sections in the chapter will discuss the use and benefits of many of these derivatives.

Fig. 6

The following are some of the reactions that are possible with amino acids:

Table 3

	Reaction of Carboxyl Group
1. Esterification* (peptide synthesis)	$\xrightarrow{R'OH}$ $H_2NCHRCOOR'$ Amino acid ester
2. Amidation (derivatization)	$\downarrow NH_3$ $H_2NCHRCONH_2$ Amino acid amide
3. Decarboxylation (derivatization)	$\xrightarrow[\text{or enzyme}]{\text{Heat}}$ H_2NCH_2R Amine
4. Reduction (derivatization)	$\xrightarrow{LiBH_4}$ $H_2NCHRCH_2OH$ Amino alcohol

Table 4

As formulators it is important to keep these possible reactions in mind.

	Reaction of α-Amino Acids
1. Chelation** (assay, identification, purification)	$\xrightarrow{Cu^{++}}$ Amino acid - copper complex
2. Hydantoin formation (derivatization)	$\xrightarrow{KCNO}$ Hydantoin
3. Azlactone formation (derivatization)	*N*-acetly amino acid $\xrightarrow{(CH_3CO)_2O}$ Azlactone
4. NCA formation (peptide and polyamino acid synthesis)	$\xrightarrow{COCl_2}$ *N*-carboxy amino acid anhydride
5. Diketopiperazine formation (derivatization)	$\xrightarrow{Heat}$ Diketopiperazine
6. Oxidation (assay, other applications)	$\xrightarrow{H_2O_2Fe^{3+}}$ $RCOOH+CO_2+NH_3$ $\xrightarrow{NaOCl}$ $RCHO+CO_2+NH_3$ $\xrightarrow{Enzyme}$ $RCOCOOH+NH_3+H_2O_2$ α-keto acid $\xrightarrow{Ninhydrin}$ $RCHO+NH_3+CO_2$

*Methylesterification, ethylesterification and benzylesterification are usually performed.
**Copper(II) glycine is used in threonine synthesis.

4.3.4.2 THE APPEARANCE OF AGING OF SKIN AND HAIR

We all see the signs of aging. Skin that is wrinkled, dry, and fragile. Hair that breaks easily, is dull and thinning. Sometimes these signs appear with the accumulation of years. Sometimes they appear as a result of disease, or of abuse, such as unprotected chronic tanning. Medical conditions must, of course be addressed by the appropriate physician. The personal care formulator can be and is equipped to formulate products that mitigate the appearance of aging and in some cases (e.g. the use of sunscreens) can prevent, the damage to a large extent before it occurs.

What are the antagonists of skin and hair?

Free radicals top the list. Free radicals are produced in all cells and in macrophages in large amounts. They support the immune system and are important for health. However, an excess of free radicals can attack healthy cells and tissue. Free radicals are highly reactive molecules that can damage cell membranes, lipids,

proteins, and DNA.[10] Species such as singlet oxygen, superoxide, lipid peroxides, hydrogen peroxide, and hydroxyl radicals can and do have significant effects on the skin and the hair. Formulations that counter the buildup of reactive oxygen species (ROS) go a long way towards mitigating the appearance of aging.

a. Skin

The skin is the largest organ of the body and is responsible for protecting it from environmental impacts. At the same time the skin sends a signal regarding the health and attractiveness of the person. Sagging skin, flaking dry skin, loss of elasticity, dullness—these are some of the signs of aged skin and are seen as unattractive.

The signs of aged skin can be caused by a number of things. These include, but are not limited to the accumulation of years, environmental damage, illness, poor diet, lack of exercise, and lack of sleep. Personal Care/Cosmetic Science can address these issues. Of course in the case of illness and disease the root cause must only be addressed by the proper medical professional, but even in this case personal care/cosmetic science can help the patient look and feel better while they are undergoing treatment and also afterwards. A good example of this is the American Cancer Society's "Look Good, Feel Better" program on how personal care/cosmetic science can help patients undergoing treatment.

There is significant evidence that topically applied amino acids penetrate into the epidermis. This was demonstrated by Maibach, et al.,[11] Coderch et al.,[12] Ruland et al.,[13] and others. But what effect do they have on the appearance of the skin?

The effect of absorbed amino acids into the skin results in a moisturiziation of the *stratum corneum*, thereby making the skin smoother and more supple.

It has also been shown that in the case of atopic dermatitis (dry, flaky, itchy skin), the amount of every standard amino acid normally present in the skin is significantly reduced, as is the total amount of these amino acids—which can be seen from the following graph.

Figure 7

The mechanism for this aspect of atopic dermatitis may be the decrease in amino acid metabolic conversion into metabolites in hyperkeratosis. Some of the metabolic pathways may be shown as in the following table.

Amino acid metabolism in stratum corneum (horny layer)

	Metabolic pathway
PCA	Glu → γ-Glu-AA → PCA (γ-Glutamyl-AA synthetase; γ-Glutamyl cyclotransferase)
UCA	His —NH3→ UCA (histidase)
Orn Cit	Arg —Urea→ Orn —carbamoyl phosphate, Pi→ Cit (arginase; ornitine carbamoyltransferase)
Ala	Asp —CO2→ Ala (aspartate 4-decarboxylase)

(S. Marstein, (1980): H. P. Baden & M. A. Pathak, (1967)
P. G. Crounse & S. Rothbera. (1961)

Figure 8

The rate of conversion to metabolites is greatly decreased in hyperkeratosis skin.

Rates of conversion to amino acid metabolites greatly decrease in induced hyperkeratosis. (SLS closed patch)

(J. Koyama, *J. Soc. Cosmet. Chem.*, **35**, 183 (1984))

Figure 9

The topical application of amino acids to the skin helps to mitigate the dryness, flaking, and itching of the skin and restores a more natural and healthy amino acid balance, again making the skin appear more youthful.

b. Wrinkling

Of all the things that adversely affect the skin and cause the appearance of aging under "normal" circumstances, free radicals and reactive oxygen species are arguably the worst offenders. Free radicals and reactive oxygen species (ROS) induced by environmental causes and UV radiation can overwhelm the skin's natural antioxidant defenses. This can lead to wrinkling, collagen breakdown, the loss of elasticity, and a myriad of diseases associated with oxidative stress.[14] It has been shown that topically applied antioxidants have a positive effect on the appearance and health of the skin.[15]

The work of Podda et al. was focused on vitamin C, vitamin E, ascorbic acid, tocopherol, and lipoic acid and showed that these small-sized antioxidants penetrated the skin with a positive effect. Amino acids are similarly small molecules and have indeed been shown to penetrate the skin. Cysteine (and cystine, since the S-S bond is easily broken), methionine, taurine, and N-acetyl cysteine are powerful antioxidants that will penetrate the skin. The tape-stripping work of Coderch et al. on threonine, isoleucine, and lysine shows that amino acids penetrate to 16 strippings, while Kawasaki, Sakamoto, and Maibach, working with glycine, sodium glutamate, proline, and threonine, show that there is penetration to 10 strippings.

Additionally, the prevention of ultraviolet light-induced wrinkles may be accomplished by the application of zinc pyrrolidone carboxylate prior to UV exposure.

Zinc PCA does this by interfering with the production of activator protein -1, AP-1. UV radiation initiates the formation of AP-1, which induces the production of collagenase. Collagenase causes the degradation of collagen, which then causes a wrinkle. If the cycle is broken, that wrinkle formation is stopped to a good extent. It has been shown in *in vitro* testing on human fibroblasts that 3 ppm zinc PCA inhibited 69.3% of AP-1 production and that 30 ppm of zinc PCA inhibited 77% of AP-1 production. It is expected that in *in vivo* work, somewhat more of zinc PCA would be required to produce the same effects.

Another cause of wrinkles is a lower rate of collagen production. If collagen production slows, the appearance of wrinkles increases. It has been shown that the topical application of an amino acid cocktail featuring a 1:1:1 molar ration of glycine: proline: alanine doubles the rate of collagen production. A 3:2:1 molar ratio of those same amino acids increases collagen production fourfold.[16] This goes a long way to reduce the appearance of wrinkles and is noticeable for as long as the application of the amino acid mixture is used.

Lysine has been reported to have a positive cosmetic effect on wrinkle formation. It is known that lysine is needed for the production of collagen and that lysine-poor diets may increase the development of wrinkles. The thought is that

since nutritional lysine supplementation has a positive effect on collagen production and it has been shown that topically applied amino acids also have a positive effect, then lysine topically applied may also help with the reduction of wrinkles. Other amino acids that have an effect on skin wrinkling are creatine, carnitine, arginine, and methionine. In these cases it has been reported that these amino acids stimulate regeneration of connective tissue.

Fine lines may be temporarily removed by the application of highly hygroscopic materials that supply moisture to the *stratum corneum*. Sodium PCA and proline are two candidates that have been shown to moisturize skin very efficiently. This moisturization "plumps" the skin slightly, removing the fine lines to a greater or lesser extent. Additionally, amino acid derivatives such as phytosterol/decyltretradecyl myristoyl methyl meta-alaninate binds in the order of 25 times its own weight in water, which provides a reservoir for the skin to use. Other analogs of this material such as phytosterol/behenyl/octyldodecyl lauroyl glutmate hold six times their own weight in water.

c. Elasticity

The topical application of amino acids and derivatives can increase the elasticity of the skin. Pig skin treated with sodium PCA has been tested using a Fudo rheometer. In this case, after treatment with a humectant, the rheometer measures the millimeters of skin elongation until it breaks.

<Method>
A sheet of pig skin (3mm*30mm) dipped in 10% each humectant solution for 1 hour was dried for 2 hours under 25°C / 40%RH. Then the elongation of each pit skin was measured until breaking.

<Result>

The effect of various humectant as softener for skin

Figure 10

Sodium PCA increases the elongation of the skin dramatically over that of untreated and just water-treated skin. It also shows better results than other common humectants. Similarly, amino acid cocktails comprised of sodium PCA, betaine, serine, glycine, glutamic acid, alanine, threonine, and proline have similar elongation effects.

A demonstration of the effects of sodium PCA treatment on the skin is as follows:

Sodium polyaspartate treatment increases elasticity a good deal. A 10% active ingredient treatment of sodium polyaspartate allows the skin to stretch 5.75 mm, while a 10% Active Ingredient treatment of hydrolyzed animal collage permits the skin the stretch 1.5 mm, and no treatment 1.25 mm. The measurements are done on a Fudo rheometer at 25°C and 40% RH. In this test a specimen is placed between two grips and the instrument stretches the specimen at constant rate until breaking. The length of the specimen at breaking is recorded and sometimes the breaking force is also recorded. This instrument is similar to a universal testing machine used in tensile strength measurements.

d. Clarity

<Method>
To induce the scaly skin. 1% SDS was applied to the forearm for 24 hours closed patch. After patch removal, each area was washed with the mild skin cleanser. 2 µl /cm2 of samples were applied to the test areas once a day for 3 days. Water holding capacity of the skin was measured quanitatively using skin surface hygrometer (Skicon 200, IBS INC.). Conductance values were measured at 25 °C. 40 RH%.

<Result>

Figure 11

The use of alpha hydroxy acids and beta hydroxyl acids has become almost commonplace to clarify the skin. In clarifying the skin the dead and dull skin cells at the surface are removed; a process that allows the healthy and "bright" skin cells to be seen. These materials are very good keratolitics that consume the dead surface skin cells, allowing the skin to look fresh and clear. A keratolitic is a substance that softens the skins keratin and allows the dead dull skin cells to fall off or be easily removed. One difficulty with alpha or beta hydroxyl acids is that these materials are very acidic and produce a stinging sensation. Their stinging can be reduced by adjusting the pH, typically form 3 to a pH of 4.5. However, the use of normal neutralizing agents such as NaOH of fatty amines to bring the pH to 4.5 will also reduce the clarifying effect of the alpha and beta hydroxy acids. The amino acid arginine, an alkaline amino acid, has been shown to have the ability to raise the pH of the alpha or beta hydroxy acid product without reducing its efficacy.[17, 18]

e. Hydration:

Hydration of the skin is a key to a youthful appearance.

It has been shown that there is a linear correlation between the amount of amino acids present in the *stratum corneum* (SC) and the degree to which the skin is hydrated. The greater the amount of amino acids present, the greater the hydration.

(Y. Kumano, *Fragrance Journal.*, **79**. 64 (1986))

Figure 12

Since fully hydrated skin is soft and supple, the increase in hydration due to the presence of amino acids makes the skin softer and more pliable as is youthful skin.

The skin's *stratum corneum* contains within the corneocytes the natural moisturizing factor (NMF) that helps the skin retain moisture and suppleness. This NMF is comprised of 40% amino acids, pyrrolidone carboxylic acid 12%, lactates 12%, urea 7%, ammonia, uric acid, glucosamine, creatinine, citrates, various salts, glucose, organic acids, peptides, and others totaling about 28% according to Spier and Pascher.[19]

The composition of the amino acids in the NMF is illustrated in the following graph.

Figure 13

As mentioned above, there is a direct correlation between the amounts of amino acids present in the SC and the degree of skin hydration. The more amino acids present, the greater the hydration and the greater the suppleness of the skin.

Derivatives of amino acids can facilitate hydration or the mitigation of damage to the NMF. It has been shown that washing with a simple cleanser containing 10% active ingredient sodium lauryl ether sulfate will remove about 62% of the amino acids in the SC and ~56% of PCA present in the SC relative to the initial amounts present. Soap will remove about 70% amino acids and 62% PCA again relative to the initial amounts present. A wash with 10% active substance of sodium lauroyl glutamate, a glutamic acid derivative, washes out only 52% amino acids and 39%

PCA of those initially present. This shows that the retention of amino acids and PCA can be influenced by the character of the surfactant employed to cleanse the skin. Similarly, the application of an amino acid–derived emollient can reduce the trans-epidermal water loss, thereby increasing hydration of the skin as reported by Ishii.[20]

Figure 14

Where cholesteryl/behenyl/octyldodecyl lauroyl glutamate (golden line), and Type 3 ceramide (red line), petrolatum (black line), and untreated (blue line). The phytosterol analogs of the glutamic acid–based compounds show similar results. The timeframe is Initial, the Zero hour post-damage, then one, two, and three days after application of the treatment.

L-proline is unique among the amino acids in its ability the increase moisturization, especially in the presence of leucine and isoleucine.

Figure 15

The graph above, Figure 15, contrasts the absorption of humectants at high-humidity and low-humidity conditions over the course of time. The humectants were initially dried to constant weight and then exposed to high, 65% RH over 15 days with the water weight uptake measured. At the end of day 15 the humectants were exposed to low, 35% RH for an additional 15 days with the water weight loss measured. The first case measures the greatest hydration from atmospheric moisture that can be expected to be attained while the second case measures the moisture retention under dry conditions.[21]

It can be easily seen that sodium PCA and sodium lactate absorb the greatest amount of moisture under high-humidity conditions but that L-proline retains the greatest amount of moisture relative to its initial hydration at low humidity. The low-humidity retention of moisture is important, since this is the condition that dries out the skin and causes it to be itchy and cracking.

Leucine and isoleucine establish a lipid bi-layer with water bound to L-proline. This bi-layer inhibits moisture loss from the skin.[22]

Figure 16

f. UV Damage

Amino acids and their derivatives are useful in mitigating the effects of UV radiation on the skin. It is important to note that these materials are not replacements for good sunscreens/sun blocks but augment their effects and protection.

Zinc PCA has been shown to prevent the formation of activator protein-1, which is caused by incident UV radiation. This inhibition prevents to a good extent the formation of collagenase and damage to collagen and elastin in the skin. Other amino acids such as cysteine, methionine, taurine, glutathione, and n-acetyl cysteine are powerful antioxidants and free-radical scavengers. When topically applied they augment the skin's natural defenses against reactive oxygen species induced by UV radiation and other environmental factors.

Amino acid derivatives like the ester isopropyl lauroyl sarcosinate are outstanding solvents for organic UBV absorbers and allow easy incorporation of these materials into a formulation and help inorganic UV blocks spread uniformly across the skin substrate. Lauroyl lysine is another derivative that helps inorganic UV blocks deposit uniformly on the skin. It also has the advantage of delivering a soft lubricious skin feel.

Materials such as phytosterol/octyldodecyl lauroyl glutamate and phytosterol/behenyl/octyldoddecyl lauroyl glutamate are excellent wetting agents for pigments used as UV blocks, such as micronized titanium dioxide and zinc oxide. These also allow a more uniform deposition across the skin.

4.3.4.3 HAIR

The function of amino acids in the hair is much less well understood than it is in the skin. Of course amino acids are constituents of keratin and an integral component of the cell membrane complex (CMC), which "glues" the cells of the cortex of the hair together.

We all start with healthy hair but with the modern propensity for washing, perming, and coloring, we damage it thereby causing breakage, dullness, loss of elasticity, and roughness. This damage shows up as lower tensile strength, cuticle damage, and components such as collagen, cholesterol, amino acids, fatty acids, and ceramides leaching out of the hair shaft. What are needed are materials that help repair the hair and/or mitigate the degree of damage in the first place. Amino acid derivatives can have a positive effect on the hair.

a. Breakage

The reduction in tensile strength from perming and bleaching results in severe hair breakage. It has been shown that the tensile strength of permed hair is only 95% that of normal healthy hair and the tensile strength of bleached hair is only 90% of

that of normal healthy hair. This reduction in tensile strength may seem inconsequential, but damage and breakage indeed occur.

Fig.17

In various tests it has been found that the addition of amino acid derivatives such as phytosterol/octyldodeyl lauroyl glutamate mitigates the damage done to hair from bleaching followed by shampooing. The testing procedure employed was to first take tensile strength readings on untreated hair in order to establish a baseline. Thereafter, hair was bleached with ammoniated peroxide in one case, bleached and shampooed with a 10% active sodium lauryl ether sulfate shampoo was employed in the second case, and in the third case the hair was bleached, and shampooed with a shampoo containing 0.5% phytosterol/octyldodecyl lauroyl glutamate. The untreated hair was normalized to 100% for comparison. The findings of these tests showed that bleached hair lost 5% of its tensile strength in the bleaching process, and that bleached and shampooed hair lost approximately 13% of its tensile strength. However, when 0.5 % of the amino acid derivative phytosteroyl/octyldodecyl lauroyl glutamate was added to the shampoo, the tensile strength of the hair returned to that of bleached only hair (95% of normal).

Similar effects were noted in permed hair that was first permed using an ammonium thioglycolate solution and then neutralized with a sodium bromide solution. Tensile strength measurements were made on permed hair in one case, on permed and sodium laureth sulfate–shampooed hair in a second case and on permed hair shampooed with phytosterol/octyldodecyl lauroyl glutamate added to the shampoo at 0.5% active. The results showed that permed hair retained only 75% of the tensile strength of normal hair and that shampooing further increased the damage so

that the hair retained only 65% of its tensile strength. By contrast, the addition of the amino acid derivative prevented further damage from shampooing, allowing the hair to retain about 78% of its normal tensile strength.[23]

In additional tests hair was bleached with ammoniated peroxide, then conditioned with a commercial conditioner. The hair that was bleached and not conditioned had 85% of the tensile strength of normal unbleached hair, while the hair that was beached and conditioned with a commercial conditioner had 88% of the tensile strength of normal hair. The addition of 10% phytosterol/octyldodecyl lauroyl glutamate to the commercial conditioner restored the hair to 93% of its original normal tensile strength. The addition of phytosterol/octyldodecyl lauroyl glutamate and amino acid derivative to a shampoo or conditioner restores chemically damaged hair nearly to its original pre-damaged state.

Sodium polyaspartate is another useful amino acid derivative to help mitigate hair breakage through the loss of tensile strength. Hair was permed as before with an ammonium thioglycolate solution neutralized with a sodium bromide solution. Tensile strength measurements were made on untreated haired, permed hair, and permed hair on which a 1.5% active tonic of sodium polyaspartate was sprayed. The results are evident on the following chart.

Figure 18

The permed hair treated with the sodium polysapartate solution had its tensile strength significantly restored.

Treating permed hair with an amino acid solution has been shown to improve the tensile strength at 20% extension measured in water. Hair again was permed in the usual way using a thioglycolate solution neutralized with sodium bromide and tensile strength measurements were made. Treating permed hair with histidine and also with phenylalanine improved the tensile strength of the hair to almost that of normal untreated hair.[24]

b. Dullness

Dull, lifeless hair is one sign of aged hair. Many materials have been added to conditioners, shampoos, and styling products to increase the sheen and luster of the hair to enhance an attractive look. Materials such as oils, esters, and silicones are commonly used and do a good job. The addition of amino acid derivatives can augment the positive effects on sheen and luster in the hair.

An amino acid cocktail composed of sodium PCA, PCA, sodium lactate, arginine aspartic acid, glycine, alanine, serine, valine, proline, threonine, isoleucine, histidine, and phenylalanine has been shown to increase the sheen and luster of hair.

Fig 19

The first tress is untreated hair, the second tress is treated with 2% of the amino acid cocktail, the third tress is treated with 5% of the amino acid cocktail.[25] As can easily be seen from the photograph In Figure 19 there is a dramatic enhancement of sheen and luster at the 2% level and a small gain at the 5% level over the 2% level.

The amino acid derivative lauroyl arginine, an amphoteric surfactant, has been shown to increase the luster of hair. In this case the lauroyl arginine helps align the hair fibers uniformly and reduces frizz. The more aligned hair fibers are more reflective as an artifact of the alignment increase in sheen and luster.

Fig. 20

The left side of the half-head is hair untreated with lauroyl arginine, which shows frizz and some dullness. The right side of the half-head is treated with lauroyl arginine, showing more uniformly aligned hair fibers and better luster. It should be noted that a combination of 1% amino acid cocktail mentioned previously and 0.1% lauroyl arginine in a conditioner have shown remarkable benefits in field tests where 68% of the subjects said that they saw an immediate improvement in their hair after one application.[26]

c. Elasticity

The ability of hair to bounce and move lends itself to a picture of youth and vigor. In the 1960s there was a TV commercial that depicted a young woman running through a pastoral field with her hair bouncing and swinging as she ran. This was supposed to depict the essence of beautiful hair.

A measurement of bounce is bending strength in which hair fibers are cleaned, then treated with conditioning agents, and a force is applied transversely across the fibers.

Method: Hair was washed with a SLES solution and then treated with a cationic surfactant solution. Bending strength was then measured 25C 40% RH.

Figure 21

STAC= Stearyl Trimethyl Ammonium Chloride
DSTMAC=Distearyl Dimethyl Ammonium Chloride
CAE=PCA Ethyl Cocoyl Arginate

The more force required to bend the fibers, the greater the bending strength and the greater the bounce. In the above chart, Figure 21, stearyl trimethyl ammonium chloride (STAC), and distearyl dimethyl ammonium chloride (DSMAC) are contrasted with the amino acid conditioning agent PCA ethyl cocoyl arginate (CAE). It is clear that the force required to bend the hair fibers treated with CAE is greater than that required to bend STAC- or DSMAC-treated fibers. The amino acid–derived conditioning agent provides more bounce to the hair. By combining materials such as this with those previously noted that increase the hair's tensile strength, there is a positive effect on hair elasticity.

d. Roughness

Smooth hair feels good to the touch and reflects light giving the hair good shine and luster as seen earlier. There are many materials that can be used to produce smoothness in the hair.

Hair was washed in a simple shampoo:

	%
SLES	15.0 active
Humectant	15.0 active
Water	qs to 100%

The humectants: 1,3 Butylene Glycol, Glycerin, PCA-Na

The hair was rinsed and allowed to dry at ambient

temperature and humidity.

- Sensory evaluation done n= 6

Figure 22

In this test the hair was washed with a simple sodium laureth sulfate shampoo and the shampoo in which humectants were added. The hair was then rinsed and allowed to dry at room temperature. Sensory evaluations were then conducted. Hair treated with glycerin and 1,3 butylene glycol produced a higher degree of roughness to the finger than the SLES-washed hair. Sodium PCA on the other hand produced a feeling of greater smoothness.

This can also be demonstrated using dynamic frictional coefficient measurements as in the following chart. "The dynamic coefficient of friction, also called the kinetic or sliding coefficient of friction, or friction coefficient (symbol μ or f, dimensionless), is a measure of how large the friction forces are which act between two solids."[27] In this case the hair is treated with the humectant and the combing force is measured via a rheometer.

Fig. 23

Hair was washed in a simple shampoo:

	%
SLES	1.0 active
Humectant	0–1.0 active
Water	qs to 100%

The humectants: 1,3 butylene glycol, glycerin, PCA-Na

The hair was rinsed and allowed to dry at ambient temperature and humidity.

Frictional coefficient was then measured.

Here again the hair treated with sodium PCA is much smoother than hair treated with either 1,3 butylene glycol or glycerin.

PCA ethyl cocoyl arginate has been shown to repair the cuticle smoothing the hair surface. This can be seen in SEM micrographs of damaged hair without and with CAE treatment.

Fig. 24

Top: SEM observation of a 1% SLES-washed and tied hair

Bottom: SEM observation of a 1% SLES-washed, 1% CAE-treated (dipped 4–5 minutes), tied hair.

CAE helps repair mechanical surface damage to the hair shaft.

Arginine is another useful amino acid to smooth the hair. In the following chart, hair was washed with 1% sodium lauryl ether sulfate and 1% sodium lauryl ether sulfate plus 0.1% arginine, all on an active-substance basis. The wet dynamic friction coefficient clearly shows that the hair washed with SLES and arginine is smoother than that washed with SLES alone.

Figure 25

Arginine is an alkaline amino acid and can be used to neutralize styling gel polymers. In the following series of tests, arginine is shown to have a positive effect on smoothness and texture of styled hair.[28]

4.3.4.4 FORMULATION FOR SKIN CARE

a. Cleansers

Facial Cream Cleanser

Ingredient	**% (w/w)**
Sodium Stearoyl Myristoyl Glutamate	20.00
Sorbitol	20.00
Stearyl Alcohol	1.5
Disodium Cocoamphodipropionate	5.00
Zinc PCA	0.10
Glycine	0.36
L-Proline	0.45
L-Alanine	0.15
Preservative	Qs
Deionized water	Qs

Combine ingredients except the sodium myristoyl glutamate with mixing and heat to 75°C. Slowly add the sodium myristoyl glutamate, maintaining temperature and controlled mixing. Mix until uniform at 75°C, then allow to cool to room temperature.

		Formulation No. 1	Formulation No. 2	Formulation No. 3	Formulation No. 4	Formulation No. 5	Formulation No. 6
Pemulen®* TR-1† (BF Goodrich)		0.50	0.50	0.50			
Carbomar* (Carbopol®* 980‡)					025	0.25	0.25
L-Arginine		0.42			0.21		
Triethanolamine			0.35			0.18	
2-Aminomethylpropanol				0.22			0.11
Purified water		99.08	99.15	99 28	99.54	99 57	99.64
pH		5.65	5.51	5.68	6.13	6.09	6.11
Viscosity (No. 4, 30 seconds)	12 rpm	22570	25170	22820	24170	25270	28020
	30 rpm	11670	12270	11760	12380	13660	15410

* : Acrylates / C10 - 30 Alkyl Acrylates Crosspolymer
† : Acrylates / Alkyl Methacrylates Copolymer (BF Goodrich)
‡ : Carboxy Vinyl polymer (BF Goodrich)

Table 5, 6, 7

1. Evaluation at setting (curler)*		Formula-tion No. 1	Formulation No. 2	Formulation No. 3	Formulation No. 4	Formulation No. 5	Formulation No. 6
Immediately after	Setting power	Slightly strong	Slightly strong	Strong	Strong	Strong	Strongest
	Texture	Light	Heavy	Slightly heavy	Light	Heavy	Light
	Elasticity	Strong	Strong	Strong	Strong	Strong	Strong
After 1 day	Sagging	Weak	Slightly Strong	Weak	Weak	Slightly strong	Weak
	Smoothness at brushing	Good	Good	Good	Good	Good	Slightly bad

2. Evaluation at application to straight hair**		Formulation No. 1	Formulation No. 2	Formulation No. 3	Formulation No. 4	Formulation No. 5	Formulation No. 6
At application	Spreadability	Good	Slightly bad	Normal	Good	Normal	Normal
	Smoothness	Good	Creaky	Creaky	Slightly good	Creaky	Most creaky
	Wetting feeling	Strong	Slightly Strong	Strong	Slightly Strong	Slightly Strong	Strong
	Dryness	Rapid	Slightly slow	Slightly rapid	Rapid	Slightly slow	Slightly rapid
Before brushing after 1 day	Hardness	Slightly strong	Strong	Strong	Slightly weak	Slightly strong	Slightly strong
After brushing after 1 day	Smoothness	Good	Bad	Slightly bad	Good	Bad	Slightly bad
	Creakiness (feeling of coating)	Less	Slightly strong	Slightly strong	Less	Slightly strong	Slightly strong
	Texture	Slightly light	Slightly heavy	Slightly heavy	Light	Slightly heavy	Slightly light
	Dryness of the tip of hair	Less	Slightly less	Slightly less	Slightly less	Slightly strong	Strong

* : Condition of curling (National CURLUN 18 EH947 (Matsushita Electric Works Ltd.) (Curler: large (Blue))

Make a bundle of hair (about 2 x 4cm) of a constant volume using a wig, and apply 0.5g of the sample all over centering around the tip of hair. Immediately after application, curl the hair with a curler, allow to stand a room temperature for 20 minutes, remove the curler cautiously.

**: Condition at application to straight hair

Make a bundle of hair (about 2 x 4cm) of a constant volume using a wig, and apply 1.0g of the sample all over centering around the tip of hair. At this time, apply the sample while spreading so that the bundle of hair may be uniform straightly. After application, allow to stand at room temperature for 1 day and confirm the setting power, the smoothness of combing, etc.

Pearl Bath and Shower Cleanser

Ingredient	**% (w/w)**
Potassium Cocoyl Glycinate	30.00
Lauramide MEA	1.50
Lauramidopropyl Betaine	30.00
Propylene Glycol	1.50
Glycine	0.40
Amino Acid Cocktail (Sodium PCA, Glycine, Glutamic Acid, Alanine, Lysine, Arginine, Threonine, Proline, Betaine, sorbitol	0.55
Alanine	0.55
Sodium PCA	0.15
Glycol Distearate	1.00
Citric Acid (20% aq soln)	2.00
Preservative	QS
Deionized Water	Qs

Sebum Control Facial Cleanser

Phase	**Ingredient**	**%(w/w)**
A	Didodium Cocoamphdiacetate	8.00
	Lauryl Glucoside	9.00
	Disodium Cocoyl Glutamate and Sodium Cocoyl Glutamate	20.00
	Glyceryl Laurate	3.00
	Sodium PCA	2.00
	Zinc PCA	1.00
	Deionized Water	QS
B	Lauroyl Lysine	1.00
	Deionized Water	2.80
	Sodium Hydroxide, pellets	0.20
C	Preservative	QS
D	Citric Acid	To pH 5.7 +/– 0.2

Combine phase A and begin mixing and heating to 75°C. Carefully combine phase B mix until clear (pH should be > 13.3). When phase A is uniform, carefully add phase B to phase A, maintaining mixing. Cool to room temperature. Add phase C and adjust pH (phase D).

Cold Process Cream Cleanser

Phase	Ingredient	% (w/w)
A	Xanthan Gum	0.30
	Deionized Water	42.70
B	1,3 Propane Diol	28.00
	Glycerin	4.00
C	Lauroyl Lysine	0.50
	Sodium Hydroxide pellets	0.10
	Deionized Water	1.40
D	Sodium Myristoyl Glutamate	15.00
	Sodium Cocoyl Glycinate	5.00
E	Lauryl Glucoside	1.50
	Preservative	QS
F	Citric Acid	To pH 7.0 +/– 0.2

Combine phase A with mixing at room temperature until gum is completely hydrated and phase is clear and uniform. Add phase B to phase A. Carefully combine phase C and when clear and uniform (pH should be > 13.3) add to phase A. Add phases D and E. Adjust pH (phase F). Maintain controlled mixing throughout.

Pump Facial Cleanser

Ingredient	% (w/w)
Sodium Cocoyl Alaninate (30%)	30.00
Coco-Betaine	14.00
Disodium Cocoamphodipropionate	15.00
Sodium PCA	1.00
Zinc PCA	0.1
Glycine	0.36
Proline	0.45
Alanine	0.15
Preservative	QS
Deionized Water	QS
Citric Acid	To pH 5.7 +/– 0.2

Combine all ingredients with mixing at room temperature. Adjust pH.

b. Moisturizers

Facial Moisturizer

Phase	Ingredient	% (w/w)
A	Grape Seed Oil	7.50
	Sesame Seed Oil	3.00
	Isopropyl Lauroyl Sarcosinate	15.00
	Lauroyl Lysine	1.50
	Alpha Tocopherol	0.50
	Phytosterol/Octyldodecyl Lauroyl Glutamate	5.00
B	Deionized Water	QS
	Xanthan Gum	0.40
	C12-13 Alkyl Glyceryl Hydrolyzed Hyaluronate	0.10
	Perilla Seed Extract	0.10
	Litchi Seed Extract	0.10
C	Sodium PCA	1.00
	Zinc PCA	1.00
D	Sodium Stearoyl Glutamate	3.60
E	Preservative	QS
F	Citric Acid	To pH 5.5 +/– 0.2

Combine phase A ingredients at room temperature with stirring. Charge batch vessel with water of phase B and begin controlled propeller mixing. Dust Xanthan into phase B and allow to completely hydrate. When clear, add phase C and phase D to phase B. Add phase A to phase B, maintaining mixing. Add phase E. Adjust pH (phase F), all at room temperature.

Light Moisturizer

Phase	Ingredient	% (w/w)
A	Vegetable Oil	25.00
	Phytosterol/Octyldodecyl Lauroyl Glutamate	3.00
	Isopropyl Lauroyl Sarcosinate	10.00
	Lauroyl Lysine	5.00
	Tocopheril	0.50
B	Deionized Water	QS
	Xanthan Gum	0.50

Phase	Ingredient	% (w/w)
C	Sodium Steroyl Glutamate	2.60
D	Preservative	QS
E	Citric Acid	To pH 7.0 +/– 0.2

Combine phase A ingredients with mixing. Charge batch vessel with water phase B and begin propeller mixing. Slowly add Xanthan to water, maintaining mixing. When completely hydrated, add phase C to phase B. Add phase A to phase B, maintaining mixing. Add phase D to phase B and adjust pH (phase E), all at room temperature.

Lotion

Phase	Ingredient	%(w/w)
A	PEG-10 Hydrogenated Castor Oil	0.25
	PEG-20 Hydrogenated Castor Oil	2.25
	Phytosteroyl/Octyldodecyl Lauroyl Glutamate	1.00
	Mineral Oil	9.00
	Glycerin	10.00
B	Acrylates/C 10-30 Alkyl Acrylate Crosspolymer	0.5
	Deionized Water	49.50
C	Hydroxylethylcellulose	0.07
	Deionized Water	6.93
D	Arginine	0.40
	Deionized Water	3.60
E	Sodium PCA	1.00
	Amino Acid Humectant Cocktail: Betaine, Sodium PCA, Sorbitol, Serine, Glycine, Glutamic Acid, Alanine, Lysine, Arginine, Threonine, Proline	1.00
	Butylene Glycol	12.00
	Deionized Water	QS
	Preservative	QS

Dissolve part B and C respectively. Heat and mix part A to 80°C. Heat part B and C to 70°C. Add part B and C to A with agitation. Cool to room temperature with agitation. Add part D to ABC with agitation. Add part E to ABCD with agitation.

c. Serums

Phase	Ingredient	% (w/w)
A	Hydroxypropyltrimonium Hyaluronate	0.05
	Deionized Water	QS
B	Sodium PCA	1.00
	Amino Acid Cocktail: Sodium PCA, Serine, Glycine, Glutamic Acid, Alanine, Lysine, Arginine, Threonine, Proline, Betaine, Sorbitol	1.00
	Hydroylzed Hyaluronic Acid	0.10
C	Aloe Vera Extract	0.50
	Honeysuckle Extract	1.00
	Parsley Extract	1.00
	Rosemary Extract	1.00
	Green Tea Polyphenols	0.50
D	Preservative	QS

Mix phase A until dissolved; add phase B, then phase C and phase D.

Toner Mist

Phase	Ingredient	% (w/w)
A	Trehalose	0.25
	C12-13 Alkyl Glyceryl Hydrolyzed Hyaluronate	0.05
B	Deionized Water	89.30
	Glycerin	3.00
C	Amino Acid Cocktail: Sodium PCA, Sodium Lactate, Arginine, Aspartic Acid, PCA, Glycine, Alanine, Serine, Valine, Lysine, Threonine, Proline, Isoleucine, Histidine, Phenylalanine	1.50
	Saccharide Isomerate	2.00
	Extract mixture: Propylene Glycol, Water, Echinacea Angustifolia Extract, Centella Asiatica Extract, focus Vesiculosus Extract, Trigonella Foenum-Graecum Seed Extract	1.00
	Sakura Flower Extract	0.20

Phase	Ingredient	% (w/w)
	Bresenia Schreberi Leaf Extract	0.50
	Phenylehtanol, Ethylhexyl Glycerin	0.5
	Sodium Caproyl Prolinate	1.70

Combine phase B and add phase A ingredients in sequential order with thorough mixing until dissolved. Add phase C.

Facial Skin Toner

Ingredient	%(w/w)
Witch hazel Extract	25.00
Amino Acid Cocktail: Betaine, Sodium PCA, Sorbitol, Serine, Glycine, Glutamic Acid, Alanine, Lysine, Arginine, Threonine, Proline	1.00
Glycine	0.36
Proline	0.45
Alanine	0.15
Aloe Concentrate 40X	0.25
Chamomile Extract	0.29
e-Polylysine	1.00
Deionized Water	QS
Citric Acid	To pH 6.0 +/– 0.2

Combine ingredients at room temperature with mixing. Adjust pH.

4.3.4.5 HAIR CARE

a. Shampoos

Conditioning Shampoo with Water-Dispersible Lauroyl Lysine

INCI Name	*% w/w*
Ammonium Lauryl Sulfate	25.0
Sodium Laureth Sulfate	12.0
Disodium Cocoyl Glutamate and Sodium Cocoyl Glutamate	10.0
Glycol Distearate	2.5
Dimethicone	2.0
	3.0

INCI Name	*% w/w*
Cocamide MEA	1.50
Cocamidopropyl Betaine	7.0
Polyquaternium 7	1.0
PEG-80 Glyceryl Cocoate	1.0
Sodium PCA, Sodium Lactate, Arginine, Aspartic Acid, PCA, Glycine, Alanine, Serine, Valine, Lysine, Threonine, Proline, Isoleucine, Histidine, Phenylalanine	2.0
Lauroyl Arginine	0.1
Glyceryl Laurate	1.6
Zinc PCA	0.6
Phenoxyethanol and 3((2-ehtylhexyl)oxyl) 1,2-Propanediol	0.7
Aqua	Qs
Citric Acid	To pH 5.5

Combine all ingredients (except euxyl) with mixing while heating to 75°C. Allow to mix at 75°C for 15 minutes, then allow to cool to 50°C and add the euxyl. Continue cooling to room temperature while maintaining mixing. Adjust the pH with citric acid.

Powder Shampoo

INCI Name	*% w/w*
Sodium Lauroyl Glutamate	15.00
Sodium Myristoyl Glutamate	15.00
Lauroyl Lysine	1.00
Hydrolyzed Hyaluronic Acid	0.10
PCA	1.00
Talc	23.00
Sucrose	22.70
Maize Starch	22.00
Lauroyl Arginine	0.20

Combine in a tumble mixer.

Zinc PCA Shampoo

INCI Name	*% (w/w)*
Sodium Laureth Sulfate	35.0
Cocamidopropyl Betaine	15.0
PEG-7 Glyceryl Cocoate	2.0
Polyquaternium-7	0.2
Sodium PCA	2.0
TEA-Cocoyl Glutamate	16.0
Lauryl Lactate	1.5
	Qs
Aqua	QS
Zinc PCA	0.6

Combine all ingredients and adjust the pH with citric acid.

b. Conditioners

Natural Conditioner

INCI Name	*% (w/w)*
Isopropyl Lauroyl Sarcosinate	20.0
Lauroyl Lysine	2.0
Phytosterol/Octyldidecyl Lauroyl Glutamate	6.0
Vitis Vinifera (Grape) Seed Oil	6.0
α-Tocopherol	1.0
Aqua	Qs
Xanthan Gum	0.4
Lauroyl Arginine	0.2
Hydrolyzed Hyaluronic Acid	0.2
Sodium PCA, Sodium Lactate, Arginine, Aspartic Acid, PCA, Glycine, Alanine, Serine, Valine, Proline, Threonine, Isoleucine, Histidine, Phenylalanine	2.0
Sodium Stearoyl glutamate	5.0
Phenoxyethanol, 3[(2-ethylhexyl)oxy] 1,2-Propanediol	1.0
Citric Acid	Qs to pH 6.0 +/– 0.2

Charge batch vessel with water (phase b) and begin mixing. Slowly add remaining ingredients of phase B maintaining mixing and ensure complete hydration. Add phase C to phase B. Combine phase A in a separate vessel and add to phase B maintaining mixing. Add phase D. Adjust pH.

Leave On Conditioner

Phase	Ingredient	% (w/w)
A	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0.4
	Deionixed Water	39.60
B	Arginine	0.37
	Deionized Water	19.63
C	Butylene Glycol	3.00
	PEG-40 Hydrogenated Castor Oil	0.20
	PEG-40 Hydrogenated Castor Oil PCA Isostearate	0.20
	Phytosterol/Octyldodecyl Lauroyl Glutamate	0.05
	Isopropyl Lauroyl Sarcosinate	0.20
D	Dimethicone and Laureth-4 and Laureth-23	5.00
E.	Preservative	QS
	Alcohol	10.00
	Fragrance	QS
F	Amino Acid Cocktail: Sodium PCA, Sodium Lactate, Arginine, Aspartic Acid, PCA, Glycine, Alanine, Serine, Valine, Proline, Threonine, Isoleucine, Histidine, Phenylalanine	1.00
G	Disodium EDTA	0.02
	Deionized Water	QS

Dissolve part A and B separately with stirring at RT. Add part C to part A with stirring. Add part B to AC and heat to 60°C. Dissolve part D at 60°C, then add ABC to part D carefully and emulsify. Add part E with stirring. Cool to 50°C and add part F with stirring. Cool to 45°C and add part G with stirring.

CONCLUSION

Amino acids are very functional materials, both topically applied and through oral supplementation. The uses and patents of amino acids are growing year by year as can be seen from the following chart.

Figure 26

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GLOSSARY

1. Corneocyte: An outer skin cell

2. Dynamic Frictional Coefficient: The dynamic coefficient of friction, also called the kinetic or sliding coefficient of friction or friction coefficient (symbol μ or f, dimensionless), is a measure of how large the friction forces are that act between two solids.

3. Keratolitic: The loosening or shedding of the horny layer of the epidermis.

4. Rheometer: An instrument for measuring flow, viscosity, and extensions of materials.

AHAS AND BEYOND: ANTI-AGING INGREDIENTS AND THEIR BENEFITS FOR ALL LAYERS OF THE SKIN

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ABSTRACT

The alpha-hydroxy acids (AHAs), including glycolic acid, are ingredients that have revolutionized cosmetic anti-aging and are a critical part of the cosmetic chemist's formulary. This class of small organic acids has significant reparative effects to all layers of the skin, including exfoliation of the *stratum corneum*, increased epidermal keratinocyte proliferation and differentiation, modulation of melanin production, and increase to fibroblast production of dermal matrix components, resulting in clinically proven and consumer-recognized cosmetic benefits collectively associated with anti-aging.

Second- and third-generation hydroxy acids, including polyhydroxy acids (PHAs) and bionic acids (BAs), are not only milder having increased tolerability, but also impart protective effects such as barrier strengthening, antioxidant, and matrix metalloproteinase inhibition. More recently, acetylamino sugars and acetylamino acid derivatives have arisen from the AHA research. Many of these compounds are effective at higher pH, and like the PHAs, provide significant anti-aging benefits with inherent gentleness to the skin. Formulating with hydroxy acids represents a challenge to the cosmetic chemist, as there are nuances to formulating for efficacy without irritation, and in addition, low-pH emulsions can be difficult to stabilize. However, the challenges to formulating highly effective and stable topical products containing hydroxy acids and related compounds can be overcome through careful selection of vehicle components, including thickeners, emulsifiers, formula pH, and ingredient combinations.

This chapter provides an in-depth review of the evolution and discovery of the value of AHAs as uniquely powerful anti-aging ingredients. The in-depth review of the literature, combined with numerous clinical study results and the thoughtful description of the various mechanisms proposed, is compelling. Also of interest is the description of various cogent formulation approaches to maximizing efficacy while minimizing irritation.

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4.3.5.1 INTRODUCTION

Improvement in the appearance of photo-aged skin continues to be a high priority among consumers. Photo-aged skin is the manifestation of accumulated skin damage from chronic sun exposure superimposed upon the chronological aging process. The appearance of photo-aged skin is typically defined by lines and wrinkles, irregular texture, loss of firmness and elasticity, and an increased amount and reduced uniformity of pigmentation. The alpha-hydroxy acids (AHAs) were initially discovered by Drs. Van Scott and Yu in the 1970s and found to be useful for their effects on normalizing hyperkeratotic conditions such as ichthyosis.[1] In the subsequent decades, the discoveries of the broad pleiotropic effects of AHAs on all layers of the skin resulted in an array of cosmetic clinical benefits that is unmatched in cosmetic skin care. Topical cosmetic formulations containing AHAs at low concentrations (4–10%) have been shown to improve the appearance of photo-damage by a number of quantitative clinical endpoints. Numerous investigations into the mechanisms of action of AHAs support that the cosmetic benefits are likely derived from their impact on the skin surface as well as well as stimulatory effects on keratinocytes and fibroblasts.

The first-generation AHAs gave way to second- and third-generation polyhydroxy acids (PHAs) and bionic acids (BAs), which impart comparable clinical benefits for amelioration of photo-aging to AHAs, with improved mildness and additional skin-protective effects. This stream of investigation led to the discovery of the anti-aging benefits of N-acetylamino sugars and N-acetylamino acids and derivatives. The majority of these compounds are physiologic, natural, and nontoxic. They normalize keratinization and skin structure. The compounds discussed herein have a marked effect on epidermal and dermal thickness, and stimulate dermal fibroblasts to increase synthesis of glycosaminoglycans and collagen. Those with multiple hydroxyl groups impart a humectancy benefit as well as antioxidant characteristics, and are ideal for cosmetic treatment of aging skin appearance. This latest generation of anti-aging ingredients are especially gentle, which makes them ideal for use on sensitive skin.

Successfully formulating with AHAs requires a multiplicity of considerations since the balance of efficacy and irritation can be dependent upon the type of formula employed (such as oil-in-water, water-in-oil, or water-in-silicone emulsions) and the desired pH. Further, other parameters to be considered are emulsifier

and thickener selection in order to ensure formula stability, and selection of co-actives, which work optimally at a given pH. Thus, the thoughtful formulator must balance a variety of critical parameters in maximizing performance of cosmetic formulations containing hydroxy acids.

4.3.5.2 ALPHA-HYDROXY ACIDS (AHAS)

Structural and Physicochemical Properties of AHAs

The AHAs are organic carboxylic acids, with the distinct feature of having a hydroxyl functionality group attached to the carbon unit adjacent (or in the alpha position) to a carboxylic acid moiety.

Figure 1: Alpha-Hydroxy Acid Chemistry

The AHAs are typically low-molecular-weight compounds with acidity that is modulated by the proximal hydroxyl group. AHAs are found naturally in many fruits, and have aptly been nicknamed the fruit acids.

The most commonly used AHAs in skin care today include glycolic acid, lactic acid, and citric acid. Glycolic acid, or 2-hydroxyethanoic acid, is the smallest of the AHA family, and is found in sugar cane juice. Glycolic acid has a molecular mass of approximately 76 daltons and a pKa of 3.8, approximately 1 unit lower than its non-hydroxy analog acetic acid. Lactic acid, or 2-hydroxypropanoic acid, is a 3-carbon AHA derived from milk. Lactic acid is endogenous to the body and is produced via metabolism and exercise, during which time pyruvate becomes converted by the enzyme lactate dehydrogenase. Several AHAs, such as lactic acid, have chiral carbon atoms, resulting in D and L stereoisomers. Citric acid is a polycarboxyl AHA, and is a unique hydroxy acid in that it has a hydroxyl group alpha to the 2-carboxylic acid moiety, and beta to the 1- and 3-carboxylic acid moieties. This structural configuration thereby makes it not only an AHA but also a beta-hydroxy acid (BHA).

Citric acid is naturally found in citrus fruits, and is also found in the body in the Kreb's (citric acid) cycle where it is critically involved in energy production. Citric acid also has antioxidant properties. These smaller AHAs are hygroscopic in nature and highly soluble in water. Mandelic acid, or phenylglycolic acid, is an aromatic alpha-hydroxy acid, and, as such, is considered a lipophilic AHA. As a lipophilic AHA, mandelic acid tends to not only possess anti-aging benefits, but has unique applications for oily and acne-prone skin due to the affinity of more lipophilic molecules to sebum.

Table 1: Alpha-Hydroxy Acids – Natural Occurrence and Properties

AHA	Occurrence	Structure	Molecular Weight
Glycolic acid	Sugar cane	$CH_2(OH)CO_2H$	76
Lactic acid	Sour milk, Tomato juice	$CH_3CH_2(OH)CO_2H$	90
Citric Acid	Citrus fruits, ie oranges and lemons	$HO\text{-}C(CH_2CO_2H)_2\text{-}CO_2H$	192
Malic Acid	Apples	CH_2CO_2H $CH(OH)CO_2H$	134
Tartaric Acid	Grapes	$CH(OH)CO_2H$ $CH(OH)CO_2H$	150
Methyllactic acid	Mangos	$CH_3\text{-}C(CH_3)(OH)\text{-}CO_2H$	104
Mandelic acid	Almonds	$C_6H_5CH(OH)CO_2H$	152

Discovery of AHA Benefits

The cosmetic uses of AHAs are said to date back to the times of Cleopatra, renowned queen of ancient Egypt, where the benefits of milk acids, later to be identified as lactic acid, were known at this early time for generating younger smoother skin. However, key scientific and clinical evidence for AHAs emerged in the 1970s when Van Scott and Yu discovered the effects of glycolic acid on normalizing skin keratinization dysfunctions in hyperkeratotic conditions such as ichthyosis.[1]

Through decades of clinical, histological, and cellular investigations, the beneficial effects of glycolic acid on *stratum corneum* desquamation and plasticization, epidermal proliferation and signaling, dermal matrix remodeling, and melanin production were discovered, providing strong support for the potential use of this compound for improving photo-aged skin. Initially the benefits of 25% glycolic acid for reversing both epidermal and dermal markers of photo-aging were demonstrated on photo-damaged forearms,[2] and subsequently the efficacy of lower-strength glycolic acid formulations were demonstrated.[3] *As a result, glycolic acid has become one of the most widespread cosmetic anti-aging ingredients used.*

4.3.5.3 QUANTITATIVE CLINICAL BENEFITS OF AHAS

The clinical benefits of topical cosmetic AHAs have been demonstrated in numerous randomized, double-blind, vehicle-controlled studies in both face[3,4] and photo-damaged arm[5,6] models. A few of the many clinical studies are described below.

The first such study of cosmetic strength AHAs was a 22-week, single-center study with both 8% glycolic and 8% L-lactic acid compared to a vehicle control.[3]

Products were applied monadically to the entire face and in paired comparison application to photo-damaged arms. Significant benefits to overall severity of photo-damage were observed with both glycolic and lactic acid compared to the vehicle control on the face and arm. Further, evaluation of the photo-damaged forearm provided greater sensitivity for differentiating individual aspects of photo-damage, such as the appearance of mottled hyperpigmentation, sallowness, and fine lines and wrinkles.

Another randomized, double-blind, vehicle-controlled face and neck 12-week application study compared a 5% unneutralized formulation of glycolic acid to its vehicle control.[4] In this study, the glycolic acid treatment showed significant improvement compared to the vehicle control in physician-assessed skin texture, and a strong trend for improvement to discoloration (composite score of melasma, solar lentigines, guttate hypomelanosis, poikiloderma) compared to the vehicle control.

Additional studies have shown benefits of AHAs in both paired forearm application and split-face application studies.[7-10] In addition to expert visual assessment of photo-damage, objective instrumental assessments have shown additional clinical benefits of AHAs compared to vehicle controls, and allow for sensitive measurement of their benefit at earlier time points. In 12-week photo-damaged arm studies,[5-7] healthy female subjects (n~20 per product application cell), ages 45–70 years old, with a moderate degree (grades 4–6 on a 0–9 scale) of photo-damaged skin on the forearms, were enrolled to participate in IRB-approved arm studies. Photo-damage on the forearms was defined by the appearance of "crepe-like" skin,[5] using a 0–9 scale (0 = none, 1–3 = mild, 4–6 = moderate, 7–9 = severe, with ½ point grades allowed). In addition, the arms were compared to obtain a directed difference assessment, using a 0–4 scale, of skin tone, dryness, crepey appearance, and overall photo-damage. The forearm studies were randomized 12-week, double-blinded, bilateral-paired comparison studies. Subjects applied 0.5 to 1.0 g of each product to the appropriate left/right forearm, twice daily for 12 weeks. Efficacy was assessed at baseline and after 4, 8, and 12 weeks of product application by an expert evaluator using the grading scales shown in Tables 2–4 below.

Table 2: Photodamage Intensity of Crepe-Like Appearance[5]

Numeric Grade	Category Grade	Descriptor
0	None	
1-3	Mild	Small circular fine lines; may not be obvious at first glance; see more with twisting of the skin; <25% of the lower outer forearm; usually not seen in the inner surface of the forearm
4-6	Moderate	Obvious lined pattern; may look like circular lines; may look 'puffy';> 25% coverage but not entire outer lower arm; usually seen on the inner forearm but to a lesser degree; full coverage near elbow regions, with extension of crepey pattern/lines toward the wrist
7-9	Severe	Up to 100% cover of the outer lower forearm: very obvious, well demarcated; long "puffy" (depth) longitudinal lines; inner covered

Table 3: Directed Difference Attributes for Photo-Damaged Arm Appearance[5]

Attribute	Descriptor
Crepe-like/crinkled	Loose surface, saggy/lined/wrinkled skin
Skin tone	Evenness of skin color; freckling; color of keratosis
Overall appearance	Global assessment of the condition of the area with respect ot texture, evenness of skin tone and overall surface featrues
Visual dryness	Scaling/flaking

Table 4: Directed Difference Magnitude for Photo-Damaged Arm Appearance[5]

Numeric Grade	Descriptor
0	No difference
1	Very slightly better
2	Slightly better
3	Moderately better
4	Much better

The benefits of 8% glycolic acid cream (pH 3.8) compared to a vehicle cream were assessed in repeated clinical studies using the Photo-Damaged Arm Model.[5] Improvement by glycolic acid to the crepe-like appearance of skin was statistically significant in 15 out of 16 clinical studies. The reproducibility of the magnitude of benefit imparted by glycolic acid was demonstrated within individual studies and across studies. In a similar study design, the significant improvement of an 8% glycolic acid (pH 3.8) compared to its vehicle cream was observed for crepe-like skin intensity (see Figure 2). Glycolic acid provided significantly greater improvement to crepe-like skin appearance than its vehicle after 8 and 12 weeks of use. This was also demonstrated using the directed difference evaluation of overall appearance and crepe-like appearance after 8 and 12 weeks of application, and continued improvement at 12 weeks of application. A trend was observed ($p<0.1$) for directed difference evaluation of skin tone (Figure 3).

Figure 2: Improvement (from Baseline) to the Intensity of Crepe-Like Appearance on Forearms: 8% Glycolic Acid Cream Compared to Vehicle

Figure 3: Directed Difference Evaluation of Photo-Damaged Arms: 8% Glycolic Acid Cream Compared to Vehicle

In split-face photo-damaged skin studies,[7-10] healthy female subjects, ages 30–70 years old, with moderate to severe overall facial photo-damage, were enrolled to participate in IRB-approved vehicle-controlled facial studies. Since subjects served as their own within-treatment control (e.g., applied both the AHA and vehicle cream to either side of their face), these studies required fewer subjects than monadic application studies, where independent groups of subjects evaluated either the test product or the control on the whole face. In addition, the introduction of noninvasive, objective instrumental evaluation to the clinical protocols allowed for more sensitive discrimination between the effects of the test products versus the vehicle, as well as detection of effects at earlier time points. Therefore these studies were typically a maximum of 12 weeks in duration, shorter than the earlier monadic studies, which were six months in duration.[3]

Photo-damage intensity on the face was assessed using validated expert visual scales. Subjects applied test products to their face, twice daily for up to 12 weeks. Assessment of skin condition (overall photo-damage, lines and wrinkles) was conducted by expert visual assessment and objective instrumental measurements were obtained at baseline and at monthly intervals.

In the photo-damaged facial studies, most study expert visual assessments were made using traditional photo-numeric 0–9 scales, where 0 = none, 1–3 = mild, 4–6 = moderate, and 7–9 = severe.[11–14] These scales are helpful for characterizing overall photo-damage as well as individual attributes of photo-damage, and assessments may be made using ½ point increments,[14] which allows for sensitive differentiation between technologies. In one study, 10 visual parameters were assessed using a physician global assessment scale, where 0=none, 1=mild, 2=moderate, and 3=severe. All ten parameters were summed to provide an overall score,

as well as a wrinkle and discoloration score (the sum of four out of ten parameters for each).[4]

In addition to visual assessment of photo-damage, objective instrumental assessment of skin improvement by AHAs included digital photography, ballistometry (elasticity and firmness), and 3D Cyberware™ facial scanning. The Diastron™ ballistometer is a pendulum-based, torsional instrument that can be applied to the skin at different forces, to measure initial indentation and rebound from the skin, as well as a measure of overall elasticity (damping coefficient, alpha). The 3D 3030/HIREZ Cyberware™ facial scanner uses a near-IR line projector attached to a cylindrical motion stage to compute 3-D depth information on the face in cylindrical coordinates (Figure 4). From these virtual scans of the face, the length, depth, and volumes of surfaces may be calculated. Since the development of this technique, there are now other commercially available 3-D face and body systems. Over time, these systems have continued to improve the resolution and photorealism of 3-D images.

Figure 4: Sample C3030/HIREZ Cyberware™ Facial Scanner Images

In a previously reported split-face and photo-damaged arm application study,[7] subjects applied an 8% glycolic acid cream (pH 3.8) compared to its vehicle. Ballistometer readings were collected at weeks 0, 4, and 8 using three different force settings. The ballistometer response curves were analyzed with digital filtering (Fast Fourier Transform methods) to remove noise, and then a genetic algorithm (GA) was used to identify features in the filtered response curves. Both the glycolic acid cream and vehicle cream improved skin elasticity at weeks 4 and 8. The GA was able to correctly classify an "active" response on the glycolic-treated side over a vehicle response in 17 out of 18 subjects, in both the face and arm treatment sites.

The 3-D depth and length of fine lines and wrinkles around the periorbital region were evaluated using the Cyberware™ 3030 HIREZ facial scanner in a split-face application study (P. D. Hawkins SS). An 8% glycolic acid cream (pH 3.8) was compared to its vehicle cream. Significant improvement to the length of fine lines was observed after four weeks of product application with the glycolic acid cream (Figure 5). The glycolic acid cream was statistically superior to the vehicle cream at weeks 4 and 8 for the length of fine lines and wrinkles, and at week 8

for the depth of fine lines and wrinkles. A sample baseline and week 8 image from the 2-D photographs is shown in Figure 6.

Figure 5: 3D evaluation of lines and wrinkles in the periorbital site using the Cyberware™ 3030 HIREZ facial scanner.

Figure 6: Left) Baseline; Right) After eight weeks of application using 8% glycolic acid cream (pH 3.8). After treatment, the fine lines and wrinkles are fewer and less deep in the periorbital area. The skin tone is also improved on the cheek, and the overall texture is smoother.

Consumers are also able to perceive the skin benefits imparted by AHAs over the vehicle controls. In a previously published report,[9] subjects viewed before and after photos in a randomized, double-blind 12-week multicell split-face application study. One of the treatment groups compared an 8% glycolic acid cream to its vehicle. Consumers evaluated their skin condition across multiple anti-aging attributes at the end of the study without using photos. Approximately three months later, subjects returned to view their baseline and week 12 photos, and were administered a similar questionnaire using the same attributes previously evaluated. Subjects were able perceive improvement to photo-damaged appearance, such as lines and wrinkles, evenness of color, and firm appearance, which were statistically significant in favor of the AHA cream compared to its vehicle.

The anti-aging benefits of AHA formulations over a vehicle control have been observed in multiple randomized, double-blind, vehicle-controlled studies. In both the split-face photo-damaged face and paired comparison arm models, improvement to appearance attributes has been observed for texture/lines and wrinkles as well as even tone/hyperpigmentation and overall appearance. These clinical changes correspond to the longer-term benefits observed in earlier six-month monadic use clinical studies, and translate to consumer perceived benefits, as observed in split-face application photo-damaged facial tests.

4.3.5.4 CELLULAR AND STRUCTURAL CHANGES ASSOCIATED WITH ALPHA-HYDROXY ACIDS

Several studies have been conducted to understand the modes of action by which AHAs provide their beneficial effects on skin. These studies have demonstrated that AHAs can have effects on all layers of the skin, starting with the outermost layer, the *stratum corneum*. The effects of AHAs extend through the viable epidermis, to the dermal compartment. *In vitro* studies have indicated that AHAs can have direct effects on multiple cell types present in the skin, including keratinocytes, fibroblasts, and melanocytes, which may explain, in part, the clinically observable effects on skin such as moisturization and improvement of photo-aging.

Effects of AHAs on the *Stratum Corneum*

Early studies by Van Scott and Yu[1,15] demonstrated that treatment of xerotic skin results in the normalization of *stratum corneum* (SC) exfoliation, increased plasticization, decreased formation of flaky scales, and a thinner, flexible, and more compact *stratum corneum*. An extreme example of this effect is seen in Figure 7, where thick scaly plaque skin treated with a combination of 20% alpha- and polyhydroxy acids (pH 3.8) provides a dramatic improvement to this condition.

Figure 7: Descaling effect observed with 20% AHA/PHA/bionic acid formulation: scaling on knee before (left) and after use (right)

One of the ways in which AHAs are believed to bring about these effects is by diminishing the adhesiveness of the corneal layer. AHAs are believed to modulate desquamation by altering or degrading corneosomes (desmosomes), thereby decreasing cohesion between corneocyte layers. There is general agreement that this action is restricted to the *stratum corneum*, but where exactly the AHAs exert their exfoliating effect in this epidermal layer remains unclear.

In low concentrations, for example 2–5%, glycolic acid is believed to facilitate progressive weakening of cohesion of the intercellular material of the *stratum corneum*, resulting in uniform exfoliation of its outermost layers, the *stratum disjunctum*. A study by Fartasch et al.[16] investigated the effects of topical treatment with a 4% glycolic acid formulation, pH 3.8, on the SC using electron microscopy. The lamellar body (LB) secretory system in the *stratum granulosum* (SG), and intercellular lipid lamellae in the SC in both vehicle and glycolic acid-treated samples were found to be comparable to normal human SC. The site of action of glycolic acid as applied in this study appeared to be the desmosomes.

Normally, progressive stages of degradation of the desmosomal plug are seen as a function of their precise location in the different SC layers.[17] The skin's natural degradation in the *stratum disjunctum* was accelerated in the glycolic acid-treated skin compared with vehicle-treated skin. Within the SC, enhanced desmosomal breakdown, promoting loss of cohesion and desquamation, appeared to be restricted to the *stratum disjunctum* while desmosomes of the *stratum compactum* were unaffected. In this study, glycolic acid appears to target the desmosomes without affecting the barrier structures of the *stratum corneum* since TEWL (trans-epidermal water loss) values remained unchanged. It also appears that lactic acid can stimulate ceramide synthesis,[18] which suggests that AHAs may provide improvement in skin barrier function.

Several hypotheses have been proposed with regard to the mechanisms by which AHAs affect the cohesion of the layers within the *stratum corneum* and enhance desquamation. AHAs could affect ionic bonding of the layers, inhibit enzymes such as sulfate and phosphotransferases, resulting in fewer electronegative sulfate and phosphate groups on the surface of corneocytes, thus diminishing

cohesive forces.[15] Enhanced desquamation may also be achieved via preferential acidification of the polar domains embedded within the hydrophilic lipid bilayers, or by activating by other means the acid proteases crucial for desmosomal degradation.[19] Human tissue kallikreins are a family of 15 trypsin- or chymotrypsin-like secreted serine proteases (KLK1–KLK15). Many KLKs have been identified in normal *stratum corneum* (SC) and are candidate desquamation-related proteases, particularly KLK-7.[20] AHAs could transiently change the pH of the corneum layers and activate these proteases, leading to corneosome dissolution. Topical application of a high concentration (50% @ pH 0.9) led to an initial inactivation followed by prolonged activation of cathepsin D-like protease in the upper layers. No changes in chymotrypsin-like proteinases were observed in this study.[21]

When applied to hairless mice, high concentrations of AHAs (70% glycolic acid) resulted in a loss of calcium gradient. This could lead to lamellar body exocytosis. An *in vitro* study using cultured keratinocytes suggested that glycolic acid could lower the calcium ion concentration, at least in part, through the chelating effects of the glycolic acid on the cationic ions.[22]

Effect of AHAs on the Epidermis

One of the most consistent effects observed following topical application of AHAs is an increase in viable epidermal thickness. Lavker et al.[23] reported that daily topical application of a 12% ammonium lactate formulation resulted in an increase in epidermal thickness. A thickened epidermis was also observed following 5% or 12% lactic acid application twice daily for three months.[24] Similar results were reported with a three-week application of glycolic acid.[25]

This change in the epidermis of skin could be a direct effect of AHAs on keratinocyte proliferation. When human epidermal keratinocytes were exposed to glycolic acid and L-lactic acid in cell culture, a stimulatory effect on keratinocyte proliferation was observed. The results of these studies indicated that both glycolic and lactic acids are mitogenic in nature as each significantly stimulated proliferation of keratinocytes compared to control cells.[26]

Effect of AHAs on the Dermis

In addition to the effects on the *stratum corneum* and the viable epidermis by AHAs, beneficial changes in the dermis have also been reported in multiple studies. These effects include a marked increase in glycosaminoglycans (GAGs), collagen, and dermal thickness. The studies described below used a range of concentrations of AHAs, starting from 4% to up to 50% and at varying pHs. Lavker et al.[23] demonstrated that 12% ammonium lactate, applied to normal skin in an open (four weeks) or occluded (three weeks) manner, resulted in a striking deposition of GAGs in the dermis. This AHA preparation also spared the atrophic effects of corticosteroids on the epidermis and dermis when applied in combination with clobetasol propionate.

In addition to GAGs, increased deposition of collagen has also been observed following application of AHAs. Smith et al. reported that treatment with 12% lactic acid resulted in increased epidermal and dermal firmness and thickness. This was accompanied by clinical improvement in skin smoothness and in the appearance of lines and wrinkles. In the same study, a lower concentration of AHA, namely 5% lactic acid, modulated surface and epidermal changes but no dermal change.[24]

In another study, 4% glycolic acid, pH 3.8, in a suitable formulation, was applied to forearms of ten subjects for three weeks under semi-occlusive conditions (five patches/week for three weeks). Biopsies were collected at the end of three weeks and samples were analyzed for viable epidermal thickness, pro-collagen 1 by immunohistochemistry, total collagen (Masson trichrome stain) and GAGs (colloidal iron). Four out of ten subjects showed an increase in viable epidermal thickness and in colloidal iron staining and seven out of ten showed increases in pro-collagen 1 as well as total collagen.[27] Representative images are shown in Figures 8–11.

Figure 8: Effect of topical application of glycolic acid on viable epidermis. The dorsal forearms of ten subjects were each treated with 4% glycolic acid formulation or vehicle control for three weeks under semi-occlusive patch. Treated sites were biopsied and sections were stained with hematoxylin & eosin. Viable epidermal thickness (VET) was increased in four out of ten subjects (yellow arrows). *Stratum corneum* layers were reduced in eight out of ten subjects (blue arrows). Epidermal cells became more "compact" and "organized" in subjects with increased VET.

Figure 9: Effect of topical application of glycolic acid on glycosaminoglycans (GAGs). The dorsal forearms of ten subjects were each treated with 4% glycolic acid formulation or vehicle control for three weeks under semi-occlusive patch. Treated sites were biopsied and sections were stained with colloidal iron stain. Four out of ten subjects showed increased colloidal iron deposition at DEJ, the staining pattern became thicker and more continuous (yellow arrows).

Vehicle control Glycolic acid

Figure 10: Effect of topical application of glycolic acid on pro-collagen 1. The dorsal forearms of ten subjects were each treated with 4% glycolic acid formulation or vehicle control for three weeks under semi-occlusive patch. Treated sites were biopsied and sections were stained for immunohistochemical analysis for pro-collagen-1, a biomarker for new collagen synthesis. Five out of ten subjects showed increased pro-collagen-1 staining level at papillary dermis (yellow arrows). There was a significant induction of pro-collagen-1 at papillary dermis and dermal-epidermal junction, indicating a beneficial biochemical effect of topical AHA treatment.

Vehicle control Glycolic acid

Figure 11: Effect of topical application of glycolic acid on total collagen. The dorsal forearms of ten subjects were each treated with 4% glycolic acid formulation or vehicle control for three weeks under semi-occlusive patch. Treated sites were biopsied and sections were stained with Masson trichrome stain. Seven out of ten subjects showed increased intensity of fiber-forming collagen staining, indicating a beneficial biochemical effect of topical AHA treatment.

In the pivotal 8% AHA clinical study by Stiller et al.,[3] where the clinical improvement to photo-damaged skin after 22 weeks was first reported, histological changes in skin were also observed.[28] In this study, biopsies at baseline and week 22 were obtained and immunohistochemistry for pro-collagen 1 was performed. Slides were blindly evaluated for changes in pro-collagen staining, and an increase in pro-collagen 1 staining intensity relative to baseline was observed in all three groups—vehicle, glycolic, and lactic acid-treated skin samples. While no significant difference was observed between the groups, there was a significant

correlation between collagen synthesis and changes in clinical photo-aging, suggesting that clinical improvement after AHA/sunscreen treatment may be due, in part, to increased collagen synthesis. Examination of the samples using electron microscopy revealed that dermal density and papillary dermal collagen structures increased in all three groups, correlating with immunohistochemistry results. Increase in anchoring fibrils and dermal density correlated with clinical improvement. This was the first time electron microscopy results demonstrated treatment-induced structural changes in photo-damaged skin, that were consistent with clinical improvement.

Bernstein et al.[29] performed a study employing a higher concentration of AHA, 20% glycolic acid, twice a day for three months, and demonstrated an increase in collagen gene expression and additionally, increases in epidermal and dermal hyaluronic acid production. In addition to glycolic and lactic acids, other AHAs, such as citric acid, having clinical benefits for photo-aging skin, have also been studied for their effect on dermal matrix components. Bernstein et al.[30] observed that 20% citric acid application for three months resulted in increased viable epidermal thickness and dermal glycosaminoglycans in AHA-treated skin, compared to vehicle-treated site.

A study by Ditre et al.[2] used even higher concentrations of AHAs, e.g., 25% glycolic, lactic or citric acid and demonstrated that topical application of these AHA lotions for an average of six months caused a 25% increase in skin thickness relative to placebo. This increase could be attributed to the increased thickness of the epidermis as well as the dermis, and papillary dermal changes included increased acid mucopolysaccharides, improved quality of elastic fibers, and increased density of collagen. Induction of epidermal cell proliferation, correction of epidermal basal cell atypia, enhanced melanin pigment dispersal, and a return to a more undulating rete pattern at the epidermal-dermal junction were also reported.

Mechanism of Action Studies *In Vitro*

In order to further understand the dermal remodeling action of AHAs observed *in vivo*, several studies looked at the effects of AHAs on fibroblasts *in vitro*. Human dermal fibroblasts exposed to glycolic and L-lactic acids in cell culture secreted significantly higher levels of pro-collagen 1, and this response was dose dependent.[26] Two other studies[31,32] also reported similar effects, using ^{3}H-proline labeling, suggesting that AHAs have a stimulatory effect on collagen at the protein synthesis level, and glycolic was found to be superior to malic acid in stimulating collagen. It has been suggested that the net increase in collagen observed *in vitro* may be, in part, due to a primary effect on fibroblast proliferation.[33]

Okano et al.[34] investigated the effect of glycolic acid on the dermal matrix metabolism of keratinocytes and fibroblasts using *in vitro* and *ex vivo* systems.

Glycolic acid was found to directly accelerate collagen synthesis by fibroblasts in a dose-dependent manner. Keratinocytes treated with glycolic acid released higher levels of IL-1 alpha, and this effect was also observed *ex vivo*.[35] When fibroblasts were exposed to conditioned medium from keratinocytes treated with glycolic acid, they up-regulated MMP-1 and MMP3 mRNA expression. Direct treatment of fibroblasts to glycolic acid did not elicit this response. The effect on MMP expression is likely mediated by IL-1alpha, which was up-regulated by glycolic acid in keratinocytes. Rakic et al.[36] investigated the influence of mediators released from glycolic acid-treated keratinocytes on fibroblasts, and showed that IL-6 secretion from fibroblasts cultured in the conditioned medium of GA-treated keratinocytes is significantly up-regulated. This is consistent with the observation of IL-1alpha release from keratinocytes following exposure to glycolic acid, as IL-1alpha is known to induce IL-6.[37] Thus, it appears that cytokines released from keratinocytes can modulate dermal matrix degradation and contribute to remodeling.

A study by Rendl et al.[38] examined the effect of topical application of different concentrations of lactic acid on the secretion of cytokines. Lactic acid, at 1.5% or 3%, increased the secretion of VEGF (Vascular Endothelial Growth Factor) from epidermal equivalents. Secretion of angiogenin was inhibited and IL-8 was not changed. Lavker et al. recently showed that topical AHA treatment can prevent the regression of skin microvessels caused by topical corticosteroid treatment and that it appears to increase skin vascularization. The enhancement of VEGF by lactic acid could serve as a possible explanation for the effect of AHAs on the microvasculature.

Considering the observed remodeling effects of AHAs on the dermis *in vivo*, especially at higher concentrations, one may speculate that it could be a result of peeling, which, like a chemical burn, may induce a wound-healing response, initiating a multistep inflammatory reaction that activates and modulates fibroblasts. However, the studies described above suggest that the increase in collagen production is mediated by a direct action on fibroblasts that is independent of inflammatory mechanisms. In studies using animals[33] as well as humans,[2] there was a lack of inflammatory cell infiltrates observed in the dermis after glycolic and lactic acid treatment, which was correlated with a greater increase in dermal thickness than controls.

In order to understand how biochemical processes such as proliferation and dermal matrix synthesis could be impacted by AHAs, a study was conducted to investigate the effects of lactic and glycolic acids on basal metabolism of human keratinocytes and fibroblasts, specifically, with respect to changes in the Krebs cycle and oxidative phosphorylation (OP) activity. Measurement of $^{14}CO_2$ produced using appropriately labeled glucose, lactic acid, and glutamine, was used to determine Krebs cycle activity. OP was assessed by measuring oxygen uptake

using Clark electrodes. Lactic and glycolic acids were able to stimulate both CO_2 release and O_2 uptake by keratinocytes and fibroblasts. Increases in O_2 uptake coincided with increases in collagen I measured from fibroblast conditioned media. These results suggest that AHAs may function, in part, by stimulating aerobic metabolism to ultimately generate more available ATP required for cellular processes, including proliferation and collagen synthesis.[39] This phenomenon, seen *in vitro*, was also studied *in vivo*. Treatment of skin with an 8% lactic acid formulation for 1.5 hours resulted in a decrease in transcutaneous oxygen relative to vehicle cream, indicating that more oxygen was utilized by skin treated with lactic acid. Similar results were seen upon long-term treatment with 8% glycolic acid for 12 weeks. These studies support a role that AHAs may play in aerobic metabolism, generating energy to support cellular processes and photo-damage repair.[40]

To further delineate the mechanism by which AHAs such as glycolic and lactic acids induce cell proliferation, the effect of AHAs on signaling kinases ERK2, JNK, and p38 have been examined. These kinases have been implicated in response to a variety of environmental stimuli. Treatment of dermal fibroblasts with lactic or glycolic acid, even at neutral pH, led to a dose-dependent decrease in intracellular pH. In addition, activation of ERK 1/2, JNK, and p38 by serum was down-regulated by pretreatment of cells with lactic acid. This implies the involvement of signaling pathways using these kinases in the response of the cells to lactic acid, and this response is likely triggered by transient intracellular acidification.[41]

Consistent with the study described above, Denda et al.[42] observed that application of glycolic acid to a skin-equivalent model induced a pH-dependent effect on keratinocyte proliferation, which was inhibited by antagonists of TRPV1 (Transient Receptor Potential Vanilloid), an acid-sensitive ion channel. Furthermore, transient ATP release was detected in the culture medium after glycolic acid stimulation, and this was also suppressed by TRPV1 antagonists. These results suggest that one of the mechanisms of glycolic acid-induced epidermal proliferation is a growth response of basal keratinocytes to the local elevation of H(+)-ion concentration by infiltrated GA. This response is mediated by TRPV1 activation. Another recent study[43] suggested that glycolic acid causes intracellular acidification and activates TRPV3 channel. Activation of TRPV channels due to transient acidification by protons from AHAs could also be involved in the perception of stinging and burning caused by AHAs at low pH in individuals with sensitive skin.

Effects of AHAs on Melanocytes

AHAs such as glycolic and lactic acids have been reported to be effective in treating pigmentary lesions such as melasma, solar lentigines, and post-inflammatory hyperpigmentation. It is conceivable that accelerated desquamation and epidermal effects seen following topical application of AHAs could result in rapid pigment dispersion, leading to clinically observed improvement in pigmented

lesions. In addition, it has been shown that AHAs could also have a direct effect on melanin synthesis. Treatment of mouse melanoma cells with glycolic or lactic acids led to a dose-dependent decrease in melanin formation, possibly through inhibition of tyrosinase activity.[44] Thus, the benefits of AHAs in improving the appearance of pigmentary lesions may be mediated by a combination of accelerated epidermal turnover and a direct inhibition of melanin formation in melanocytes.

The above studies demonstrate that AHAs contribute to the repair of photo-damaged skin through various mechanisms, on multiple skin cell types, and the resultant cosmetic benefits are likely mediated by the combination of modifications of the skin surface, the epidermis, and the dermis. Epidermal and dermal remodeling of the extracellular matrix results in significant reversal of epidermal and dermal markers of photo-aging. Collectively, these data support the view that AHAs can alter cellular processes in a manner consistent with actives expected to improve xerotic and photo-damaged skin.

4.3.5.5 POLYHYDROXY ACIDS (PHAS)

Structural and Physicochemical Properties of PHAs

The polyhydroxy acids, aldonic acids, are a class of organic carboxylic acids that have multiple hydroxyl groups, and represent second-generation AHAs.[45]

Table 5: Polyhydroxy and Bionic Acids – Natural Occurrence and Properties

Compund	Structure	Occurrence	MW
Gluconolactone		Corn Sugar	178
Lactobionic acid		Milk Sugar	358
Maltobionic acid		Corn Sugar	358

Many PHAs are also AHAs, if one of the hydroxyl groups is positioned on the alpha carbon adjacent to a carboxylic acid group. Polyhydroxy acids are often carbohydrates found in the body and/or intermediates in carbohydrate metabolism.

PHAs are slightly larger AHAs, but with molecular weight still sufficiently low enough to penetrate into the skin. PHAs possess physicochemical and biological properties that make them extremely useful in anti-aging skin care formulations. One of the most commonly used PHAs in cosmetic formulations today is gluconic acid, or gluconolactone, which is naturally derived from corn sugar, and also found endogenously in the body as an intermediate in the pentose phosphate pathway. Other PHAs include glucuronolactone, glucoheptonolactone, and galactonolactone, which are less commonly utilized in cosmetic formulations due to availability, cost, and/or formulation challenges.

Many PHAs have the ability to form a lactone, due to loss of a water molecule between the carboxylic acid and terminal hydroxyl group. In solution or in formulations, the lactone, acid, or a combination of both forms may exist in equilibrium depending upon the local environmental factors, such as pH. The chemical structure of PHAs, which includes two or more adjacent carboxyl and hydroxyl groups, contributes to the antioxidant properties of this class of compounds. Antioxidant properties have been demonstrated via several methods including: prevention of air oxidation of anthralin, hydroquinone, or banana peel. Using these tests, several PHAs, including gluconolactone, have demonstrated their antioxidant potential.[46,47]

Cosmetic Benefits of Topical PHAs

Numerous clinical studies have demonstrated the profound effects of the PHAs, like AHAs, for the improvement of aging skin appearance, while also being very mild and gentle.[48–51] One example was a 12-week clinical study that directly compared the effect of gluconolactone to glycolic acid. In this study, gluconolactone significantly improved all aging attributes including fine lines, wrinkles, pore size, roughness, firmness, mottled pigmentation, and clarity, to an equivalent degree to the glycolic acid treatment, although with slight but significantly lower sensory irritation.[52] The chemical structures of PHAs impart hygroscopicity, allowing this class of compounds to effectively bind water, thereby contributing increased hydration.

Gluconolactone, in particular, also provides an improvement to the skin barrier. Another study by Berardesca et al.[53] demonstrated that AHAs can help enhance skin barrier function, particularly with respect to increasing resistance to topically applied irritants such as sodium lauryl sulfate (SLS). When glycolic, lactic and tartaric acids, and gluconolactone (8%) were applied topically to the volar forearm for four weeks followed by an SLS challenge, sites treated with tartaric acid and gluconolactone were particularly resistant to disruption in barrier function (TEWL) and erythema measured over 48 hours. This indicates that PHAs can improve barrier strength and provide resistance to the SLS challenge.

The PHAs also possess protective effects against harmful external and environmental stressors. Gluconolactone has been found to effectively chelate metal ions,

thereby preventing activation of metal-dependent matrix-degrading enzymes, i.e., the matrix metalloproteinases. It is well known that solar elastosis, the underlying structural damage to the dermal matrix due to chronic photo-aging, is exacerbated by UV radiation–induced elastin promoter gene activation. Bernstein et al. have shown that gluconolactone reduces UV-induced activation of the elastin promoter gene by 50%, which is comparable to the effects of other well-known protectants, ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E).[54] Gluconolactone has minimal direct UV absorbance, so therefore this photoprotective effect is provided by its antioxidant, free-radical scavenging properties.

The inherently mild nature of the PHAs, coupled with their proven clinical benefits for barrier improvement and for effacement of aging skin, makes this class of AHAs ideal cosmetic ingredients for use with sensitive skin and on facial male skin prone to shaving.

Figure 12: Improvement to skin texture and pores using a glycolic acid/gluconolactone regimen (cleanser, day cream SPF15 with gluconolactone, night lotion with glycolic acid): before (left) and after 12 weeks of use (right).

Clinical studies have demonstrated that gluconolactone is compatible with sensitive skin conditions, such as rosacea, eczema, and atopic dermatitis, providing a hydroxy acid option for those individuals who cannot tolerate traditional AHAs.[55–58] Furthermore, the conditioning benefits of gluconolactone make this an excellent co-ingredient for potentially irritating cosmetic and pharmaceutical ingredients, like the retinoids, salicylic acid, or benzoyl peroxide.[48,59,60]

4.3.5.6 BIONIC ACIDS (BAS)

Structural and Physicochemical Properties

The aldobionic acids are a third generation of AHAs, more commonly referred to as bionic acids. Bionic acids are also PHAs consisting of a monosaccharide linked to an aldonic acid. Bionic acids are oxidized forms of dimeric carbohydrates and are derived from the corresponding reduced sugars. Table 5 shows two of

the more commonly utilized bionic acids, lactobionic acid and maltobionic acid, and depicts how the bionic acids are also PHAs. Chemically, lactobionic acid is a dimer of gluconic acid ether-linked to a galactose molecule, and is derived from milk sugar. Maltobionic acid is a dimer of gluconic acid ether-linked to glucose, and is derived from corn sugars. Other bionic acids include stereoisomers such as isolactobionic acid, isomaltobionic acid, cellobionic acid, gentiobionic acid. While the bionic acids are larger in size than the more commonly used AHAs and PHAs, such as glycolic acid, lactic acid, and gluconolactone, these compounds are still sufficiently small enough to penetrate the skin at a molecular weight of approximately 358 daltons.

Like the second-generation hydroxy acids, PHAs, the bionic acids are extremely hygroscopic in nature due to the multiple hydroxyl groups in close proximity on the molecule, which aid in attracting and holding on to water. Quite uniquely, the bionic acids have the potential to form a clear gel matrix when the compounds are dissolved in water and then evaporated. It is the strong hygroscopicity of these compounds that allows the gel matrix, rich in water, to be formed. Both maltobionic acid and lactobionic acid binds water strongly, and following evaporation, the resultant gel matrix contains 29% water, and 14% water, respectively.[61,62]

Skin Benefits of Topical BAs

Lactobionic and maltobionic acids have both protective and reparative effects beneficial for the amelioration of photo-aged skin. These bionic acids are also powerful antioxidants with strong metal chelation properties.[63] Both compounds effectively inhibit matrix metalloproteinase enzymes, which are responsible for the degradation of dermal collagen and elastin. Maltobionic acid has also been shown to reduce lipid peroxidation *in vitro*, thereby imparting antioxidant and protective benefits.[64]

Numerous investigations into the role of pH of the *stratum corneum* in maintenance of SC homeostasis and integrity have been conducted. The role of pH in the SC has been shown to regulate protective effects on the skin, including barrier homeostasis, SC integrity, and antimicrobial defense, and pH is higher in impaired or inflamed skin. Elias et al.[65–67] reported that while elevation of SC pH has deleterious effects on its structure and function, both lactobionic acid and gluconolactone effectively lower SC pH and improved SC structure and function. The improvement of permeability barrier homeostasis by lactobionic acid and gluconolactone is thought to be due in part to pH-dependent increase in activity of key enzymes such as beta-glucocerebrosidase and acidic sphingomyelinase.

The bionic acids also have been shown to enhance cell turnover rates *in vivo* as measured using the dansyl chloride assay.[68] For example maltobionic acid provided an 82% increase in cell turnover in just eight days (Figure 13).

Figure 13: Enhanced Cell Turnover by Maltobionic Acid *in vivo*, as Measured in the Dansyl Chloride Assay

Lactobionic acid also impacts melanogenesis and pigmentation pathways as evidenced through a reduction in alpha-MSH–induced melanin production in B16 melanoma cells as shown in Figure 14.[69] Maltobionic acid has similar inhibitory effects on melanogenesis *in vitro*.[64] These data, taken together, suggest this class of third-generation hydroxy acids should provide benefits for smoother, more even-toned skin.

Figure 14: Inhibition of alpha-MSH induced Melanin Production by Lactobionic Acid (LBA) in B16 Melanoma Cells

The clinical anti-aging benefits associated with a topical formulation containing 8% lactobionic acid, pH 3.8, were comparable in breadth and magnitude of benefits as was seen with glycolic acid. Following 12 weeks of twice-daily usage, a significant improvement was seen in all measured signs of aging, including fine lines, wrinkles, mottled pigmentation, laxity, sallowness, pore size, clarity, and roughness. In addition, elasticity and firmness were improved as measured by pinch recoil, and skin thickness was improved as measured by digital calipers. This clinical improvement was underpinned by structural changes within the skin: namely an increase in epidermal thickness and compacted *stratum corneum*, and an increase in dermal glycosaminoglycan staining as well as a reduction in matrix metalloproteinase staining, MMP9.[63] Likewise, the anti-aging benefits of an 8% maltobionic acid, pH 3.8 topical formulation, applied twice daily for twelve weeks to the face and three times daily to one forearm, showed comparable benefits. All visually assessed signs of aging were significantly improved with maltobionic acid, as was elasticity and skin thickness as measured by pinch recoil and digital calipers, respectively. These clinically measured changes were also supported by ultra-structural changes as evidenced by a compacted *stratum corneum*, increased epidermal thickness, and dermal increase in glycosaminoglycans.[70]

One key advantage of the PHAs and bionic acids is their inherent gentleness to the skin. These PHAs do not cause sensory irritation, such as burning or stinging, or visual inflammation/redness. For example, a high-strength (20%, pH 3.8) maltobionic acid–containing cream was found to be nonallergenic and nonirritating in a repeat insult patch test.[64] Therefore these second- and third-generation AHAs are not only extremely effective in preventing and improving signs of photo-aging, they are also ideal for use on all skin types, including those with sensitive skin conditions.

4.3.5.7 FORMULATION STRATEGIES FOR OPTIMIZING HYDROXY ACID PERFORMANCE

When formulating products with alpha-hydroxy acids (AHAs), one must be cognizant of the formulation requirements of a low-pH environment needed to obtain maximum product efficacy, while maintaining acceptable product stability with minimal irritation potential. To begin, the single-most important factor that influences AHA bioavailability to the *stratum corneum*, and thus product efficacy, is the pH of the formulation. The pH of aqueous-based products determines the amount of AHA that will be present in the undissociated, free-acid form. Knowing the pH of the formulation and the pKa of the acid, one can determine the relative molar concentration of AHA present in the acid form versus the ionized, anionic form. This is of vital importance as only the free-acid, non-salt form is immediately bioavailable for penetration into skin.[45]

To that extent, it is important to have a fundamental understanding of the molecule's pKa value and determine the appropriate pH range in which to formulate, to obtain optimal product efficacy while minimizing any potential irritation. To review, the pKa value is the logarithmic measure of the acid disassociation constant, Ka. Relatively speaking, Ka is the strength of an acid when in solution. Given the magnitude of the Ka values, a logarithmic scale is commonly used, thereby giving us the pKa value.[91]

pKa=-LogKa

Taking into account that pH is a logarithmic relationship of the hydrogen ion concentration, it can be seen that as the pH is adjusted above the pKa value of the AHA, the calculated amount of free acid quickly falls below 50%. Conversely, when the pH is reduced to a value below the acid's pKa, there is a large increase in calculated free acid. When the pH is adjusted to the same level as the acid's pKa, the amount of free acid in the formulation is calculated to be approximately half of the concentration of the AHA. For example, a 10% glycolic acid formulation at pH 3.8 contains 5% free glycolic acid in the bioavailable form, and 5% in the ionized salt form.[45] Table 6 shows some commonly used AHAs and their respective pKa values:

Table 6: Commonly Used Organic Acids in Topical Formulations and pKa Values[71]

Acid	pKa Value
Glycolic Acid	3.83
Lactic Acid	3.86
Pyruvic Acid	2.49
Salicylic Acid	3.00
Mandelic Acid	3.41

Since it is very likely that an AHA formula will be developed in a low pH range, typically 3.5–4.5, in order to impart efficacy, one must consider the formulation challenges that this brings forth. To start, formulating in a low-pH environment prohibits the use of some of the more traditional ingredients commonly used in our industry, which are incompatible with acidic conditions. In addition to this, one must also consider the amount of salt that is being generated from the neutralization of the acid, as some of these ingredients do not have a high electrolyte tolerance and will not function at full capacity in a high-electrolyte environment, potentially leading to formulation instability. Small-chain esters either used as emollients or in fragrances should be used with caution given their tendency to

hydrolyze under acidic conditions, forming unwanted by-products. Thickeners requiring neutralization via an alkaline base often do not function well in these types of formulations or with limited capacity.

Acknowledging these limitations and given the growing popularity of low-pH anti-aging formulations, the cosmetic industry has done a great job at developing ingredients that function in a low pH range and with good electrolyte tolerance.

Emulsion Stabilizers and Thickening Agents

Thickeners such as smectite clays, polymers based on polyacrylic acid, and natural polysaccharides are all very useful and efficient to thicken and stabilize these low-pH formulations. Smectite clays work by building a colloidal structure to the aqueous phase of an emulsion similar to a house-of-cards formation. Salts added both directly or indirectly will typically increase the smectite's viscosity and yield value properties, although this is not without limitation. Once this structure is formed, the formulation itself becomes thixotropic in nature and will possess a yield value useful for the stabilization of the particular formulation.[72] When incorporating these clays into a formulation, it is important that these types of thickeners be added up front to the aqueous phase and allowed to mix and fully hydrate prior to the addition of any acids or electrolytes. In doing so, this will allow the smectite clay to properly set up, leading to maximum viscosity and yield-value formation.

Polymers based on polyacrylic acid are another class of thickeners that are very useful in a low-pH environment. Some of these polymers are more commonly known as Hydro Swellable Droplet (HSD) polymers and are typically supplied as pre-neutralized solutions in various carrier fluids with a high-HLB surfactant. When they come in contact with water, they instantly invert and the polymer network expands leading to an instant increase in viscosity.[73] The advantage to these polymers is that they can be produced under moderate shear, contribute to varying degrees some emulsification properties, and can be used to create novel product aesthetics such as gel creams, lotions, and balms. They have a broad pH stability profile and depending on the specific type used, have a relatively good electrolyte tolerance. However, for high-electrolyte environments, it would be advantageous to use these types of thickeners in conjunction with other low-pH thickeners for optimal product stability.

Another class of thickeners that work well to thicken and stabilize low-pH systems is acid-swellable associative thickeners. These polymers were designed to thicken and stabilize low-pH formulas while at the same time offer elegant product aesthetics. These polymers are often supplied as low-viscosity emulsions for ease of use and consist of a polymer backbone of uncharged acrylic amines. Once in the presence of an acid such as an AHA, these amines become protonated and the

polymer backbone swells, leading to a rapid increase in viscosity and yield value in the formulation.[74]

Finally, natural thickeners such as xanthan gum, cellulose ethers, starches, and guar gums also work well to help stabilize low-pH systems, albeit at a more auxiliary role. These natural polymers are extremely pH stable, impart varying degrees of yield value to the system, and are very easy to work with from a processing and formulation standpoint. Furthermore, they too offer some very unique sensory attributes to the finished formula either alone or in combination with other thickeners.

Emollients

When choosing which emollients to use in low-pH systems it is advisable to look for those with a wide pH tolerance. The use of small- and medium-chain linear esters should be minimized given their susceptibility to hydrolysis under acidic conditions and elevated temperatures. Other ingredients such as long-chain branched esters, silicone, or natural oils such as sesame seed oil, olive fruit oil, and jojoba oil to name a few are less susceptible to this type of instability and offer the formulator a lot of flexibility. However, one must be aware that even with the use of natural oils there is a potential for degradation and color shift in the finished formulation. Moreover, hydrocarbon-based emollients such as hydrogenated polydecenes, isohexadecanes, and isododecanes are versatile over a wide pH range and prove to be very useful in these types of topical formulations.

Emulsifiers

When developing traditional O/W emulsions, nonionic emulsifiers and fatty alcohols work best in stabilizing low-pH emulsions. When creating these types of emulsions, a combination of a low and high hydrophilic-lipophilic balance (HLB) emulsifier should be used along with a long-chain fatty alcohol for high-temperature stability. Initially, it is advisable to run an HLB study to determine the correct amount and ratio of emulsifiers to use in order to get maximum efficiency and stability. Furthermore, one should always employ the use of the microscope to ensure that the particle size of the emulsion is small and uniform prior to starting any formal formula stability. For W/Si and W/O emulsions, even though water is the internal phase one must be aware of the amount of salt that is being generated from the neutralization of the AHAs. Typically in these types of emulsions salt either in the form of sodium chloride or magnesium sulfate is intentionally added to help stabilize the emulsion, commonly at a level of 0.5–1%. However, if a high level of salt is generated from the neutralization of the AHAs, adding additional salt to the formula may have the opposite effect and destabilize the emulsion.

In addition to nonionic emulsifiers, there are also numerous cationic emulsifiers that are versatile across a broad pH range and with a good electrolyte tolerance used either alone or in conjunction with nonionic emulsifiers. These types of emulsifiers are often used in creams and lotions designed for dry skin applications, as the cationic charge of the emulsifier allows it to adhere to the negatively charged skin thereby offering superior skin conditioning and substantivity properties.

Amphoteric Complex for Minimizing Irritation

When developing AHA topical formulations, the effectiveness of the formulations depends on skin penetration of the AHA. This penetration is, in part, governed by the available concentration of the free AHA in the topical formulation. In order to maximize the AHA bioavailability, the formulation pH must be suitably acidic and the formulator must be aware of the acid's pKa value. For example, in a topical glycolic acid formulation formulated at a pH of 3.8, approximately 50% of the glycolic acid is in the nonionized, free-acid form, thereby making it more readily bioavailable. While it is desirable and a necessity to have a certain level of free glycolic acid available when developing anti-aging and therapeutic AHA creams and lotions, the down side is that rapid penetration of the acid is often associated with stinging and burning, especially in individuals with sensitive skin.[75]

One approach to overcoming this stinging and burning was through the development of an amphoteric complex, formed by combining the AHA with naturally occurring amino acids such as arginine and ornithine in a specific molar ratio. In this system, the amino acid forms a temporary complex with the free AHA thereby providing a slow release of the acid into the skin. In doing so, the pH of the formulation is adjusted and the potential for stinging and burning is minimized. Clinical studies have shown significant benefits of this type of amphoteric complex when compared to a non-amphoteric AHA system for reduced stinging, burning, and irritation potential.[75] As shown in Figure 15 below, a 14-day cumulative irritation study was conducted comparing a 20% glycolic acid solution adjusted to a pH of 3.5 using ammonium hydroxide versus an amphoteric complex consisting of 20% glycolic acid complexed with arginine also adjusted to a pH of 3.5. Overall, the level of irritation was greatly reduced with the amphoteric complex. The AHA solution adjusted with ammonium hydroxide took only four days to reach a moderate level of irritation, whereas the amphoteric complex, containing the same level of glycolic acid, took 11 days to achieve similar levels of irritation.[76]

Figure 15: Amphoteric AHA Formulations Have Reduced Irritation Potential in Cumulative Irritation Patch Test – 20% Glycolic Acid Solutions, pH 3.5[76]

While striving to reduce irritation and stinging should always be a primary goal in topical hydroxy acid formulations, it's important to point out that the amphoteric AHA complex achieves this without sacrificing product efficacy. In a 14-day clinical study, a test cream containing 6.3% glycolic acid/arginine complex adjusted to a pH of 3.5 was compared to an untreated control to evaluate cell turnover and exfoliation via dansyl chloride assay. The results confirmed that the amphoteric cream significantly reduced mean *stratum corneum* turnover time by 15.7% compared to the untreated control. Furthermore, the amphoteric cream also significantly reduced the mean total fluorescence score by 18.4% when compared to the untreated control,[75] further supporting the efficacy of the amphoteric AHA complex.

In summary, when developing topical AHA formulations it is important to first be cognizant of the acid's pKa value and required pH range in order to achieve maximum efficacy with minimal irritation. Complexing the alpha-hydroxy acid with a naturally occurring amino acid such as arginine or ornithine is one route to further reducing irritation potential without compromising product efficacy or aesthetics. During the product development of these types of formulations, one should always look to use a combination of emulsifiers along with thickeners and emulsion stabilizers that work well at a low pH and that have high electrolyte tolerances. Linear or short-chain esters both by themselves and within a fragrance composition should be used with caution due to their tendency to hydrolyze at the low pH and with high temperatures causing unwanted color shifts and malodors.

When monitoring the stability of these topical formulations, it is advisable to incorporate the use of a microscope to monitor the emulsion for particle size and particle-size distribution. An increasing particle size and uneven particle-size distribution is typically indicative of long-term emulsion instability and should be monitored very closely. Furthermore, given the aggressive environment of these AHA formulas, it is crucial to monitor the product viscosity at each time point, as a continuing drop in viscosity is generally indicative of product instability, especially when seen at elevated temperatures. Finally, taking into account the importance of the product's pH with respect to product efficacy and safety, it is important to monitor formula pH during stability. Undesirable increases in pH may lead to less efficacious products, while rapid reductions in pH may lead to the potential for product irritation or formula instability. To counteract this, a buffer system can be used to negate the pH shifts typically consisting of citric acid and sodium citrate.

4.3.5.8 N-ACETYL AMINO SUGARS

Amino sugars are a class of sugar molecules in which one of the hydroxyl groups on the structure is replaced with an amine group. N-Acetylglucosamine (NAG), one of the most abundant chemically neutral amino sugars found on earth today, is a component of glycosaminoglycans (GAGs), glycolipids, and membrane glycoproteins. Chitin, a highly abundant organic substance in nature found in the shells of crabs, shrimp, and in the exoskeletons of insects, is comprised of repeating units of N-acetylglucosamine. Hyaluronic acid, a large GAG comprised of 50,000 repeating disaccharide units, is made up of NAG and D-glucuronic acid.[77] N-acetylglucosamine is beneficial to the skin, likely due to its stimulatory effects on GAGs, which decrease in photo-aged skin. GAGs play an integral part in the skin as they provide cushion and help to plump it, thereby keeping the skin looking healthy and youthful.

The potential to improve skin firmness and fullness through enhanced production of GAGs and/or diminished degradation of GAGs is therefore an exciting opportunity in topical anti-aging formulations.[77,78] The advantage that NAG brings in a topical formulation is that it can be formulated at a higher, more neutral pH range and does not have the pH limitations and requirements often associated with AHAs. This also allows the product development scientist the flexibility to incorporate NAG with other low-pH–sensitive ingredients in one formulation.

Cosmetic Anti-Aging Benefits of NAG

Preliminary observations by Drs. Van Scott and Yu on several subjects showed that formulations containing 5–10% N-acetylglucosamine applied topically twice daily for four to ten weeks can increase skin thickness and improve wrinkles in photo-aged skin.[78] A 12-week controlled anti-aging clinical study with females having Fitzpatrick Skin Type I, II, or III, and mild to moderate photo-damaged skin, was conducted to demonstrate the effects of an 8% NAG cream. Clinical

evaluations included visual grading of photo-aging symptoms and irritation on the face, pinch recoil on the cheek as a measure of firmness/elasticity, and skin thickness measurements using digital calipers on the forearms. Self-assessment data were collected with a questionnaire. All attributes of photo-aging were improved by N-acetylglucosamine treatment as shown in Figure 16 below. Pinch recoil improved by 12.2% at 12 weeks, and total skin thickness increased by 16.2% compared to 3.8% on the untreated control shown below in Figure 17. Overall, 54% of the participants felt their skin looked and felt younger within two weeks, and two-thirds felt their skin noticeably improved within three weeks of product use.[77]

Figure 16: Anti-aging effects of 8% N-Acetylglucosamine used twice daily for 12 weeks in a facial anti-aging clinical study. Mean percent improvement from baseline is shown.

Figure 17: Significant increase in skin thickness using topical application of 8% N-acetylglucosamine twice daily for 12 weeks on lateral forearms in comparison to untreated control sites. Percent thickness increase from baseline as measured using digital calipers is shown.

Benefits of NAG for Acne-Prone Skin

In a controlled clinical study conducted in 2007 comparing the performance of an 8% N-acetylglucosamine gel versus a 10% benzoyl peroxide cream, N-acetylglucosamine was found to provide fast-acting acne-reducing benefits on the skin in males and females ages 13–29, with mild to moderate facial acne. Clinical evaluations were conducted for lesion counts and clinical grading, irritation and safety grading, digital photography and self-assessment. Overall, N-acetylglucosamine quickly reduced the number of acne lesions showing good effects within the first two weeks and continuing over the eight-week treatment period. The early benefits of NAG were outperformed by BP for lesion counts at the later time points (weeks 4 and 8) (Figure 18). However, N-acetylglucosamine statistically outperformed BP for skin tolerability and mildness parameters. Self-assessed cosmetic advantages in favor of NAG being less drying and less stinging were also observed.[77]

Figure 18: Improvement to Inflammatory Acne Lesion Counts following eight-week twice daily use of N-Acetylglucosamine or Benzoyl Peroxide

Figure 19: Improvement to Texture & Clarity following use of 8% N-Acetylglucosamine, twice daily for eight weeks

The importance of this study is that it demonstrates the benefit of combining N-acetylglucosamine with topical anti-acne therapeutic agents to enhance the therapeutic effect of the topical agent and the cosmetic benefits to the skin while at the same time improving overall tolerability.

Pigmentation and Skin-Smoothing Benefits of NAG

N-acetylglucosamine is very effective in reducing melanin and exfoliating existing pigmented spots on the skin. The mechanism of action is through the inhibition of tyrosinase, the key element in the formation of melanin. Specifically, the reduction of melanin is believed to be through the inhibition of the glycosylation of tyrosinase. In human skin, glycosylation is critical for the conversion of inactive tyrosinase to active tyrosinase.[79]

In a dansyl chloride cell-turnover study, N-acetylglucosamine was found to increase natural cell turnover relative to a glycolic scid solution. Mechanistically, research has shown that NAG interacts with glycoproteins involved in corneocyte binding and intercellular cohesion and that topical NAG can reduce skin flakiness, increase dansyl chloride cell-turnover rates, and normalize *stratum corneum* exfoliation.[77] A cell-turnover study was conducted comparing the cell turnover rates of 8% solutions of N-acetylglucosamine, glycolic acid, and a control. The results of this study, illustrated below in Figure 20, show that N-acetylglucosamine significantly reduces mean fluorescent scores compared to the untreated, thereby supporting the hypothesis that N-acetylglucosamine enhances cell turnover and desquamation.

Dansyl Chloride cell turnover model measures the disappearance of skin stain (Dansyl Chloride) with a Wood's Lamp

Figure 20: Dansyl Chloride Cell-Turnover Rates of 8% Solutions of NAG, Glycolic Acid and Untreated Control

NAG Synergy with Retinoids

Studies have also shown a synergistic effect between N-acetylglucosamine and retinoids in the production of hyaluronic acid. Research by Sayo et al. demonstrated synergy between N-acetylglucosamine and retinoic acid on the production of hyaluronic acid in human keratinocytes. It was shown that a significant increase in HA production was seen in the NAG-treated media after two days, and after three days there was a 2.5-fold increase in HA. Conversely, retinoic acid–treated media exhibited HA production within the first 24 hours and then leveled off. While both materials enhanced keratinocyte HA production via different pathways and at different times, combining both of these ingredients offers one the ability to have both a faster and more sustained HA production.[80]

N-acetylglucosamine, a chemically abundant naturally occurring amino sugar, has many benefits in topical formulations. Due to its ability to stimulate GAG synthesis, NAG is often incorporated into anti-aging skin care formulations for its plumping and firming properties. Its ability to work synergistically with topical anti-acne therapeutic agents to enhance the therapeutic effect of the topical agent along with the cosmetic benefits makes it a smart choice for anti-acne applications. Finally, the ability of NAG to inhibit glycosylation of tyrosinase and therefore its activity, as well as its exfoliation capabilities, make it a great addition to topical formulations designed to reduce pigmentation and for overall skin brightening and even skin tone. From a formulation perspective, NAG offers greater flexibility than traditional AHAs in that it does not require the product to be formulated in an acidic pH range in order to be efficacious and therefore it does not have the formulation constraints that one would come to expect with low-pH AHA systems.

NAG can be effectively incorporated into product regimens combining other AHAs, PHAs, as well as cosmetic retinoids. A 16-week double-blind, vehicle-controlled clinical study was recently conducted on 69 Caucasian women (active group, n=44; vehicle group, n=25), aged 45–65, with mild to moderate photodamage, to demonstrate the benefits of a high-potency, multimechanism skin care regimen containing the AHA, PHA, BA, aminosugar ingredients described herein.[81] The active treatment regimen consisted of morning use of cleanser (gluconolactone/maltobionic acid), eye cream (N-acetylglucosamine), and an SPF20 day cream (N-acetylglucosamine/retinol); and evening use of the same cleanser; eye cream, and a night cream (glycolic acid/gluconolactone/maltobionic acid). The vehicle-treatment regimen consisted of the same vehicles as the active group, however, with all cosmetic benefit ingredients removed. Clinical evaluation consisted of expert visual assessment of all signs of photo-aging (0–9 scale with half-point increments), pinch recoil, skin thickness via ultrasound imaging, and digital calipers, silicone replicas, digital photography, self-assessment, and safety assessment. The regimen was tolerated very well during the study with minimal reports

of irritation. The active regimen showed profound benefit over the vehicle group on all assessed visual and instrumental measures as described in Table 7. The benefits were perceived by the participants and are evident in the before-and-after photos as shown in Figures 21 and 22.

Table 7: Improvement in Visual Signs of Aging following 16-week, twice-daily usage of a high-potency regimen (AM: Cleanser, Eye Cream, Day Cream SPF20; PM: Cleanser, Eye Cream, Night Cream). Regimen contained a combination of glycolic acid, gluconolactone, maltobionic acid, N-acetyl glucosamine, and was compared to a vehicle control group[82]

	1-Grade Improvement (Responders, %)		2-Grade Improvement (Responders, %)	
	Active (N=44)	Vehicle (N=25)	Active (N=44)	Vehicle (N=25)
Clarity	100%	100%	77%	20%
Dyschromia	86%*	24%*	14%*	0%*
Sallowness	89%*	24%*	39%*	0%*
Fine Lines	91%*	48%*	30%*	0%*
Wrinkles	55%*	16%*	11%	0%
Laxity	98%*	48%*	41%*	0%
Visual Roughness	100%	96%	86%*	36%
Tactile Roughness	100%	100%	100%	84%
Pore Size	61%*	12%	2%	0%
Eyelid Crepiness/Wrinkling	89%*	48%*	18%*	0%
Lifted Appearance of Eyelid/ Droopiness	86%*	52%*	14%*	0%
Overall Appearance	93%*	72%	9%	0%

*Active group outperformed Vehicle group, $P < 0.05$.

Week 0 Week 16

Figure 21: Educed forehead laxity and wrinkling following use of a high-potency skin care regimen (Cleanser, Eye Cream, Day Cream SPF20 (AM) or Night Cream (PM)) containing a combination of glycolic acid, gluconolactone, maltobionic acid, and N-acetylglucosamine, twice daily for 16 weeks

Week 0 Week 16

Figure 22: Reduced pigmentation following use of a high-potency skin care regimen (Cleanser, Eye Cream, Day Cream SPF20 (AM) or Night Cream (PM)) containing a combination of glycolic acid, gluconolactone, maltobionic acid, and N-acetylglucosamine, twice daily for 16 weeks

4.3.5.9 N-ACETYL AMINO ACIDS

The most recent advance related to the hydroxy-acid class of compounds has been the identification of novel N-acetyl amino acid derivatives. Many hydroxy acids are structurally similar and/or derived from amino acids. While the physicochemical properties of amino acids may differ from hydroxy acids, in many cases AHAs and amino acids are structural analogs of each other. While the AHAs have a hydroxyl group in the alpha position to the carboxylic acid, the amino acids have an amino group in the same position, as seen with glycolic acid and glycine in Figure 23.

Figure 23: Structural Similarity of Alpha-Hydroxy Acid and Amino Acid

There have been extensive studies of the many amino acids and derivatives that comprise the skin's natural NMF, namely their impact on *stratum corneum* formation and functionality, and skin moisturization. However, research on derivatives of amino acids, including N-acetyl amino acids, esters, and amides, and their effects on improvement to aging skin is more recent.

Many N-acetylamino acids are naturally occurring biopeptides, found in the body. For example, N-acetylserine and N-acetyltyrosine are found in melanocyte-stimulating hormone (MSH) and endorphins, respectively.[83] N-acetylcysteine, which is used medically as a mucolytic agent and to counteract acetaminophen overdose, is also a potent antioxidant and serves as a pro-drug to L-cysteine, a precursor to the biologic antioxidant, glutathione. N-Acetyl proline and N-acetylhydroxyproline convert to proline and hydroxyproline, respectively in the skin, and are two of the key amino acid components of collagen, along with glycine and lysine.[84] N-acetylhydroxyproline has also been shown to improve barrier function.[85]

Most recently, the benefits of N-acetyltyrosinamide for improvement to the signs of photo-aging have been described. N-acetyltyrosinamide increases hyaluronic acid expression in human dermal fibroblasts,[86] and collagen-1 and elastin expression in aged human dermal fibroblasts.[87] These dermal changes suggest the potential for this ingredient to deliver clinical improvement for photo-aging skin, which has been observed in several clinical studies. One eight-week pilot clinical study, on eight women aged 40–65 years, assessed a two-step application of N-acetyltyrosinamide to the arms[87] designed to mimic early development work on this ingredient, which showed a rapid skin-plumping effect when evaluated under occlusive conditions.[88] The two-step regimen included: Step 1 gel containing N-acetyltyrosinamide; Step 2 occlusive vehicle cream was used for the first four weeks and for the last four weeks also contained the test compound. After eight weeks of this treatment regimen, skin thickness increased by 13% as measured by digital calipers, and was supported by an increase in collagen and GAGs, as observed via histological assessment of skin.[86] In a separate four-week vehicle-controlled study on 32 females aged 35–59, the potential for a formula containing N-acetyltyrosinamide to improve skin properties associated with aging was evaluated using ballistometry and Optical Coherence Tomography (OCT). After two and four weeks of twice-daily use, unlike the vehicle-treated sites, skin treated with N-acetyltyrosinamide showed a significant improvement to multiple ballistometer measures, suggesting an improvement to the skin's biomechanical properties. The N-acetyltyrosinamide treatment also showed an improvement to epidermal thickness as evidenced by OCT.[87] These rapid improvements to skin thickness and viscoelastic properties, coupled with matrix-building benefits observed *in vitro*, suggest N-acetyltyrosinamide can contribute to the amelioration of deeper skin wrinkles.

The benefit to target deep lines on the face from N-acetyltyrosinamide in combination with complementary matrix-building ingredients was demonstrated in a 16-week double-blind vehicle-controlled clinical study of a topical two-step formulation regimen on 70 women (active group, n=47; vehicle group, n=23). The target areas evaluated in this study included glabellar line, nasolabial folds and crow's-feet wrinkles. The active treatment regimen included a Step 1 Serum containing N-acetyltyrosinamide, N-acetylhydroxyproline, and a low concentration of glycolic acid, followed by application of a Step 2 cream containing complementary matrix-building ingredients, N-acetylglucosamine, triethyl citrate,[89] and palmitoyl oligo and tetra peptides (Matrixyl®, Sederma Inc.). The vehicle-treatment regimen applied a Step 1 vehicle gel followed by a Step 2 vehicle cream. Both groups used an all-over daytime moisturizer and night cream that did not contain any cosmetic anti-aging ingredients. The active regimen provided statistically significant improvements to deeper lines and wrinkles versus the vehicle as early as four weeks for some attributes, and a dramatic improvement after 16 weeks of use, including: 10.3% improvement to nasolabial folds (versus 0.9% for the vehicle), 7.7% improvement to glabellar lines (versus 1.3% for the vehicle), 20.3% improvement to under-eye wrinkles (versus 5.8% for the vehicle), and 17.8% improvement to crow's feet (versus 7.9% for the vehicle).[90] An example of the effect of this two-step treatment containing N-acetyltyrosinamide is shown below after 24 weeks on perioral rhytides (Figure 24).

Figure 24: Reduced appearance of perioral rhytides following use of a two-step regimen twice daily for 24 weeks: Step 1 (N-acetyltyrosinamide, N-acetylhydroxyproline, low level of glycolic acid) followed by Step 2 (N-acetylglucosamine, triethyl citrate, and palmitoyl oligo and tetra peptides).

CONCLUSION

The discovery of the hydroxy acids and related compounds, and their profound effects on the amelioration of photo-aged skin appearance, has provided the cosmetic formulation chemist a myriad of technology options to meet the broad need of consumers seeking younger-looking skin. The first generation alpha-hydroxy

acids (AHAs), including glycolic acid, have been proven to deliver cosmetic anti-aging benefits and shown to impact *stratum corneum* desquamation, epidermal proliferation and differentiation, dermal matrix synthesis, and melanin production through various mechanisms of action.

The AHAs' efficacy and proven safety profile make them a mainstay in cosmetic anti-aging formulations, and the cosmetic industry has sufficiently advanced the requisite formulation ingredients to allow for stable, aesthetically pleasing formulas that balance efficacy with minimal irritation. The second- and third-generation polyhydroxy acids (PHAs) such as gluconolactone, and bionic acids (BAs), such as lactobionic and maltobionic acids, offer protective benefits in addition to their mild but effective anti-aging benefits. N-acetylamino sugars, such as N-acetyl glucosamine, which are neutral compounds, open the door to combinations with low-pH–sensitive benefit ingredients such as retinoids. Furthermore, a combination product regimen containing AHAs, PHAs, BAs, and an N-acetylamino sugar demonstrates how both product and ingredient synergy can achieve significant cosmetic anti-aging effects. Lastly, the N-acetylamino acids and derivatives, such as N-acetyltyrosinamide, offer complementary dermal matrix-building benefits and have been shown to deliver improvement to even the more difficult aging characteristics of deep lines and wrinkles. As a class, the AHAs and related compounds are multimechanistic and highly useful cosmetic ingredients for the improvement to photo-aged skin appearance.

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GLOSSARY

Corneosomes: Proteins that bind the corneocytes in the stratum corneum.

Fibroblast: A type of cell that synthesizes the extracellular matrix and collagen, the structural framework for animal tissues, and plays a critical role in wound-healing.

Glycosaminoglycans: Long sugar carbohydrate chains found in numerous cells in the human body. Their main function is to hold moisture within our skin cells and maintain skin integrity by providing volume, elasticity, and firmness.

Ichthyosis: A family of genetic skin disorders characterized by dry, scaling skin that may be thickened or very thin.

Immunohistochemistry: An assay that shows specific antigens (or proteins) in tissues by the use of markers that are either fluorescent dyes or enzymes.

Keratinocyte: Keratinocytes are the most common type of skin cells. They make keratin, a protein that provides strength to skin, hair, and nails.

Lentigines: A condition marked by small brown patches on the skin, typically in elderly people.

Melanocytes: The pigment-producing cells in the skin.

Melanogenesis: The production of melanin by melanoctyes.

Melasma: A very common patchy brown, tan, or blue-gray facial skin discoloration, almost entirely seen in women in the reproductive years. It typically appears on the upper cheeks, upper lip, forehead, and chin of women 20–50 years of age

Mitogenic: Any substance or agent that stimulates mitotic cell division.

Monadic clinical study design: Study subjects use one product to test on its own, and the effects of the product are compared to the skin condition at baseline. Often, between–test group comparisons are made to compare the effects of multiple products (or a product versus a placebo/vehicle).

Mucopolysaccharides: Also known as glycosaminoglycans. Complex polysachharides that contain amino groups and are found in the epidermis and dermis.

Oil-in-Water Emulsion: An emulsion where oil is the internal dispersed phase and water is the external continuous phase

Photo-aging: The manifestation of accumulated skin damage from chronic sun exposure superimposed upon the chronological aging process. The appearance of photo-aged skin is typically defined by lines and wrinkles, irregular texture, loss of firmness and elasticity, and an increased amount and reduced uniformity of pigmentation.

Pigmentation: Pigmentation is a common skin condition, where some areas or patches of skin turn darker in color. It is usually caused when there is too much of the pigment, melanin, on the surface of the skin. This condition can affect people of all races.

Poikiloderma: A skin condition that consists of areas of increased and decreased pigmentation, prominent blood vessels, and thinning of the skin, creating redness.

Sallowness: A yellowish hue or complexion

***Stratum Corneum*:** The outermost of the five layers of the epidermis, largely responsible for the vital barrier function of the skin.

***Stratum Disjunctum*:** The outermost layer of desquamating keratinized cells of the *stratum corneum*.

Thixotropic: The property exhibited by certain systems of becoming fluid when stirred or shaken and returning to the semi-solid state upon standing.

Tyrosinase: An enzyme occurring in many organisms that is a catalyst in the conversion of tyrosine to the pigment melanin.

Water-in-Silicone Emulsion: An emulsion where water is the internal dispersed phase and silicone is the external continuous phase

Water-in-Oil Emulsion: An emulsion where water is the internal dispersed phase and Oil is the external continuous phase

Yield Value: The initial resistance to flow under stress. Yield value is a property critical to achieving certain physical characteristics such as suspension and emulsion stabilization.

CYTOKINES, GROWTH FACTORS, AND STEM CELLS: NEWEST APPROACHES TO YOUNGER-LOOKING SKIN

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ABSTRACT

As the population grows, there is a particular increase in the growing numbers of middle-aged and elderly people, the so-called "baby boomers." A major need among this group is a continued increase in the desire for younger-looking skin. Areas of particular concern include: loss of elasticity, wrinkles, irregular texture, pigmentation, and dryness (1,2). This desire has led to the development of **cosmeceuticals**, a term first coined by Albert Kligman, MD, which, from a regulatory view in the U.S. lie in an unresolved schism between "cosmetics" and physiologically altering pharmaceuticals (APIs-active pharmaceutical agents).

This chapter takes a close look at several selected and highly promising types of cosmeceuticals including: Cytokines, Growth Factors, & Stem Cells.

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4.3.6.1 AGING

Aging occurs via two mechanisms. These are termed intrinsic and extrinsic aging.

Intrinsic aging is inevitable and results in thinned skin, fibroblast reduction, and thinning blood vessels (3). Collagen is particularly affected, as its synthesis steadily declines with age (4). Likewise, elastin also declines with age (4,5).

Extrinsic aging primarily results from the effects of UV damage. Other causes of this type of aging include environmental factors such as smoking, pollution, and poor nutrition (3,6). This type of damage causes an up-regulation and overstimulation of epidermal and dermal cells. It leads to a deterioration of both collagen and elastin as well as other components of the dermal extracellular matrix.

Aged skin shows a decrease in extracellular matrix proteins, increased collagen degradation, and decreased fibroblasts (7). Furthermore, there is a reduction in the immune response, wound repair, and fiber synthesis (8). Free radicals, which are often caused or increased by extrinsic factors, activate matrix metalloproteinases (MMPs) leading to extracellular matrix (ECM) degradation (9). Additionally, free radicals inhibit the tissue inhibitors of these enzymes. The goal of cosmeceuticals is to mitigate some of these effects of aging.

Effective cosmeceuticals must be able to penetrate into and throughout the *stratum corneum* while maintaining their effectiveness. They also must have visible benefits without impacting the skin's barrier function (10). There has been a recent surge of new cosmeceuticals and this chapter discusses the functions, limits, and benefits of some of the most promising types employed in anti-aging formulations. As stated in the abstract, these include: growth factors, cytokines, and stem cells. These newer cosmeceuticals are being utilized to enhance or restore homeostasis of the body by aiding in impacting the skin's own natural mechanisms involved in such processes as wound repair and extracellular matrix development.

4.3.6.2 GROWTH FACTORS AND CYTOKINES

Many growth factors are involved in wound-healing, both chronic and acute. These have been introduced into the cosmeceutical world based on the hypothesis that the aging process of skin is similar to that of a chronic wound (9). Their ability to

increase fibroblast and keratinocyte proliferation within the dermis, thus inducing ECM formation, supports this idea (7). Aging skin has reduced amounts of fibroblasts as well as decreased levels of growth factors. By supplementing these normal growth factors, we may allow for the *natural repair* of skin. This hypothesis opens up fertile ground for future studies involving anti-aging therapies for skin.

Growth factors are produced and secreted by many cell types of the skin, including fibroblasts, keratinocytes, and melanocytes. Included within these secreted growth factors are those that regulate the immune system, also known as cytokines. **Cytokines** are also involved in skin repair (11). Many growth factors are involved in wound repair, and thus most of the topical products contain a combination of growth factors. It is important to combine growth factors and cytokines as they work together and regulate each other throughout the healing process.

One of the safety concerns with growth factors is their size and ability to penetrate the epidermis. While growth factors and cytokines are very large hydrophilic molecules that do not easily cross the skin barrier, it has been proposed that absorption can occur via hair follicles, sweat glands, and compromised skin (12–14). Once absorbed into the epidermis, communication is able to occur between epidermal cells and cells of the dermal layers (9). Elsewhere in this book is an extensive chapter on anti-aging approaches for hair employing the living part of hair—the follicle. The chapter delves into the biological mechanisms of hair follicles and growth out of the scalp and will generate much insight into current progress in reversing graying of hair as well as reducing or minimizing hair loss.

Growth factors regulate cell growth and thus have a potential for carcinogenic transformation of cells (9). This is proposed to be largely based on the presence of vascular endothelial growth factor, VEGF. Receptors for VEGF are present on some types of melanoma cells (15). However, research on increased VEGF in melanoma has shown conflicting results to date. One study has shown an increase in melanoma cell proliferation when cells were combined with VEGF, while another failed to do so (16,17). An FDA investigation of a growth factor product determined that very large concentrations of growth factors, much higher than the levels found in topical cosmeceutical products, are required for this potential (9). Furthermore, because of the large size, minimal amounts of growth factors are entering the skin. Thus, topical application of growth factors is unlikely to have any effect on cancer growth (15).

Topical transforming growth factor – beta 1 (TGF-β1) has been shown to restore damaged skin and increase epidermal thickness, as shown by histologic studies (18). The topical application of this growth factor also showed a reduction in damage to the skin caused by the sun, especially among patients with more severe photo-damage. Furthermore, an increase in collagen fibers demonstrated new collagen production. There was a decrease in the appearance of wrinkles (19).

Patients reported improved skin elasticity, texture, hydration, lines, and wrinkles, along with decreased skin tightness (18).

One source for growth factors, cytokines, and matrix proteins is neonatal dermal fibroblasts, which secrete over 110 of these combined products (20). A study of applications of these products shows a statistically significant decrease in wrinkles and fine lines along with an increase in epidermal thickness following topical application to Fitzpatrick skin types II and higher (21). These effects were found to last at least three months following the discontinuation of treatment. Improvements were also shown in skin roughness and shadows following topical application to mild to severe photo-damaged skin. This was particularly evident among those with more severe photo-damage (22).

Processed skin-cell proteins (PSP) from cultured fetal dermal fibroblasts contain a mixture of over 100 growth factors and cytokines (3). Fetal fibroblasts have been used for wound-healing in the pediatric population and have been shown to produce complete closure (23). These fibroblasts have altered growth factor components that are associated with the scarless repair seen in fetal skin (24).

After two months of twice-daily application, reduction of wrinkles in both the periorbital and perioral areas were seen. Reduced dark under-eye color was noted, as well as improved texture and firmness. These effects may have resulted from increased collagen production of the skin under the eyes. Thicker skin aids in the ability to cover the underlying vessels responsible for the appearance of dark circles (25). Improvements became statistically significant after 30 days of treatment and continued to improve for the remainder of the six weeks. Cheek skin was also noted to be tighter and firmer.

Growth factors are safe and have not been associated with any risks, although there is a potential for allergic reaction in hypersensitive patients (15). Topical application produces increased collagen generation, thickening of the epidermis, and improvements in skin texture and wrinkles (21).

4.3.6.3 STEM CELLS

A stem cell is one that can divide and proliferate, constantly renewing itself, and can subsequently be differentiated. Stem cells and their extracts are being studied for their potential use in cosmetic products such as serums and creams. These can renew, generate, and repair skin. Stem cells can be used as cosmeceutical ingredients. In this case they are extracted from animal or plant sources and then cultured in the lab to yield extracts of the stem cells. These extracts often contain various growth factors and other cytokines involved in dermal repair. Sources include human adipose tissue, sheep and cow placentas, and plant sources such as Swiss apple seed, which is well known to be a rare type of apple that does not turn brown on sitting in air (no oxidation) (26).

Stem cells derived from humans for dermatologic use arise from human adipocytes. Adipose tissue is abundant in mesenchymal stem cells, which show an ability to regenerate damaged skin (27). Currently, adipocytes harvested during procedures such as liposuction are being injected into the skin, but *in vitro* studies of conditioned media of adipose-derived stem cells (ASC-CM) have shown potential benefits when applied topically. Growth factors produced in adipose-derived stem cells (ASCs) can promote collagen synthesis by dermal fibroblasts (28). Furthermore, ASCs promote wound-healing, improve wrinkling, and inhibit melanogenesis (29–31). They can be used to replace lost soft tissue through the induction of ASCs into adipocytes (32).

ASCs stimulate collagen synthesis and decrease MMP-1 levels, which breaks down collagen. They also stimulate the migration of dermal fibroblasts resulting in a reduction in wrinkles and protect fibroblasts from oxidative stress induced by solar UVB irradiation (27,31). ASC-CM significantly enhanced antioxidative mechanisms (31). ASCs also are able to lighten skin in a dose-dependent manner (30) and are therefore of high interest in Eastern societies that value skin whitening. Anti-inflammatory properties result from the secretion of cytokines that modulate lymphocytic activities (27). Furthermore, inhibition of activities of cell death–inducing enzymes was exhibited by pretreatment with ASC-CM (31), promoting its cell survival capabilities (29,31).

Whitening effects of ASC-CM result from the down-regulation of tyrosinase and tyrosinase-related protein 1 (TRP1), enzymes involved in melanin production.

Stem cells derived from plants include the previously mentioned Uttwiler Spatlauber apple tree from Switzerland as well as jasmine and lilac seeds (26). This particular apple tree was chosen because of its prolonged storage properties resulting from a resistance to oxidation. The hypothesis for this phenomenon is prolongation of longevity when mixed with a person's endogenous stem cells. These plant-derived cells have been shown to reverse or delay senescence, the natural process of aging. Fibroblasts were incubated with 2% stem-cell extract following induction of senescence using hydrogen peroxide treatment. This was identified by an up-regulation or neutralization in several genes important for cellular growth and proliferation. Likewise, isolated hair follicles maintained in growth medium were also shown to have a slightly prolonged growth phase by four days (26).

Clinically, products containing 2% Uttwiler Spatlauber stem-cell extract have been shown to significantly reduce wrinkles in as little as two weeks (26). However, studies are required to determine the exact mechanisms behind these results as well as the duration of effectiveness. While still lacking rigorous data of extensive human studies and research on stem cells, interest continues to rise.

It is worth mentioning that some companies use the term plant "stem cells" for extracts from the actual stem of the plant. These extracts usually don't contain any undifferentiated biological cells, but often have antioxidant properties. However, these types of "stem cells" have not been reviewed in this chapter.

CONCLUSION

Cosmeceuticals are a safe and effective way to prevent and improve the undesired effects of skin aging. There are various agents that can be applied to address wrinkles, fine lines, and hyperpigmentation. They exert local effects, without systemic absorption. They are also generally well tolerated, and no major adverse effects have been observed. Many different options exist, including growth factors, cytokines, and stem cells, which have joined the cosmeceutical armamentarium. These newer options consist of endogenous proteins and enzymes whose function and presence in the skin declines with age, often due to both internal and external epigenetic stressors. Applying topical formulations containing these products helps restore homeostasis within the skin, thereby contributing to preventing and reversing the common signs of aging.

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ANTIOXIDANTS IN COSMETICS FOR ANTI-AGING

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ABSTRACT

It's ironic that the oxygen we need to survive is also the catalyst for the production of free radicals. The job of antioxidants is to "quench" free radicals, meaning that they neutralize their electrical charge and prevent the free radical from taking electrons from other molecules. Antioxidants can slow down some of the physical signs of aging by minimizing wrinkles and preserving skin's natural "glow." The mechanisms by which skin damage occurs generally involve (1) direct oxidative alterations of physiologically critical molecules, including proteins, lipids, carbohydrates, and nucleic acids, along with modulation of gene expression and the inflammatory response; and (2) indirect oxidative response first by stimulating production of oxidase enzymes and then generating radicals with the alteration of physiologically critical molecules. This chapter is focused on addressing three key areas: (A) major causes and consequences of UV-induced photo-aging to skin, (B) role of endogenous antioxidant defense system, and (C) use of conventional and nonconventional antioxidants for skin protection and reversal of signs of aging.

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4.3.7.1 BACKGROUND

Antioxidants are used widely, but rarely defined. They are used in food to stop rancidity; in polymer synthesis to control polymerization during manufacturing and to protect polymer (plastics) against UV damage; and as a nutritional supplement for a wide variety of health benefits. In personal care, antioxidants are used to protect formulation ingredients and to protect skin from sun-induced damage as well as reversing aging signs. An antioxidant is defined by Haliwell and Gutteridge as "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate"[1].

Aging is a natural phenomenon; it happens to all of us, and more signs of aging show up the longer we live. Antioxidants can slow down some of the physical signs of aging by reducing wrinkles and preserving skin's natural "glow." Use of "antioxidant for anti-aging" alone cannot provide the results we are looking for. A positive outlook on life and an optimistic perspective do contribute to beauty, both inside and out. We also need to take a holistic approach regarding the health and beauty of skin.

Free radicals promote beneficial oxidation that produces energy and kills bacterial invaders, but in excess—through accumulation—they can disturb cell structure, resulting in cellular damage in protein, fat, and DNA molecules. This is believed to contribute to aging, as well as various other health problems, because the human body's functions depend on these biomolecules.

The skin is at risk of photo-oxidative damage due to the generation of singlet oxygen, hydroxyl radicals, hydrogen peroxides, and other reactive oxygen species

(ROS). The most severe consequence of photo-oxidative damage is skin cancers. Less severe photo-aging changes result in wrinkling, scaling, dryness, and uneven pigmentation consisting of hyper- and hypopigmentation [2–4]. The mechanisms by which skin damage occurs generally involve (1) direct oxidative alterations of physiologically critical molecules, including proteins, lipids, carbohydrates, and nucleic acids, along with modulation of gene expression and the inflammatory response, and (2) indirect oxidative response first by stimulating production of oxidase enzymes and then generating radicals with the alteration of physiologically critical molecules.

This chapter is focused on three key areas: (A) major causes and consequences of UV-induced photo-aging to skin, (B) the role of endogenous antioxidant defense system and (C) the use of conventional and nonconventional antioxidants for skin protection and reversal of signs of aging.

4.3.7.2 CAUSES OF UV-INDUCED CHEMICAL AND BIOCHEMICAL CHANGES IN SKIN

Photosensitizer and Reactive Oxygen Species (ROS)

Singlet oxygen is generally accepted to be the first ROS formed from triplet excited states of endogenous photosensitizers. There are many endogenous chromophores in human skin, which in the presence of UVA radiation can generate ROS. Porphyrins (protoporphyrin, coproporphyrin, and uroporphyrin), flavins (riboflavin), quinone (ubiquinone), and the pyrimidine nicotinamide cofactors (nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, NADH and NADPH) are examples of common photosensitizers in mammalian cells [5]. The identification of the epidermal UVA-absorbing chromophore trans-urocanic acid that quantitatively accounts for the action spectrum of photo-aging has been reported [6]. The excited photosensitizer subsequently reacts with oxygen, resulting in the generation of ROS including superoxide anion radical and singlet oxygen. Superoxide anion radical and singlet oxygen are also produced by neutrophiles that are present in increased quantities in photo-damaged skin, and contribute to the overall pro-oxidant state. Superoxide dismutase (SOD) converts superoxide anion radical to hydrogen peroxide. Hydrogen peroxide is able to cross cell membranes easily and, in conjunction with Fe^{2+}, generates highly toxic hydroxyl radicals. Both singlet oxygen and hydroxyl radical can initiate lipid peroxidation. Many organic sunscreens also act as triplet sensitizers that convert harmless triplet oxygen into the highly reactive singlet oxygen [7]. As a consequence of their high reactivity, ROS react nonspecifically with nearly every cellular target and may damage DNA, proteins, lipids, and carbohydrates [8].

While ROS are short-lived and extremely reactive, RNS are longer-lived and more specific in the reactions they undergo [9]. Nitrogen oxide (NO) reacts with superoxide to form the peroxynitrite anion, resulting in oxidative stress. This results in the release of cytochrome c from the mitochondria to the cytoplasm and the subsequent conjugation of the cytochrome c with caspase-9 and Apaf-1 to form apoptosomes that activate caspase-3 and caspase-7. Activated caspase-3 then activates caspase-2, -6, -8, and -10, resulting in apoptosis [10].

The balance between cell cycle arrest and damage repair on one hand and the initiation of cell death, on the other hand, could determine if cellular or DNA damage is compatible with cell survival or requires cell elimination by apoptosis. Defects in these processes may lead to hypersensitivity to cellular stress, and susceptibility to DNA damage, genomic defects, and resistance to apoptosis, which characterize cancer cells [11].

Oxidase Enzymes

Several oxidoreductases have been identified as potential sources of superoxide in mammalian cells [12]. These include cyclooxygenase, lipoxygenase, cytochrome P450 enzymes, nitric-oxide synthase, xanthine oxidase, mitochondrial NADH:ubiquinone oxidoreductase (complex I) , and NADPH oxidase (Nox). Unlike other oxidoreductases, NADPH oxidase is a distinct enzymatic source of cellular ROS generation, because this enzyme is a “professional” ROS producer [13] whereas the other enzymes produce ROS only as by-products along with their specific catalytic pathways.

NADPH oxidase is a major source of UVA-induced ROS in keratinocytes [14] and fibroblasts [15]. NADPH oxidase catalyzes the one-electron reduction of O_2 to superoxide anion (O_2^-) using NADPH as an electron donor. Valencia and Kochevar have demonstrated that the mechanism for activation of Nox1 is mediated by an increase in intracellular calcium. Ceramide, which has been proposed to mediate responses to UVA in human keratinocytes, also activated NADPH oxidase. These results indicate that UVA activates Nox1-based NADPH oxidase to produce ROS that stimulate PGE2 synthesis, and that Nox1 may be an appropriate target for agents designed to block UVA-induced skin injury. Glucose can also stimulate production of NADPH oxidase [16].

Free Iron, Copper, and Calcium

In mammalian cells, the level of iron-storage protein is tightly controlled by the iron-regulatory protein-1 at the post-transcriptional level. This regulation prevents iron acting as a catalyst in reactions between ROS and biomolecules. It has been shown that both UVB and UVA can cause biological damage in exposed tissues via iron-catalyzed oxidative stress [17, 18]. The iron content is substantially elevated in the sun-exposed skin of healthy individuals [19]. The underlying

mechanism appears to be the UVB-induced formation of superoxide radical anion and its attack on ferritin, resulting in the release of free iron [20]. Furthermore, a superoxide anion radical can react with hydrogen, which again enters the Fenton reaction. Interestingly, Reelfs et al. identified the immediate iron release as a key modulator of the activation of NFκB in fibroblasts after UVA-irradiation [21].

The key question is the availability of catalytic amounts of iron and copper in the skin. UV light and sweat are the two dominant sources for iron and copper [19]. Water is also a source for iron in the skin. It is also easy to see from these data how athletes following an intensive training might become anemic due to loss of iron. Iron-chelating agents have been shown as protectants against UV radiation–induced free radical production [22].

UVA exposure of skin has been shown to increase intracellular calcium [Ca^{2+}] in fibroblasts [23], which activates Nox1 thereby increasing the ROS that stimulate a further increase in calcium. The ROS formed initiate production of additional ROS from oxidation of mitochondrial and possibly other membranes and induce synthesis of PGE_2, an inflammatory mediator after UVA irradiation, by a Ca^{2+}-dependent mechanism [14].

Antioxidants Act as Pro-Oxidant

When a general use of antioxidants is advocated, it is often disregarded that these compounds not only function as antioxidants, but (intrinsically) have pro-oxidant action, especially in the presence of transition metals. There is pro-oxidant action even in well-known antioxidants, such as vitamin C (ascorbate), vitamin E (tocopherols), glutathione, and proanthocyanidins (from pine and grape). The pro-oxidant activity of vitamin C results from the reduction of Fe^{3+} to Fe^{2+} and its reaction with H_2O_2 to generate OH radical [24]. Pro-oxidant effects are not unique to vitamin C; they can be demonstrated with many reducing agents in the presence of transition metal ions, including vitamin E, glutathione, and several plant phenolics. Thus if vitamin C's pro-oxidant effects are relevant, the pro-oxidation effects of these other reductants may also be expected to occur [22].

High concentrations of vitamin E accelerate lipid auto-oxidation *in vitro* [25, 26]. Other authors also reported pro-oxidant effects *in vitro* for α-tocopherol [27, 28]. It is quite possible that α-tocopherol can generate tocopheroxyl radicals on skin under UV radiation and may thereby act as a pro-oxidant. Indeed, adverse biological effects of α-tocopherol are documented [29]. A pro-oxidant activity of carotenoids has also been reported [30]

Pro-oxidant properties of antioxidants are not all that bad. For example, oxidation of proline to hydroxyproline, the key building block of collagens, is catalyzed by ascorbic acid due to its pro-oxidant activity. It is the excessive production of ROS that causes irreversible skin damage.

4.3.7.3 ANTIOXIDANTS IN THE DEFENSE SYSTEM OF THE SKIN

In order to counteract the harmful effects of reactive oxygen species (ROS), the skin is equipped with antioxidant defense systems consisting of antioxidant defense enzymes and non-enzymatic antioxidants, forming an "antioxidant network." The antioxidant network is responsible for maintaining the equilibrium between pro-oxidants and antioxidants. However, the antioxidant defense can be overwhelmed by increased exposure to exogenous sources of ROS. Such a disturbance of the pro-oxidant/antioxidant balance may result in oxidative damage to lipids, proteins, and DNA. Thus, a comprehensive and integrated antioxidant skin defense mechanism is considered to be crucial for protecting this organ from ROS, and consequently for preventing the aging process of skin [31].

Antioxidant Defense

Antioxidant defenses are comprised of a number of species [1]:

(1) **Low-molecular-weight antioxidants that scavenge ROS and RNS.** These antioxidants can be subdivided into two groups: endogenous (synthesized in the body) and exogenous (derived from the diet). Human skin contains both lipophilic [vitamin E (tocopherols and tocotrienols), ubiquinones (coenzyme Q), and carotenoids], and hydrophilic [(vitamin C (ascorbate), glutathione (GSH), and uric acid (urate)] antioxidants. Vitamin E, vitamin C, and carotenoids are derived from the diet, whereas the other three are synthesized *in vivo*.

(2) **Enzymes that catalytically remove free radicals and other ROS.** Examples are the enzymes superoxide dismutase (SOD, converts superoxide to hydrogen peroxide and oxygen), catalase (converts hydrogen peroxide to water and oxygen), glutathione peroxidase (GPx, converts hydrogen peroxide to water and oxygen).

(3) **Proteins that minimize the availability of pro-oxidants** such as iron ions, copper ions. Examples are transferins, metallothionein.

(4) Proteins that protect biomolecules against oxidative and other damages, e.g., heat shock proteins.

The composition of antioxidant defense differs from tissue to tissue and cell type to cell type and even from cell to cells of the same type. On a molar basis, vitamin C is the predominant antioxidant in skin; its concentration is 15-fold higher than glutathione, 200-fold higher than vitamin E, and 1,000-fold higher than ubiquinones [32]. Concentrations of antioxidants are higher in epidermis than dermis; sixfold for vitamin C and glutathione, and twofold for vitamin E and ubiquinones. Some antioxidants are also present in the *stratum corneum*, such as vitamin E, which is the predominant antioxidant in the human *stratum corneum* [33].

In aged and photo-aged human skin, the components of the antioxidant defense system are regulated differently. In the epidermis, SOD and GPx remain

completely unchanged, whereas catalase and glutathione reductase tend to increase. In the dermis, of the four antioxidant enzymes examined by Rhie et al., only catalase activity was significantly decreased [31]. Non-enzyme antioxidants such as α-tocopherol, ascorbic acid, and glutathione were decreased in the epidermis and/or dermis. The decreased antioxidant capacity of aged skin may cause an increased accumulation of ROS, which will affect cell-signaling pathways leading to skin aging [31].

Ideally, an antioxidant or an antioxidant blend that can quench radicals and nonradicals, chelate free iron, copper, and calcium, inhibit activities of NADPH oxidase and other oxidase enzymes, and stimulate antioxidant defense system, is able to provide true broad-spectrum skin protection.

Skin aging entails drastic changes in the extracellular dermal matrix (ECM) and dermal-epidermal junction (DEJ) areas. In order for an antioxidant to provide anti-aging benefits, it has to work on two fronts: Protect ECM and DEJ from further degradation due to the sun-induced generation of matrix-degrading metalloprotease (MMPs) and stimulate synthesis of ECM and DEJ proteins to provide the bulk of the dermis lost due to the photo- as well as chronological-aging.

4.3.7.4 CONSEQUENCES OF UV-INDUCED CHEMICAL AND BIOCHEMICAL CHANGES IN SKIN

Matrix-Degrading Metalloprotease

Matrix Metalloprotease (MMPs) are involved in the remodeling and degradation of extracellular matrix (ECM) proteins, such as collagens, elastins, fibronectin, and proteoglycans, both as part of normal physiological processes and in pathological conditions. At this time 24 different MMPs have been identified and classified [34].

Several studies carried out by researchers using dermal fibroblast cells show that both UVA and UVB cause a four- to fivefold increase in the production of MMP-1 and MMP-3 [35–37]. Brennan et al. have shown by punch biopsies of human skin after UV irradiation that MMP-1 rather than MMP-13 as the major collagen-degrading enzyme responsible for collagen damage in photo-aging [38]. In contrast, the synthesis of tissue-inhibitory metalloprotease-1 (TIMP-1), the natural inhibitor of matrix metalloprotease, increases only marginally. This imbalance is one of the causes of severe connective tissue damage resulting in photo-aging of the skin. Many of the molecular alterations observed following UV irradiation occur in sun-protected chronologically aged human skin *in vivo*.

Extracellular Matrix (ECM)

The skin consists of two main layers, epidermis and dermis, separated by the basement membrane. The ECM is the material that forms the bulk of the dermis,

excluding water and cells. Proteins and complex sugars form most of the dermal ECM and they are arranged in an orderly network fibers and ground substances. ECM is a compilation of different macromolecules organized by physical entanglements, opposing ionic charges, chemical covalent bonding, and cross-linking into a biomechanically active polymer. These matrices provide a gel-like form and scaffolding structure with regional tensile strength provided by collagens, elasticity by elastins, adhesiveness by structural glycoproteins, compressibility by proteoglycans – hyaluronans, and communicability by a family of integrins, which exchanges information between cells and between cells and the dermal extracellular matrix.

From a biological point of view in aged skin, significant histological changes occur within the dermis accompanied by an increased degradation of ECM through up-regulation of MMP production as well as a decrease in collagen content and synthesis [39, 40]. This reduction of collagen levels results in skin thinning and increased fragility.

Although collagen content decreases, collagen synthesis in sun-damaged skin appears to remain similar to that of sun-protected sites [41]. Thus, evidence suggests that the decrease in collagen content in photo-damaged skin results from increased collagen degradation, by matrix metalloprotease, without significant changes in collagen production. The skin repercussion on the degradation of the ECM proteins may then be revealed in many ways depending on age, genetic predisposition, lifestyle, and of course, on the general health status of the individual.

Dermal and Epidermal Junction (DEJ)

Structural modifications of the superficial dermis during the aging process draw a parallel with considerable alterations of dermal epidermal junction (DEJ). The epidermis forms an undulating appearance, with intermittent regular protrusions of the epidermis layer (rete pegs) into the upper layers of the underlying dermis. One of the major morphological features of aged skin is a flattening of DEJ with the loss of rete pegs and reduplication of the lamina densa [42]. Flattening of the rete pegs makes the skin more fragile and easier for the skin to shear. This process also decreases the amount of nutrients available to the epidermis by decreasing the surface area in contact with the dermis, also interfering with the skin's normal repair process. Thus, DEJ changes are considered as crucial markers of skin aging [43].

At the structural level, DEJ consists of four distinct zones between epidermis and dermis: 1) plasma membrane and hemidesmosomes belonging to basal keratinocytes, 2) lamina lucida containing laminin 5, 3) lamina densa mostly consisting of type IV collagen, perlecan, and nidogen, and 4) a sublamina fibrillar zone containing anchoring fibrils of type VII collagen [44].

Several of these components have been shown to be altered and reduced in aged skin. Collagen VII, which is responsible for anchoring the basement membrane onto the dermal matrix, decreases with aging [45]. This reduction has been shown to represent an important biochemical marker of wrinkles. During aging, the papillary dermis tends to be reduced, which is related to decreased expression of pro-collagen 1 and 3 [46] and fibrilin 1 [47]. Collagen IV content decreased with age over 35 years. The epithelial basement membrane thickness increased significantly with age and there is an inverse correlation between these two parameters [48].

4.3.7.5 USE OF CONVENTIONAL AND NONCONVENTIONAL ANTIOXIDANTS FOR SKIN PROTECTION AND REVERSAL OF SIGNS OF AGING

Direct application of antioxidants on to skin has the advantage over oral administration because targeting antioxidants to the area of skin needing the protection is easier to achieve. It seems desirable to add low-molecular-weight antioxidants to the skin reservoir by applying antioxidants topically as they protect skin against oxidative stress. A review of the protective effects of topical antioxidants in humans has been published [2, 49].

a. Conventional Antioxidants

Tocopherols and Its Derivatives

Tocopherols are a mixture of four lipid-soluble tocopherols (α, β, γ, and δ) and four lipid-soluble tocotrienols (α, β, γ, and δ). Tocopherols and tocotrienols differ only in their prenyl side chain. The chromanol head of each is identical with α, β, γ, and δ-isomers, each containing an essential hydroxyl group necessary for antioxidant activity. Photochemically, natural tocopherols are not very stable [50]. The relative antioxidant activities of tocopherols in lipid systems is α > β > γ > δ-isomers [51].

Tocopherol is depleted during the oxidative stress and cannot be regenerated in the absence of a co-antioxidant. Tocopherol is important for protecting the lipid structures of the stratum corneum proteins from oxidation. However, topically applied α-tocopherol is rapidly depleted by UVB radiation in a dose-dependent manner. The photoprotective effect of tocopherol and its esters has been studied extensively. Topical application of tocopherol has shown significant reduction in oxidative damage induced by UV-irradiation [52].

Tocopherol esters, particularly tocopherol acetate, succinate, and linoleate, were shown to be promising agents in reducing UV radiation–induced skin damage [53]. Their protective effects, however, are less pronounced as compared to tocopherol. This is quite understandable because tocopherol esters need to be

hydrolyzed first during skin absorption to show antioxidant activity. It seems that the bioconversion of α-Tocopherol esters to α-Tocopherol is slow and occurs only to minor extent.

L-Ascorbic Acid and Its Derivatives

L-ascorbic acid is involved in many biological processes such as collagen synthesis, antioxidation, intestinal absorption of iron, and metabolism of some amino acids [1]. An essential function of L-ascorbic acid is to act as a cofactor for the hydroxylation of proline and lysine residues in collagen, a major protein component of the body. L-ascorbic acid also increases transcription of pro-collagen genes and stabilizes pro-collagen mRNA [54]. L-ascorbic acid improves epidermal barrier function, apparently by stimulating sphingolipid production [55].

Unfortunately, L-ascorbic acid is not stable in aqueous solutions, even at neutral pH at room temperature (~25°C). To solve this problem, a number of stable synthetic derivatives have been developed such as sodium (SAP), magnesium ascorbyl phosphate (MAP), and ascorbyl glucoside (AG). Ascorbic acid also acts as a pro-oxidant in the presence of transition metals [22]. Unfortunately, SAP and MAP have to be formulated at basic pH (>7.00) due to their instability in the acidic system. A chelating agent needs to be included in the formulation to prevent degradation of MAP and SAP. A water-soluble analog of vitamin C, ascorbic acid 2-O-α-D-glucoside, has been shown to have improved stability and efficacy [56]. Also, AG is stable in an acidic environment and is much easier to formulate with than SAP and MAP.

Recently, several human clinical studies have been reported on the use of vitamin C at high percentage levels (5% or above) and its beneficial effect on skin [57, 58]. Topical application of 5% vitamin C cream was an effective and well-tolerated treatment. It led to a clinically visible improvement of the photo-damaged skin and induced modifications of skin relief and ultra-structure, suggesting a positive influence of topical vitamin C on parameters characteristic for sun-induced skin aging.

Carotenoids

Carotenoids are a class of lipophilic compounds of plant origin that contain an extended system of conjugated double bonds; β-carotene and lycopene are the most prominent compounds. β-carotene, α-carotene, lycopene, β-cryptoxanthine, lutein, and zeaxanthine are major carotenoids in human skin, and their levels differ between various skin areas [59]. It was demonstrated that a single exposure to solar-simulated UV light lowers the skin lycopene level by 31 to 46%, whereas the same UV dose has very little effect on the β-carotene level [60]. However, repeated exposure to UV light also depleted the β-carotene level.

Carotenoids have been reported to react with virtually any radical species likely to be encountered in a biological system [61]. β-carotene has been supplemented

prior to sun exposure in order to prevent sunburn. The protective effects are related to the antioxidant properties of the carotenoid. The data obtained in different studies on this topic have been reviewed by Stahl and Sies and appear to be conflicting [62]. There is some clinical evidence of increased skin cancer incidence in smokers supplemented with β-carotene [63]. β-carotene oxidation products formed by smoke may be responsible for pro-carcinogenic effects in human [64].

Plant Polyphenolics

Polyphenolics comprise a wide variety of natural products of plant origin. Almost all of them exhibit a marked antioxidant activity. Typical examples are oligomeric catechols, flavonoids, monomeric and oligomeric flavan-3-ols (condensed tannins), gallo- and ellagitannins (hydrolysable tannins), xanthones, and lignans. The tannins are considered superior antioxidants as their eventual oxidation may lead to oligomerization via phenolic coupling and enlargement of the number of reactive sites, a reaction that has never been observed with flavonoids themselves [65]. Many of these plant polyphenolics are consumed in the diet and are believed to have beneficial health effect for human beings. Administrations of different plant extracts, particularly flavonoids, have been reported to reduce acute and chronic skin damage after UV radiation exposure [49]. Polyphenolics are deeply colored and inherently unstable compounds due to aerial oxidation; this allows them to function in redox reactions.

Tea Polyphenols

Tea from the *Camellia sinensis* is consumed by more than two-thirds of the world's population. Tea is the most popular beverage next to water. Tea is a potent source of polyphenols, comprising 30 to 35% of the dry weight of the leaf. During processing, tea leaves are progressively fermented to produce green tea, oolong tea, or black tea. Major ingredients in green tea are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), gallocatechin, and catechin. All these polyphenols are potent antioxidants and are capable of quenching superoxide radical, hydroxyl radical, peroxyl radical, singlet oxygen, and hydrogen peroxide. Several comprehensive reviews on tea polyphenols are available [2, 66].

Topical application of green tea polyphenols reduced UV-induced erythema and sunburn cell formation in human skin [66]. Tea polyphenols protected human skin from UV-induced langerhans cell depletion [66]. EGCG when applied topically reduced UVB-induced inflammatory responses and infiltration of leukocytes in human skin [67]. Standardized extract of green tea polyphenols also protected against erythema, and c-Fos and p53 induction after PUVA phototoxic injury to human skin [67]. Application of green tea polyphenols to skin reduced UVB-induced pyrimidine dimer formation in both epidermis and dermis [68].

Emblica Antioxidant

Emblica antioxidant is a standardized water-based extract of *Phyllanthus emblica* (*syn. Emblica officinalis*) fruits. The product is defined to the extent of well over 50% in terms of its key bioactive components and has acceptable color for topical application at about 0.5% level. The low-molecular-weight (<1,000) hydrolyzable tannins, namely emblicanin A and emblicanin B, along with pedunculagin and punigluconin, are the key ingredients in emblica antioxidant [69]. Emblica is a hydrolytically as well as photochemically stable antioxidant [69, 70]. While most antioxidants go from an active to an inactive role, emblica antioxidant utilizes a multilevel cascade of antioxidant compounds, resulting in a prolongation of activities [70].

Emblica has an excellent free-radical and nonradical quenching ability [69, 70], strong chelating ability to iron and copper (no pro-oxidative activity) [70], and significant matrix metalloprotease-inhibitory activity [69]. Emblica has been shown to reduce UV-induced erythema [69] to human skin. *In vitro* study using human skin fibroblasts and chondrocytes, Chaudhuri et al. [69] showed that emblica antioxidant increases the synthesis of collagenic proteins significantly. Human clinical trials have shown that emblica has a strong skin-lightening and even-toning effect [71] and has the ability to reduce fine lines and wrinkles [72].

Resveratrol

Resveratrol (3,5,4'-trihydroxy stilbene) is a polyphenolic antioxidant. It is thought to be responsible for many health benefits attributed to red wine. It is found in more than 70 species of plants. One of these plants, *Polygonnum cuspidatum*, an ingredient in traditional Asian medicines, is the major source of natural resveratrol. Synthetic resveratrol is also available commercially. Unfortunately, resveratrol is not photochemically stable.

Resveratrol has been the subject of intense interest in recent years due to a range of unique anti-aging properties. Resveratrol is an activator of the silent information regulation 2 homolog 1 (SIRT1) and is known to extend lifespan and improve metabolic disease. In a widely publicized report, researchers at Harvard Medical School have demonstrated that resveratrol activates a "longevity gene" in yeast that extends life span by 70% [73]. The role of resveratrol in prevention of photoaging has recently been reviewed and compared with other antioxidants [74]. Several oxygenated analogs of resveratrol have also been shown to have good to excellent antioxidant activity [74, 75]. Resveratrol also stimulates the antioxidant system by up-regualting glutathone, glutathione peroxidase, and glutathione reductase [76].

Bakuchiol

Bakuchiol (Phenol, 4-[1E,3S]-3-ethenyl-3,7-dimethyl-1,6-octadinenyl) is a novel phenolic compound with a monoterpene side chain. Bakuchiol belongs to a

rare group of terpenoids in which the aromatic ring system is derived from phenylpropane unit [77]. Bakuchiol has been the subject of intense interest in recent years due to a range of unique skin protective properties—inhibition of mitochondrial lipid peroxidation [78]; inhibition of hypoxia-inducible factor-1 (HIF-1) and nuclear factor-k-B [79]; and inhibition of inducible nitric oxide synthase [80]. Bakuchiol has also been shown to have anti-tumor potential [81]. However, commercialization of this product was hampered due to the difficulty in removing skin-sensitizing furano coumarins. Chaudhuri et al. were able to develop psoralene-depleted bakuchiol (trade named Sytenol® A) with about 95% purity, which is free of sensitization potential as demonstrated by human repeat insult patch test and photo toxicity test [82, 83]. Bakuchiol is photochemically and hydrolytically stable and was found to possess free-radical scavenging activity against a wide range of reactive oxygen species, which is much higher than vitamin E. Comparative gene expression profiling of bakuchiol and retinol has been shown to modulate genes associated with reversing aging signs, namely, dermo-epidermal junction, matrix proteins, cell adhesion molecules, cytoskeletons, tight junction, epidermal differentiation, and with skin protection, namely, antioxidant defense enzymes (up-regulation), pro-inflammatory enzymes (down-regulation), DNA repair genes (up-regulation) [83, 84]. In a clinical study, topical application of a 1% lotion of bakuchiol for seven days before UV exposure completely eliminated erythema versus placebo [82]. Bakuchiol has also been shown clinically to be very effective in improving the condition of acne-affected skin [85] and reducing the signs of aging [84].

b. Other Photoprotectants

Retinoids

Retinoids are a class of compounds consisting of four isoprenoids units joined in a head-to-tail manner. All retinoids are derived from a monocyclic parent compound containing five carbon-carbon double bonds and a functional group at the terminus of the acyclic portion. They are vitamins, because retinol is not synthesized in the body and must be derived from the diet. Vitamin A is used as the generic descriptor encompassing retinol, and retinal and retinoic acid. The main circulating form of vitamin A in the blood is retinol; epidermis stores it as retinyl esters [86]. The epidermis can be easily loaded with high amounts of vitamin A by topical application of either retinol or retinyl. Retinol, however, is sensitive to light, oxygen, and heat and is difficult to formulate with [87]. Retinol can easily be stabilized against photo-oxidative and singlet oxygen environments by using bakuchiol [88].

Topical retinoids, namely retinoic acid (tretinoin), have been proven to prevent and repair clinical features of photo-aging; these processes are facilitated by an ability to prevent loss of collagen from, and stimulate new collagen formation

in, the papillary dermis of sun-exposed skin [89]. Fisher et al. have shown that pretreatment of skin with retinoic acid inhibits UV induction of matrix metalloproteases [90]. A recent randomized, double-blind, vehicle-controlled clinical study (elderly subjects, mean age 87 years, 24 weeks' treatment) using 0.4% topical application of retinol lotion revealed that there were significant differences between retinol-treated and vehicle-treated skin for changes in fine wrinkling scores. As measured in a subgroup, retinol treatment significantly increased glycosylaminoglycan expression and pro-collagen I immunostaining compared with vehicle [91]. With greater skin-matrix synthesis, retinol-treated aged skin is more likely to reverse sun-induced damage along with improved appearance. Several good reviews on the topical application of retinoids have been published [92, 93].

Alkylresorcinols

Alkylresorcinols (ARs), a group of phenolic lipids, exist in the human diet in whole-grain rye and wheat [94]. ARs were reported to have antitumor, antibacterial, antifungal, and antiparasitic activities. These effects of ARs were attributed to membrane-modulating effects due to the interactions of their alkyl tails with phospholipids and/or proteins and to antioxidant effects of the phenolic hydrogen [94, 95]. ARs are weak antioxidants, but excellent inhibitors of Fe^{2+} or H_2O_2-induced oxidation [96]. Anticarcinogenic properties of ARs were related to induced DNA strand scission caused by incorporation of the AR chain in the double helix of DNA [97].

Lower alkylresorcinol, namely 4-hexylresorcinol (HR), has a long history of human use, well over 80 years. HR is one of the key active ingredients in throat lozenges. HR is included in the U.S. pharmacopoeia and is considered to be safe and effective in use as an anti-browning agent and GRAS status for use in food [98].

4-hexylresorcinol and resveratrol have recently been shown to have protective effect against oxidative DNA damage in human lymphocytes induced by H_2O_2. Both 4-hexylresorcinol and resveratrol have been shown to induce an increased glutathione, glutathione peroxidase, and glutathione reductase [76]. UVA irradiation induces immediate loss of reduced glutathione (GSH) in both melanocytes and keratinocytes. UVB irradiation of keratinocytes caused instant reduction of reduced GSH and impaired plasma membrane stability. On the other hand, in melanocytes, UVB had no effect on GSH level or plasma membrane stability, although increased apoptotic cell death and reduced proliferation was detected. These results may have implications in the induction of malignant melanoma and nonmelanoma skin cancer [99]. 4-hexylresorocinol also has anti-glycation effect [100].

4-hexylresorcinol has excellent tyrosinase and peroxidase/H_2O_2-inhibitory activity, which is many fold superior than hydroquinone, kojic acid, or licorice extract [101, 102]. High-purity 4-hexylresorcinol (>99%) for topical applications

is available commercially under the trade name Synovea® HR for skin-lightening and skin-protection purposes [102]. It is quite conceivable to assume that 4-hexylresorocinol in combination with broad-spectrum photostable sunscreen may provide improved cell protection due to stimulation of cell-protective glutathione and glutathione enzymes and address sun-induced pigmentation problems.

Antioxidant-Photostabilizers

A photostabilizer having built-in antioxidant functionality—diethylhexyl syringylidene malonate—has been designed by Chaudhuri and its utility in stabilizing avobenzone has been demonstrated [103]. Besides being an excellent photostabilizer, it is also a very good antioxidant and its activity has been demonstrated to be superior to vitamin E [103]. Recently, Chaudhuri has developed yet another unique antioxidant-photostabilizer based on 2,4-pentanedione chemistry [104], namely, 3-(4-hydroxy, 3-methoxybenzylidene)-2,4-pentanedione (HMBP) and 3-(3, 4, 5-trimethoxybenzylidene)-2,4-pentanedione (TMP). Both are excellent stabilizers for avobenzone and other photosensitive compounds. HMBP protects skin by quenching radicals and nonradical due to its broad-spectrum antioxidant activities, whereas TMP protects skin by inhibiting key oxidase enzymes [104].

It is quite conceivable to assume that antioxidant-photostabilizers in combination with photostable broad-spectrum sunscreen would provide a significant reduction in the generation of free radicals, thereby protecting skin from the onslaught of reactive oxygen species. Its commercial utility has been demonstrated by Meyer et al. by including one of these antioxidant-stabilizers in the Coppertone product line [105].

Meyer et al. were able assess antioxidant efficacy in models that better mimic actual use conditions on human skin [106]. This method uses lipids removed from the skin on broad pieces of tape that served as the substrates for subsequent exposure to UV radiation. This study reveals several striking features: (1) both water- and oil-soluble materials can protect lipids against UV radiation-induced peroxidation; (2) vitamin E protects skin lipids in a dose-dependant manner; (3) vitamin E in combination with emblica provides synergistic improvement in protection of skin lipids; (4) some materials, such as N-acetyl cysteine, *Rosemary officinalis* oleoresin, *Rosa gallica* extract, *Thermus thermophillus* ferment, and the bioactive photosynthetic complex from green plants increase rather than decrease lipid hydroperoxide levels, acting as pro-oxidants as opposed to antioxidants.

To study the effects of ultraviolet radiation on free-radical generation and the role this plays in skin damage, Meyer and Hanson employed a two-photon laser fluorescence-imaging microscope. Using the technique, it was shown that the *stratum corneum*—the skin's main protective barrier against environmental assault—generated a tremendous number of free radicals when exposed to ultraviolet light. The free radicals caused considerable damage to both the cytoplasm

and the lipid matrix. The cytoplasm of the lower epidermis was also dramatically damaged. While typical SPF-30 sunscreen offered only 39% reduction in free-radical damage, the addition of 0.5% vitamin E and 0.1% emblica as antioxidants to the SPF-30 sunscreen reduced the generation of free radicals by 73% [105, 106]. Result clearly demonstrates that judicious use of antioxidants in broad-spectrum sunscreen enhances skin protection beyond that afforded by sunscreen alone.

Combination of Antioxidants

The antioxidant network is a complex system and is interlinked [2]. Thus, enhanced photoprotection and anti-aging benefits can be achieved by topically applying an appropriate combination of antioxidants. Topical application of a single antioxidant does not take into account the dynamic interplay of multiple antioxidants. Photoprotection involves the synergistic interplay of several antioxidants. The effect of topical antioxidants after UV irradiation is less obvious, whereas the photoprotective effect of topical antioxidants applied before UV exposure has been well recognized [53].

Co-application of vitamin E and vitamin C provided a much more pronounced photoprotective effect as compared to the application of a single antioxidant [53]. Even further improvement in photoprotection resulted when co-application of melatonin together with vitamin E and vitamin C was topically done [107]. Lin et al. [58] have shown that the combination of 15% L-ascorbic acid and 1% alpha-tocopherol provided significant protection against erythema and sunburn cell formation. L-ascorbic acid or 1% alpha-tocopherol alone also was protective but the combination was much superior. In addition, the combination of vitamins C and E provided protection against thymine dimer formation.

Topical application of a formulation containing 15% L-ascorbic acid, 1% alpha-tocopherol, and 0.5% ferulic acid has been shown to protect human skin (*in vivo*) against damage induced by solar-simulated UV radiation. Skin protection was determined by evaluating erythema and sunburn cells, and immunohistochemically for thymine dimers and p53. Additionally, UV-induced cytokine formation, including interleukin (IL)-1alpha, IL-6, IL-8, and IL-10, and tumor necrosis factor-alpha, were evaluated by real-time polymerase chain reaction. Antioxidant combination provided significant and meaningful photoprotection for skin by all methods of evaluation [108].

CONCLUSION

ROS increases with aging due to the reduced activity of the antioxidant defense enzymes. Rhie et al. have shown that decreased activity of catalase in the dermis, and the decreased levels of non-enzymic antioxidants in epidermis and dermis of photo-aged and aged human skin *in vivo* [31]. This imbalance of pro-oxidant/

antioxidant ratio is believed to cause an age-associated increase in oxidative stress (ROS) in aged skin. Consequently, ROS, such as hydrogen peroxide, in aged skin may increase and be accumulated; these ROS will affect signaling pathways and finally lead to aged and photo-aged skin *in vivo*. Thus, modulation of endogenous antioxidant defense mechanisms, quenching radicals, and nonradicals and chelating free-transition metals using antioxidants may offer a good strategy for the treatment and prevention of aging and photo-aging in human skin.

Skin aging entails drastic changes in the extracellular dermal matrix (ECM) and dermal-epidermal junction (DEJ) areas [42–44]. In order for an antioxidant to provide anti-aging benefits, it has to work on two fronts: Protect ECM and DEJ proteins from degradation due to the sun-induced generation of MMPs, and stimulate synthesis of ECM and DEJ proteins to provide the bulk of the dermis lost due to the photo- as well as chronological- aging. Thus, stimulation of ECM and DEJ proteins and inhibition of MMPs may provide a good strategy for the treatment and prevention of aging and photo-aging in human skin.

It would seem reasonable to assume that photostable broad-spectrum sunscreens in combination with broad-spectrum antioxidants will continue to be the major weapon in this preventative and restorative anti-aging campaign. In order to function as photoprotective agents, antioxidants have to be present at the right place in the right concentrations at the right time. Topical application of photoprotectants should supplement the natural antioxidant protection present in skin, and provide supplemental reserves as oxidative stress depletes antioxidant stores. Ultimately, protection of the skin by sunscreens in combination with antioxidants can only be as effective as their adequacy of application to the skin surface in the everyday setting permits.

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ANTI-AGING PATHWAYS

FUNDAMENTALS OF SKIN ANTI-AGING OVERVIEW

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Aging is a process that is natural for everyone.

This section provides a comprehensive and integrated description and approach to the problems associated with the process aging. A diverse group of scientific researchers have been brought together in this section to describe their different points of view and help you integrate their ideas on aging. Their perspectives and lines of inquiry vary widely; their various lines of inquiry extend from genomics to apoptosis and everything in between.

Gene therapy and longevity science are the driving innovation factors in preventive anti-aging treatment. Anti-aging is expanding to encompass protecting stem cells, protecting/repairing DNA, and activating sirtuins, as well as targeting age-related inflammation, modulating sirtuins—thereby boosting cell longevity and anti-aging benefits.

The impacts of stress, free radicals, and glycation all have an effect on cell longevity. The same glucose molecules that provide energy for our cells also react with proteins. These proteins also include the skin's collagen, which results in the formation of Advanced Glycation End products (AGEs), known to contribute to loss of skin elasticity, production of wrinkles, inflammation, and accelerated aging.

There are a number of hypotheses that explain aging in terms of progressive failures in cellular mechanisms leading to accumulated damage. The **telomere theory** of aging says that the faster your telomeres shorten, the faster you age—biologically speaking. Other "theories" of aging are also appealing, but much research will be necessary before they can be accepted as valid.

Nowhere is cellular dehydration more important than in the skin. A healthy diet that includes hydrating foods and deficient nutritional supplements are known to increase cell vitality. This concept is known as “beauty from within” and involves the key knowledge that ingestible (nutri-cosmetics) help protect the skin internally from the damaging free radical activity.

Sleep deprivation contributes to adverse skin effects, proving that sleep is imperative for recharging the body and helping to reset its metabolic functions, repair damaged cells, and eliminate accumulated waste.

Apoptosis is a critical biological process in understanding aging. This process is essential in the development of embryos and the immune system as a protective mechanism against abnormal cells.

Reversal of cellular senescence is strongly believed to be an important function of anti-aging products.

Our long-time assumption that aging is a process of deterioration that continues without stopping is now challenged by epigenetic science. It’s no wonder that genomics is becoming regularly used by the skin care industry.

Extending life will be of little value unless ways can be found to maintain good health into old age. At this writing, sadly, we are not close to unraveling the mystery known as the aging process; at present there is not one unified theory of aging. Regulations aside, the integration of skin anti-aging processes with internal organ and bodily aging may be combined to create future challenges for all researchers.

THEORIES OF AGING SKIN ANTI-AGING: AT THE TIPPING POINT

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ABSTRACT

The skin is the largest, most complex immune organ. The skin is exposed to both endogenous and environmental oxidizing agents leading to its premature aging and impaired cellular function. All vital organs begin to lose some function as you age. Aging changes have been found in all of the body's cells, tissues, and organs. These changes affect the functioning of all body systems. Aging is common to all living organisms, yet there is no universal agreement as to its biological mechanisms. If the underlying cause of aging can be determined, it might be possible to interfere with the process, thus extending human longevity. To date more than 300 molecular biological mechanisms have been described in an effort to explain human aging. Biologists define aging as a genetic process associated with morphological and physiological process-functional changes in cellular and extracellular components aggravated by injury throughout life and resulting in a progressive imbalance of the control and regulatory systems of the organism, including hormonal, autocrine, neuro-endocrine, and immune homeostatic mechanisms (1). In short, aging is a process in which both intrinsic and extrinsic determinants lead progressively to a loss of structural integrity and physiological function (2). Aging theories basically can be separated into two fundamental groups. DNA damage theories advocate that aging is caused by accumulated damage to DNA, which in turn inhibits cells' ability to function and express the appropriate genes. This leads to cell death and overall aging of the skin. Another group is in favor of built-in breakdown theories, which advocate that aging is a direct consequence of genetic programming. The causes for aging are directly built into the genome and cellular structure as a sort of molecular clock. This chapter will examine a few mainstream theories of aging.

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5.1.1 THEORIES OF AGING:

a. Wear and Tear Theory (immunological theory)

This theory assumes that the body and its cells are damaged by constant use, causing minor damage to cells, which remain unrepaired. There are changes in the immune system as it begins to wear out and the body is more prone to infections and tissue damage. As the system breaks down, the body becomes more apt to have autoimmune reactions in which the body's own cells are mistaken for foreign material and are destroyed by the immune system. It is generally agreed that the body and its cells are damaged by abuse, neglect, and overuse. The organs such as liver, stomach, kidneys, and skin are worn down by toxins in our diet, environment, excessive consumption of fat, sugar, caffeine, alcohol, nicotine, ultraviolet rays, and by many other physical and emotional stresses to which we subject our bodies every day. Wear and tear is not confined to organs, but also takes place at the cellular level (1). As a result, the system becomes worn or damaged, adding more stress on other organs and causing a downward spiral in body functioning. These are nonreversible physical or chemical changes within cells that gradually alter the cellular function. This theory supports the concept that aging is a programmed process. Each cell

has a specific amount of metabolic energy available to it, and the rate at which this energy is used determines an individual's length of life. Besides depletion of available energy, this theory includes other contributing factors, such as effects of accumulation of harmful by-products of metabolism and of faulty enzymes due to random errors. This is one of the oldest and most outdated theories of aging.

b. The Neuro-Endocrine Theory

The neuro-endocrine system is a complicated network of biochemicals that govern the release of hormones by the hypothalamus, located in the brain. The hypothalamus controls and instructs other organs and glands to release their hormones. Aging endocrine glands secrete a progressively reduced level of hormones leading to decline in the body's ability to repair and regulate itself, and the circadian rhythms of hormone in the blood become progressively more irregular. Furthermore, hormone effectiveness is also reduced due to the receptors' downgrading.

c. The Genetic Control Theory

Life-span determining genes are inherited and are called longevity assurance genes, and they determine the process of aging. Furthermore, aging is genetically programmed by some sort of neurological center that functions as a biological clock. It specifically focuses on the genetic programming encoded within our DNA. We are all born with a unique genetic code, and a predetermined tendency to certain types of physical and mental functioning. The genetic inheritance has a great deal to tell about how long we live. Each of us has a biological clock ticking away, set to go off at a particular time. When that clock goes off, it signals our body first to age and then to die. The timing of this genetic clock is subject to enormous variation (2). Certain cell components specifically limit the number of divisions of that particular cell type. This raises the question: How can there be lifelong division of many cell types in the body if each cell type is capable of only a limited number of cell divisions? Well, there are present in the body several generations of dormant "renewal cells" in each tissue that begin dividing at different rates. These are, in effect, reserve embryonic cells. Only when these are used up, the tissue begins to show signs of aging. It is safe to speculate that aging results from the loss of capacity to divide, rather than the finite division capacity of cells, which is rarely, if ever, reached in the human body. Thus the reaction of at least some cells in the body is different than when they are cultured. Other functional losses occur in the cells, producing physiological dysfunctions. This causes the cells to show aging changes before they reach their finite limits to divide.

d. The Free Radical Theory

Free radicals are unstable molecules that damage most other kinds of molecules in the body. This starts a chain reaction that disrupts cell function, accelerating the aging process. This is the most commonly held theory of aging. Free radical theory was discovered in 1954 by Dr. Denham Harman. "Free radical" describes any molecule that has a free electron, which causes the rogue molecule to react with the healthy molecule in a destructive manner. It is based on the fact that ongoing chemical reactions in the cells produce free radicals. In the presence of oxygen, these free radicals cause the cells of the body to break down. While free radicals are required for physiological functions, they also attack the structure of our cell membranes, generating metabolic waste products like lipofuscins. Such toxic accumulations interfere with cell communication, disturb DNA, RNA, and protein synthesis, lower the energy levels, and generally impede vital chemical processes. An excess of lipofuscins in the body manifests as a darkening of the skin in certain areas, also known as "aging spots." Lipofuscins in turn interfere with the cells' ability to repair and reproduce themselves. Cell metabolism is disturbed by free radicals, and this is partly responsible for the aging of our cells (3). As time goes on, more cells die or lose the ability to function, and the body soon ceases to function as a whole. Essentially, free radicals cause irreversible damages such as: DNA damage, cross-linking of proteins, and formation of age pigments.

e. Mitochondrial Theory

The mitochondria organelles are found in most cells and are considered to be the weakest link in aging. The role of mitochondria is to utilize oxygen in the production of energy. Many researchers have linked mitochondrial DNA damage to virtually all degenerative diseases. With time, toxins, or by-products of energy generation, accumulate in the mitochondria. This poisons the mitochondria and allows it to make less and less energy. This is known as the mitochondrial theory of aging. The primary cause of mitochondrial damage is its exposure to oxygen radicals. Free radicals or oxygen radicals on the other hand are by-products of mitochondrial metabolic functions, which then attack and damage their own DNA. Failure of cellular/mitochondrial DNA repair mechanism is usually assumed to play a role in cellular aging. Mitochondria are one of the easiest targets of free radical injury because they lack most of the defenses found in other parts of the cell. Although mitochondria do suffer damage from free radicals, such damage seldom triggers wholesale cell death. The connection between free radical damage to the mitochondria and the broader aging process remains unclear.

f. Waste Accumulation Theory

As cells age, they accumulate large amounts of various by-products of normal cellular metabolism. These include free radicals, aldehydes, histones, and lipofuscins, which when accumulated to a certain level can interfere with normal cell function, ultimately killing the cell. Lipofuscins are yellow-brown pigments common in many types of aging cells. The cells most commonly found to contain lipofuscin are nerve and heart muscle cells, both very critical to life (4). Lipofuscins are actually a form of cellular garbage. Waste accumulation theory states that gradual accumulation of inert substances such as lipofuscins in cells interferes with normal functioning of the cell, by causing deleterious changes in cellular components including proteins and nucleic acids. Aging cells are less efficient in repairing these damages, which cause irreversible changes that lead to more damage and aging.

g. Hayflick Limit Theory

Hayflick theorized that the aging process was controlled by a biological clock contained within each living cell, and there are a preset number of possible rejuvenations in the life of a given cell. When cells die at a rate faster than they are replaced, organs do not function properly, and they are soon unable to maintain the functions necessary for life. Human fibroblast cells (lung, skin, muscle, heart) have a limited life span. They divide approximately 50 times over a period of years and then suddenly stop. Dr. Hayflick showed that each time a cell divides, it duplicates itself with poorer quality than the time before and this eventually leads to cellular dysfunction, aging, and death. Cell death, however, is the natural order of the life of the skin. There are four important elements a cell needs: nourishment, proliferation, function, and protection. Researchers have found that nutrition seems to have an effect on the rate of cell division, concluding that overfed cells divide much faster than underfed cells (5).

h. Death Hormone Theory

Aging begins at birth; each species has its own average longevity and aging is programmed in each species. The site of the localized aging chronometer (timekeeper) is the hypothalamus, which is located in the base of the brain. The hypothalamus contains centers that control the production of growth hormones by the pituitary gland, as well as the development and activities of the gonads (thyroid, adrenal). Hormones and neurons carry out the information originated from this center, but the ability of the organism to transmit the message declines with age. This could be because of decrease in nerve conduction rates, or change in structure and amount of hormones, or receptors for nerve impulses or hormones may become less capable of reacting appropriately to incoming impulses or molecular messages. Thymus, a

lymphoid gland, may be involved in some manner in the programmed regulation of aging. It atrophies at the onset of adolescence, implying that aging occurs more readily in the absence of thymus. Some research excludes the role of the central nervous system in programmed aging. Each normal cell type has a finite number of divisions and then dies; e.g., fibroblasts taken from an embryo divide 50 times, but if taken from adult, only divide 20 times. These suggest that cell death is an inherent property of the cells, rather than being controlled by the hypothalamus or some other aging chronometer. It is speculated that as we age, the pituitary gland begins to release DOCH (decreasing oxygen consumption hormone), which inhibits the ability of cells to use thyroxin, a hormone produced by the thyroid. Thyroxin governs basal metabolism, the rate at which cells convert food to energy. The metabolism rate brings on and accelerates the process of aging (2).

i. Caloric Restriction Theory

Caloric restriction extends life span in yeast, flies, worms, mice, and primates. Sirtuins have been implicated in the anti-aging effects induced by calorie restriction in diverse species. The mechanism of sirtuin activity does not appear to operate through antioxidant pathways. Researchers have concluded that SIR2 appears to stop the aging process by stopping production of waste material in the cell, thus allowing the cell to work better longer. It was demonstrated that this gene played a role in extending longevity during periods of caloric restriction in several different species. But it so happens that humans do not have SIR2; they have a gene called SIRT1. Both SIR2 (Silencing Information Regulator 2) and SIRT1 seem to work the same way in the body. The role of sirtuins is to help cells live longer during periods of severe caloric restriction. SIRT1 has been shown to be involved in this process by slowing down cellular metabolism to conserve life. They are basically responsible for repairing DNA within the body and suppressing certain genes. This suppression, called gene silencing, is very important because if the wrong gene becomes activated, the cell's function could be destroyed. A gene is the basic physical and functional unit of heredity. Genes are made up of DNA, and act as instructions to make molecules called proteins. In humans, genes vary in size from a few hundred DNA bases to more than 2 million bases. The Human Genome Project has estimated that humans have between 24,000 and 25,000 genes. As we age it becomes harder for both SIR2 and SIRT1 (sirtuins) to conduct several tasks simultaneously, and the gene-silencing function becomes obsolete; as a consequence humans develop chronic aging diseases such as cancer, Alzheimer's, and diabetes. It seems caloric restriction is indeed effective in extending life span because it helps sirtuins work better within the body. It is widely agreed that a diet restricted in calories, but otherwise rich in nutrients, dramatically extends the life span of experimental animals. Over two thousand studies have confirmed the

effectiveness of calorie restriction in a wide variety of species. Calorie restriction or energy restriction can improve longevity and health in experimental organisms. Studies in humans also demonstrate reduced risk factors for major diseases when they are on calorie restriction diet. But longevity should not be confused with ideal skin health. People who practice calorie restriction are not necessarily a picture of youth. The focus of their life is aimed at getting away from food slavery. While many members of caloric restriction societies have lived active lives at age 100, these individuals do not look young. Their skin is not smooth, pink, taut, and lovely, but rather sallow, wrinkled, and aged. In fact, persons who actively engage in calorie restriction look older than their stated age when evaluated on facial appearance alone (16). Calorie restriction retards several aspects of the aging process in mammals, including mortality, tumorigenesis, and physiological decline, and helps delay the onset of disease as well. After years of experiments and research on longevity, Dr. Walford has developed a high-nutrient low-calorie diet demonstrating that undernutrition can dramatically retard the functional aging process. An individual on this program would lose weight gradually until a point of metabolic efficiency was reached for maximum health and life span. This is a promising theory for extending life span.

j. The Cross-Linking Theory

This theory of aging is also referred to as glycosylation theory. In this theory, it is the binding of glucose to protein (a process that occurs under the presence of oxygen) that causes various problems. Once this binding has occurred, the protein becomes impaired and is unable to perform effectively (7). Proteins (building blocks of the cells) are composed of peptides. Protein denaturation is irreversible (e.g., a cooked egg is denatured and its physical state is changed permanently). This denaturation is caused by the formation of cross-links between peptide strands, which causes structural and functional changes in protein. Cross-links are chemical bonds that develop between hydrogen atoms in the peptide. The cross-linkage theory states that, with age and formation of new cross-links, the structure of some proteins in the cells is irreversibly altered, leading to organ/system dysfunction (e.g., enzymes and collagen). Enzymes are organic catalysts. They accelerate the rate of chemical reactions (digestion, metabolism, etc.). Collagen is metabolically inert supportive tissue, located between cells, composed of protein fibers embedded in an intercellular matrix. Collagen structural changes are responsible for the loss of blood vessel elasticity that occurs with age. Also what occurs is an increase in collagen deposition with aging, leading to excess fibers (fibrosis). Elastic fibers in skin degenerate with age and are replaced by collagen fibers that are less flexible and hence the elasticity of skin decreases with age. With age, proteins, DNA, and other structural molecules develop abnormal bonds (cross-links) to one another.

These abnormal bonds decrease the mobility of proteins, and the accumulation of these damaged proteins in the cells wreaks problems. The cross-linking of the skin protein collagen has been shown to be responsible for wrinkling and other age-related changes in skin.

k. The Telomerase Theory

DNA strands, which are found at the ends of chromosomes, are called "telomeres." They contain noncoding DNA material and prevent constant loss of important DNA from chromosome ends. They stabilize and maintain genetic integrity of the chromosomes (DNA) and regulate cellular life span. Most theorists believe that DNA damage is central in the aging process. With age, telomeres progressively shorten and their functioning decreases, most likely accelerating aging. They are linked to a wide range of degenerative diseases. Telomeres protect chromosomes from end-to-end fusions, inappropriate repair, and degradation. Telomeres are maintained by telomerase. Telomerase malfunction or its absence can result in telomeres' progressive loss, which leads to instability and senescence. Every time our cells divide, telomeres are shortened, leading to cellular damage and cellular death associated with aging. This theory was born from the surge of technological breakthroughs in genetics and genetic engineering. Telomeres are sequences of nucleic acids extending from the ends of chromosomes, and act to maintain the integrity of our chromosomes. Every time our cells divide, telomeres are shortened, leading to cellular damage and the cellular death associated with aging. Researchers have discovered that the key element in rebuilding our disappearing telomeres is the enzyme telomerase, which is found only in germ cells and cancer cells. Telomerase appears to repair and replace telomeres, which manipulate the "clocking" mechanism that controls the life span of dividing cells. Future development of telomerase inhibitor may be able to stop cancer cells from dividing and presumably may convert them back into normal cells (8, 9).

l. Glycation Theory

Glycation can be described as the binding of the protein molecule to a glucose molecule resulting in the formation of damaged protein structures. Many age-related diseases, such as arterial stiffening, cataracts, and neurological impairment are attributable to glycation (10). During glycation or glycosylation, glucose molecules stick to proteins and create a molecule called advanced glycosylation end products (AGEs). When the AGEs stick to other proteins, they can cause abnormal cross-links between the proteins. These abnormal cross-links reduce the elasticity in the proteins, causing breakdown in tissues that weaken structures within our body. In the skin, glycation accounts for accelerated aging, yellowing, stiffness,

and decreased circulation. This process also affects skin elasticity and has been linked to protein clumps in Alzheimer's patients.

These destructive glycation reactions render proteins in the body barely functional. As the degraded proteins accumulate, they cause cells to emit signals that induce the production of inflammatory cytokines. Glycation is progressive and destructive. One of the compounds that is capable of stimulating NF-kB production is the AGEs. Excessive accumulation of AGEs over time contributes to changes in the structure and function of cells, tissues, and organs. Glycation theory focuses primarily on genetic influences in the aging process and specifically the role that DNA plays in premature aging. Our DNA and certain proteins cross-link to each other over time.

m. Mutation Accumulation and DNA/RNA Damage

Most theories demonstrate that DNA damage is central in aging processes. Decay of the integrity of chromosomal DNA will cause a loss of function. DNA damage refers to chemical changes in the structure of DNA molecules, producing structural abnormalities that cause dysfunction of DNA. Mutations are any kind of changes in the polynucleotide sequence. Both interfere with gene expression, which is proposed as a possible mechanism of aging and many degenerative diseases.

n. Deficient Immune System/Autoimmune Theory

Autoimmune theory suggests that with advancing age the immune system is no longer able to distinguish foreign proteins from the body's own proteins. Cellular senescence in humans causes the cells to stop replicating themselves through the process of mitosis, resulting in cellular degradation (apoptosis) over a period of time. Senescence is attributed to defects in the immune system. With age, the system's ability to produce necessary antibodies that fight disease declines. The body experiences increased frequency of infections and the immune system becomes self-destructive and reacts against itself.

o. Inflammation Theory

It is the free radicals that initiate attack on the cell plasma membrane, producing arachidonic acid by an enzyme called phospholipase A2. This enzyme is always triggered when the skin is traumatized. Arachidonic acid becomes oxidized into chemicals that result in inflammation. Once inflammation is initiated, it produces more free radicals, continuing this cycle and accelerating aging in all organ systems including the skin. External factors that trigger inflammation are cigarette smoke, UV radiation, air pollution, pro-inflammatory diet, weakened immune system, stress, and lack of sleep. Through the body's inflammatory response, it combats

infections and clears away damaged tissue and residues of other oxidative processes. Excess inflammation results in an accelerated rate of aging. Inflammation at the cellular level causes free radicals to form that damage collagen and elastin in the skin, leading to wrinkle formation and sagging skin. As we age, there is an increase in systemic inflammatory cytokines (destructive cell-signaling chemicals) that contribute virtually to every degenerative disease (11, 12). While inflammatory cytokines can cause agonizing pain, as in arthritis, they also disrupt the lining of our arteries, mutate DNA, and degrade brain cells (13, 14). In rheumatoid arthritis there is a buildup of cytokines that include tumor necrosis factor-alpha (TNF-a), interleukin-6, and NF-kappaB (NF-kB), which are known factors in contributing to inflammation. In aging people with multiple degenerative diseases, there is an elevated blood level of C-reactive protein indicating the presence of an inflammatory disorder (15). Aging destroys the delicate balance between destructive and protective inflammatory responses.

CONCLUSION

The most published and scientifically established theories are free radicals (ROS), mitochondrial dysfunction, altered hormone levels, and telomere theory. The theory of glycation processes (AGEs) is predominant in medical literature, mainly due to its impact on the treatment of diabetes. Unfortunately, the inevitable conclusion of this chapter is that the jury is still out regarding mechanisms of aging. For centuries, people have sought various ways to induce immortality. As we age, our skin, like so many of our organs, also slows down, and eventually succumbs to the ravages of chronic oxidant stress and inflammation. Skin aging is a continuous process determined by the additive influences arising from intrinsic aging, ultraviolet radiation, the environment, and lifestyle factors. Lost moisture in the stratum corneum leaves the skin surface pale and dry with fine wrinkles. Although the search for a pacemaker of age-related changes continues, the bottom line is that all proposed mechanisms can be up-regulated by some other—unknown or known—mechanism. The large number of aging theories is proof that our understanding of aging is still far from perfect. To quote David Rollo: "In any field of science, the true degree of understanding of aging is inversely proportional to the number of explanatory theories that prevail." Even so, and since there are more doubts than answers in gerontology, we should not discard these theories easily. Life, and marveling over life and death as we do in gerontology, is a game of probabilities.

Some theories have gathered more evidence than others and hence may be more promising foci for future research and for developing anti-aging interventions. Aging is largely a mysterious process. No one really knows how and why people change as they get older. Some theorists view aging as a predetermined process controlled by genes. However, no single process can explain all the changes

of aging. Aging is a complex process that varies in how it affects different people and different organs. Most gerontologists feel that aging is due to the interaction of many lifelong influences. These influences include heredity, environment, culture, diet, exercise, past illness, etc. Each person ages at a unique rate. No one theory adequately explains the process of growing old. Aging processes are better explained if a number of these theories are integrated. There are numerous theories behind what causes signs and physical effects of aging. Many theories of aging are interlinked. Age-related changes do not occur uniformly in individuals; rather they are controlled jointly by genetic and environmental factors, which makes the finding of a universal theory of aging that much more difficult, and makes it certain that we are all aging without knowing the limit of human life span. It is unrealistic to think that the clock could be turned back on many of life's activities that lead to aging. Actually, nothing has ever been shown to reverse cellular time. The only alteration that has ever been shown to slow cellular time is caloric restriction. A 40–60% reduction in caloric intake has prolonged the lives of yeast, worms, and monkeys. Caloric restriction has not been shown to slow aging and delay the onset of diseases in humans (16). More research must be done to clarify the exact mechanisms relating to aging and oxidative stress. We do not know of any way to delay the aging process, much less stop or reverse it. Living longer does not necessarily mean that the fundamental process of aging has been slowed down. It is possible to delay some of the many effects of aging; e.g., if you avoid unprotected exposure to the sun, you might delay skin aging, and a balanced diet could lower the incidence of heart disease. In fact we live longer now, because deaths caused by infectious diseases have gone down. Presumably, future research will yield better theories or determine ways to better test the current theories experimentally.

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THE CELLULAR WATER PRINCIPLE

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ABSTRACT

For the present, aging is an inescapable component of human life and an important part of all human societies. It reflects not only biological changes that occur, but cultural and social conventions, as well. While the personal care and cosmetic industry primarily have concentrated on undesirable changes in the skin's appearance, the skin is but one of six bodily organs and these changes are far more encompassing. To fully begin to understand skin aging one must take a more in-depth view of aging of the body overall.

The phenomenon of aging can be classified either as **adaptive**, where aging is considered a beneficial trait in its own right, or **nonadaptive**, with the implication that it is detrimental or at least neutral. Knowledge, life experience, and wisdom grow and expand over time, but some other age-related changes arise as well. These include, but are not limited to: a decrease in intracellular water, gradual loss of muscle and bone mass, reduction in height, and a lower metabolic rate. Other changes include lower reaction times, declines in certain memory functions, declines in sexual activity, a functional decline in senses such as audition, olfaction, and vision. Still other changes include declines in kidney, pulmonary, vascular and immune functions, declines in exercise performance, and multiple endocrine changes.

This chapter will focus on the cellular water–related changes and how they manifest themselves in the aging process.

Although the volume of water in the body is indisputably important, it is the location and mechanisms by which water is both compartmentalized and utilized by tissues that more accurately define whole body health. Traditionally, it was thought that tissues compartmentalize water into two separate environments: intracellular and extracellular. Intracellular water, which is located within the cytosol of the cells, is expressed as cell water volume. Cell water volume must be maintained at a steady state for proper metabolic functions and healthy, youthful appearance.

Extracellular water—water located outside the cell membranes of tissues—can be viewed as a reservoir for the maintenance of intracellular water volume during periods of hypohydration. Stores of extracellular water can be further divided into three sublocations: (i) within the interstitial spaces in tissues with densely packed cells (e.g., epidermis, heart, liver); (ii) within the extracellular matrix of tissues with loosely packed cells (e.g., dermis, eyes, brain, kidneys); and (iii) so-called edematous water in inflamed or injured tissue. **Edematous** water, which manifests as bloating, puffiness, and swelling, may be considered "wasted water" because it is not available to maintain cellular water volume.

Improving (i.e., normalizing) and, more preferably, optimizing, the intracellular and extracellular tissue water content, and thereby achieving and maintaining cellular water homeostasis, may provide marked improvements in metabolic processes, health, and appearance of the dermis and overall health. As such it forms the core of what will be referred to in this review as the Cellular Water Principle.

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THEORIES OF AGING AND CELLULAR WATER

5.2.1 WHAT IS AGING, FROM A PHYSIOLOGICAL PERSPECTIVE?

In a way, human aging is obvious to everyone: if we compare young and old persons, macroscopic differences are evident. Even in ancient Greece, the phenomenon of aging was described from an empirical perspective. According to Aristotle the human body is composed of the four main components of the world: humid, dry, warm, and cold. Humid and warm prevail while we are young; the dry and cold take predominance when we get old (8).

In past decades, aging was defined by several researchers as a time-dependent irreversible degeneration that accumulates until it becomes life threatening. Aging was not seen as a linear function of time. Each species has a threshold age after

which the aging process accelerates until death intervenes. (9) Grimley defines senescent changes as de novo structural and functional alterations that are not part of the developmental program (10).

Hyflick defines aging as an increase in molecular disorder (increase in entropy) (6). It is a stochastic process, and occurs systematically after reproductive maturity when fixed size in adulthood is reached. Escalating loss of molecular fidelity ultimately exceeds repair and turnover capacity and increases vulnerability to pathology or associated diseases. Hyflick's stand is that aging is not gene driven but a random, thermodynamically driven process. (6, 11) Milne's premise is that senescence processes, or biological aging, are stochastic and cumulative, and operate from very early life. The lifelong progression of "senescent diseases" appears to be influenced by conditions *in utero*, and the first signs of these may be observed from at least infancy. (11)

The evolutionary definition of human aging suggests that the mechanisms of aging involve progressive accumulation of unrepaired somatic damage of various kinds. This premise implies that life span is influenced by a diverse repertoire of genes that regulate somatic maintenance functions; it is also possible that there exist purely deleterious late-acting mutations that may have escaped the forces of natural selection. In this view, many of the age-related diseases in humans may share common causative mechanisms with each other and with "normal" aging itself. The process of aging probably begins early in life, even before birth, although the outward signs do not become apparent until later on in life. (2)

Miller defined aging as a process that converts healthy adults into frail ones with diminished reserves in most physiological systems and an exponentially increased vulnerability to most diseases and death. (7) King's definition of aging is more specific: "Aging is a genetic physiological process associated with morphological and functional changes in cellular and extracellular components aggravated by injury throughout life and resulting in a progressive imbalance of the control regulatory systems of the organism, including hormonal, autocrine, neuro-endocrine, and immune homeostatic mechanisms". (12)

Probably the most comprehensive phenomenological definition of aging was created by Dr. Bernard Strehler. He suggested that the aging process has four main characteristics (13):

1. destructive (functional ability decreases in time);
2. progressive (irreversible, an ever ongoing process);
3. intrinsically determined (bound to internal characteristics of living beings, and does not depend exclusively on any external factor);
4. universal (all species show aging phenomena, and individuals of a given species display a more or less uniform pattern of aging).

A totally satisfactory definition of aging will exist once we have a fuller understanding of the details of the processes explored and presented here in the many theories of aging as well as those that have yet to emerge. We present here a distilled essence of what is hypothesized today and encourage the reader to use this information as a foundation for future work and thinking about this important subject so relevant to us all.

5.2.2 WHY DO WE AGE?

At present, no consensus exists over what causes aging. Many theories have emerged to explain what changes lead to human aging. Various authors have tried to create a logical systemic overview, including Strehler, Platt, Esposito, Harrison, Hyflick, etc. (2, 13, 14, 15). The classification of the aging theories created by Esposito in 1983 seems to point out the approaches that are of importance even nowadays. In general, he divided theories into three groups: causal, systemic, and evolutionary. (14)

The foundation of the causal explanations of aging is the assumption that the effects of sporadic, stochastic physicochemical changes may accumulate in complex biological systems and cause the appearance of aging phenomena. Some of the well-recognized causal theories are: the theory of somatic mutations, the theory of genetic mutations, the wear and tear theory, the cross-linking theory (i.e., glycation), and the accumulation theory, etc. (14, 15, 16).

The basis of the concept of the systemic theories is that aging is a consequence of interactions between various systems (organs) of vital importance in the organism. A few of the many systemic explanations are: the program theory of aging, the theory of expired programs, the theory of autoimmunization, and organic explanations of aging (14, 15).

Evolutionary theories consider aging as an adaptive process. They mostly favor the existence of the pleotropic genes able to determine the specific aging rate for each species, too. So far, interpretations are highly speculative. (2, 15)

It is interesting to note how many different, fully developed theories of aging we have, and yet we still have not arrived at a unified synthesis that represents a fully satisfying “ultimate” one. Could it be that we need to have a stronger ability and willingness to better synthesize existing knowledge before we attempt to upgrade it?

5.2.3 WATER LOSS AND MEMBRANE HYPOTHESIS OF AGING

Water is essential to life. Life can be described as a process during which a highly hydrated state of fertilized oocytes, embryos, newborns, etc. is transformed into a gradually more and more dehydrated one. (13) This process is useful until the

organism reaches its optimal performance requiring a given amount of enzymes, muscle fibers, collagen, neurofilaments, etc. Unfortunately, a dehydration tendency exists and continues with time and other factors, resulting in a further accumulation of intracellular dry mass that has serious consequences. First, this process slows down and stops growth of the organism. Further, the increase in physical density of cell colloids compromises the basic functions of the cells because it increases the damaging efficiency of the free radicals and the *in situ* enzyme catalytic rate constants, all of which are strongly dependent on the density of their microenvironment. (18, 19)

The Membrane Hypothesis of Aging, created by Nagy, elaborates the role of the plasma membrane in differentiation and aging. Structural and functional alterations of every cell's plasma membrane occur throughout life. This is due to free radical–induced cross-linking of proteins and lipids, molecular damage, and residual heat formed during each discharge of the resting potential. Replacement of the damaged plasma membrane components by de novo synthesis is continuous, but a certain accumulation of residual damage is inevitable. Implicational functional changes in cells are: a gradual decrease in potassium permeability with an increase of intracellular potassium content and a consequent colloid condensation; a loss of intracellular water and an increase of intracellular dry mass content, with consequent inhibition of enzymatic activity and decreases in the rates of RNA and protein and gene synthesis; and the accumulation of waste products in cells (lipofuscin). (13, 18, 19)

5.2.4 THE SCIENCE OF CELLULAR WATER

As we ponder the question, "Is aging a disease?," we note that regardless of what causes aging or disease, the final common pathway is water loss—not only in the cell and plasma membrane but also in the connective tissue. The regulation of cellular hydration and volume is important for maintenance of the cell function. (20) When our cells are not fully hydrated they cannot function at their optimal level, and that leads to aging. Deterioration of the cells ultimately leads to death, but before that come disorders and diseases. Studies show that elderly persons, especially if diseased, display reduced intracellular water. (21)

Effectively, total body water (TBW) is distributed between two compartments: extracellular water (ECW, all fluids outside the cells) and intracellular water (ICW, water inside cells). Extracellular water is further divided into three compartments: interstitial (bathes all cells), plasma, and transcellular water (includes such fluids like cerebrospinal, intraocular, pleural, peritoneal, and synovial fluids, and digestive secretions). (22) A common belief is that drinking eight,

ten or more glasses of water a day is the answer to maintaining appropriate levels of hydration. The Water Principle is not just about drinking water; it is about getting water into the cells and connective tissue and keeping it there so that every cell can function to its full capacity. The author's clinical experience indicates that this dehydration and deterioration of our cells can be due to a wide variety of factors including: a deficiency in our diets of vital cell and connective tissue-building and hydrating nutrients (e.g., glucosamine, amino acids, phosphatdylcholine / lecithin, essential fatty acids, minerals, water- and fat-soluble vitamins, trace minerals, sirtuin activators, and phytonutriens). **The organism is continually repairing itself.** One of the main mechanisms is the cells and connective tissue regeneration. We must supply the body with the right raw materials for it to repair and regenerate.

In the last five years we have had more than 1500 patients, 25–85 years old, who, besides other health concerns, wanted "to look better and feel better." Many of them already had some age- related changes and diseases. As part of our approach to achieving their goals, we recommended a comprehensive dietary supplement regimen. Bioelectrical Impedance Analysis (BIA) was used as a diagnostic tool for total body hydration and metabolic activity (RJL Systems, Quantum II). Results of TBW, ICW, ECW, basal metabolic rate (BMR), and phase angle (PA) were collected before starting with dietary supplementation. Phase angle (arctangent of the ratio of reactance over resistance) is indicator of cells' vitality. Lower PA is consistent with either cell death or a breakdown of the cell membrane. Higher PA is consistent with large quantities of intact cell membranes (23). All living substances have PA. Phase angle is a predictor of outcome and indicates the course of disease or increase in health. (24, 25, 26, 27) As we age, our PA decreases. (28)

We have noticed an increase in ICW, BMR, and PA in many patients who were on nutritional supplementation program as recommended and came for follow-up after four or more weeks. Patients with increased PA reported many other improvements, such as lower blood pressure, decreased insulin intake, decreased cholesterol, improved blood count, decreased swelling/edema, decreased joint pain, improved digestion, weight loss, decreased PMS symptoms, decreased perimenopause symptoms, improved sleep, better memory, increased calmness and sense of relaxation, increased motivation and improved self-esteem, smoother, firmer, and better-hydrated skin, etc.

Retrospectively, we extracted results of only those patients who were generally in good health but interested in "looking better and feeling better" and were on a dietary supplement regimen continually for 12 weeks while having

their usual diet and exercise routine. We found 40 of them. Their results were encouraging. Among 40 patients, 26 (65%) had increase in PA, 23 (57.5%) had increase in ICW, and 22 (55%) had increase in BMR. The correlation between increase in PA and ICW was highly statistically significant ($p<0.01$). Correlation between increase in BMR and PA was statistically significant as well ($p<0.05$). Interestingly, all patients with increased ICW had decrease in ECW and the same or insignificantly higher TBW results—water has changed compartments (ICW+ECW=TBW).

To provide all the nutrients necessary to restore cell membranes and connective tissue is the optimal way to treat the cause (all structural and functional changes induced by genetic and environmental factors) with a consequent positive effect on the aging process. Our question was, "How to create an optimal overall dietary supplement?" We were looking for optimal levels of nutrients to increase cells' vitality as measured by ICW and PA (optimal anti-aging nutritional supplement regimen). Our clinical experience has shown that dietary supplementation can play an important role in achieving positive alteration of the process of aging.

CONCLUSION

Many potential therapies for age-related change and diseases are scientifically considered in this chapter, e.g., calorie restriction diet, different anti-aging pharma, and gene therapy (i.e., telomere restoration). The concept of Cellular Water and the Water Principle Hypothesis is the result of a long clinical experience and research driven by a strong wish to bring our patients closer to healthy and happy aging.

Many researchers have tried to define "Successful Aging." Rowe and Kahn emphasize the following criteria: low risk of disease and disease-related disability, high mental and physical function, and active engagement in life. (29) Glass defines successful aging as something beyond health and longevity; it is rooted in a broader definition of the "good life" in late life. (30) The Water Principle should be a basis of what this author calls "Inclusive Health," and it is even beyond a "good life." We have to approach our patients with individualized, optimal topical (skin care regimen), internal (nutrition—Figure 1, exercise and other factors of healthy lifestyle) and emotional care (psychological and social balance, thus the "comfort foods" included in the nutrition recommendations in Figure 1). **Every individual should have a possibility of restoring and preserving inclusive health from earliest age.**

Figure 1: Nutrition Recommendations from Dr. Murad based on these principles.

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ANTI-SENESCENCE: ACHIEVING THE ANTI-AGING EFFECT BY MANAGING CELLULAR FUNCTIONS

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ABSTRACT

This chapter focuses on one of humanity's greatest fears in life: old age, wrinkles, and skin aging. While the primary focus has heretofore been on the treatment of women, we see an accelerating shift towards enhanced interest by men as well. This overall trend offers stimulation for new product development that works based upon a continued search for understanding and thwarting the inevitability of the aging phenomenon. This is not a boring, knee-deep science filled with sky-high concepts, and this chapter is not meant to induce sleep.

Approaches to the beautification of facial skin includes lightening of skin color, smoothing of wrinkles and fine lines, reduction of surface disfigurements and blemishes, and reduction or disappearance of dark spots and circles around the eyes. These have been consumer-desirable attributes since ancient times. An arsenal of anti-wrinkle, anti-aging, skin brightening, skin-tone enhancement, and dermabrasion treatments have now become available for skin anti-aging cosmetics. While such treatments offer a plethora of marketing opportunities, they also tend to confuse the consumer, relative to their benefit claims. Consumers thus tend to select multiple products to obtain their combined benefits. In reality, however, such practice may cause an overload of ingredients on skin, thereby reducing their perceived efficacy.

A "single-bullet" treatment (a bundle of ingredients that perform multiple functions) that provides a highly desirable facial glow for the multinational consumer has been a focus of current active research for some time. The first such single-bullet treatment is based on anti-senescence, a fundamental approach to retarding aging described in recent patents by Gupta and Walker (1).

Aging at the cellular level is characterized by oxidative stress, inflammation, and cell senescence (2). Cells are the fundamental structure composing our bodies, and cellular senescence in humans causes the cells to stop replicating themselves through the process of mitosis. This process results in cellular degradation over a period of time, thus contributing to the aging process. Cellular senescence also initiates oxidative stress and inflammation. Such changes range from those affecting cells and their function to the whole organism. The management of cellular senescence is thus essential for any single-bullet skin anti-aging regimen (3).

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5.3.1 ROLE OF CELLULAR SENESCENCE AND APOPTOSIS IN SKIN AGING

Higher organisms contain two types of cells: postmitotic cells, which never divide, and mitotic cells, which can divide. Postmitotic cells include mature nerve, muscle, and fat cells, some of which persist for life. Mitotic cells include epithelial and stromal cells of organs such as the skin. Normal mitotic cells do not divide indefinitely. The process that limits the cell division number is termed cellular or replicative senescence. Senescent keratinocytes and fibroblasts appear to accumulate with age in human skin. Moreover, senescent cells express genes that may have pleiotropic effects, such as degradative enzymes and inflammatory cytokines. Thus, relatively few senescent cells might compromise skin function and integrity. Moreover, by altering the tissue microenvironment, senescent cells may also contribute to the rise in ailments that occur with age, such as cancer, diabetes, and arthritis (4).

Cellular senescence is the phenomenon by which normal cells lose the ability to divide and produce new cells. It differs from apoptosis, which is the process of programmed cell death that occurs in multicellular organisms. Aging causes distinctive changes in cells and certain cellular molecules. Two cellular processes, apoptosis and cellular senescence, may be examples of antagonistic pleiotropy wherein the action of a gene results in multiple competing effects, some beneficial but others detrimental to the organism. (Pleiotropy is the phenomenon where one gene controls for more than one phenotypic trait in an organism. Antagonistic pleiotropy is when one gene controls for more than one trait where at least one of these traits is beneficial to the organism's fitness and at least one is detrimental to the organism's fitness.) Both processes are essential for the viability and fitness of young organisms, but may contribute to aging and certain age-related diseases (5) and their possible treatment via chaperone proteins (6). The apparent result of both, the cellular senescence and the apoptosis, is the net loss of cells. With chronological aging the rate of both the apoptosis and the senescence increases. Intrinsic skin aging is characterized by the progressive accumulation of senescent cells, thinning of the epidermis, decline in cell-to-cell adherence, reduction of collagen content, formation of fine wrinkles, and gradual loss of elastic material (7). The treatment of these signs of skin aging—for a "forever young" physical appearance—has been one of the foremost areas of consumer interest.

5.3.2 ROLE OF ENZYME DYSFUNCTION IN SKIN AGING

Cellular senescence has been ascribed to dysfunction of certain key enzymes and biological functions of the cell that have a direct role in the chronological process of skin aging, as illustrated below. A discussion of a select number of these follows.

SKIN AGING

Cellular Senescence No Cell Division	**Apoptosis** Programmed Cell Death

- **Oxidative Stress** — **Loss of Cells**
- **Cellular Inflammation**
  (Inflammatory Cytokines)
- **Proteasomes Malfunction**
- **Enzyme Dysfunction**
  MMP Activation
  Tyrosinase Activation
- **Advanced Glycation End Products (AGES)**
- **Mitochondrial Free Radical Theory of Aging (MFRTA)**
- **Superoxide Dismutase (SOD) Inhibition**
- **Immunosenescence** (Loss of Immune Response with Age)
- **Peroxisomes**

a. Oxidative Stress and Free Radicals

The proof for the participation of oxidative stress in senescence are specific diseases that to some extent are gender dependent and appear more frequently in males or females (8). Redox active metals like iron (Fe), copper (Cu), chromium (Cr), cobalt (Co), and other metals undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radicals and nitric oxide in biological systems. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species (ROS) overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, inflammation, protein modification, and other effects.

Paradoxically, two major antioxidant enzymes, superoxide dismutase (SOD) and catalase, contain as an integral part of their active sites metal ions to battle against toxic effects of metal-induced free radicals (9).

The role of free radicals has long been proposed as a cause for the aging process. Oxidative stress is considered a major factor for altering many physiological processes and enzymatic activities during aging and is also known to play a major role in the development of several age-dependent diseases. Oxidative damage caused by free radicals includes mitochondrial dysfunction, dopamine auto-oxidation, α-synuclein aggregation, glial cell activation, alterations in calcium signaling, and excess free iron. Alterations in transcriptional activity of various pathways, including nuclear factor erythroid 2-related factor 2, glycogen synthase kinase 3β, mitogen-activated protein kinase, nuclear factor kappa B, and reduced activity of superoxide dismutase; catalase and glutathione are also the result of oxidative damage caused by free radicals. Paraoxonase 1 (PON1) is an anti-atherosclerotic enzyme that mainly prevents accumulation of lipoperoxides and inhibits the lipid oxidation in low-density lipoproteins (10). A declining endogenous defense system during senescence dictates the need for supplementation with exogenous antioxidants.

Reactive oxygen species (ROS) are thought to have deleterious effects on T-cell function and proliferation. Low concentrations of ROS in T-cells are a prerequisite for cell survival, and increased ROS accumulation can lead to apoptosis/necrosis. Antioxidants such as glutathione, thioredoxin, superoxide dismutase, and catalase all serve to balance cellular oxidative stress. T-cell redox states are also regulated by expression of various vitamins and dietary compounds (11).

b. Peroxisomes

These are ubiquitous organelles that perform such functions as hydrogen peroxide metabolism and β-oxidation of fatty acids. Reactive oxygen species produced by peroxisomes are a major contributing factor to cellular oxidative stress, which is supposed to significantly accelerate aging and cell death. The role of peroxisomes in aging and cell death has recently been reported (12).

c. Immunosenescence

The immune response declines with aging (immunosenescence), which is primarily due to T-cells' decline. Resveratrol has been found to be capable of counteracting immunosenescence, thereby leading to cellular rejuvenation (13).

d. Advanced Glycation Endproducts (AGEs)

Reducing sugars can react non-enzymatically with the amino groups of proteins to form Amadori products. These early glycation products undergo further complex

reaction such as rearrangement, dehydration, and condensation to become irreversibly cross-linked heterogeneous fluorescent derivatives, termed advanced glycation endproducts. The formation and accumulation of AGEs have been known to progress in a normal aging process and at an accelerated rate under diabetes. This is one of the reasons that diabetic skin is more prone to excessive dryness, eczema, and other clinical issues (14). There is evidence that interaction of AGEs with a Receptor for Advanced Glycation Endproducts (RAGE) elicits oxidative stress generation. The interaction between RAGE and its ligands is thought to result in pro-inflammatory gene activation and subsequently evokes inflammatory, thrombogenic, and fibrotic reactions.

e. Proteasomes in Cellular Anti-Senescence

Normal human fibroblasts undergo a limited number of divisions in culture and progressively they reach a state of irreversible growth arrest, a process termed replicative senescence. The proteasome is the major cellular proteolytic machinery, the function of which is impaired during replicative senescence. Recent work suggests that proteasome malfunction may contribute to the aging process in human skin and that the maintenance of normal proteasome activities could delay skin aging. Proteasome activities changed with age in a biphasic manner, decreasing significantly up to 50 years of age and showing no significant change between 50 and 78 years of age. The decreases in proteasome activities were accompanied by reduced levels of proteasomal peptidase activities coupled with increased levels of both oxidized and ubiquitinated proteins in senescent cells. Recent work also suggests that mitochondrial and proteasomal activities are interdependent (15).

The body constantly produces proteins and degrades proteins that are no longer needed or are defective. The production and destruction of proteins, called protein turnover, is a constant, ongoing process that is crucial for tissue renewal. A well-nourished person synthesizes nearly one pound of protein per day. Proteins that are broken down balance this protein gain. The process of protein breakdown, called proteolysis, is essential to cell survival. Numerous proteolytic systems exist in mammalian cells, the most important of which are the lysosomes, the ubiquitin-proteasome pathway, and enzymes called calpains. Lysosomes are small cell components that contain specific enzymes (proteases), which break down proteins. In the ubiquitin-proteasome pathway, proteins that are to be degraded are first marked by the addition of ubiquitin molecules and then broken down by large protein complexes called proteasomes.

Calpains are proteases that are involved in several physiological processes, including the breakdown of proteins that give cells their shape and stability. The ubiquitin-proteasome system is now considered the major system involved in intracellular protein degradation. Two major components of this system are (1) three

enzymes that add a small protein called ubiquitin onto substrate proteins destined for degradation, and (2) the proteasome, a rather large cellular particle composed of several smaller protein subunits, which executes the actual proteolysis. By degrading short-lived regulatory proteins, the ubiquitin-proteasome system controls basic cellular processes such as cell division, cell signaling, and gene regulation. The system also removes misfolded, damaged proteins, and in certain immune cells it breaks down foreign proteins into pieces called antigenic peptides, which can then be transported to the cell surface to induce an immune response.

Ubiquitin is a small protein that occurs in most eukaryotic cells. Its main function is to mark other intracellular proteins for destruction, known as proteolysis. Several ubiquitin molecules attach to the condemned protein (polyubiquitination), and polyubiquitinylated protein then moves to a proteasome, a barrel-shaped structure where the proteolysis occurs. Ubiquitin can also mark transmembrane proteins (for example, receptors) for removal from the membrane.

Ubiquitin consists of 76 amino acids with two sequentially linked glycine moieties at the carboxyl terminal and has a molecular mass of about 8500 atomic mass units (amu). It is highly conserved among eukaryotic species: Human and yeast ubiquitin share 96% amino acid sequence identity.

The process of marking a protein with ubiquitin consists of a series of steps; (i) Activation of ubiquitin—the carboxyl group of the terminal glycine of ubiquitin binds to the sulfhydryl group -SH of an ubiquitin-activating enzyme E1. The sulfhydryl group is a cysteine residue on the E1 protein. This step requires an ATP molecule as an energy source and results in the formation of a thioester bond between ubiquitin and E1; (ii) Transfer of ubiquitin from E1 to the ubiquitin-conjugating enzyme E2 via trans (thio) esterification; (iii) Then, the final transfer of ubiquitin to the target protein can occur either directly from E2 (this is primarily used when ubiquitin is transferred to another ubiquitin already in place, creating a branched ubiquitin chain) or via an E3 enzyme, which binds specifically to both E2 and the target protein. The target protein is usually a damaged or nonfunctional protein that is recognized by a destruction-targeting sequence. Ubiquitins then bind to a lysine residue in the target protein via the transformation of thioester bond into an iso-peptide bond, eventually forming a tail of at least four ubiquitin molecules. The resulting ubiquitin-linked protein, called ubiquitin-protein conjugate, then can be recognized and degraded by the proteasome into peptides. This is the typical way to mark specific proteins for proteolysis.

A functional proteasome (also called 26S proteasome) is composed of a smaller barrel-shaped core and two “caps” that are attached to each end of the core. The proteasome core consists of four stacked rings containing two types of subunits, all facing into a central cavity. These subunits together have at least five distinct proteinase activities that cleave proteins at different sites. The “caps” at each end

of a proteasome perform a regulatory function. Each cap is composed of multiple subunits with numerous functions. These subunits recognize the ubiquitinylated protein, cut off the ubiquitin chains from this protein, thereby "unfolding" the protein, and open the channel inside the proteasome core so that the protein can enter the channel for degradation; and (iv) Finally, the marked protein is digested in the 26S-proteasome into small peptides, amino acids (usually 6–7 amino acid subunits). Although the ubiquitins also enter the proteasome, they are not degraded (despite their protein structure) and may be used again.

Proteasomes are large multi-subunit protease complexes, localized in the nucleus and cytosol, which selectively degrade intracellular proteins. Proteasomes play a major role in the degradation of many proteins that are involved in cell cycling, proliferation, and apoptosis. The ubiquitin-proteasome pathway is well documented. Intracellular proteolysis is the most recently discovered regulatory system of cellular physiology. Everything from cell division, development, and differentiation to cellular senescence has a proteolytic component. There is no simpler way to stop a physiological process than to destroy one of the components of a pathway in a controlled fashion. The discovery of the role of ubiquitin in the proteolytic pathway earned Aaron Ciechanover, Avram Hershko, and Irwin Rose the 2004 Nobel Prize in Chemistry. Several books have become available that further

reveal the importance of ubiquitins in human biology and human disease control, some of which are referenced in (15).

A wide variety of neurodegenerative disorders are associated with the accumulation of ubiquitinylated proteins (if they are not further degraded by proteasomes) in neuronal inclusions, and also with signs of inflammation. In these disorders, the ubiquitinylated protein aggregates, which will be seen as a foreign body by the immune system, may themselves trigger the expression of inflammatory mediators, such as cyclooxygenase 2 (COX-2). Impairment of the ubiquitin-proteasome pathway may contribute to this neurodegenerative and inflammatory processes. Products of COX-2, such as prostaglandin J2, can, in turn, increase the levels of ubiquitinylated proteins and also cause COX-2 up-regulation, creating a self-destructive feedback mechanism.

The disruption of the ubiquitin-proteasome pathway can result from damaging events, such as aging-induced decrease in proteasome function, and production of neurotoxic molecules from mutations. A dysfunctional ubiquitin-proteasome pathway may then cause proteins that are normally turned over by this pathway to aggregate and form inclusions. One of the mechanisms by which the abnormal accumulation of ubiquitinylated proteins may mediate neurodegradation is by triggering an inflammatory response. Inflammation is a natural defense against diverse insults, intended to remove damaging agents and to inhibit their detrimental effects. Treatment of neurons with proteasome inhibitors, oxidative stressors, or cyclopentenone prostaglandin J2 elicits accumulation of ubiquitinylated proteins and cytotoxicity in a concentration-dependent manner. These agents also increased the neuronal levels of COX-2 and prostaglandin E2. COX-2 is the pro-inflammatory and inducible form of cyclooxygenases, which are enzymes that catalyze the rate-limiting step in the biosynthesis of prostaglandins, prostacyclins, or thromboxane A2 from their precursor arachidonic acid.

Cyclooxygenases are bifunctional hemoproteins that catalyze the cyclooxygenation of arachidonic acid to PGG2 followed by the hydroperoxidation of PGG2 to PGH2. Specific enzymes, such as reductases, isomerases, and synthases, then convert PGH2 to other PGs (prostaglandins) and thromboxane A2. Reactive oxygen species (ROS) produced during this biosynthetic pathway are known to contribute to tissue damage. The pro-oxidant effect of prostaglandin J2 could be mediated by its cyclopentenone ring, which contains an alpha-beta-unsaturated carbonyl group that can react with a sulfhydryl group of cysteine residues in glutathione and cellular proteins to inhibit ubiquitin isopeptidase activity. This may also contribute to the accumulation of ubiquitinylated proteins. This toxic positive feedback may create a self-destructive mechanism that contributes to the neurodegenerative process. Neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, found to be associated with

the accumulation of ubiquitinylated proteins in neuronal inclusions, also exhibit signs of inflammation.

The modulation of the ubiquitin-proteasome pathway can be achieved in several manners, including (i) the inhibition of thioester bond formation between ubiquitin and cysteine moiety of the ubiquitin-activating enzyme (E1, E2, or E3), (ii) the inhibition of iso-peptide bond formation between ubiquitin and lysine moiety of target protein, (iii) the inhibition of ubiquitin-proteasome complex, (iv) acceleration of proteolysis by ubiquitin-proteasome complex (acceleration of proteasome ligase, E3, action), (v) selective inhibition of cyclooxygenase enzyme, (vi) use of thiol-reducing antioxidants, (vii) caspase inhibitors, and (viii) use of molecular chaperones to attenuate the accumulation of ubiquitinylated proteins. The molecular chaperones could thus be highly beneficial in the reduction of inflammation caused by accumulating ubiquitin-proteasome complex, which could be useful for the treatment of skin aging, inflammation, ulcer and wound healing, and enzyme malfunction–related ailments, and this aspect is the focal point of the present invention.

Ubiquitin contains 76 amino acids (one-letter code: MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG), of which gly-gly dipeptide is at the carboxy-terminal. It is believed that by chemically blocking the above gly-gly binding site, the attenuation of the accumulation of ubiquitinylated proteins may be possible.

f. Mitochondrial Free Radical Theory of Aging (MFRTA)

Aging is associated with cellular deterioration. Most cellular components, including mitochondria, require regeneration throughout the life span. Mitochondria are susceptive to damage as they are a source of oxidative stress in cells. Dysfunction of either biogenesis or fission/fusion of mitochondria is associated with aging. Mitochondria are considered the most important cellular sources and targets of free radicals. They are also a source of signaling molecules that regulate cell cycle, proliferation, and apoptosis.

The promotion of mitochondriogenesis is critical to prevent aging. MFRTA has been one of the most influential ideas over the past 50 years; this theory proposes that aging is caused by damage to macromolecules by mitochondrial-reactive oxygen species (ROS). MFRTA is supported by the accumulation of oxidative damage during aging along with comparative studies demonstrating that long-lived species or individuals produce fewer mitochondrial Reactive Oxygen Species (mtROS) and have lower levels of oxidative damage. Mitochondrial function regulates the rate of aging, and the two underlying—ROS-dependent and ROS-independent—mechanisms by which mitochondria can affect longevity remain unclear.

The role of different ROS (superoxide, hydrogen peroxide, and hydroxyl radical), both as oxidants and as signaling molecules, is a complex process whereby ROS-dependent and ROS-independent mechanisms interact to determine the maximum life span of species and individuals. Physiological free radicals such as superoxide and nitric oxide are involved in senescence and aging development. They have been studied, and the mechanisms of processes mediated by these radicals suggest that being themselves mostly harmless species, superoxide and NO are precursors of really reactive species hydroxyl radicals and peroxynitrite, which are the initiators of aging. The potential of intervention with antioxidants and other antiradical agents to suppress aging and senescence and expand the life span is thus feasible (16). An increasing number of studies on long-lived vertebrate species, mutants, and transgenic animals have seriously challenged the pervasive MFRTA. Theories alternate to MFRTA have been proposed. A well-developed theoretical framework is required to understand the molecular basis of aging. Such a framework is a prerequisite for the development of clinical interventions that will constitute an efficient response to the challenge of age-related health issues. Recent experimental evidence both supports and refutes the MFRTA (17).

5.3.3 ANHYDROBIOSIS AND SKIN AGING

Aging is the result of a gradual failure of physiological and/or biochemical pathways that culminates with the death of the organism. The causative factors of aging remain elusive, despite the increasing number of theories that try to explain how aging initiates. Interestingly, aging cells show an increase in intracellular water volume. All cells have a crowded cytoplasm, where the high concentration of macromolecules creates an environment that excludes small molecules such as water. In this crowded environment, water can be found in two states—termed low-density water (LDW), which shows low reactivity, and high-density water (HDW), which is highly reactive. LDW predominates in a macromolecular-crowded environment, while HDW is found only in microenvironments within cytoplasm.

The failure in the water homeostasis mechanisms changes the equilibrium between LDW and HDW, increasing the concentration of intracellular HDW. Being reactive, HDW leads to the generation of reactive oxygen species. The cell becomes less prone to repair damage when the concentration of HDW increases, resulting in aging and finally death. Interestingly, some biological mechanisms (e.g., anhydrobiosis) reduce the concentration of intracellular water and prolong the life of cells, possibly via a cellular anti-senescence biochemical mechanism. Some organisms adapt to persistent and severe stress by reversibly adjusting life-death balance to a new equilibrium, e.g., anhydrobiosis ("life without water") enables survival in a quiescent state of extreme desiccation. Aging is characterized

by declining response and increasing vulnerability to stress. In this sense, anhydrobiosis and related biological mechanisms could be used as a platform to study new anti-aging therapies via cellular osmoprotective agents (18). For example, topical application of carnitine in dry eye offers osmoprotection and modulates immune and inflammatory responses (19).

5.3.4 OSMOPROTECTION, CELLULAR ANTI-SENESCENCE, AND SKIN ANTI-AGING

This part of the chapter describes novel osmoprotective peptides as cellular anti-senescence agents for skin anti-aging applications.

Water is a key element for life. However, some organisms have evolved an amazing adaptation (osmoprotection) that allows them to survive under complete dehydration conditions for months or years, until water is present again, at which time they resume their metabolism and growth. Anhydrobiosis ("life without water") is found throughout all biological domains, from the hot bubbling mud flats of Yellowstone and thermal vents in the deep seas to frozen Antarctica, which includes several species of eubacteria, archea, some fungi, certain invertebrate species, and "resurrection plants." Cellular anti-senescence agents act through compatible solutes to prevent cellular damage.

Osmoprotection, a recently recognized new therapeutic methodology, offers new solutions for topical problems that relate to skin aging, including collagen synthesis, intracellular inflammation prevention, antioxidant protection, free radical scavenging, de novo DNA synthesis, control of wrinkles and fine lines, skin brightening, age spot reduction, and acne (19). Cells must maintain an optimal balance of water to stay plump and healthy. Imagine a face drawn on a balloon and filled with water. The face will look plump and youthful as the water keeps pressure on the balloon's inside surface. But if the water were to slowly leak (osmosis) out of the balloon, the drawn face would increasingly look wrinkled, tired, saggy, and aged.

Similar loss of internal cellular pressure due to water loss causes osmotic stress resulting in appearance of skin aging. Figure above published by permission of GCI Magazine, (20).

a. Hyperosmarity, Inflammation, and Cellular Senescence:

There are several reports suggesting that hyperosmolarity induces inflammation, which is one of the main causatives of cellular senescence. Schwartz et al. (21) have shown that there is a link between hyperosmolarity and inflammation by assessing osmolarity values *in vivo* during inflammation, and they have compared the inflammatory potential of different osmotic agents and studied the long-term consequences of hyperosmolarity on cell fate. The exposure of cells to the different compounds, whatever their molecular weight, has no effect on the secretion of cytokines as long as the osmolarity is below a threshold of 300 mOsm. Higher osmolarities result in the secretion of pro-inflammatory cytokines (Interleukin-8, Interleukin-6, Interleukin-1beta, and Tumor Necrosis factor-alpha).

Abolhassani et al. (22) report that hyperosmolarity can induce pro-inflammatory cytokine responses. Inflammation appears to be the simple consequence of a shift of methylation of Protein Phosphatase 2A, which in turn activates Nuclear factor-kappa B (NF-kappaB). The production of inflammatory cytokines causes the acceleration of aging process.

Sarkar et al. (23) report that a direct relationship exists between aging and increasing incidences of chronic diseases. In fact, with most age-associated diseases individuals manifest an underlying chronic inflammatory state as evidenced by local infiltration of inflammatory cells, such as macrophages, and higher circulatory levels of pro-inflammatory cytokines, complement components, and adhesion molecules.

Consequently, treatment with anti-inflammatory agents provide symptomatic relief to several aging-associated diseases, even as remote as Alzheimer's or Parkinson's disease, indicating that chronic inflammation may play a substantial role in the pathogenesis of these disease states. The molecular mechanisms underlying this chronic inflammatory condition during cellular senescence are presently unclear.

Cellular damage by oxygen free radicals is a primary driving force for aging and increased activation of redox-regulated transcription factors, such as NF-kappaB, that regulate the expression of pro-inflammatory molecules, has been documented in aged animals/individuals versus their young counterparts. Human polynucleotide phosphorylase (hPNPase [old-35]), an RNA degradation enzyme shown to be upregulated during differentiation and cellular senescence, may represent a molecular link between aging and its associated inflammation. HPNPase (old-35) promotes reactive oxygen species (ROS) production, activates the NF-kappaB pathway, and initiates the production of pro-inflammatory cytokines such as IL-6 and IL-8. In these contexts, inhibition of hPNPase (old-35) may represent a novel molecular target for intervening in aging-associated chronic diseases.

b. Chemical Basis of Hyperosmarity:

Chambers et al. (24) disclose that human urine is cellular anti-senescence for enteric bacteria, permitting E. coli to grow with high concentrations of NaCl and other salts and even higher concentrations of sucrose and mannitol but not urea. Two major cellular anti-senescence compounds in urine have been identified: glycine betaine, and proline betaine. The presence of glycine and proline betaines in human urine may reflect a cellular anti-senescence role for the kidney.

Chambers et al. (25) further report that glycine betaine is believed to be the most active naturally occurring osmoprotectant molecule for Escherichia coli and other bacteria. It is a dipolar ion possessing a quaternary ammonium group and a carboxylic acid group. To examine the molecular requirements for cellular anti-senescence activity, dimethylthetin was compared with glycine betaine. Dimethylthetin is identical to glycine betaine except for substitution of dimethyl sulfonium for the quaternary nitrogen group. Dimethylthetin was found to be about equally as effective as glycine betaine in permitting E. coli to grow in hypertonic NaCl, and both compounds were recovered almost completely from bacterial cells grown in the presence of hypertonic NaCl. 3-Dimethylsulfonioproprionate, an analog of dimethylthetin observed in marine algae, and 3-Dimethylsulfonio-2-methylproprionate were found to be less active. Dimethylthetin may prove useful as a molecular probe to study betaine metabolism and as a model for the development of antibacterial agents.

In modern medicine, dry eye syndrome is one of the most common disorders encountered in daily ophthalmological practice. Hyperosmolarity of the tear film is one of the key pathogenetic factors in the development of a commonly subclinical

inflammation of the ocular surface, the lacrimal gland, and the tear film in dry eye syndrome. Cellular anti-senescence agents prevent a hyperosmolar tear film from damaging the ocular surface. Future applications of anti-senescence agents in the treatment of dry skin condition are envisioned. While use of humectants and emollients has been a common practice, the application of osmoprotective anti-senescence agents in skin care has practically been unknown to modern cosmetology.

The agents that are known to provide osmoprotection in nonmammalian organisms and plants include trehalose, maltose, sucrose, palatinose, cellobiose, gentiobiose, turanose, sorbitol, calcium chloride, certain amino acids (such as proline and alpha-glutamate), alpha-D-mannopyranosyl-(1-->2)-alpha-D-glucopyranosyl-(1-->2)-glycerate, Di-myo-inositol 1,1'-phosphate, N(gamma)-acetyl-2,4-diaminobutyrate (NADA), ectoine, glycine betaine, carnitine, pipecolic acid, dimethylsulfoniopropionate, dimethylsulfonioacetate, peptones, taurine, and taltrimide.

Rosas-Rodriguez et al. (26) report enzymes involved in osmolyte synthesis, especially oxidative stress affecting osmoregulation in renal cells. Kidney medulla cells are exposed to a wide range of changes in the ionic and osmotic composition of their environment as a consequence of the urine-concentrating mechanism. During antidiuresis NaCl and urea concentrations increase and an efficient urinary concentrating mechanism is accompanied by medullar hypoxia. Medullar hypotonicity increases reactive oxygen species, a byproduct of mitochondria during ATP production. High intracellular ionic strength, hypoxia, and elevated ROS concentration would have deleterious effects on medulla cell function. Medulla cells respond to hypertonicity by accumulating organic osmolytes, such as glycine betaine, glycerophosphorylcholine, sorbitol, inositol, and taurine, the main functions of which are osmoregulation and osmoprotection.

Sagot et al. (27) report the dipeptide N-acetylglutaminylglutamine amide that was discovered in the bacterium Sinorhizobium meliloti grown at high osmolarity, and subsequently shown to be synthesized and accumulated by a few osmotically challenged bacteria. This key finding has hitherto been untapped.

Gouffi et al. (28) report that sucrose, trehalose, maltose, cellobiose, gentiobiose, turanose, and palatinose are very unusual osmoprotectants for Sinorhizobium meliloti, because these compounds, unlike other bacterial osmoprotectants, do not accumulate as cytosolic osmolytes in salt-stressed S. meliloti cells. Rather, these compounds were catabolized during early exponential growth, and contributed to enhance the cytosolic levels of the two endogenously synthesized osmolytes: glutamate and the dipeptide N-acetylglutaminylglutamine amide.

Talibart et al. (29) report the fate of exogenously supplied glycine betaine and the dynamics of endogenous osmolytes that were investigated throughout the growth cycle of salt-stressed cultures of strains of Sinorhizobium meliloti, which differ in their ability to use glycine betaine as a growth substrate, but not as an

osmoprotectant. Glycine betaine is only transiently accumulated as a cytoplasmic osmolyte, which virtually prevents the accumulation of endogenous osmolytes during the lag and early exponential phases of growth. Then, betaine levels in stressed cells decrease abruptly during the second half of the exponential phase. At this stage, the levels of glutamate and the dipeptide N-acetylglutaminylglutamine amide increase sharply so that the two endogenous solutes supplant glycine betaine in the aging culture, in which it becomes a minor osmolyte because it is progressively catabolized. Ultimately, glycine betaine disappears when stressed cells reach the stationary phase.

Dominguez-Ferrera et al. (30) report the disaccharide trehalose is a well-known osmoprotectant, and trehalose accumulation through de novo biosynthesis is a common response of bacteria to abiotic stress.

Perez-Arellano et al. (31) report that glutamate kinase, an enzyme involved in osmoprotection in plants and microorganisms, catalyses the first and controlling step of proline biosynthesis.

Lynch et al. (32) report a biopolymer that has been shown to facilitate efficient delivery of trehalose, a bioprotectant normally impermeable to the phospholipid bilayer, into ovine erythrocytes. Cellular uptake of trehalose was found to be dependent on polymer pendant amino acid type and degree of grafting, polymer concentration, pH, external trehalose concentration, incubation temperature, and time.

Kitko et al. (33) report diverse osmolytes, including NaCl, KCl, proline, or sucrose, contribute to cytoplasmic pH homeostasis in E. coli, and increase the recovery from rapid acid shift. Osmolytes other than K+ restore partial pH homeostasis in a strain deleted for K+ transport.

Iturriaga et al. (34) report trehalose, a nonreducing disaccharide that is widely distributed in Nature and has been isolated from certain species of bacteria, fungi, invertebrates, and plants that are capable of surviving in a dehydrated state for months or years and subsequently being revived after a few hours of being in contact with water. This disaccharide has many biotechnological applications, as its physicochemical properties allow it to be used to preserve foods, enzymes, vaccines, cells, etc. in a dehydrated state at room temperature.

Jorge et al. (35) report the discovery of a new solute, whose structure was established as alpha-D-mannopyranosyl-(1-->2)-alpha-D-glucopyranosyl-(1-->2)-glycerate(MGG). The level of MGG increased notably with the salinity of the growth medium up to the optimum NaCl concentration. At higher NaCl concentrations, however, the level of MGG decreased, whereas the levels of proline and alpha-glutamate increased about fivefold and tenfold, respectively. MGG plays a role during low-level osmotic adaptation of Petrotoga miotherma, whereas alpha-glutamate and, to a lesser extent, proline are used for osmoprotection under salt stress. Fernandez et al. (36) report that mannosylglucosylglycerate (MGG),

recently identified in Petrotoga miotherma, also accumulates in Petrotoga mobilis in response to hyperosmotic conditions and supraoptimal growth temperatures.

Jung et al. (37) report amino acid transport is a ubiquitous phenomenon and serves a variety of functions in prokaryotes, including supply of carbon and nitrogen for catabolic and anabolic processes, pH homeostasis, osmoprotection, virulence, detoxification, signal transduction, and generation of electrochemical ion gradients.

Garcia-Estepa et al. (38) report N(gamma)-acetyl-2,4-diaminobutyrate (NADA), the precursor of the compatible solute ectoine, to function as an osmoprotectant for the non-halophilic bacterium Salmonella enterica serovar Typhimurium.

Pintsch et al. (39) have shown that cells steadily face changes of the external osmolarity, to which they have to adapt. To withstand a steep increase in osmolarity, eukaryotic cells activate responses like "regulatory volume increase," accumulation of compatible osmolytes, and stimulated expression of stress proteins.

Recently, an exception from this scheme has been identified: Dictyostelium cells protect themselves against hyperosmolarity by largely rearranging cellular proteins, whereas no "regulatory volume increase," no accumulation of compatible osmolytes, and no change of the expression pattern of the most abundant proteins were observed. Among the translocated proteins identified, cytoskeletal proteins appear to be predominant. In particular, the rearrangement of actin and myosin II to the cell cortex beneath the plasma membrane was shown to constitute a pivotal element of osmoprotection in Dictyostelium. In this process the distribution of the actin-associated protein Hisactophilin (histidine rich; 31 histidine out of 118 amino acids), actin-binding protein from Dictyostelium discoideum was investigated in order to gain a better insight into the osmoprotective mechanism of the cell. Hisactophilin was found to be enriched in the cytoskeletal fraction of wild-type cells exposed to hyperosmotic stress. Hisactophilin is both translocated to the cytoskeleton and phosphorylated during hyperosmotic stress in Dicytostelium.

5.3.5 NEW PEPTIDE DERIVATIVES FOR ANTI-SENESCENCE AND SKIN ANTI-AGING

a. Chemical Discovery:

Sagot et al. (27) cite discovery of dipeptide N-acetylglutaminylglutamine amide as the key mediator in osmoprotection provided a lead. With a relationship between skin aging and senescence having being established (7), the new derivatives of glutaminylglutamine amide were prepared as osmoprotective anti-senescence agents for skin anti-aging topical applications (1).

In a first series of the derivatives of aloesin (one of the key osmoprotective active agents in aloe; Aloe vera) with amino acids, peptides and amino sugars have been reported (40).

For example, the reaction of aloesin with the dipeptide amide, glutamylglutamine amide, forms isomeric compounds of formula (I):

(I).

For topical applications it became clear that more bioavailable forms of the dipeptide amide that could be delivered via both water and oil phases of an emulsion formulation (such as a skin lotion or cream) were desirable. The compound of general formula (II) has recently been reported (1) and addresses the conditions of mammalian skin degradation resulting from cellular senescence and hyperosmarity (excessive skin dryness, for example):

(II).

The examples of general formula (II) include:

(III),

(IV),

(V),

(VII), and

(VIII).

b. Formulation Methodology:

A wide variety of compositions can be formulated with the peptide derivatives described above in various cosmetic and pharmaceutical consumer products utilizing a variety of delivery systems and carrier bases. Such consumer product-forms include body and hand lotions, skin creams, liquid soaps, bar soaps, bath oil bars, shaving creams, shampoos, aftershaves, sunscreens, hair conditioners, permanent waves, hair relaxers, hair bleaches, hair detangling lotion, styling gel, styling glazes, spray foams, styling creams, styling waxes, styling lotions, mousses, spray gels, pomades, shower gels, bubble baths, hair-coloring preparations, conditioners, hair lighteners, coloring and noncoloring hair rinses, hair-grooming aids, hair tonics, styling waxes, band-aids, and balms. Of these, skin care lotions and creams that are left on skin (leave-on) are preferred over rinse-off formulations such as shampoo and liquid soaps.

The delivery systems for formulations represent traditional water and oil emulsions, suspensions, colloids, microemulsions, clear solutions, suspensions of nanoparticles, emulsions of nanoparticles, or anhydrous compositions.

Consumer- and marketing-preferred ingredients such as skin cleansers, cationic, anionic surfactants, nonionic surfactants, amphoteric surfactants, and

zwitterionic surfactants, skin- and hair-conditioning agents, vitamins, hormones, minerals, plant extracts, anti-inflammatory agents, collagen and elastin synthesis boosters, UVA/UVB sunscreens, concentrates of plant extracts, emollients, moisturizers, skin protectants, humectants, silicones, skin-soothing ingredients, antimicrobial agents, antifungal agents, treatment of skin infections and lesions, blood microcirculation improvement, skin redness reduction benefits, additional moisture absorbents, analgesics, skin penetration enhancers, solubilizers, moisturizers, emollients, anesthetics, colorants, perfumes, preservatives, seeds, broken seednut shells, silica, clays, beads, luffa particles, polyethylene balls, mica, pH adjusters, and processing aids can also be included.

It is preferred to formulate these compositions in a pH range of 4.5 to 6.5. These peptide derivatives can be added directly in either hot or cold process formulations. When adding to cold process formulations it is advisable to first check the solubility of these peptide derivatives in cold base. If poor solubility is observed, then a presolubilization in one of the liquid components in which these peptide derivatives are soluble is advised. Heat may be applied, as necessary, for presolubilization.

The illustrative examples follow.

Anti-Aging Cream:

Ingredients (weight percent). (1) Water 55.9 (2) Dicetyl Phosphate (and) Ceteth-10 Phosphate 5.0 (3) Glyceryl Stearate (and) PEG-100 Stearate 5.0 (4) Phenoxyethanol 0.7 (5) Chlorphenesin 0.3 (60) Titanium Dioxide 0.2 (7) Sodium Hydroxide 0.5 (8) Magnolol 0.2 (9) Boswellia Serrata 0.5 (10) Cetyl Dimethicone 1.5 (11) Tetrahydrocurcuminoids 0.5 (12) Shea butter 2.0 (13) Ximenia oil 1.0 (14) Niacinamide Hydroxycitrate 2.2 (15) Ethyl Lactate 15.0 (16) Niacinamide Salicylate 4.0 (17) Compound of formula (III) 0.1 (18) Paeonol 1.5 (19) Carnosine 0.1 (20) Cyclomethicone, Dimethicone Crosspolymer 2.0 (21) Arbutin 0.5 (22) Polysorbate-202.0 (23) Polyacrylamide 2.0.

Procedure. Mix (1) to (15) and heat at 70º to 80ºC till homogenous. Cool to 40º to 50ºC. Premix (16) to (22) and heat, if necessary, to a solution and add to main batch with mixing. Cool to room temperature and add (23) and mix. An off-white cream is obtained.

Anti-Inflammatory Transparent Gel:

Ingredients (weight percent). (1) Ethyl Lactate 96.0 (2) Hydroxypropyl Guar 1.0 (3) Ximenia Oil 0.1 (4) Compound of formula (IV) 1.0 (5) Magnolol (and) Honokiol 0.2 (6) Paeonol 0.5 (7) Compound of formula (VII) 0.2 (8) Fragrance 1.0.

Procedure. Mix (1) and (2) and heat at 50º to 60ºC till clear. Cool to 40º to 45ºC and add all other ingredients and mix. Cool to room temperature. A transparent gel-like product is obtained.

5.3.6 CONSUMER PERCEPTION AND MARKETING OF ENZYME BIOLOGY-BASED SKIN CARE PRODUCTS

It's a jungle out there!

Marketing buzzwords, unclear product benefit claims, and a constant flow of "new and improved" anti-aging products with flashy advertisements and celebrity endorsements—all lead to an ever-growing consumer confusion and product dissatisfaction over claimed performance. A current popular marketing mode is to showcase a list of "percentage claims" bullet points, such as "87% of users say they saw fewer lines in 4 weeks." These claims are based on the users' own observations, looking in their own mirrors. The use of a good moisturizer alone, on a regular basis, can cause a consumer to sense lines are diminished! Is it the moisturizing ingredients, or the featured anti-aging peptide, that is actually causing line softening in a short time frame? Is this confusing or misleading to the consumer at times? Regardless, this "immediate result" marketing mode is rapidly training consumers to expect fast results from their anti-aging products.

The most enduring skin change comes from patiently coaxing cells to perform at an optimal level via biology-based ingredients such as enzymes. This slower, cumulative inductive method strengthens the cells' own intrinsic protection against premature senescence. However, is there an easy solution to help consumers, as well as skin care professionals, develop a better understanding of modern cosmetology that is based on biology and cellular function versus more superficial concept products?

Explaining enzyme biology to consumers and skin professionals certainly offers challenges. The consumer perception of enzymes is typically related to laundry applications for spot and stain removal and papaya fruit for digestion! Professionals are mainly aware of topical use of fruit enzymes like papain and bromelain for gentle exfoliation and smoothing. Providing all groups an objective, easy-to-understand how, what, and why of various enzyme mechanisms that harmoniously operate in concert in skin-aging processes would be a formidable task. Perhaps a campaign that first helps them understand that the best anti-aging skin care ingredients just may perform more like a plodding, dull workhorse, than a fast, exciting racehorse, in order to achieve the goal of delayed cell senescence.

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GLYCATION, PROTEASOME ACTIVATION, AND TELOMERE MAINTENANCE

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ABSTRACT

Theories of why we age are numerous and no general consensus has resulted from the many years of research. A review article of the various theories of aging (Huber and Hess 2010) lists ROS (free radicals), mitochondrial dysfunction, telomere shortening, and glycation as currently held opinions on why we age.

This chapter briefly describes the two latter mechanisms, focusing on aspects of skin physiology, and then reports some of the latest developments of cosmetic ingredients designed to alleviate and, wherever possible, reverse the aging symptoms that are connected to these causes. As one reads through the literature from various databases (PubMed, Google Scholar, Kosmet), it becomes clear that not one of the various "concepts" often singled out by cosmetic research communication stands truly alone as a target for anti-age activity. Stem cells, sirtuins and hormesis, proteasome and mitochondrial activity, ROS, telomere length, glycation, proteolysis, inflammation, and melanogenesis, all hang together in complex interplays, making any classification of research articles (or ingredient-data brochures) to one or the other topic rather frustrating. It is even worse for finished products, which, except for very specific cases, mix these ideas and properties in often quite arbitrary fashion, depending on the current market and competitive situation. We cannot even really distinguish the two main categories of anti-age cosmetics of "prevention" and "treatment," as many ingredients possess activities that promise and deliver both aspects, as a review of the relevant literature in peer-reviewed and in technical trade journals shows. A typical example:

Blood circulation becomes poor due to aging or a disturbed daily rhythm, which leads to skin problems such as dark eye circles and lip disorders. In this study, we focused on developing Piper Longum Fruit as a cosmetic ingredient for

improving poor blood circulation. . . . Piper Longum Fruit extract promoted blood circulation by the topical application on human skin. In addition, Piper Longum Fruit extract showed various activities such as promotion of keratinocyte proliferation, increase of filaggrin synthesis, inhibition of melanin synthesis and anti-glycation. These results suggested that Piper Longum Fruit extract might be a useful ingredient for cosmetics. (Kiso 2011)

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5.4.1 GLYCATION

As so many other topics, glycation (and anti-glycation) are recurring themes in cosmetic research. As our understanding progresses, new ideas of how to fight glycation and/or how to reverse the process once initiated are investigated and tested. Scientists at Estée Lauder some time ago called anti-glycation *the* tool to fight aging (Maes et al. 2007). They then soon also became interested in the proteasome, the sirtuins, then the stem cell concepts, etc. As we shall see, though, all these ideas are somewhere connected.

Glycation is a non-enzymatic process whereby various sugar molecules react nonspecifically with proteins (and other macromolecules). This phenomenon

increases with age, in all types of tissue, particularly in people with diabetes, and leads to degradation of enzymatic vital functions. These include brittleness in collagen and elastin fibers, leading to wrinkles, decreased elasticity, and reduced efficacy of cellular mechanisms. Ingredients with antiglycation activity attempt to prevent this sugar-attachment to proteins; some progress has even been made with partial reversal of the glycation reaction, not only *in vitro* but also in some clinical studies. For example, skin aging has long been associated particularly with exposure to sunlight, but glycotoxins from the diet or even from cigarette smoke (Cerami et al. 1997) are at least as important a factor in skin aging. The term glycotoxins comprises a heterogeneous group of molecules, many derived from sugars, which bind to the different components of the cell and change their qualities or properties.

Our body needs sugar, glucose, to produce its energy. Glucose, however, is not entirely used by the body and some binds directly to protein amine groups, nucleic acids, or lipids and creates Advanced Gyration Endproducts (AGEs). These compounds were described for the first time by Maillard in 1912.A brief review of the field is given by Draelos and Pugliese (2011).

AGE formation involves several successive stages (see Figure 1). In the early stages, the aldehyde group of a reducing sugar such as glucose forms an unstable bond with the protein amine, known as a Schiff's base, which rapidly rearranges to produce an Amadori compound. These two formations are reversible. The next, later, stage leads to the formation of almost irreversible advanced glycation endproducts, which accumulate over time in tissues.

At the Amadori compound stage, the sugar can deprotonate and create several highly reactive compounds that themselves become glycotoxins: glyoxal (GO) or its methylglyoxal derivative (MGO), 3-deoxyglucosone (3-DG), and fructosamines (Thornalley et al. 1999).

Figure 1: Simplified mechanism of formation of AGEs

Glyoxals and 3-DG can in turn bind to free protein amines and produce different AGEs such as carboxymethyl-lysine (CML), carboxymethyl-arginine (CMA), carboxymethyl cysteine (CMC) , pentosidine, and pyrraline. These reactive chemical groups can also be formed by glucose auto-oxidation. Other sugars such as ribose or xylose, although present in smaller amounts, are far more reactive than glucose and fructose and also produce AGEs, which have only been shown to be toxic *in vitro* in the absence of reliable *in vivo* demonstration methods. Experimentally, these products are formed very quickly (Wei et al. 2009).This leads to the formation of many altered proteins (particularly those with a long half-life), whose functional, enzymatic, and structural properties are also changed with serious consequences on the cell or body. SOD and catalase are among the enzymes most often cited.

Collagen, for instance, has a half-life of 15 years and therefore contains three times more carboxymethyl-lysine (CML), carboxyethyl-lysine (CEL), and pentosidine at age 80 than at age 20 (Page on et al. 2010).

Mizutari (1997) studied the influence of UV exposure and showed that UV-induced oxidation accelerates CML formation in actinic elastosis of photo-aged skin, demonstrating once more that the various influences on aging complement and reinforce each other (oxidation, UV irradiation, glycation . . .).

Glycated collagen also promotes apoptosis, both after injection and in contact with cultured fibroblasts. This may be due at least partly to overproduction of H_2O_2 by methylglyoxal (MGO) and glyoxal (Sejersen & Rattan 2009), which would then explain the acceleration in the senescent phenotype of these cells. The AGEs also activate inflammatory mechanisms that themselves are pro-oxidizing, further fueling the aging process.

Although the process of glycation occurs throughout the body, and affects all proteins, a study by Kueper et al. (2007) shows that the cytoskeletal protein vimentin is a specific and primary target of glycation in skin, leading to reduced contractile function in fibroblasts. Fibroblasts in the skin's dermis create collagen and the elastic fibres necessary for young looking skin. An interesting analysis of glycation specifically occurring in the stratum corneum taking into account environmental factors is presented by Gomi et al. (2010).

Many more interesting scientific studies on glycation and the skin can be found in the literature. Their detailed presentation here would go beyond the scope of this chapter and book.

a. Measurement of AGEs

Knowing how detrimental the formation of AGEs is for the body, it had been an important challenge to measure and quantify the amount of glycation occurring in an organism. For many years, diabetologists used skin biopsies and histological AGE

labeling to estimate the severity of a patient's state. This very invasive method had then been replaced by measurement of blood-glycated haemoglobin, which is less invasive and can, for example, be repeated if the patient is in a hospital. More recently, an instrument called AGE-Reader™, which measures AGEs present in the skin completely painlessly by autofluorescence, has become available. The AGE-Reader™ (DiagnOptics Technologies) is the first medical device allowing non-invasive measurement of AGE accumulation. The volunteer's skin is exposed to light at a wavelength of 370 ± 50 nm, which excites functional fluorescent groups, particularly those in the AGEs. The reflected light is transmitted selectively by a 50 μm diameter fiber and measured by spectrophotometry (Lutgers et al. 2006). In practice, the volunteer places his/her forearm on the instrument and measurements are taken through a 1 cm^2 window.

From such studies, a remarkable fact has come to light: there is a strong linear correlation between AGE-Reader™ readings and the age of the person measured; to a point where a significant increase in (arbitrary) units on the instrument readings of 0.023 per year of subject age can be read on a graph; even somewhat different regression lines are obtained for smokers or diabetics (Koetsier et al. 2010).

Maes and his team went further in calculating apparent age by using both the autofluorescence of skin combined with facial skin parameters to predict the efficacy of cosmetic products and to assess the predisposition to accelerated aging (Dicanio et al. 2009, Corstjens et al. 2008).

b. Prevention and/or Reversal of Glycation/Glycoxidation

Is there a way to prevent glycation, in general, and on the skin in particular? And even better: Is there a way to reverse it, to eliminate glycated structures or to "deglycate" them? Surprisingly—in view of earlier documents that call glycation an "irreversible" deterioration of macromolecules—there have been at least partially successful attempts to fight this particular aspect of aging.

Of course, reducing sugar intake in our diet and consuming food rich in antioxidants are first measures of prevention. Not using monosaccharides (honey . . .) in cosmetic formulas would be another logical consequence.

More to the point of the present chapter: AGEs are not formed immediately, glycation proceeds in two phases, the first stage is rapid, the subsequent more slowly. The early forms of AGEs are generated either directly or indirectly, via glyoxal or methylglyoxal. Cells have an active mechanism based on an enzyme system called Glyoxalase -1 and -2, which detoxifies the glycotoxins via glutathion GSH (Rabbani & Thornalley 2009). Experimental overexpression of glyoxalase-1 in diabetic animals increases their lifespan, whereas any reduction in its activity reduces survival.

As is so often the case with the organism's defense mechanisms: the very systems (enzymes) that exist to protect cells and organs are equally affected by the very agents they are designed to inactivate. The efficacy of antioxidant enzymes (SOD, catalase, glutathion peroxidase) is decreased by oxidative attack (and by glycation!), and the glyoxalase enzymes are equally subject to these destructive processes: glycation products tend to reduce glyoxalase activity.

Diabetes is a major and widespread disease, justifying extensive pharmacological research in preventing and treating it. Cosmetic research, interested in the aspects of skin aging, has indirectly benefited from the learnings and has generated a great number of studies and research projects attempting to prevent and/or reverse skin-based glycation/glycoxidation. In the following paragraphs, we shall briefly present some of the ideas and results obtained in recent years.

Given the fact that many papers on the mechanisms of glycation mention oxydation as an essential element in the chemical reactions of glycation/glycoxidation (e.g., Baynes 1991), it is not surprising that many if not most anti-glycation proposals are based more or less consciously on antioxidant activity. The other major idea is to supply "sacrifice" substances that compete with the reactive sugar molecules, thus preventing their attachment to proteins. Aspirine for example attaches acetyl groups to the amines of lysine, thus blocking the reaction sites (Watala et al. 2005).

A great number of plant extracts with more or less defined content of specific active substances, but consistently tested for antioxidant activity, are thus proposed as "anti-glycation" anti-age "actives." The AGE-Reader™ technology, not yet being widely used, appears to be best suited to substantiate the anti-glycation efficacy based on *in vitro* studies.

Schmid et al. (2002) relate the effects of a combination of grape seed procyanidines and a-g-d-tocopherol on AGE and Amadori production with bovine serum albumin incubated with glucose. Aminoguanidine, a well-known benchmark for anti-glycation activity, was used as control. Inhibition of AGE formation achieved 90% with the antioxidant mixture.

Pauly et al. (2011) and Jeanmaire (2011) showed *Manilkara multinervis* leaf extract[1] to be active in both *in vitro* and *ex vivo* protocols; the same team (Jeanmaire et al. 2005) had already investigated the bark extract of *Pterocarpus marsupium Roxb*. on its activity in isolated hair follicles, assuming that glycation contributes to hair loss and baldness. A clinical study appeared to confirm the hypothesis.

Yashihiki (2012) focused on UME extract (Japanese Prune mume, often presented as a health drink) for improving skin dullness. This result mirrors the radiance improvement demonstrated *in vivo* by the cosmetic topical use of fermented black tea (Kombucha, the traditional Mongolian beverage),[2] tested in similar ways.

Further plant-derived *in vitro* tested anti-glycation ingredients are puerarin and chlorogenic acid (Gasser et al. 2011), sunflower (*Helianthus annuus*) seed cake extract[3] (Lenaers 2007), an antioxidant series of garlic (*Allium sepa*) skin, *Illicum religiosum* (bark and wood), rosemary leaf extract, *Origanum officinals*, etc. (Kim 2003), *Panshanbheda* and pomegranate flower and rose apple leaf (Kanazawa 2012), *Paenia suffruticosa* and *Sanguisorba officinalis* (Okano 2001).

Additional interesting papers on glycation were presented at the 2010 IFSCC congress in Buenos Aires. Fitoussi et al. (2010a) were interested by the aforementioned observation on vimentin being a prime target of glycation (Kueper 2007) and carried out a study to show the ability of a *Chrysophyllum* extract to prevent the glycation phenomenon and the glycation consequences in human skin fibroblasts. On cultured normal human fibroblasts, the authors show that by inhibiting type I collagen and vimentin glycation, *Chrysophyllum* extract retards the cellular aging process and enables dermal fibroblasts *in vitro* to retain their motility and their important contractile power.

D'Arcangelis et al. (2010) sought to study the consequences of lowering pH from 7.0 to 3.5 on protein glycation *in vitro*. Reaction of reducing sugars with bovine serum albumin (BSA) or a Gly-Lys dipeptide to produce fluorescent glycation products was decreased proportionally with decreasing pH. The decrease observed as the pH is lowered to <7.0 is amplified by the addition of glycation inhibitors, aminoguanidine, or Bay cedar extract, which further lower the accumulation of fluorescent products in an additive manner.

This group of researchers had previously shown that this Bay cedar extract acted by stimulating the skin's own cellular defense/repair system composed of fructoseamine-3-kinase (FN3K), an apparent de-glycation system. An observed increase in FN3K after Bay cedar extract incubation led to partial restoration of glycation-related damages (Federova et al. 2008).

A rather surprising approach is described by Kim et al. (2012),using gold nanoparticles, was shown to prevent collagen glycation, albeit again just *in vitro*.

Other nonplant-based approaches to inhibiting glycation should be mentioned:

Rather than just preventing oxidative steps, scientists from Exsymol developed Decarboxy Carnosine (Carnicine), which acts as a transglycation agent able to detach sugars from proteins and scavenging them on the NH2 group of beta-alanine (Lafitte 2008, Nicolay 2010). Although interesting, this original approach still requires field-testing in a clinical trial.

Monsan (1998, 2001) was probably the first to try oligopeptides composed of lysine and arginine only, obtained by enzymatic synthesis[4] as sacrifice molecules to prevent glycation of the skin's proteins. Much more recently, Guglielmini et al. (2012) described an azeoloyl-tetrapeptide[5] that possesses antioxidant and anti-glycation

activity. The peptide reduces AGE formation in fibroblasts by ≈50% and ROS in keratinocytes by similar amounts. A vehicle-controlled clinical study documents improved skin parameters (wrinkles, elasticity) after six weeks of treatment.

Merino (2010) took a step in a different direction and measured glycation on DNA, rather than proteins, also using a peptide (Tripeptide 9 Citrulline).[6] The preparation chelates harmful copper ions and prevents the reaction of methylglyoxal with DNA *in vitro*.

Draelos et al. (2009) carried out one of the first cosmetically inspired clinical studies on diabetic skin, using several parameters to judge the potential beneficial effect of a topical product containing C-xyloside and blueberry extract. C-xyloside was chosen as a GAG synthesis stimulator and blueberry extract (an antioxidant) for its anti-glycation potential. Dermatological scoring of wrinkles, fine lines, smoothness, skin tone, sagging, etc. was coupled with the measurement of AGE associated skin autofluorescence. Most of the scores improved significantly over 12 weeks, including skin moisture (Corneometry, $p<0.001$), which in itself might be the cause of the overall improvement. Unfortunately no control formula without the "actives" included was used in the study. The AGE autofluorescence values did not change significantly over the time studied, a fact that might be explained by the self-imposed tough challenge of using diabetic skin. It was previously mentioned that the skin appears to have an intrinsic defense and repair system for glycation-related phenomena. Deglycating enzymes (called Amadoriases) have been evidenced in vertebrates (Monnier and Sell 2006), but the discovery has not yet led to development of true AGE-breakers. Alagebrium®[7] once considered to be a promising prescription drug with evidence for being able to revert AGE formation, but has not succeeded in establishing a market. Tada et al. (2008) presented intriguing possibilities for achieving the breakage of cross-linked proteins using YAC extract (Artemisia princeps). On the other hand, Matsuura et al. (2006) studied plantago side and penathydroxy flavanones, which cleave a-diketones and AGE cross-links *in vitro*.

The most recent offering to reduce the AGE related symptoms and skin damages, is again plant based: An extract of *Albizia julibrissin*.[8] Numerous endpoints were measured extensively both *in vitro*, *ex vivo* (3D model), and in vehicle-controlled clinical trails, including the skin's autofluorescence *in vivo* (Mondon et al. 2012).It is important to understand that in almost all studies described in the previous paragraphs, the observed anti-glycation effect was followed in protocols of "prevention."As the following data show, the Albizia extract provides both protective (preventive) and curative (reversal) activity.

In all following cellular and skin model experiments, a sufficient preincubation period of the cells and/or the *ex vivo* skin with methylglyoxal **preceded** the contact with a control medium or the *Albizia*-containing medium. Thus, glycation and the catalytic production of AGEs had already been set in motion before any preventive

and/or curative action of the plant extract was initiated. This is a more challenging and more reality-relevant protocol than simultaneous incubation of cells or skin with MGO (the "aggressor") and the active ingredient (the "protector").

c. In vitro data

An extract of *Albizia julibrissin* (called the "night sleeper" in Persia) was prepared by lixiviation. In screening assays it turned out that this particular extract inhibited the glycation of BSA (bovine serum albumin) with glucose by close to 50%.

From this initial observation, further studies were undertaken to investigate the mechanisms and efficacy of anti-glycation activity. Carboxymethyl-lysine (CML) and Carboxyethyl-lysine (CEL) are typical AGE products of collagen aging and glycation *via* a reaction with methylglyoxal (a first oxidized derivative of sugar). Treating fibroblasts in culture with MGO leads to a strong reduction in cell viability (–44%) and a huge increase in CML (+124%, $p<0.01$). When the cells are **subsequently** in contact with the *Albizia* extract for five days and CML levels are again checked in comparison to untreated cells, they decrease by about 43% in the *Albizia*-contacted cells.

d. Ex vivo data on explants

Table 1

product	AGEs (AFU*)	% change; significance
MGO, then vehicle cream	9.56± 1.30	baseline
MGO, then 4% cream	7.33± 1.40	–23.4%; $p<0.01$

* AFU: arbitrary fluorescence units

Human abdominal skin explants from a 34-year-old woman were placed in contact with MGO dissolved in the survival medium. After the glycation phase, the skin (n=5/case) received an application of a cream containing 4% of the *Albizia* extract preparation or the vehicle cream (both of which were used in the following clinical tests). Topical applications were administered once per day for 4.5 days. The skin sections were cut with a microtome before being labeled with a fluorescent anti-AGE antibody. Ten photos/cases were taken and analyzed on image analysis software. The results (Table 1) show that AGE labeling was located particularly in the dermis, an area rich in stable matrix proteins, and significantly reduced in the treated skins.

This is confirmed by the quantification of glyoxalase-1 synthesis in these MGO-treated fibroblasts where a Western blot analysis shows a 56% drop in detoxifying glyoxalase-1 in the control media, whereas the *Albizia*-treated cells maintain their initial glyoxalase-1 level.

We have previously mentioned Vimentin as a major target of glycation. MGO-incubated and then vehicle-treated skin samples showed a drastic reduction in Vimentin, whereas *Albizia* extract prevented/repaired this effect and maintained Vimentin staining 75% higher ($p<0.01$), which in a parallel experiment preserved fibroblast contractile capacities (data not shown).

e. In vivo studies:

44 panelists with stressed skin (as determined by AGEs present in adhesive strippings) were enrolled in randomized vehicle-controlled clinical trials on three subgroups. Endpoints measured were autofluorescence (AGE-Reader™), AGE content in tape strippings of the forearm, skin fatigue analysis using the Reviscosimeter™ method, and a daily and weekly self-assessment of facial fatigue every morning. Creams with either 2% (day cream) or 4% (night cream) of the *Albizia* preparation were tested against the identical vehicle without the plant extract. Application sites were the two forearms; frequency of application was twice a day over a two-month period.

Fig. 3: Decrease in AF values after two months' treatment

The graph shown in Figure 3 indicates the difference in AGE-Reader™ Autofluorescence (AF) values between the *Albizia*-treated skins and the vehicle-treated ones, after two months. AF values are converted into AGEs, glycated collagen, or Pentosidine according to Lutgers et al. (2006), Greven (2010),and Samborski (2011) and can further be interpreted in age differences based on a large datafile linking AF values to skin age (Koetsier 2010).

Similar significant results were obtained with the Reviscosimeter method. Self-assessment (scoring of various facial features such as baggy eyes, dark circles, and

drawn faces every morning at wakening, in front of a mirror), established the visible benefits of a novel cosmetic ingredient to combat aging and skin fatigue both *via* preventive and curative treatment of glycation, and confirmed that *Albizia* is not without reason called "night sleeper," as skin treatment with this extract appears to give similar results as a good "beauty sleep."

The example presented here shows, on the basis of the extensive *in vitro*, *ex vivo*, and clinical data, that an approach to simultaneous immediate skin beautifying and long-term physiological skin care is possible and justifiable. The measurable results on the three panels of volunteers are in good correlation with the proposed *in vitro* mechanisms of activity of the ingredients formulated in these preparations.

f. Conclusion

The study of glycation and the fight against this age-related phenomenon in skin care has come a long way. There is still much room for further discoveries and development of increasingly efficacious ingredients, especially now that easily measured values can be obtained in clinical studies, which after all are the final judge of cosmetic efficacy.

5.4.2 THE PROTEASOME

a. Introduction

The skin is exposed to many external stresses: UV, oxidizing agents, pollution, and biological agents, inducing more or less chronic micro-inflammatory effects and the formation of noxious products in or around the epidermal cells, including pre-inflammatory mediators and carbonylated proteins, lipoperoxides, and lipofuscin, which disturb cellular physiology and homeostasis, adversely affecting mitochondrial respiration, membrane integrity, enzyme activity, and purification mechanisms such as the proteasome. Accumulation of these noxious products, which can be seen in a simple keratinocyte culture, (see Figure 4) can be assessed both from their harmful effects and also from their ability to absorb light and reduce epidermal transparency.

Figure 4: Keratinocyte cultures without (left) or with (right) accumulations of noxious products

Fig 5: A view down the central core of the proteasome complex

Aging is of course another factor contributing to this phenomenon. Accumulation of these products of oxidative processes disturbs cellular metabolism and homeostasis. Among them are oxidized proteins and protein aggregates. On the other hand, in a functioning metabolic system, oxidized proteins are constantly being degraded, mainly by the proteasome. During aging, however, proteasome activity declines and the ability to degrade oxidized proteins is attenuated (Petropoulos et al. 2000, Widmer et al. 2006). Maintaining the activity of the proteasome is therefore one of the targets of present cosmetic research, as its increased activity would supplement the cells' anti-aging defense armamentarium.

The proteasome is a cylindrical complex containing a "core" of four stacked rings around a central pore. It is composed of a number of protein units (Peters et al. 1994) with at least three distinct proteolytic-active centers. Its precise functioning and the factors regulating its activity are highly complex and cannot be discussed in detail here; for details see Suga et al. (1993) and Brégégère et al. (2006). In fact, the proteasome is part of the UPS (ubiquitin-proteasome system). Ubiquitin is a 76 amino acid globular protein that is first attached to those structures destined for degradation by the proteasome. The proteins thus ubiquitinated then enter the proteasome channels where they are broken down into more easily eliminated fragments.

b. Cosmetic approach to proteasome activity

Given the extreme complexity of proteasome (and UPS-related) regulation, and the rather only recent interest in the subject, only a number of rather general attempts to incorporate "proteasome activation" into active ingredient concepts have been published. Interestingly, a number of studies linked proteasome activity to questions of glycation, as glycated proteins (see previous section) need elimination from cells and extracellular space.

Domloge et al. (2009) looked at the expression of the 20S proteasome and of ubiquitin in aged keratinocytes *in vitro* and determined changes in the expression and synthesis as a function of various stresses, including glycation. They also studied the effects of an (undisclosed) "active ingredient" on enhancing proteasome 20S and ubiquitine expression. The experiments conducted revealed (in this *in vitro* model) that the active ingredient was able to increase proteasome expression and enzymatic activity, keratinocyte proliferation, and decreased senescence. Glycated 3D skin models (*ex vivo*) appeared with improved morphology after active treatment.

In a follow-up publication, Imbert et al. (2010) describe the activity of a dimerized tripeptide in this protocol, confirming the maintenance of the UPS system by this treatment with the peptide. The results remain at the *in vitro* and *ex vivo* level.

Belot et al. (2009) present calcitrol as an effective anti-aging ingredient preventing UV induced senescence, DNA damages, and SA-b-galactose; DNA array experiments also indicate the activation of proteasome and overexpression of ARE[9] genes. This should stimulate research into vitamin D-mimetic substances, given the fact that EU regulation excludes the use of vitamin D in cosmetic products.

In an indirect approach, Kueper et al. (2010) show that diglycerol phosphate DGP (found in *Archeoglobus fulgidus*, a marine prokaryote) possesses numerous protective properties, including the stimulation of HSP27 synthesis and release. This heat-shock protein is directly associated with 26S proteasome, from which fact the authors estimate that DGP helps—besides all other benefits described in the paper—to act on clearing out damaged proteins *via* this mechanism.

A peptide complex extracted from delipidated avocado pulp[10] was shown also to have multifaceted activity in various *in vitro* models (Baudouin et al. 2010). The extract stimulates collagen I and elastin expression, inhibits MMP1 activity, activates LOX and LOXL enzymes, has anti-oxidant properties, and increases proteasome expression in young and older skin cells. A vehicle-controlled clinical test indicates a replenishing of the epidermis, but the observed effect is certainly due to the conjunction of all mentioned *in vitro* mechanisms; it is difficult to conclude on the specific proteasome activation contribution.

At the same event (IFSCC congress in Buenos Aires), Fitoussi et al. (2010b) presented data on a *Cochlearia* extract on artificially aged (hydrogen peroxide stressed) fibroblasts in which proteasome activity is increased over baseline, resulting in a decreased level of protein oxidation.

Obermayer and colleagues (2011) developed a blend containing olive leaf extract, tonic extract from Jujube, and a tightening polysaccharide Levan,[11] said to activate the proteasome. By reactivating the proteasome, the symptoms of premature skin aging such as wrinkles, skin hydration, and skin renewal are markedly improved, leaving skin visibly and perceivably younger, according to the author.

Figure 6: Autophagy—backup for the proteasome under conditions of stress (Ding et al. 2008)

The UPS system is, however, not unique in helping cells to get rid of unwanted debris and noxious accumulation. A research team from Silab (Boudier et al. 2010) became interested in the alternative and complementary system called autophagy. Indeed, there are two major systems in eukaryotic cells that degrade cellular components: the ubiquitine-proteasome and the autophagy pathways. At the basal level, the proteasome is involved in degradation of short-lived soluble proteins, while autophagy is a dynamic process that essentially eliminates long-lived proteins (the majority of cellular proteins) and altered organelles such as mitochondria, which are sources of reactive oxygen species and DNA mutations. Under conditions when the proteasome is overwhelmed or itself damaged by oxidation, by glycation and breakdown, autophagy takes over for the proteasome to compensate for its failure. The autophagic machinery ensures the degradation of soluble altered proteins and also maintains its role in elimination of insoluble proteins, oxidized lipids, and damaged organelles.

This proteolytic or cellular "self-eating" system, of detoxification may serve as a target for the development of detoxifying active ingredients in cosmetics. The authors (Aymard et al. 2010) describe the studies in detail to demonstrate the idea:

Human keratinocytes were treated for three hours with increasing but sublethal concentrations (125μM, 250 μM, and 1,000μM) of H_2O_2. Under these conditions of H_2O_2 treatment (125 and 250 μM), no apoptotic or necrotic pathways were induced, suggesting that potential detoxification pathways might be activated. Determination of the level of oxidized proteins following oxidative stress under these sublethal conditions showed that immediately after moderate oxidative stress, the level of oxidized proteins increased in a dose-dependent manner. However, in a step called "recovery," the level of oxidized proteins returned rapidly to the level of the control cells, posing the question whether the autophagic system or the proteasome (or both) was active during this cell recovery step.

c. The study of the LC3-II protein

A specific marker of autophagy found that after 1.5 hour of recovery, expression of LC3-II significantly increased, particularly with the H_2O_2 treatment at 250 μM. This result, combined with an increase in the number of lysosomal compartments observed in skin cells under the same treatment conditions, supported the hypothesis that these skin cells activated the autophagic system to eliminate oxidative damage. In confirmation of this observation, a specific inhibitor of the autophagic system under these oxidative conditions limited the return of the level of oxidized proteins to the level similar to that of control cells, indicating that autophagy was the main mechanism of detoxification in the study. An extract of a fermentation broth of the fungus *Candida saitoana*[12] was shown to increase LC3 expression by ≈20%, a similar decrease in oxidized proteins and lipids and a measurable improvement of skin tone, radiance, and complexion (Pitman 2009).

One of the targets of proteasome research is to help reducing glycation phenomena in the skin. The *Albizia julibrissine* extract[13] described in the previous section on glycation has also been tested in this context.

In one study, bovine serum albumin (BSA) and pre-formed glycated (BSA-AGE) were placed in contact for a week with the *A. julibrissin* extract to detoxify the protein. More or less deglycated BSA was left in contact with fibroblasts for three days and the persisting proteasome activity was measured.

Table 2: Change in proteasome activity after contact with AGE proteins

	Proteasome activity*	% Change; significance	
BSA control	971+ 64	baseline	–
BSA-AGE control	813 +91	–16%; p=0.01	2nd baseline
BSA-AGE + Albizia extr.	944+59	–	+16% ; p<0.05

* Proteasome activity in ΔUA/min/106 cells

The extract has no effect on the proteasome under basal conditions.

Table 2 demonstrates that BSA-AGE placed in contact with cells reduces the activity of the cell-clearing system, confirming published findings and showing that the extracellular glycotoxins can change the actions in the cell.

It also shows that the *A. julibrissin* extract helps to maintain the equilibrium of proteasome activity, avoiding intracellular accumulation of toxic waste products.

For this product and most of the others described, the observed clinical effects (improved radiance, skin luminosity, and transparency) can only be partially ascribed to the proteasome stimulation/maintenance, in view of the numerous other *in vitro* observed anti-glycation activities.

d. Caveat

Stimulating the activity of the proteasome in keratinocytes *via* topical application of appropriate ingredients, some of which were briefly presented above, may seem an interesting idea for cosmetic purposes: diminishing the accumulation of (more or less visible) noxious debris that can lead to visible dulling of skin tone as well as preventing the loss of proteasome efficacy in stressed and aging skin are laudable goals. As with a few other ingredients used in anti-age concepts, some caution is in order, especially in view of a number of literature references that—in cases and conditions far removed from cosmetic application—indicate proteasome inhibition to be a desirable goal: Elliot et al. (2003) mentions proteasome inhibition as a new anti-inflammatory strategy; increased proteasome activity is also tied to cancer, for instance *via* its rapid degradation of p53, whose apoptose-inducing property is considered important in controlling

the proliferation of mutated DNA (Bégrégère et al. 2006). Bégrégère mentions many more intricacies of proteasome regulation from which all sorts of conclusions one way or another may be drawn, although none relate to skin or cosmetics in particular. Should we then stay away from stimulating proteasome in the skin? Not necessarily. Koziel et al. (2011) describe the interplay between mitochondrial and proteasome activity in aging skin, where they observed intra-individual co-regulation of mitochondrial and proteasomal activities in all human fibroblast strains tested, suggesting that both systems are interdependent. The authors also found accordingly that pharmacological inhibition of the proteasome led to decreased mitochondrial function, whereas inhibition of mitochondrial function in turn reduced proteasome activity. Given that we aim to maintain and/or stimulate mitochondrial potential in skin cells, this correlation is of interest. And finally, a paper by ando et al. (2009) shows that the Ubiquitin-Proteasome System might be of use in skin pigmentation, as tyrosinase degradation could be accelerated with increased proteasome activity.

5.4.3 TELOMERES

a. Introduction

Oxidative degradation is at the basis of most phenomena involved in tissue aging: glycation, protein carbonylation, lipid peroxidation, mitochondrial deterioration, and DNA lesions are all tied to specific or random oxidation of these functional molecules.

In addition to this basic understanding of a principal cause of aging, the theory of telomere shortening as a contributing factor has received considerable attention. The importance of telomeres was revealed to the general public by the 2009 Nobel Prize awarded to Blackburn, Szostak, and Greider for their work on the subject.

As to how importantly telomere length is involved in aging, the jury is still out. Telomeres are regions of highly repetitive DNA fragments of approximately fifteen thousand bases at the end of a chromosome that function as a cap to protect the chromosome ends, and as a disposable buffer. Every time chromosomes are replicated, the DNA polymerase complex is incapable of replicating all the way to the end of the chromosome; if it were not for telomeres, this would quickly result in the loss of vital genetic information, which is needed to sustain a cell's activities. It is however not only this "biomechanical" fact that contributes to telomere shortening: certain physiological influences (obesity, disease, smoking, oxidative stress) increase the annual number of bases of the G-tail lost up to fivefold! The telomerase enzyme, however, is able to restore some of these lost sequences. The size of the telomere for a given cell type and species is an indicator of cell age (Epel et al. 2004).[14] Protecting both telomeres and telomerase may thus be useful

in slowing down the aging process, and a few active ingredients have been developed in recent years.

While activating the proteasome and reversing glycation can be thought of as "curative" anti-aging concepts, protecting telomers and telomerases is more of a "preventive" age-retarding approach for which it is more difficult to substantiate claims in clinical studies, even if interesting *in vitro* results are promising.

b. Telomere length and aging

Duan et al. (2005) showed that the regular use of low doses of H_2O_2 simulates prolonged oxidative stress *in vivo*. For cells exposed to H_2O_2, there is a discontinuation in growth and a senescent-type phenotype. In parallel, accumulation of DNA damage, reduction in repair capabilities, and shortening of the telomeres are observed. All those findings are in line with those of Passos et al. (2007), showing a link between oxidative stress and telomere shortening. Thus, for normal cells, the shorter the telomeres, the more the cell has aged, until the telomeres reach a critical size at which the cell no longer divides and becomes senescent (Ben-Porath & Weinberg 2004). Interestingly though, a paper by Yokoo et al. (2009) indicates that a hormesis-like effect of trace hydrogen peroxide as well as pro-vitamin C's antioxidant activity are able to slow the age-dependent telomere shortening in human skin keratinocytes.

Similarly, psychological stress has an influence on telomere length. Thus, in mothers of sick children, the duration of the child's disease is positively correlated with the quantity of oxidized molecules in the urine and negatively correlated with telomere length (Epel et al. 2004). An interesting observation was described in a paper by Flores et al. (2008), in which the authors correlate stem-cell-ness (the property of being a "stem cell") with telomere length. The longer the telomere chain, the more "stem-cell-like" behavior is displayed by the cells. This allows Flores to conclude "that telomeres shorten with age in different mouse stem cell compartments, which parallels a decline in stem cell functionality, suggesting that telomere loss may contribute to stem cell dysfunction with age." Cosmetic research is likely to pick up on such statements.

A delicate equilibrium exists between the shortening and lengthening of the telomere sequences, the latter being induced by telomerase. Telomerase is a special type of enzyme, composed of a ribonucleo protein complex (for details see Botchkina, Xu, and Blackburn 2004) capable of adding hexanucleotide repeats onto the ends of linear chromosomal DNA. However, telomerase is largely repressed in proliferating cultured human fibroblasts or after a few passages of culturing of primary human keratinocytes. The normal senescence of such fibroblasts and other human cell types in culture can be overcome by forced expression of telomerase in

these cells. The lifespan of the cells is extended and they grow healthily, generally without evidence of impairment to DNA checkpoint pathways. Blackburn and others clearly correlate telomere length and telomerase expression and activity with improved health and life span of cells. Taylor (1996) studied the amounts of telomerase in various skin tissues and found the enzyme not only in malignant cell types (melanoma, basal cell carcinoma) but also in sun-damaged and psoriatric lesional skin, albeit at lower levels. Even in neonatal foreskin keratinocytes, telomerase was expressed constitutively. The author concludes that higher levels of telomerase in sun-exposed and inflammatory skin lesions suggest environmental factors contributing to modulating telomerase activity.

Han et al. (2009) investigated the relationship between telomere length and susceptibility to cancer. When telomeres shorten to a critical length, the cells undergo apoptosis, senescence, or become genomically unstable. Shorter telomere length has been linked to an increase risk of some cancers and, in fact, UVB exposure creates a high frequency of pyrimidine dimers in telomeric regions whereas UVA-induced cellular oxidative stress on the other hand may lead to telomere shortening. Both of these events can be potentially carcinogenic. The data presented in this study gave conflicting results that could result from the diversity of the skin cells in epidermis and the differing roles and functions orchestrated by the cell types.

Bickenbach (1998) also detected telomerase activity in proliferating transit amplifying cells, which indicates that telomerase is not restricted to stem cells or "stemness." One important regulating factor in epidermal telomerase expression is the concentration of calcium: as epidermal cells progress in their path of differentiation, following the calcium gradient to the top of the skin (Denda et al. 2003), their telomerase activity is progressively downregulated.

Further evidence comes from a study on human hair follicles (Ramirez 1997). Cells of renewal tissues (e.g., skin, intestine, blood) require an extensive proliferative capacity and express telomerase activity, most likely to prevent rapid erosion of their telomeres during cell proliferation. In anagen hair follicles, high levels of telomerase activity were found almost exclusively in the bulb-containing fragment of the follicles, with low levels of telomerase in the bulge area (intermediate fragments) and gland-containing fragment. In comparison, catagen follicles had low levels of telomerase activity in the bulb-containing fragments as well as in other compartments. Such observations indicate that, in anagen hair follicles, the fragments containing cells actively dividing (e.g., transient amplifying cells) express telomerase activity, whereas fragments containing cells with low mitotic activity—for example, quiescent stem cells—express low levels of telomerase activity. This could be of interest for research into hair (re-)growth.

A more cautious opinion is expressed by Boukamp (2004), who asks what the telomere/telomerase hypothesis means for aging of the skin? As fibroblasts hardly ever proliferate and consequently do not suffer from critical telomere shortening, it remains questionable whether *de novo* activation of telomerase in these cells would help to prevent or at least postpone aging of the skin. The situation may be different for keratinocytes, but further studies are needed. Nevertheless, a number of research teams have begun to develop cosmetic concepts and ingredients based on the premise: "In order to limit premature cell aging and senescence, it appears essential to delay telomere shortening."

c. Senescence

Cellular senescence is defined as the ultimate and irreversible loss of the replicative capacity of a cell (Hayflick & Moorhead 1961; Passos et al. 2007). It is thus a very advanced stage of aging and is accompanied by a set of profound structural and biochemical changes relative to younger cells.

Replicative senescence has been studied *in vitro* over long time periods (often several months). The cultured cells are regularly detached from their base and replaced in culture to induce multiplication until the number of cells thus obtained is smaller than the initial number. And it takes ever longer, for cells in culture, to double their number, this slowdown marking the beginning of replicative senescence. In addition to the reduction in the number of cells, senescence may be visually observed: fibroblasts become larger.

Furthermore, specific assays of senescence markers, which increase or emerge, enable evaluation of cell status. Senescence-associated b-galactosidase (SA-bgal) is an example of a senescence marker that increases (Dimri et al. 1995) while telomere length decreases with age.

However, the study of replicative senescence becomes problematic when molecules are to be tested. The very long exposure times make that type of study laborious. Another manner of studying cell aging is to induce weak oxidative stress, most frequently in a repeated manner, using cell cultures. These methods, which vary depending on the authors and targets under study, are referred to as Stress-Induced Premature Senescence (SIPS) (DeMagalhaes et al. 2002; Duan et al. 2005). The two types of senescence share common characteristics in terms of cell morphology and physiological changes.

Essentially, this opens two possible paths to preserve telomere length: the classical antioxidant activity preventing oxidative degradation of DNA, of lipids and of enzymes needed for metabolism and replication, including telomerase; and the more innovative but more difficult search for increasing telomerase expression and activity without inducing malignancy.

d. Cosmetic ideas on telomere maintenance

It appears that it is possible to maintain telomere length against the natural tendency of their shortening. Xu et al. (2009) showed that oral supplementation with multivitamins led to 5% longer telomere chains in the persons so treated compared to non-users of the supplement. If it works orally, why not topically?

Another oral supplement made the headlines, being featured even in *Scientific American* (Kendrick 2009) and in *HAPPI* magazine (Geria 2011). A mystery molecule named TA-65 extracted from *Astragalus* is claimed to have been tested and to show lengthening of telomeres *via* telomerase activation. The only peer-reviewed article on this ingredient appeared in 2011 (de Jesus et al.). Whereas cosmetic products with *Astragalus* extracts are available, it is doubtful that TA-65 (whatever it is) is at present used in topical applications and documented as effectively increasing telomere length in humans.

Makpol et al. (2010) tested the above-mentioned antioxidant pathway on telomere length and telomerase and found that g-tocotrienol increased fibroblasts' viability with optimum dose of 80 μM for young cells (21-year-old donor) and 40 μM for both medium-old (40-year-old donor) and old (68-yr-old donor) fibroblasts. Exposure to H_2O_2 decreased cell viability in a dose-dependent manner, shortened telomere length, and reduced telomerase activity in all age groups. In young and old cell types, pretreatment with g-tocotrienol prevented shortening of telomere length and reduction in telomerase activity. In cells of the 40-year-old donor, telomerase activity increased while curiously no changes in telomere length were observed. Also, post-treatment of g-tocotrienol did not exert any significant effects on telomere length and telomerase activity. Thus, these data suggest that g-tocotrienol protects against oxidative stress–induced cellular aging by modulating the telomere length, possibly *via* telomerase.

A *Narcissus tazetta* bulb extract, obtained from material in a dormant state, was shown to slow down cell-turnover and consequently slow the aging process of the epidermis, thus "sparing" the telomeres (Sorgenfrey 2006).

Delving into more detail, a research group at Vincience became interested in the protein family called shelterins, in particular the Telomeric-Repeat Binding Factor-2 (TRF-2 protein), which contributes to telomere stabilization Imbert (2011). With approaching senescence, this protein is less expressed; an "active" ingredient dubbed IV10.008, composed of hydrolyzed soy and wheat protein, limited the loss of TRF-2 expression in artificially induced senescent fibroblasts as well as decreasing double-strand breaks in DNA (Bergeron et al.2012). A review of the topic is presented by Imbert (2012), who insists particularly on the need to

study the various proteins that bind to the telomere tails, rather than focusing on telomerase. Whereas the length of telomeric DNA is maintained by the enzyme telomerase, six telomere-associated proteins—TRF1, TRF2, POT1, RAP1, TIN2, and TPP1 in mammalian cells—have been shown to form a complex known as the telosome, or shelterin complex, that is essential for telomere function (Huawei 2008).

Using a novel *in vitro* tool (activation of the TERT promoter, thus quantifying telomerase stimulation or inhibition: Armegnol et al. 2010) to study the phenomena around telomere length and function, Siekiersky et al. (2010) discovered a plant extract of *Scutellaria baicalensis Georgi* standardized in baicalin, which delays telomere erosion and increases lifespan of fibroblasts without causing malignant cell transformation. In a daring extrapolation from *in vitro* data showing that fibroblasts treated with the *Scutellaria* extract underwent 10% more cell duplications than the ≈50 standard ones (Hayflick limit), the authors suggest that the plant extract might delay cellular senescence and rejuvenate skin by ten years. . . . A vehicle-controlled clinical test on human volunteers showed that the application of the *Scutellaria* extract in a cream on the forearms improved surface roughness (micro depressionary network) and skin elasticity.

According to the company, a recent offering, red algae galactans, limits the deterioration of telosomes and limits telomere shortening. In tests, it significantly reduced stress-induced telomere shortening by 65%. Expression of the aforementioned essential proteins for telosomes, POT1 and TPP1, is reduced in senescent human fibroblasts. The galactans significantly limited the age-related decrease in the POT1 expression by 100% and TPP1 expression by 89%.

Two synthetic substances (geranylgeranylacetone = GGA and geranylgeranylpropanol = GGP) were studied by Lintner et al. (2007) and Fournial et al. (2011) with respect to their ability to delay senescence and/or prevent telomere degradation. Full genome DNA array studies, replicative and stress-induced senescence, mitochondrial membrane potential, telomere length, and cytoskelettal functions were studied for one or the other or both molecules, both analogs of geranylgeranyl phosphate and a similar structure called Vitamin K, important biomolecules and cofactors (Wiemer et al. 2011).

The network of interconnected gene activation matches the profile of a product that repairs, and protects against, cellular oxidative lesions by stimulating selenoproteins (thioredoxine), stimulating the enzymes involved in the protection and repair of mRNA, DNA, and telomeres, and harmonizing epidermal turnover by equilibrating growth and differentiation (stem cells), and lipid metabolism.

Figure 7: Gene network activated by GGA

Fibroblasts are shown to survive hugely more mitosis cycles after GGA incubation; re-epithelialization is more rapid after strong irradiation. Catalase activity is protected, and sunburn cells are diminished. Clinical results demonstrate decrease in TEWL, rosacea, sunspots, and pore size, increase in moisturization, firmness, and anisotropy. Direct and indirect (antioxidant) protection mechanisms seem plausible explanations, although more specific evidence of telomere protection and maintenance *via* telomerase or shelterin complex activity is still needed. The two molecules clearly have powerful protective and repair activities.

Table 3: Telomere length (in kilobase pairs): GGP helps maintain telomere length in senescent cells.

		Concentration	Average length (in kbp)	Change	Change
Proliferating cells	-	-	10,66 ± 1,80	baseline	-
Cells in pre-senescent state (low or absent proliferation rate)	Control	-	6,90 ± 0,44	–3,76kbp; *p<0,05*	baseline
	GGP	1,65ppm	8,35 ± 0,75	–2,31kbp; *ns*	+1,45kbp; *p<0,05*

A last item of interest (and future cosmetic research) concerns the protein progerin. Progeria, a devastating premature aging disease, is caused by a point mutation in the lamin A gene (LMNA), resulting in a mutant lamin A protein known as progerin. Recent studies have demonstrated that progerin is also produced at low levels in normal human cells and tissues. Cao et al. (2011) have now shown in normal human fibroblasts that progressive telomere damage during cellular senescence plays a causative role in activating progerin production. Interestingly, elevated progerin production was not seen during cellular senescence that does **not** entail telomere shortening. Taken together, these results suggest a synergistic relationship between telomere dysfunction and progerin production during the induction of cell senescence, providing mechanistic insight into how progerin may participate in the normal aging process. It can be expected that cosmetic ingredients inhibiting progerin synthesis or activity are being investigated and proposed in the near future.

CONCLUSION

Glycation, proteasome activity, and telomere protection, although separated into three sections in this chapter, the reader of the abundant literature (academic, industrial) on these subjects must realize that the separation is artificial and arbitrary. Many more details need to be explored for further understanding (and thus increased efficacy of treatment) of these particular aspects of skin aging. Exciting times are ahead.

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14. Indeed, we lose about 50 bases per year, which would give us a theoretical maximal life span of 300 years if no other influence on telomere length existed, such as oxidative shortening and telomerase-initiated repair and no other aging process intervened!
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SIRTUINS AND SKIN

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ABSTRACT

In this chapter, we discuss the role of sirtuins in various cellular processes, with a particular emphasis on their impact upon aging and skin. By this means we point to their relevance for the development of new cosmetic products designed to modulate sirtuin activity and their application to anti-aging skin care.

Although sirtuins (SIRT) have only recently been discovered, much research has been devoted to their study, and the picture that is now emerging in cell biology shows that they are molecules that play critical roles in many epigenetic and metabolic pathways. Sirtuins are evolutionarily well-conserved proteins found throughout nature and, in mammalian cells, seven isotypes have been identified. They function primarily as NAD-dependent deacetylases, although two of the seven have ribosyl transferase activity. Further, sirtuins are localized in subcellular compartments where they participate in organelle-specific biochemical reactions. For example, in the nucleus, deacetylation of histones by increased levels of SIRT 1 condenses chromatin structure, which facilitates gene silencing. In this way, an organism can protect itself during times of caloric restriction by limiting gene activity, which conserves energy. Since caloric restriction has been associated with increased longevity, this aspect of sirtuin up-regulation has become a parameter for developing new strategies in aging research. Additionally, current research has shown that changes in sirtuin expression can have immediate effects on cellular energy and DNA damage repair. In this review, we discuss sirtuins in various cellular processes with a particular emphasis on aging and skin, including their relevance to new cosmetic products designed to modulate sirtuin activity.

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5.5.1 INTRODUCTION TO SIRTUINS

Increasing evidence has demonstrated that sirtuins are a family of enzymes that play a critical role in many diverse cellular reactions. Although only recently discovered as post-translational modifiers (1), their presence has already been established in areas that include epigenetic gene silencing, transcriptional signaling, energy metabolism, and aging (Figure 1). Sirtuins are found throughout nature. Their function and amino acid sequence are well conserved and indicate the evolutionary importance of these molecules. Beginning with the discovery of a mutation in a yeast protein called Silent Information Regulator 2 (Sir2), which normally keeps the mating-type genes, known as HM loci genes, transcriptionally silent but when mutated leads to errant mating (2), the concept of gene silencing by sirtuins took shape (3). Further, when it was discovered that overexpression of Sir2 led to increased lifespan (4), research on sirtuin activity and reduced nutrients in relation to aging began. Although not without controversy, one study showed that sirtuin overexpression did not lead to increased lifespan in *C. elegans*, possibly due to the genetic background of these organisms (5). However, by adjusting the genetic controls of these organisms, Viswanathan and Guarente still showed increased lifespan, although to a lesser degree than originally reported (6). This chapter specifically focuses on mammalian sirtuins with a particular emphasis on skin, aging, and applications to skin care products. For more extensive reviews on the biology of sirtuins, the reader is referred to Sauve et al. (7) and Haigis and Sinclair (8).

Figure 1: Sirtuins influence aging and life span through various modes of cellular activity.

In mammalian cells, seven sirtuin (SIRT1-7) homologs have been identified and they are distributed in several subcellular compartments. SIRT1 and 6 are localized in the nucleus, whereas SIRT 3, 4, and 5 are in the mitochondria. SIRT2 is cytoplasmic and SIRT7 has been reported to occur in the nucleolus. Sirtuins are NAD+ (nicotinamide adenine dinucleotide)-dependent Class III deacetylases that remove an acetyl group from a lysine side chain of its protein substrate (9). Upon reaction, the acetate moiety is transferred to its NAD+ cofactor to form 2'-O-acetyl-ADP ribose with the release of nicotinamide (10) and is illustrated in Figure 2. 2'-O-acetyl-ADP ribose is a unique reaction product formed as a result of sirtuin action. Alternatively, the ADP portion of NAD+ is added to the lysine side chain and nicotinamide released in an ADP-ribosylation reaction (Figure 3; ref. 10). In both reactions, nicotinamide is enzymatically regenerated back to NAD+. However, as nicotinamide levels rise, nicotinamide can also begin to inhibit sirtuin activity by a feedback inhibition mechanism that acts by binding to a pocket in the catalytic site and preventing further deacetylation (11).

Since sirtuins are dependent on NAD+, cells that also use as the major electron acceptor in ATP synthesis, this allows sirtuins to be uniquely sensitive to a cell's metabolic state and helps explain why sirtuins can respond to caloric restriction. In many organisms, for example in *C. elegans*, there is evidence that caloric restriction increases longevity by slowing aging mechanisms that are regulated by sirtuin expression (12). The importance of this fact cannot be overstated in the field of skin care product development. Understanding how to prolong the look of younger skin by effectively supporting sirtuin activity has now become a realistic objective in cutaneous research.

Figure 2: Deacetylation of a target protein by a sirtuin and transfer of the acetate moiety to form 2'-O-acetyl-ADP ribose. Free nicotinamide (NAM) is

enzymatically regenerated back to NAD+ by Nampt and Nmnat. Used with permission from *Journal of Cell Science*, Nakagawa and Guarente (10).

Figure 3: ADP-ribosylation of a target protein by sirtuin. Free nicotinamide (NAM) is enzymatically regenerated back to NAD+ by Nampt and Nmnat. Used with permission from *Journal of Cell Science*, Nakagawa and Guarente (10).

5.5.2 ORGANELLE-SPECIFIC BIOCHEMISTRY OF SIRTUINS

The two most studied organelles for mammalian sirtuin activity are the nucleus and the mitochondria, and these will be discussed in this section.

Nucleus

Epigenetic silencing occurs in the nucleus by the action of SIRT1. In this reaction SIRT1 deacetylates histone proteins that comprise a larger complex called the nucleosome, helical DNA that winds itself around in what is commonly referred to as the "beads on a string" model of DNA structure (13). When histones are acetylated, chromatin structure is more polar due to the repellant nature of these electrostatic groups, leading to a more open conformation that facilitates gene expression. As sirtuin activation is increased by environmental changes that, for example, may limit nutrient availability, deacetylation reduces chromatin polarity followed by condensation and gene silencing. This effect is illustrated in Figure 4 (14). Resveratrol, a natural molecule derived from grape skins, has attracted a lot of attention because of its ability to increase longevity as a function of sirtuin

activation in several organisms, including yeast (15), and is discussed further in a subsequent section.

Figure 4: Schematic representation of gene silencing by sirtuin deacetylation. Used with permission from *Current Opinion in Genetics and Development*. Adapted from Flaus and Owens-Hughes (14).

In addition to gene silencing by histone deacetylation, sirtuins can also control gene expression through deacetylation of transcriptional signaling factors. An intriguing example of this mechanism is in the area of circadian rhythm, which, as described elsewhere in this volume by Pernodet and Pelle (16), may represent a new treatment strategy for skin care. Two circadian clock proteins, CLOCK and BMAL-1, form a heterodimer that binds to gene promoters in order to coordinate specific gene expression at the appropriate time of the diurnal cycle. CLOCK, which has acetyltransferase activity, first binds to DNA and introduces polar acetyl groups on the nucleosome, thereby opening up its conformation. This allows BMAL-1 to heterodimerize with CLOCK, which in turn acetylates BMAL-1. SIRT1 can control the action of this cycle by deacetylating BMAL-1, which then prevents accessory protein binding and limits the activity of the CLOCK/BMAL-1 complex (17). In this way, sirtuins, which also follow a diurnal pattern (16), can influence the circadian rhythm of skin cells.

SIRT1 has also been shown to activate the important regulatory and tumor suppressor protein p53. As a result, sirtuins can respond to genotoxic stress by allowing

p53 to slow the cell cycle in order for DNA repair to occur or, alternatively, if the damage is too extensive, to initiate apoptosis (18). Further, transcriptional signals that respond to oxidative stress, such as forkhead box protein (FOXO), are attenuated by SIRT1, which contributes to cell survival (19).

SIRT6, the other sirtuin present in the nucleus (20), is associated with DNA damage repair, glucose metabolism, and inflammation. SIRT6 is physically bound to chromatin and aids in DNA base excision repair (21). It has also been reported to be part of a repair complex that re-anneals DNA double strand breaks (22). Glycolytic genes are also affected by SIRT6 activity. Under conditions of nutritional stress, SIRT6 will deacetylate histones associated with promoter regions of glycolytic genes and destabilize transcriptional regulators that shift glycolysis away from mitochondrial respiration in order to conserve energy (23). Animal studies, as carried out by Mostoslavsky et al. (21), have also shown that, when the SIRT6 gene is missing, an aging phenotype is clearly visible including graying and the loss of subcutaneous fat. Since SIRT6 has also been discovered to reside in conjunction with telomeres (24), future research in skin aging will surely be focused in this area.

Mitochondria

To date, most mitochondrial sirtuin activity appears to be involved in energy metabolism. Since 20% of all mitochondrial proteins are estimated to contain acetyllysine (25), this indicates an enhanced probability for post-translational modifications by sirtuins. For example, SIRT3 activates acetyl CoA synthetase by deacetylation leading to increased oxidative phosphorylation (26) and is illustrated in Figure 5. In contrast, caloric restriction leads to up-regulation of SIRT4, which inhibits glutamine dehydrogenase by ADP-ribosylation, and to a reduction of ATP synthesis (27). SIRT3 and 4 activities balance each other because the product of glutamine dehydrogenase is α-ketoglutarate, an intermediate in the tricarboxcylic acid cycle leading to increased ATP. Thus, mitochondrial sirtuins exert another level of control in cellular metabolism exclusive of genomic regulation. Work by Qiu et al. (28) has also recently demonstrated a connection between reactive oxygen species and mitochondrial sirtuins by showing that deacetylation of superoxide dismutase 2 (SOD2) by SIRT3 is required for its activation and its ability to scavenge superoxide anions. Lastly, although much less is known about SIRT5, new research has suggested that it specifically deacetylates carbamoyl phosphate synthetase, which would then place SIRT5 in the regulation of the Urea Cycle (29).

Figure 5:_This figure illustrates how sirtuins affect metabolism. SIRT3 deacetylates acetyl CoA synthetase (AceCS2), which activates the enzyme to produce acetyl CoA, leading to increased metabolism and ATP production via the TCA cycle. Used with permission from *Cell Metabolism*, Schwer and Verdin (36).

5.5.3 SIRTUIN RESPONSE TO ENVIRONMENTAL CHANGES

It is now well established that caloric restriction contributes to the longevity of many organisms (30). At the biochemical level, research has shown that sirtuins play a significant role in response to nutritional stress and that it senses this by NAD+ availability. Our laboratory has now taken this research in a new direction and has begun to explore the effects of other forms of environmental stress on sirtuin activity. In Dong et al. (31), we found that after cell starvation and synchronization, normal human

epidermal keratinocytes (NHEK) expressed SIRT3 and SIRT4 mRNA transcripts inversely proportional to each other as expected when analyzed by RT-PCR. However, when exposed to low, non-cytotoxic levels of UVB (10 mJ/cm^2), we observed an abrogation of this expression. These results are shown in Figure 6. Additionally, we observed decreased levels of ATP, whereas hydrogen peroxide levels increased. These findings are significant for skin because it demonstrates how exposure to sunlight may affect the necessary energy requirements for skin and may also expose the skin to higher levels of oxidative stress.

Figure 6: After overnight starvation, normal human epidermal keratinocytes (NHEK) were repleted in normal media and the expression of mRNA transcripts for SIRT3 and 4 were analyzed by RT-PCR. A) SIRT3 expression increased whereas SIRT4 decreased. B) 10 mJ/cm^2 UVB disrupted expression of SIRT3 and 4 (from Dong et al. (31).

Another form of pollution that is becoming a worldwide environmental problem is the formation of tropospheric ozone as a result of the interaction between sunlight and auto exhaust emissions. In McCarthy et al. (32), we exposed NHEK to environmentally relevant levels of ozone (ca. 0.4 ppm, 30 min) and then assayed for the expression of SIRT3 by Western blot analysis, normalized to actin. We observed a decrease in SIRT3 after ozone exposure, and these data show how sirtuin activity may be compromised when exposed to this type of environmental trauma. These results demonstrate again how energy metabolism may be affected in skin or oxidatively challenged by the lack of SOD2 activation. Our research demonstrates that the effects of environmental challenge, in addition to caloric restriction, need to be taken into consideration when studying cutaneous sirtuins and formulating these types of skin care products.

5.5.4 APPLICATION OF SIRTUINS TO ANTI-AGING SKIN CARE

The search for sirtuin activators is currently an active area of investigation in anti-aging research, as well as in the cosmetics industry. In addition to finding activators that possess biological activity, the cosmetics industry must also use ingredients that are safe, small enough to enter the stratum corneum, and remain active and stable in a cosmetic formulation. One molecule that we have studied that appears to fulfill most of these criteria is resveratrol. Resveratrol is a small-molecular-weight molecule that has been reported to activate SIRT1 (see Figure 4) without having to challenge cells by starvation (15). Thus, resveratrol may represent an ingredient able to increase sirtuin levels and induce gene silencing. Other molecules that mimic resveratrol are also being developed by others that possess even higher levels of activity than resveratrol (33). However, recent analytical data have also shown that the increased sirtuin activity that had been reported in the literature for resveratrol may be an artifact attributed to the way resveratrol interacts with sirtuins and the fluorescent molecule used in the activity assays (34). Nevertheless, resveratrol has been shown to increase lifespan in metazoans independent of these assays (33) and to improve the health of skin cells.

On the other hand, a skin care product designed to inhibit sirtuin activity may also be useful. Since SIRT1 promotes differentiation in keratinocytes (35), this relationship may eventually be found to be true for full-thickness skin. If so, then the inclusion of sirtuin inhibitors in skin care products for the purpose of improving certain hyperproliferative skin conditions, such as psoriasis, may be another area for future product development. In this case, nicotinamide, which has a low molecular weight and is non-cytotoxic, is an excellent candidate for this application.

In the last decade, aging research has suddenly progressed at an accelerated pace, due in large part to the discovery of sirtuins. Sirtuin activity affects longevity

and health primarily through gene silencing and reduced energy metabolism and, since they utilize NAD+, sirtuins provide a biological mechanism for understanding lifespan extension by caloric restriction. Moreover, in those animals that were deficient in sirtuins, an accelerated aging phenotype was clearly visible. Bringing these new advances in aging research to the study of dermatology and then applying these findings to the development of new and effective skin care products, although challenging, is now an achievable goal for attaining younger-looking skin.

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GLOSSARY

Well-conserved protein: protein sequence that has only diverged slightly during evolution due to its importance to an organism

Histone: nucleoprotein associated with DNA that is a target for epigenetic control by sirtuins

Deacetylase: an enzyme that removes acetyl groups from proteins

Promoter region: area of a gene that signals activation or repression of that gene

Gene silencing: deacetylation of histone proteins by sirtuins, leading to repression of gene activity

2'-O-acetyl-ADP ribose: unique reaction product produced by sirtuins when an acetyl group is transferred to nicotinamide adenine dinucleotide

EPIGENETICS OF SKIN AGING

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ABSTRACT

In this chapter, we review the profound implications that the rapidly emerging field of **epigenetics** holds for the cosmetic scientist, beginning with an explanation of basic epigenetic principles followed by the role epigenetics plays in the aging process, the impact nutraceuticals and gene-regulating ingredients have on the aging process, and the significant contributions epigenetics can make towards the future of next-generation skin care.

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INTRODUCTION

Our understanding that genes can be switched on and off is rather new to geneticists, but the finding that **gene expression** occurs in response to an immense variety of environmental signals—a phenomenon known as "epigenetics"—is currently revolutionizing all sciences where gene expression comes into play. Medicine, gerontology, botany, toxicology, nutrition, psychology, dermatology, and the cosmetic and personal care sciences are all on the verge of being transformed by discoveries in epigenetics. With over 100,000 papers published between 1992 and 2011, epigenetics is emerging as a paradigm-shifting, rapidly developing branch of genetics that is revolutionizing the way biologists view **gene regulation**, **protein expression**, and **noncoding DNA** that was once thought of as **"junk" DNA**.

Figure 1.3

Epigenetics explains how plants know when to bloom, why a gentle touch results in deep relaxation, how cancers form, and how cancer types can be passed from a grandparent to a grandchild. Epigenetics also offers an explanation as to why identical twins don't always share the same diseases or show signs of aging at the same rate, and why a skin cell looks and acts differently than a brain, bone, or muscle cell. This difference occurs even though they all start from one cell, the

zygote—the first cell of the body. More common than gene **mutations**, epigenetic aberrations that occur in response to environmental signals are being linked to an array of diseases and disorders. These include diabetes, asthma, obesity, Progeria, lupus, rheumatoid and osteoarthritis, metabolic disorder, cardiovascular disease, Down's Syndrome, Parkinson's disease, Alzheimer's, autism, schizophrenia, bipolarism, depression, anxiety, addiction, post-traumatic stress disorder, inflammation, immunosuppression, various symptomologies of aging, and all types of cancers, to name just a few on a quickly expanding list.

In this chapter, the fundamental concepts of epigenetics are described, followed by issues of concern to cosmetic and personal care scientists. These issues include a basic explanation of epigenetics and its mechanisms, how these mechanisms shift with age, and the possible positive influences nutraceuticals and gene-regulating ingredients may have in skin care. *For anyone developing next-generation skin care, familiarity with basic epigenetic concepts will prove vital in the future.*

5.6.1 THE HUMAN GENOME PROJECT GIVES BIRTH TO THE EPIGENETIC REVOLUTION

Exploration of the **genome**—the hereditary DNA sequences of protein-coding genes and noncoding DNA required to make an organism—consumed most of the second half of twentieth-century genetics. At the end of the century an international team of researchers began the **Human Genome Project** (HGP) with the ultimate goal of mapping and understanding all of the **genes** required to make and operate a human. Before the project's completion in 2003, conventional wisdom held that all genetic functions and heredity were specified by the information encoded in an individual's DNA sequence—the pattern of four **nucleotide base pairs** (cytosine-guanine, thymine-adenine) from which the three billion rungs on the 23 pairs of the twisted, ladder-shaped set of human chromosomes are formed. All genes are made up of segments of these base pairs arranged in different ways and in different lengths. Acquired changes in cell behavior, including alterations that lead to aging and disease, were thought to be due to genetic mutations, described as unrepaired damage to the DNA sequence accumulated over time. But this turned out not to be true, for one of the gifts of the HGP was the discovery that DNA mutations are not as common as originally believed. It is now known that 1) too few mutations exist to account for the wide variety of human diseases, and 2) the ability of the environment to influence or promote disease and aging does not generally involve mutations (1).

Because of the vast complexity of the human body, by the end of the HGP geneticists expected to identify between 50,000 and 120,000 genes, each one featuring the code for a different protein. Yet a modest 20,500 genes were discovered (2), amounting to less than 2 percent of the entire human genome. Unfortunately, and in view of the early information available, the remaining 98 percent of the

genome was cast off as "junk DNA" because not much was known about what it did or why it existed. Later, researchers would find that among this 98 percent were sequences preceding, following, and even in the middle of genes—sequences that govern when, where, and how much of a gene is expressed. Even more intriguing than how little of the genome was devoted to genes, researchers learned that one gene can hold the code for up to 1,000 proteins, making the estimated number of possible proteins produced in the human body between 250,000 to one million (3).

How was it possible that so few genes can produce so many proteins yet only a relatively few proteins are produced at any given time? It seemed obvious that something was happening at the genetic level to direct protein production, but what it was had yet to be unveiled. This "something" could also explain how more than 220 cell types, each type with its own agenda designed to service the tissue where it is found, contain the same genome passed down from the body's original cell, the zygote. Questions began to arise among geneticists, such as, "If genes are the same in all cells, what causes DNA to be expressed differently? What causes cell **differentiation** from the zygote to various types of **stem cells** to, finally, **terminal cells** unique to specific tissues? And what causes 98 percent of genes to shut down after the gene is no longer needed by the cell?" Those studying aging and disease asked how twins, with exactly the same genome, age at different rates and develop different diseases? Along the same lines, cosmetic and personal care scientists might ask, "How can one person respond to a new ingredient or product with astounding results while others show no response?" *None of this can be explained by identifying the 20,500 genes in the human genome.*

So what is the answer to all of the questions posed above? The answer, given the present state of knowledge, is that epigenetic chemical modifiers (aka: epigenetic tags), collectively known as the **epigenome** due to their placement "on" or "above" ("epi") the genome, are now known to be responsible for gene expression and repression. These modifications occur throughout all stages of an organism's development, in response to environmental factors such as exposure to toxins or chronic stress, and are implicated in diseases including cancer. Once thought to occur only in response to the environment, including the environment surrounding and within an individual cell, it is now known that the epigenome is in place to "run" the genome, playing genes the way that fingers play piano keys.

Even though a key exists and has the potential to sound a musical note, no sound is made until a finger presses down, either hard or softly, to cause a sound to emerge. In the same way, genes exist and have the potential to create proteins but until the epigenome gives the direction to be played, a gene is not expressed and its resulting protein isn't synthesized. The gene is "silent," made mute by epigenetic mechanisms. In an adult somatic cell (a body cell as opposed to a cell used for organism reproduction), 98 percent of the genome is silenced (4). If they weren't, all genes within every cell

would be available for expression. This would result in a wild cacophony of proteins that would wreak havoc in every cell in the body because all cells would be the same, expressed at the same time like an orchestra with no maestro. Life as we know it, where groups of unique cells work together to form and operate an organism, would not exist. This is why, where all cells contain the same set of genes, epigenomes vary from cell type to cell type and even from cell to cell. And, while genes take hundreds of generations to evolve in response to their environment, most epigenetic mechanisms respond quickly to environmental changes, sometimes within seconds. These responses can be stopped, and usually are, when the stimulus is no longer present. But some persistent or particularly harmful stimuli like endocrine disruptors, poor nutrition, or severe stress can entrench epigenetic changes for a lifetime or even for generations. *Epigenetics is a young science but it is already clear the epigenome learns from its experience.*

5.6.2 EPIGENETICS DEFINED

Epigenetics is a rapidly emerging branch of biology. Epigeneticists study the vast array of molecular mechanisms that affect the gene activity continually taking place inside cell nuclei (5). In broad terms, these mechanisms are involved in deploying the genetic programs required for the many processes operating during the life span of a cell. Among them are **cell lineage development**, **genomic stability**, **stress response**, pathological conditions, and adaptation to the environment (6). More specifically for the purposes of the cosmetic and personal care developer, epigenetic mechanisms are now known to play important roles in stem cell differentiation (7), olfaction (8), hair growth (9) and graying (10), wound healing (11), inflammation (12), hyper- and hypopigmentation (13), **endogenous antioxidant** synthesis (14), DNA repair (15), aging (16), cell longevity (17), **senescence** (18), **apoptosis** (cell death) (19), allergies (20), and skin diseases such as eczema (21), psoriasis (22), vitiligo (23) and, most likely, acne and rosacea.

Epigenetic mechanisms do not alter DNA sequences but they do change the shape and chemical behavior of the molecules that form DNA. These changes alter gene expression, determining when genes are turned up or down or turned on or off completely. Though varied, epigenetic mechanisms are typically stable and reversible while, at the same time, capable of **dynamic** and wide-ranging fluctuations, able to both respond to and direct changes in gene expression. Core epigenetic operators are chemical tags that attach to the chromosomal complex known as **chromatin**, a structure composed of **DNA**, **nucleosome**, and **RNA** molecules that will be discussed in more detail later in this chapter. When present, these tags either block the expression of genes or make them available to be read by **transcription factors** that work with RNA to translate the genetic code into a protein.

Epigenetic mechanisms occur as a response to environmental messaging, either from the macroenvironment of the organism or the microenvironment of the

cell. Until recently, only those **heritable** mechanisms passed between generations of cells and organisms have been considered epigenetic, but transient forms of gene expression, such as **histone modification**, are now regarded as epigenetic in their effect. In species that experience a diverse range of environmental conditions, whether they are animals, plants, insects, yeasts, or bacteria, epigenetic mechanisms cause key genes required for survival to be expressed differently throughout the population, thus improving the chances for individual survival (24)—and therefore the survival of the species.

5.6.3 TWO PRIMARY EPIGENETIC MECHANISMS

Research over the past few years has focused on two primary molecular mechanisms believed to participate in most epigenetic events:

FIGURE 1.4. ***Epigenetic mechanisms*** are affected by several factors and processes including development *in utero* and in childhood, environmental chemicals, drugs and pharmaceuticals, aging, diet, and certain cosmetic ingredients. DNA methylation is what occurs when methyl groups tag DNA and activate or repress genes. Histones are proteins around which DNA can wind for compaction and gene regulation. Histone modification occurs when the binding of epigenetic factors to histone "tails" alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA to be activated. All of these factors and processes can have an effect on people's health and well-being.

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1) DNA **methylation** and 2) **chromatin remodeling**, which includes histone modification. Others, such as those mechanisms utilizing several types of **small noncoding RNAs** (ncRNA) called miRNA, siRNA, and piRNA, as well as **long noncoding RNA** (lncRNA) (25), are not as well characterized at this time although they are evolving areas of investigative focus.

All epigenetic mechanisms work either singly or, more often, collaboratively in systems of **crosstalk** that direct cellular development and differentiation in **embryonic cells**, **stem cells**, and **somatic cells**. These mechanisms act as a cell's memory, allowing it to respond and adapt to its environment, ensuring it knows where it belongs as well as where it has been, the proteins it has produced (or not produced), the threats it has encountered, and how it survived. Further, epigenetic mechanisms determine when a cell should reproduce, how long it should live, and when it should die. In short, epigenetic mechanisms control how, when, how much or how little, and under what circumstances a cell's genes are expressed. Because of these feats, *the epigenome, the totality of a cell's epigenetic memories, is, quite literally, the "master" of the genome*.

1. DNA methylation

The first epigenetic mechanism, discovered almost 100 years ago (26), DNA methylation is also the most researched and understood of the two mechanisms discussed here. It is thought to be the missing, and perhaps main, link between genetics, aging, disease, and the environment (27). Distinct patterns of DNA methylation allow stem cells to differentiate and populate specific tissue types. DNA methylation patterns are known to change with age and are unique to specific disease states. As with **single nucleotide polymorphisms** (SNPs), which are small deviations in the base pairs that form the rungs on the DNA ladder, identifying DNA methylation patterns and understanding the impact of alterations in these patterns promises to significantly advance our ability to control and affect the aging process and diagnose and treat human disease.

DNA methylation occurs when specialized enzymes known as **DNA methyltransferases** (DNMTs) add a **methyl group** to a cytosine molecule that makes up one half of a rung on the DNA ladder. **S-adenosyl methionine (SAM)** acts as the methyl donor for most if not all DMNT activities and will be discussed later in this chapter.

Figure 1.5. Schematic representation of DNA methylation, which converts cytosine to 5'methyl-cytosine via the actions of DNA methyltransferase (DNMT). DNA methylation typically occurs at cytosines that are followed by a guanine (i.e., CpG motifs).
NOTES: SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine.

Three active DNMTs have been identified in mammals: DNMT1, DNMT3A, and DNMT3B. All three are involved in **de novo** (new) placement of methyl groups on cytosines, especially in early embryo development where they set up the pattern of methylation that will follow into adulthood (28). DNMTs are also vital for establishing DNA methylation after exposure to methyl donors from the environment. In addition to de novo methylation, the maintenance methyltransferase, DNMT1, the most abundant of the DNMT family, adds methyl groups during cell division to corresponding DNA when one strand is already methylated. This process works throughout the life of the organism to ensure methylation patterns are passed from mother to daughter cells during replication (29).

The addition of the methyl group alters the shape of the cytosine to such an extent that it is invisible when a **transcription protein** seeks it out during DNA transcription, the process that occurs when sections of DNA are copied onto RNA. If located in an area vital to gene **transcription**, or if an area features numerous methylated cytosines (known as **hypermethylation**), the entire gene can be shut down. This process occurs almost exclusively on **CpG regions** (cytosine linked by one phosphate to a guanine that makes up the other side of the rung on the DNA ladder). CpG regions tend to cluster in **CpG islands** located near gene-transcription start sites known as **promoter regions**. In normal tissues, less than 3 percent of promoter CpG islands are methylated (30), which causes the associated gene to

be silenced. Approximately 80 percent of the remaining CpG sites, though sparsely scattered throughout the genome, are hypermethylated (31), a cumulative process that becomes more prevalent with each successive level of stem cell differentiation when genes that are no longer needed are shut down.

Figure 1.6. DNA methylation can silence gene expression. When cytosines in gene promoter regions are unmethylated (image 1), transcription factors (TF) can initiate the copying of a gene to mRNA. When cytosines are methylated (attachment of CH3) (image 2), access to the gene promoter region is hidden from the transcription factor. Transcription of mRNA does not occur. The gene is silenced.

During aging, this pattern is gradually reversed, resulting in sporadic but higher incidences of methylation in CpG islands, especially those upstream of

developmental genes (32), **housekeeping genes**, and genes involved with **cell cycle regulation**, **tumor-cell invasion**, **apoptosis**, **metabolism**, **cell signaling**, and **DNA repair** (33). These age-related changes are accompanied by a global loss of methylation (hypomethylation) in non-island CpG sites (34), mainly in genes with multiple copies (35); (36). Indeed, global hypomethylation appears to be the most important feature of aging cells and tissues (37). Certain age-associated **DNA methylation signatures** are so consistent across multiple tissue types they provide an almost perfect correlation of 0.96 with chronological age, making them remarkably accurate calculators of cellular age (38); (39). When measured by **correlation**, determination of DNA methylation age is about 80 percent more accurate than the **telomere** length-based clock currently used to determine cellular age. Software intended to calculate DNA methylation age can be accessed online. If DNA methylation proves to be the epigenetic clock of the aging process, this software may be used to identify critical **biomarkers** for evaluating rejuvenating interventions, including those developed for use in personal care.

Moving beyond the aging process, a broad array of age-related diseases, such as those of an autoimmune, neurodegenerative, or cardiovascular nature, are associated with abberations in established DNA methylation patterns. These aberrations are even more widespread during **carcinogenesis**, causing deviant DNA methylation to be a major contributor in **tumorigenesis** by shutting down **tumor suppressor genes**. Investigation has now shown these genes are turned off in almost all cancers (40), giving cancer researchers a target for new therapies that avoid the devastating side effects of chemotherapy and radiation. Cancer drugs such as 5-Aza-2'-deoxycytidine have been developed to degrade methyl-attaching DNMTs after they are formed, thereby inhibiting the enzyme's ability to shut down tumor suppressor genes. Unfortunately, these drugs must be incorporated into the genome of the cell, which can cause epigenetic mutations to be passed to surviving daughter cells (41) if the cell does not die. Newer drugs that prevent the synthesis of DNMT1 are currently being developed, but at this time it is not clear if targeting DNMT1 alone will be sufficient to reactivate tumor suppressor genes. If production of DNMT1 *is* shut down, concerns arise regarding the appearance of unwanted and widespread effects in other areas of the genome where DNMT1 is needed. Only thorough, long-term, and careful examination of these effects can clarify the answers to these concerns. This caution is valid not only for cancer therapies, it holds true for personal care applications as well.

At the time this chapter is being written, researchers at the Cancer Science Institute of Singapore and the Harvard Stem Cell Institute are announcing they have found a novel ncRNA that binds to DNMT1 and prevents DNA methylation of the CEBPA tumor suppressor gene associated with acute myeloid leukemia, lung can-

cer, and other types of cancer (42). This is the first demonstration of such an effect and is based on the team's earlier findings that certain RNAs inhibit methylation.

Because aberrations in DNA methylation have dire consequences, including aging, predisposition to cancers, and other diseases passed from one generation of organisms to the next, efforts like the Human Genome Project are currently underway to identify all areas of DNA methylation in the human genome, collectively known as the human **methylome**. Teams around the world with interests in specific cell types, tissues, diseases, and disorders are also identifying methylome traits unique to their subject of specialty. It is hoped that as these efforts come to fruition, we will be better able to direct the biochemistry that corrects methylome aberrations found in aging, skin disorders, cancers, and diseases of all types, including those induced by **ecotoxicants** and exposure to aggressive agents such as UV, smog, pesticides, and tobacco smoke.

2. Chromatin remodeling and histone modification

Chromatin is composed of three main molecules:

a) **DNA**, the structure that forms the genome;
b) **Nucleosomes**, clusters of eight histone proteins around which DNA is wound like thread onto a spool; and
c) **RNAs** that first receive a transcribed version of genetic DNA and then translate the DNA code into a protein. Another function of some RNAs may be to identify where and how densely epigenetic tags are placed on chromatin (43), (44).

Chromatin exists in two states of **condensation**.

Figure 1.7. Chromatin is found in two states of compaction: *euchromatin* (transcriptionally competent) and *heterochromatin* (transcriptionally silent). Epigenetic events such as DNA methylation and histone modification may alter chromatin between these states. Copyright © 2014, American Biotechnologist

Genes located in loosely condensed or "open" regions known as **euchromatin** are easily accessed by gene-reading proteins called **transcription factors** and are therefore more actively expressed than tighter **heterochromatin** regions where genes are repressed, or "silenced" from transcription. Changing chromatin's state of condensation is known as **chromatin remodeling**.

Tissue-specific variations in euchromatin and heterochromatin gene regions allow for the development of over 220 cell types in the human body, even though all cells in the body feature virtually the same DNA sequences. In **terminally differentiated cells**, such as **keratinocytes**, an estimated 98 percent of the genome is maintained in a heterochromatic state, ensuring the cell does not produce proteins that were required farther back in the originating stem cell line.

Telomeric repeat sequences found on the ends of chromosomes are composed of hypermethylated heterochromatin to ensure chromosome stability (45), suggesting epigenetic regulation may be important in telomere maintenance (46), (47). However, it has been observed that telomeric heterochromatin, once thought to be permanently silent, can be transcribed (48) (49), indicating heterochromatin regions are not a static, closed entity (50) as previously thought.

Indeed, it appears chromatin's structure and underlying biochemistry support properties that are **random** and **dynamic** (51). It is now apparent that chromatin is not rigid, but is continuously remodeled and redistributed along the chromatin chain, allowing heterochromatin regions to "breathe." Sometimes these behaviors counter the aging process and extend the life span of the organism. However, over time, they may contribute to the breakdown of nuclear, cell, and tissue function, advancing the aging process and age-associated diseases (52).

Histone modifications are a more complex type of epigenetic mechanism than DNA methylation. Not only do histones structurally organize DNA when they form the protein spools called nucleosomes, but they are also subjected to modifications that alter **transcription protein binding sites**, DNA methylation, and **DNA damage responses** (53). These modifications usually occur on **amino-terminal tails** that extend from each histone in the spool.

First discovered in the mid-1990s, some histone modifications, including **phosphorylation**, **sumoylation**, and **ubiquitination**, are transient in that they are associated only with temporary changes in gene expression. Others, such as **acetylation** and methylation, are more stable. In fact, histone methylation patterns can survive from one cell generation to the next and from organism to organism (54).

Following is a list of well-known histone modifications, the enzymes responsible, and their effects on gene expression.

Acetylation. **Histone acetyltransferase** (HAT) brings about histone acetylation, causing DNA "threads" to become loosely wound around nucleosome

"spools." This relaxes chromatin into the open formation of euchromatin, making DNA available for gene expression and transcription into proteins. When histones are deacetylated by **histone deacetylases** (HDACs), chromatin tightens into heterochromatin, increasing the likelihood that genes in this region are silenced and unavailable for transcription into proteins. At least 18 HDACs have been identified in humans (55). The most well known and broadly studied are the seven members of the **sirtuin** family, especially **SIRT1**. When gene expression is altered because of an imbalance between HAT and HDAC activity, progression in aging and age-related diseases occur (56). Transcription proteins known to be modified by sirtuins include the **cell cycle regulatory gene**, **p53**, the apoptosis-regulating **FOXO genes**, of which FOXO3a is thought to play a role in protection from oxidative stress, and the **TATA-box binding protein TAF1** (57) (58), which plays a central role in mediating promoter responses to various gene activators and repressors. As a result of this activity, sirtuins most likely are involved in modulating cell survival under stress and cellular senescence (59) among other age-related processes.

Methylation. **Histone methyltransferases** (HMTs) add methyl groups to histones. Histone methylation varies throughout the nucleosome (60), resulting in gene expression or repression depending on the position and number of methyl groups on individual histones (61). At one time histone methylation was thought to be irreversible (62) as these sites are passed between cell generations, but discovery of such enzymes as **peptidylarginine deiminase 4** (PAD4) (63), **lysine-specific demethylase 1** (LSD1) (64), and **Jumonji domain-containing hydroxylases** (JHDMs or JMJDs) (65) has proven, at least in some instances, histone methylation is reversible.

Phosphorylation. Histone phosphorylation is critical in gene transcription, chromosome condensation (formation of heterochromatin), DNA repair, and cell death (66).

Ubiquitination and **sumoylation**. Structurally similar, the role of these proteins is not well understood. Sumoylation is most likely linked to repressing gene transcription while ubiquitination can assist in activating or repressing gene transcription depending upon where in the nucleosome it is associated (67).

DNMTs and histone-modifying enzymes work jointly with **chromatin remodeling factors**, such as the gene-silencing **Polycomb-group proteins** (PcG), **cyclin-dependent kinases** (CDKs) (68), and the **Nucleosome Remodeling Deacetylase** (NuRD) complex (69), a group of associated proteins with both histone deacetylase and **ATP-dependent chromatin remodeling** actions that are diminished with age.

5.6.4 EPIGENETIC LINKS TO AGING

Aging is a complex process controlled by environmental and genetic factors (70). These factors lead to **genomic instability** and epigenetic destabilization and modifications that ultimately throw the organism out of balance (homeostasis), resulting in a compromised stress response and an elevated risk of disease (71). Indeed, it appears that epigenetic changes, more than genetic mutations, drive aging and disease in the second half of life.

Because of this, the epigenetic basis of aging is becoming one of the most popular topics for exploration by researchers concerned with finding keys to reversing the aging process and extending human longevity. Among the most common research topics are the epigenetic links to known or suspected contributory factors to the aging process. These include, but are not limited to:

- Oxidative damage (ROS, RNS, RCS) (72)
- Shortened and dysfunctional **telomeres** (73)
- Mutation accumulation in nuclear and mitochondrial DNA (74)
- Alterations in molecular pathways (75)
- Mitochondrial dysfunction (76)
- Altered intercellular communication (77)
- Decline of stem cell functions and populations (78)
- Altered differentiation of stem cell dependent self-renewing tissues (79)
- Cell senescence (80) and apoptosis (81)
- Alterations in **systemic hormonal factors** (82)
- Loss of **proteostasis** (83)
- **Up-regulation** of genes with negative impacts on aging, such as the family of inflammatory **NFkB transcription factors** (84)
- **Down-regulation** of genes with positive contributions towards maintaining "youthful processes," such as the sirtuin family of longevity genes and the endogenous antioxidant regulator **Nrf2** (85)

After reviewing this list, it can be construed that aging is due to the decline of elements and activities required to maintain the "processes of youth" while those that advance aging become increasingly more pronounced. Simply stated, during aging everything good goes down and everything bad goes up.

Contributing to our understanding of the epigenetic connection to aging is the recent identification of 150 "long life" **variants** of 70 genes known as Human Longevity-Associated Genes (LAGs) (86). Believed to be rare in normal individuals, these gene variants are associated with longevity in centenarians, where they have been identified in almost 80 percent of this group. While the long-lived seem to have just as many disease-associated genetic variations as everyone else, epi-

genetic expression of these LAG variants may somehow cancel or take priority over these variations to compress disease and disability into the last few years of life (87). LAGs have recently been identified in **protein interaction networks** (PINs)

Figure 1.8. Protein Interaction Network (PIN) for Human Longevity-Associated Genes.

Reference: Glessner JT et al. 2013. Copy Number Variations in Alternative Splicing Gene Networks Impact Lifespan. *PLoS ONE* 8(1): e53846; doi: 10.1371/journal.pone.0053846 where approximately 50 interaction partners have been recognized (88). Although age-associated DNA methylation favors genes with exceptionally low **connectivity** to other genes (89), when connected to LAGs they become part of an unexpectedly large network of gene communities that play key roles in dictating complex aging processes. Understanding the effects of these various interactions may begin to provide an explanation for how LAGs operate in gene networks and pathways. Further study into the effect epigenetics plays in LAG expression could lead to advances in personalized approaches to longevity and anti-aging.

While beyond the scope of this chapter, it is important to emphasize that virtually all of the molecular mechanisms associated with the aging process are currently under investigation by various researchers to determine links associated with age-related epigenetic modifications. Although research in these areas is just a few years along and, as yet, is poorly understood in its consequences, it is already becoming evident that epigenetic mechanisms are fundamental to all known aging processes and are therefore targets for controlling and even reversing changes in gene expression that occur with age (90). For, unlike genetic mutations, epigenetic mechanisms appear to be reversible and, as a result, may prove to be receptive to environmental influences provided by personal care ingredients. Therefore, cosmetic and personal care formulations seeking to maintain youthful epigenetic patterns while working to reverse the patterns common in aging cells should deliver greater benefits than products of the past.

5.6.5 EPIGENETICS AND AGING SKIN

Epigenetic modifications at specific **CpG** sites support the fact that aging is coordinated in specific cell groups at different rates and in different ways depending upon the tissue (91). Skin is a particularly suitable model for epigenetic study due to its prolonged environmental exposure and relative ease to biopsy. Upon close examination, skin cells appear to share a high degree of epigenetic uniformity (92).

Changes due to epigenetic modifications acquired with age, a process known as **epigenetic drift** (93), are also readily apparent. Typical changes in aging skin include fine lines and wrinkling, dehydration, barrier damage, loss of elasticity, dermal thinning, loss of volume around the mouth and above the brows, hyperpigmentation, and age spots. Exacerbated by UV, tobacco smoke, and other environmental elements, these characteristics are the result of decreased epidermal thickness, disorganized corneocytes, depletion of stem cells in **epidermal niches**, lower levels of collagen types throughout the epidermal-dermal junction and demis, changes in vascularity, reduction in Langerhans cells, thus impacting the immune system, and melanocyte populations impacting pigmentation, typically by overexpression of pigmentation and **lipofuscin** (94), (95). Given the connection between the environment and skin aging, it is only a matter of time before the epigenetic mechanisms underlying the biological events specific to aging skin are disclosed.

Because **Monozygotic (MZ)** twins share identical DNA, they are excellent models to determine the environment's impact on the human epigenome, especially when comparing age-related epigenetic alterations in skin. Several well-known

photographic studies showing comparisons of DNA methylation patterns have been conducted on these individuals to exemplify the dramatic visible differences that occur when epigenetic drift is exacerbated by exposure to UV, smoking, stress, diet, and alcohol use.

Figure 1.9. Methylome comparisons between 3-year-old and 50-year-old identical twins at chromosomes 1, 3, 12, and 17. Yellow bands note similar distribution of DNA methylation, as indicated in the 3-year-old methylomes, while

significant changes in the 50-year-old methylomes are seen by the presence of hypermethylation (green) and hypomethylation (red).

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Figure 1.10. Epigenetic drift in young twins. Changes in gene expression caused by epigenetic drift can begin early in life. These identical twins not only differ in height, but one has asthma while the other does not.

© Reuters. See http://www.thestar.com.my/story.aspx

Figure 1.11. Effects of hormone replacement therapy. Twins, age 71. Twin B had 22 more years of hormone replacement therapy than twin A, resulting in a 1.2

lower BMI among other visual differences. Perceived age difference: 7.25 years. Study by Dr. Bahman Guyuron, Department of Plastic Surgery, University Hospitals Case Medical Center.

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Figure 1.12. Effects of smoking and suntanning. Twins, age 52, Ontario, Canada. Twin A has smoked half a pack a day for 14 years and has 7 times more sun exposure (8-10 weeks a year for 30 years) than her twin. Perceived age difference: 6.25 years. Study by Dr. Bahman Guyuron, Department of Plastic Surgery, University Hospitals Case Medical Center.

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Figure 1.13 Effects of smoking. Twin A has never smoked while Twin B has smoked for 29 years. Note the loose, baggy skin around her eyes, the hollow cheeks, forehead lines, large pores, and lines around her lips. Study by Dr. Bahman Guyuron, Department of Plastic Surgery, University Hospitals Case Medical Center.
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Figure 1.14. Effects of smoking. This twin pair is unique among twins. Not only did they spend their first two decades together but spent their adult years at the same type of job and lived in the same latitude, so their sun-exposure history is well-matched. However, the marked variation between them is that Twin A smoked approximately 52.5 packs of cigarettes a year while Twin B never smoked.

Doshi DN, Hanneman KK, Cooper KD. Smoking and skin aging in identical twins. Arch Dermatol. 2007 Dec;143(12):1543-6.

Figure 1.15. Effects of smoking. Twins, age not available. Both twins smoke; however, Twin B smoked for 14 years longer, as seen by more aging of facial fea-

tures, including undereye bags, more sagging of the upper eyelids, stronger lines around the mouth, on the chin and cheeks, larger pore size, and more hair loss at the hairline. Study by Dr. Bahman Guyuron, Department of Plastic Surgery, University Hospitals Case Medical Center.

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Figure 1.16. Effects of smoking, suntanning and lower weight. Twins, age 61. Twin B smoked 16 years of her life, has spent as much of her "life in the sun as possible," and has a Body Mass Index (BMI) 2.7 points less than her twin. Twin A has never smoked and has "as little exposure to the sun as possible." Perceived age difference: 11.25 years. Study by Dr. Bahman Guyuron, Department of Plastic Surgery, University Hospitals Case Medical Center.

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Illustrating a lifetime of poor choices and aggressive environments, these photos are visual indicators that genes known to help maintain the processes of youth are turned off by as yet unidentified environmental switches while those that advance aging are turned on. Further studies are required to understand the implications of epigenetic differences as compared to DNA mutations in these individuals.

5.6.6 EPIGENETICS MECHANISMS IN DNA DAMAGE AND REPAIR

Accumulated DNA damage resulting from environmental exposure is believed to be one of the leading contributors to cellular aging, resulting in **cell senescence** and ultimately cell death (96). To counteract DNA damage, cells possess an arsenal of enzymatic tools capable of remodeling and repairing DNA (97). These enzyme repair mechanisms are members of the **DNA Damage Response** (DDR) pathway that has evolved to address specific types of DNA damage. Mi-

spaired DNA bases, the "rungs" of the DNA ladder, are replaced with correct bases by **mismatch repair** (MMR) (98), and small chemical alterations of DNA bases are repaired by **base excision repair** (BER) where excision enzymes "cut out" damaged bases and replace them with the original sequence (99). More complex damage, such as **intrastrand cross-links** that occur on only one strand of DNA and UV-induced **cyclobutane pyrimidine dimers** (CPDs), are corrected by **nucleotide excision repair** (NER) (100). CPDs form most often at DNA methylation sites and are responsible for a considerable portion of the mutations induced by sunlight in mammalian skin cells (101). In all forms of successful DNA repair the damaged rungs of the DNA ladder are replaced with the original sequences. Unfortunately, valuable epigenetic tags that were on the original sequence may be removed during repair, potentially contributing to alterations in key gene expression (102).

DDR repair tools must be precisely regulated, because each in its own right can wreak havoc on DNA **integrity** if misused or allowed to access DNA at the inappropriate time or place (103). Epigenetic mechanisms rank among the many strategies utilized by cells to recruit and activate the right factors at the right time and place to repair DNA damage. Although the highly condensed structure of heterochromatin appears to protect DNA from mutations, it also presents a barrier to DDR enzymes, which must access DNA base pairs to perform repairs.

The **DDR pathway** also regulates processes that require multiple layers of decisions (104). These include working with the tumor suppressor p53 gene to activate repair pathways while delaying cell division, guiding a cell towards senescence or apoptosis if repair is unsuccessful, initiating heightened immune surveillance, and activating DNA protection as part of the tanning response (105). In the yeast *Saccharomyces cerevisiae*, the DDR pathway can also be triggered without DNA damage when histone-linked **sensor proteins** are tethered to chromatin (106). This latter finding may lead to development of skin care ingredients that can delay cell division while promoting DNA repair as well as enhancing or stimulating other beneficial DDR pathway functions.

5.6.7 COSMETIC INGREDIENTS AS EPIGENETIC MODIFIERS

Unlike genomic mutations, epigenetic alterations associated with aging are potentially reversible. This unique capacity presents targets for new and novel treatments aimed at reversing aging, dry skin, acne, rosacea, inflammation, hyperpigmentation, vitiligo, alopecia, and other skin conditions totally or partially driven by gene expression. Epigenetic modulation is already utilized to treat diseases such as melanoma and other cancers, so it is not a far reach to predict that skin care products will be intentionally employed as vehicles for epigenetically active

ingredients just as they are currently employed to deliver a variety of ingredients capable of altering gene expression.

Not surprisingly, innovative ingredient developers have already introduced several epigenetically active ingredients to cosmetic chemists. Two of these ingredients are described as being capable of up-regulating Nrf2 (107) (108), although it is not known if these genes must be located in euchromatic regions or if the ingredient causes heterochromatin to open in order to induce transcription. Another ingredient, utilizing a plant meristem cell suspension extract from plants of the genus *Oryza*, has been shown *in vitro* to reduce DNA methylation patterns in aged human dermal fibroblasts to patterns more characteristic of young, un-aged cells (109). This material appears to be the first patented ingredient in the United States intended for use in cosmetics for the control of an epigenetic mechanism.

To be clear, it is not necessary for an ingredient to be proven as epigenetically active for the ingredient to assert epigenetic activity. If one accepts epigenetics as those processes that modulate gene expression, virtually any ingredient shown to modulate gene expression falls, by default, into the realm of an epigenetic modifier. To remove any doubt as to this activity, it is simply a matter of identifying the ingredient's epigenetic mechanism. One or more will be found.

Many questions arise when working with epigenetically active ingredients in personal care. These include, but are not limited to:

1) When is it important to reverse an epigenetic tag? How do we know?
2) Once epigenetic activity is shown to occur, does the activity also alter gene expression and protein expression? Does a detectable and desired change occur in the skin? Can this change be seen or felt by the consumer?
3) Since each cell group has a unique epigenome that ages at its own rate, do epigenetically active ingredients affect various cell groups differently?
4) Does it make a difference *when* a cell is exposed to an epigenetically active ingredient? Day or night exposure, seasonal exposure, cell cycle exposure, or exposure during the development of the organism? This latter concern is of most importance when considering the **teratogenic** effects of a cosmetic ingredient during fetal gestation.
5) Does an epigenetically active ingredient vary in its effects depending upon the cell group? Can the effect on one cell group be positive while the effect on another cell group is negative? Can this be controlled?
6) When affecting one gene, are others also affected? Is the effect negative, positive, or unknown?
7) Does an epigenetically active ingredient need to penetrate the stratum corneum to assert its effect?
8) How quickly do epigenetic changes occur? How long do they endure?

9) Do epigenetically active cosmetic ingredients require a change in treatment approach? For instance, does an epigenetically active ingredient need to be used daily to produce the desired results? Can it be used several times, then discontinued once the epigenetic change has occurred? Should it be used sporadically to maintain results?
10) What types of testing need to be conducted to determine the safety and toxicity of epigenetically active ingredients?

5.6.8 NUTRIEPIGENETICS: HOW DIET ALTERS THE EPIGENOME

Nutrition is second only to the environment in epigenetic impact (110). The study of how nutrients and other chemicals in our diet affect the epigenome is named *nutriepigenetics*.

Among the most important epigenetic nutrients are those involved in the *one-carbon metabolic pathway* (aka the "folate-methioine cycle"), which describes the folate-dependent single-carbon reactions essential to **amino acid metabolism**, including those pathways that lead to the biosynthesis of DNA, RNA, membrane lipids, and neurotransmitters. Key nutrients in one-carbon metabolism are folate, riboflavin, pyridoxine, cobalamin, choline, betaine, and methionine. These nutrients provide the methyl groups required for both DNA- and histone methylation. Each is a known methyl donor and **cofactor** involved in **SAM-substrated methylation** used by DMNTs to methylate DNA. In one famous example of how nutrition impacts gene expression, Randy Jirtle, Ph.D., and his group at Duke University Medical Center in Durham, North Carolina, demonstrated that four nutritional supplements—B12, folic acid, choline, and betaine from sugar beets—when fed to pregnant mice altered the coat colors of their offspring by methlyating the mouse **agouti** gene (111). Even though both sets of mice were identical, this action gave rise to normal mice with brown coats instead of the yellow coats that arise when the agouti gene is expressed.

More importantly, the supplements lowered the offspring's adult susceptibility to obesity, diabetes, and cancer that occurs with agouti expression. Subsequent studies of Jirtle's agouti mice have been conducted over the years, all of which continue to support the importance of nutrition in the neonatal environment and on into adulthood. Niacin, pantothenic acid, biotin, and other water-soluble B vitamins play important roles in histone modification (112). Niacin is a **substrate** of SIRT1, which functions as an HDAC (113). Panthothenic acid contributes to the formation of acetyl-CoA, which is the source of acetyl groups

in histone acetylation. Biotin is covalently bound to histones in a process called "histone biotinylation." Eleven biotinylation sites have been identified in humans. Decreased histone biotinylation correlates with a decrease in life span and stress resistance in fruit flies (114). While the biological importance of histone biotinylation in humans is just beginning to be understood, it is known that certain variants play critical roles in the cellular response to DNA breaks, resulting in apoptosis (115).

Figure 1.17. Agouti mice—genetically identical, physically different. Chemicals and additives that enter our bodies can also affect the epigenome. Bisphenol A (BPA) is a compound used to make polycarbonate plastic. It is in many consumer products, including water bottles, cosmetic containers, and is a liner in tin cans. When pregnant yellow agouti mothers were fed BPA, more yellow, unhealthy babies were born than normal. Exposure to BPA during early development had caused decreased methylation of the agouti gene, leaving the mice vulnerable to obesity, diabetes, and cancer later in life. However, when BPA-exposed, pregnant yellow mice were fed

methyl-rich foods, the offspring were predominantly brown. The maternal nutrient supplementation had counteracted the negative effects of exposure.

Dolinoy D.C., Huang D., Jirtle R.L. (2007). Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. PNAS, 104: 13056-13061

Molecular modeling studies with other dietary compounds, such as α-lipoic acid, **metabolites** of tocopherol and **conjugated linoleic acids** (116), and garlic organosulfur compounds provide support for their role as HDAC inhibitors (117). Other natural compounds target DNMTs, histone modifications, and levels of noncoding RNAs. Naturally occurring dietary polyphenols, such as resveratrol, curcumin, quercetin, and catechins, exert antioxidant and anti-inflammatory properties by modulating different pathways, such as NF-κB, **mitogen activated protein kinase-dependent signaling pathways**, and activation of class III HDACs known as sirtuins (118). Sirtuins have been shown to regulate a variety of processes, including inflammation-immune function, cellular senescence and aging, stress resistance, cell survival, apoptosis, proliferation, differentiation and metabolism, stem cell pluripotency, **cell cycle** regulation, and **circadian rhythms** (119). Polyphenol activation of SIRT1, the best characterized and well studied of the seven members of the human sirtuin family, is beneficial for regulation of oxidative stress, inflammation, cellular senescence, **autophagy**/apoptosis, autoimmunity, metabolism, **adipogenesis**, circadian rhythm, and **mitochondrial biogenesis** (120). SIRT1 has also been found to regulate the activity of **heat shock factor 1** (HSF1), a transcription factor that responds to the presence of damaged cellular proteins by elevating **heat shock protein** expression, the molecular **chaperones** required for **protein folding** (121). With age, SIRT1 is known to decrease, which may explain why protein **denaturation** and **misfolding diseases** associated with aging increase (122).

Trans-resveratrol is one of the first molecules to be identified as having an age-fighting epigenetic effect. Found in the skin of red and purple grapes, berries, peanuts, and Japanese knotweed, this polyphenol may extend life span and reduce or delay aging and age-related diseases by activating class III HDACs (sirtuins) and regulating **oncogenic** and tumor suppressor **microRNAs** (123).

Genistein, a **phytoestrogenic** isoflavone found in fava beans, soybeans, and kudzu, is a demethylating agent that inhibits DNMTs and HDACs while activating HATs (124). This phytoestrogen has been shown to induce a **dose-dependent** inhibition of DNMT activity stronger than that of the soy isoflavones biochanin A and diadzein (125).

Epigallocatechin-3-gallate (EGCG), the major polyphenol found in green and white tea and to a lesser extent black tea, carob, cranberries, pecans, pistachios, and hazelnuts, has been extensively studied as a demethylating agent. It is thought to generate **SAH**, a potent inhibitor of DNA methylation (126), and is a direct inhibitor of DNMT1 and HATs. EGCG has also been found to modulate miRNA expression in **HepG2 cells** (127) and is capable of inhibiting cell growth and inducing apoptosis in cancers of dissimilar origins through both its antioxidant and epigenetic effects (1).

Sulforaphane (SFN) decreases the protein levels in DNMTs and inhibits the formation of HDACs. This water-soluble sulfur-containing **isothiocyanate** derivative is found in cruciferous vegetables, including broccoli and broccoli sprouts, cress and cress sprouts, horseradish, wasabi, daikon, canola, kale, turnip, brussel sprouts, mustards, and arugula, some of which are extracted for use in cosmetic formulas. SFN is a well-known cancer preventative agent that has been shown to regulate Nrf2, a key regulator of antioxidant defense systems, suggesting SFN may exert its chemopreventive effect in part via epigenetic modification of the Nrf2 gene (129). SFN is also known to boost **phase II enzymes** required by cells for the excretion of **xenobiotics** and other **ecotoxicants**, most likely via the epigenetic modification of **drug processing genes** (DPGs) (130).

Curcumin (diferuloylmethane), the yellow pigment in turmeric, inhibits DNMT1, HDACs, HATs, and miRNAs and modulates a variety of important transcription factors (TFs). Curcumin's HDAC activity is more potent than the well-known HDAC-inhibitors valproic acid and sodium butyrate and results in increased levels of acetylated histone (131). Conversely, HDAC2, whose activity is critical to the success of corticosteroid anti-inflammatories and which is impaired by tobacco smoke extract, is restored by curcumin (132). Because curcumin exerts diverse effects on different HDAC subtypes, further research is required to understand the chemical's mechanism of expression on these enzymes (133).

Diallyl sulfide, an organosulfur compound derived from garlic, onions, leeks, and other members of the plant genus *Allium*, inhibit HATs (134).

Vitamin A (retinol). The crosstalk between retinoid signaling and epigenetic factors is a dynamic and orchestrated process requiring a multitude of co-regulators, chromatin modifiers, and transcription machinery (135). In addition, all-trans retinoic acid (RA) is a master epigenetic regulator of gene transcription (136). A detailed discussion is required to do justice to the epigenetic effects of retinoids, placing the topic beyond the scope of this review.

Vitamin C (ascorbate) optimizes the activity of an enzyme in the **Tet** family of proteins as it acts to remove DNA methyl groups in mouse embryonic stem cells

(137). Further research is needed to determine if the same effect is attained when adult stem cells *in vivo* are exposed to vitamin C.

Vitamin D recruits histone acetylases (138) that modulate the bioactive vitamin D (VD) metabolite, 1,25- dihydroxyvitamin D3. Via its nuclear receptor (VDR) VD regulates over 1000 genes in the human genome involved in a wide array of disorders, including aging, inflammation, and cancer. It cooperates with other nuclear receptors that are influenced by HATs as well as several types of HDACs. HDAC inhibitors may contribute to normalization of VD metabolism (139).

As epigenetic alterations are now known to be both causative and contributing factors in aging, cancer, and other health conditions, natural compounds that are direct or indirect regulators of the epigenome constitute an excellent approach in nutritional prevention as well as therapy (140). Many may also offer dermal and epidermal activity when applied topically in the presence of penetration enhancers or vehicles that allow for enhanced absorption.

5.6.9 EPIGENETICS: THE UNIFYING THEORY OF AGING?

Besides disease processes in the body, the most researched area in the field of epigenetics is aging, the goals of which are extending longevity and the quality of life. Yet the biomolecular changes observed in aging cells are only beginning to be understood, let alone utilized in cosmetic science for the purposes of anti-aging. Even so, it is already clear that as the pieces of this multifaceted puzzle come together, epigenetics will prove to be the missing piece unifying the seemingly diverse theories of aging, for each theory appears to have its roots in epigenetic mechanisms. These include:

Random Damage Theory
Cross-Linking Theory
Glycation Theory
Inflammation Theory
Programmed Cell Death Theory
Mutation Accumulation Theory
Telomere Shortening Theory
Free Radical Theory
Mitochondrial Theory
Stem Cell Depletion Theory
Garbage Catastrophe Theory
Membrane Hypothesis

Indeed, the wide swath of aging theories reminds one of the blind men of India asked to describe an elephant. Each man touches a different part of the animal, then proclaims his observation as the only truth. While the elephant certainly has

the features of a snake (trunk), a rope (tail), pillars (legs), and hand fans (ears), it is not until a sighted man passes and tells the men they are describing an elephant that their efforts of description are made whole. Says the sighted observer, "All of you are right. The reason every one of you has come to a different conclusion is because each one of you has touched a different part of the elephant." And so it is with epigenetics. At the moment, though, epigenetics is the elephant in the room, composed of many moving parts without a cohesive whole. It is a nascent science, ripe with potential but not yet clear with solutions.

5.6.10 WHAT THE FUTURE HOLDS

It is obvious that epigenetics presents a major paradigm shift for cosmetic and personal care development, even though the epigenetic mechanisms directing gene expression in healthy and diseased dermis and epidermis are just beginning to be identified. Clearly, more exploration is required, yet those who have ventured forth into this territory are turning up intriguing possibilities. Epigenetic discoveries are now opening the way to developing effective therapies for a variety of disorders and diseases, that, with a little chemical creativity, can lead to the development of ingredient technologies for skin and scalp care. Gene expression and epigenetic mutations underlying the development of many pathological skin disorders and diseases have been identified (141), already opening the way for the epigenetic treatment of melanoma and psoriasis. Sun exposure, previously linked to gene mutations, has recently been associated with comparably small, but significant changes in DNA methylation patterns in the human epidermis and dermis (142), although the importance of this knowledge is not yet clear. Progress has occurred in identifying those epigenetic mechanisms involved in the control of epidermal development, specifically the transition of keratinocytes from the basal to the granular layer (143). Comparisons between young (18–24 years) and old (70–75 years) epidermal cells have revealed a set of 75 genes where DNA methylation reverses with age. Destabilization of these genes is believed to be a contributor to the decline in homeostasis that accompanies aging (144). And recently, the process of nuclear degradation and formation of keratins required for keratinocytes to evolve into corneocytes in the stratum corneum, nail matrix, and hair shaft has been associated with epigenetic mechanisms (145).

These are all first steps towards appreciating the role epigenetics plays in the skin. By understanding how a gene is programmed we should be able to understand how to reprogram it as well. Since the first decade of the twenty-first century, much progress has been made towards understanding the genetic organization of cells located in the skin, including keratinocytes, fibroblasts, melanocytes, Langerhans, T-cells, and other cells of immunity. We now know that certain genes important for maintaining a cell's youthful activity decrease

in expression while others, especially those involved in inflammation, become active or, if they are already active, are overexpressed. The epigenetic mechanisms involved in regulating these genes are obvious targets of future research, as is the understanding of epigenetic factors utilized by cosmetic ingredients to influence the expression of these genes. We can no longer think of ingredients and products as being innocuous. As research progresses, it will be discovered that virtually all ingredients that penetrate past the stratum corneum to cells in viable tissue have an epigenetic effect. Identification of the unique epigenomes in keratinoctyes, fibroblasts, melanocytes, and other cells found in the skin is underway, as is exploration into the distinct epigenetic factors common to aging, acne, rosacea, dry skin, and other skin conditions. Once complete, we will be able to satisfy not only the homeostatic requirements of an individual skin, we will be able to develop ingredients that target the epigenomic needs of individual tissues and the cells themselves—rather than painting all cells with the same broad brush of ingredients in formulaic concoctions as we do now.

A word of caution is advised here: Genes do not function in a desert. They are nodes on a path that connects them with other genes, some silent for a reason and some active for a while. This network of connectivity, known as the protein-DNA **interactome**, features transcription factors, chromatin regulatory proteins, epigenetic tags, and other groups of genes and proteins that share in the intricate biological systems that operate the human body in ways we have yet to fathom. The human sirtuin interactome is a good example of this far-reaching interconnectivity. Combined, the seven members of the human sirtuin family act as hub proteins whose connections encompass 25 percent of the entire human interactome (146), making this family of enzymes central operators in the body's metabolic network, cancer, aging, and other key mechanisms that are as yet undetermined.

No matter how good our intentions, meddling in one area without knowing how it will affect the entire network can result in catastrophes as well as cures, especially if the target gene is a hub with multiple tentacles outstretched to other genes. Therefore, we must tread lightly and research extensively when developing new personal care products with epigenetic activity.

It should also be noted here that while the study of epigenetics and genetics is important, it is their influence on protein synthesis that gives them value. Proteins are the functional elements in cells. They compose the body's structures, deliver oxygen throughout the body, remove waste, provide communication between cells and between cells and the environment, act as signaling agents, catalyze metabolic reactions, respond to stimuli, and perform a variety of other activities essential to life's processes. Without proteins, life could not exist. No

study of epigenetics and genetics can be complete without including the study of proteomics as well.

Finally, because the same epigenetic mechanisms appear to be found in all cells, cosmetic chemists can benefit from attending conferences and reading literature published in other disciplines where epigenetics is being explored. Like the crosstalk between cells, crosstalk between researchers in different fields of experience can help advance discoveries in their chosen disciplines, including the development of cosmetics and personal care.

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GLOSSARY

Acetylation. A chemical reaction that introduces an acetyl functional group into a chemical compound. Acetylation of **histones** tends to define the "openness" of **chromatin**, known as "**euchromatin**," which allows DNA to be **transcribed**.

Adipogenesis. The process by which fat cells known as adipocytes are created.

Agouti gene. When expressed, this gene produces a banded coat in mammals. In mice, this gene produces yellow coats. If one parent passes on the Agouti trait, the

mice tend towards obesity and diabetes. If both parents pass on the trait, the mice die.

Amino acid metabolism. The process by which proteins from food are broken down into their constituent amino acids in the gastrointestinal tract, passed into the bloodstream, and delivered to the liver. They are held in amino acid pools until needed by cells in other organs as energy sources or to produce proteins in cells and tissues.

Amino-terminal tail. The long tail protruding from the end of an amino acid that forms a **histone** protein. These tails are frequently the sites for enzyme modifications that alter gene regulation, which include **methylation**, **acetylation**, and **phosphorylation**.

Apoptosis. Process of "programmed cell death," also known as cell suicide, initiated by genetic and epigenetic mechanisms within the cell.

ATP-dependent chromatin remodeling. Chromatin remodeling processes that require energy from ATP to produce rapid rearrangements of the chromatin structure. Among other functions, *ATP-dependent chromatin remodeling factors* use the energy of ATP to introduce the superhelical structure into DNA.

Autophagy. The basic mechanism by which unnecessary or dysfunctional cellular components are degraded by cellular bodies known as "lysosomes."

Base pair. Two of the four **nucleotides** that form the "rungs" on the DNA ladder. Adenine (A) is always paired with thymine (T) while cytosine (C) is always paired with guanine (G). The human **genome** contains over three billion base pairs.

Base excision repair (BER). DNA repair mechanism that removes damaged bases on single DNA strands and replaces them with the appropriate bases.

Biomarkers. A molecule that allows the detection and isolation of a particular cell type. May also refer to a disease-causing DNA sequence or a sequence associated with susceptibility to a particular disease.

Carcinogenesis. The process by which normal cells become cancer cells.

Cell cycle. The ordered series of events, known as "phases," that must be completed before a cell can divide. The cell cycle includes accurate duplication of the genome during the DNA synthesis phase and segregation of complete sets of chromosomes to each daughter cell as cell division occurs.

Cell cycle regulation. The mechanisms that control or maintain the rate of speed of the cell division process known as the **cell cycle**. These mechanisms are critical to cell survival, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division.

Cell cycle regulatory gene. The genes responsible for producing proteins involved in regulation of the cell cycle. These include **p53**, **cyclins**, and **cyclin-dependent kinases**.

Cell lineage development. The process by which stem cells become more specialized via epigenetic mechanisms.

Cell senescence. The time towards the end of a cell's life when it stops replicating.

Cell signaling. One part of the complex communication system that governs cellular activities, coordinates cell actions, and enables cells to perceive and respond to their microenvironment.

Chaperones. A class of proteins found in a cell's cytoplasm that facilitates the assembly or disassembly and folding or unfolding of cellular proteins. In humans, **heat shock proteins** are chaperones.

Chromatin remodeling factors. Molecules that both activate and repress **transcription**. Also have roles outside of transcriptional regulation, such as **DNA repair**.

Chromatin. The chromosomal complex of DNA, **nucleosomes**, and RNA found within the cell nucleus of **eukaryotic** cells. Chromatin performs four main functions: 1) package two meters of DNA into the cell's nucleus, 2) strengthen DNA so it won't fragment during mitosis (cell division), 3) prevent or reduce DNA damage, 4) control **gene expression** and DNA replication. Chromatin structure varies depending upon the phase of the **cell cycle**.

Circadian rhythms. The epigenetically regulated processes that govern clear patterns of hormone production, cell regeneration, sleep, temperature regulation, skin hydration and barrier repair, DNA repair, energy (ATP) production, and other physical and behavioral changes that occur over an approximate 24-hour cycle. Also known as the body's *internal clock* or *biological clock*.

Cofactor. A nonprotein chemical compound required for the biological activity of a protein, usually an enzyme. Cofactors are considered "helper molecules" that assist proteins in biochemical transformation.

Condensation. The process of compacting chromatin to allow it to fit into the cell's nucleus.

Conjugated linoleic acids. A family of approximately 28 isomers (molecules with the same molecular makeup but different structures) of linoleic acid.

Connectivity. The functional connections between genes sharing one or more action mechanisms.

Correlation. A statistical measure that indicates the extent to which two or more variables fluctuate together.

CpG site. Region of DNA where a cytosine and a guanine **nucleotide** are placed next to one another with a phosphate between them. When a **methyl group** is attached to the cytosine, expression of the CpG site is turned off. If this occurs in a gene sequence, the gene's expression into a protein can be prevented. In mammals, 70 to 80 percent of cytosines are methylated.

CpG Island. Genomic regions that contain a high frequency of **CpG** sites. Most CpG islands are located at the beginning of a gene, known as the "**promoter region**."

CpG regions. See **CpG sites** and **CpG islands**.

Cross-linking. A form of DNA damage that occurs when molecules, such as proteins, react with two different positions in the DNA strand or in opposite strands. DNA replication is blocked by cross-links, which cause **cell cycle** arrest and **apoptosis** if the cross-link is not repaired.

Crosstalk. When one or more **signaling pathways** between cells and proteins occur.

Cyclin-dependent kinases (CDKs). Protein family that plays a role in cell cycle and transcription regulation, and mRNA processing.

Cyclobutane pyrimidine dimers (CPDs). The most common DNA lesions in human skin exposed to UVA and UVB radiation, CPDs are DNA lesions formed when thymine or cytosine bases join together in a formation called a *dimer*. Unrepaired CPDs can be mutagenic because they interfere with base pairing of nucleotides during DNA replication in the **cell cycle**. They are the primary cause of skin cancers, including melanomas.

DDR pathways. DNA damage response (DDR) and checkpoint pathways are intertwined signaling networks that arrest the **cell cycle**, and recognize and repair genetic mistakes that arise during DNA replication and transcription or via exposure to chemical and physical agents that interact with **nucleotides**.

Deacetylation. The process of removing acetyl groups from histones and other proteins.

Denaturation. The process in which proteins or nucleotides are altered, leaving only the peptide bonds between amino acids intact.

De novo. Latin for "from the beginning" or "anew." A de novo mutation is a genetic mutation is not possessed by or transmitted from either parent to a daughter cell. De novo synthesis is the formation of complex molecules from simple molecules.

Developmental genes. Genes that control or regulate development.

Differentiation. The process by which a less specialized cell (**zygote**) becomes a more specialized cell type (**keratinocyte**) due to **epigenetic** modifications in **gene expression**. Differentiation dramatically alters a cell's size, shape, metabolic activity, and signal responsiveness.

DNA (deoxyribonucleic acid). An extremely long molecule of **nucleic acid** that carries the complete set of genetic information located in a cell's nucleus. DNA consists of two long chains of **nucleotides** twisted into a double helix and joined by hydrogen bonds between complementary **bases** of adenine and thymine or cytosine and guanine. It is the primary formation in chromosomes and **chromatin**.

DNA Damage Response (DDR). The complex signaling network that includes **cell cycle** checkpoints and **DNA repair**. The DDR both affects and is impacted by chromatin structure, DNA replication, telomeres, cell growth, and the cell cycle status.

DNA methylation signature. Specific patterns of DNA methylation unique to diseases (ex: cancer types) or biological conditions (ex: dry skin).

DNA methyltransferases (DNMTs). A family of enzymes that transfer methyl groups to DNA.

DNA repair. The family of processes a cell utilizes to identify and correct DNA damage.

Dose-dependent. Refers to the effects of treatment with a drug or cosmetic ingredient. If the effects change when the amount (dose) of the substance is changed, the effects are dose-dependent.

Down-regulation. Process by which a gene or cell decreases the amount of a cellular component, such as a protein or RNA, in response to external changes.

Drug processing genes (DPGs). Genes responsible for the absorption, distribution, metabolism, and excretion of **xenobiotics**. **Phase II enzymes** are DPGs. Increasing evidence suggests DPGs are regulated by epigenetic mechanisms.

Dynamic. A process or system that is constantly changing or progressing.

Ecotoxicants. Humanmade chemicals with toxic potential that are released by human actions into the environment. Ecotoxicants have the potential to negatively impact ecosystems at relatively low concentrations.

Embryonic cells. Stem cells derived from an early-stage embryo; usually four to five days post-fertilization.

Endogenous antioxidant. Antioxidant made by the cell rather than being taken into the body.

Epidermal niche. An area, or microenvironment, where keratinocyte stem cells are located in the basal layer of the epidermis, hair follicles, and the sebaceous glands. The niche is believed to play a key role in controlling the position, fate, and regenerative potential of keratinocyte stem cells.

Epigenetic drift. The progressive deregulation of epigenetic marks, especially methyl groups, that occurs with aging and diseases associated with aging such as cancer.

Epigenetics. The study of changes in gene activity not caused by alterations in the DNA sequence. Epigenetic changes are usually **heritable**.

Epigenome. The genome-wide distribution of epigenetic marks, including all of the epigenetic changes that affect an organism's **genome**. The epigenome is involved with regulating **gene expression**, development, tissue **differentiation**, and changes in DNA sequences within the genome to reverse mutations and alter the cell's genome size. While some elements of the epigenome are stable throughout a cell's lifetime, other elements change in response to the cell's environment.

Eukaryotic. Cells that contain a nucleus where genetic material is secreted. Usually with membrane-enclosed structures known as "organelles" are also present. Animals, plants, fungi, and all other multicellular organisms are eukaryotes.

FOXO genes. Genes that belongs to the Forkhead family of transcription factors, which play important roles in regulating the expression of genes involved in cell growth, proliferation, **differentiation**, and longevity.

Gene. A specific sequence of DNA **base pairs** that is transcribed by **RNA** into a specific amino acid sequence to form a protein. Of the three million **base pairs** that make up the human **genome**, less than 2 percent form genes.

Gene expression. Process by which information from a gene is used in the synthesis of a functional gene product (protein or **RNA**).

Gene regulation. The process of increasing or decreasing **gene expression**.

Genome. The entirety of an organism's hereditary information, including protein-coding genes and noncoding sequences of DNA and RNA.

Genomic instability. A high frequency of DNA mutations.

Genomic stability. The processes required to maintain the integrity, or wholeness and original functionality, of the **genome**.

Heat shock factor 1 (HSF1). Transcription factor rapidly induced after exposure to elevated temperature and other forms of stress.

Heat shock proteins. A group of proteins induced by elevated temperatures, UV light, toxins and toxicants, infection, inflammation, and other forms or cellular stress. HSPs act as **chaperones** to fold and unfold other proteins, assist in intracellular trafficking of proteins and copying proteins denatured by heat and other stresses. HSPs can also occur under nonstressful conditions, where they simply monitor the cell's proteins, such as ensuring denatured proteins are delivered to proteosomes, the cell's "recycling" organelles.

HepG2 cells. Human liver cancer cells.

Heritable. Capable of being passed between generations, either from mother cell to daughter cells or from parent to child.

Heterochromatin. Tightly packed, or highly **condensed**, **chromatin**.

Histone acetyltransferase (HAT). Enzymes that attach an acetyl functional group (see **acetylation**) to **histone** proteins, usually resulting in *increased* **gene expression**.

Histone deacetylase (HDAC). Enzymes that remove acetyl functional groups from **histone** proteins, usually resulting in *decreased* **gene expression**.

Histone methyltransferases (HMTs). Enzymes that attach one, two, or three methyl groups to specific **histone** regions via **methylation**.

Histone modification. Covalent modifications of histone tails or cores that alter the histone's interactions with DNA and nuclear proteins.

Histone. A protein that forms a **nucleosome**.

Housekeeping genes. Genes required for the maintenance of basic cellular functions.

Human Genome Project (HGP). The international research project that 1) identified the order in which **nucleobases** appear in the human genome, 2) created a map that shows the locations of genes for major sections of all human chromosomes, and 3) produced "linkage maps" through which inherited traits, such as those for genetic disease, can be tracked over generations. It was completed in 2003.

Hypermethylation. An abundance of methyl groups attached to cytosines. Usually results in gene inactivation.

Intrastrand cross-links. Cross-linking of the same strand of DNA.

Integrity. The state of *wholeness* and original functionality of the genome or a DNA molecule.

Interactome. The chart of protein-protein or protein-**nucleic acid** interaction networks.

Isothiocyanates. A family of compounds that boost **phase II enzymes**. Derived from cruciferous vegetables, including horseradish, onions, broccoli, kale, cabbage, watercress, and nasturtiums.

Jumonji domain-containing hydroxylases (JHDMs or JMJDs). Enzyme group that removes **methyl groups** from **histones**.

Junk DNA. The now-obsolete term for **DNA** that does not code for proteins. Once believed to have no biological function, this part of the **genome** is now called **noncoding DNA**.

Keratinocyte. The primary cell type in the epidermis. Synthesizes keratin and other proteins as well as epidermal barrier lipids.

Lipofuscin. A fine granular yellow-brown pigment composed of oxidized unsaturated fatty acids, sugars and metals, including mercury, iron and copper, which remain after lysosomal digestion. May be connected to membrane and mitochondrial damage. Considered to be one of the "wear and tear" pigments that accumulate with age, lipofuscin is found in retinal, skin, nerve, and other cells.

Long noncoding RNA (lncRNA). Nonprotein coding form of RNA made up of 200 or more **nucleotides**.

Lysine-specific demethylase 1 (LSD1). Enzyme that removes **methyl groups** specifically from lysine residues of **histones**. Plays an important role in the **epigenetic** control of **gene expression**. Aberrant gene silencing due to LSD-1 overexpression is thought to contribute to **oncogenesis**.

Messenger RNA (mRNA). A large family of molecules resulting from the **transcription** of DNA by **RNA polymerase**. The resulting mRNA molecule then **translates** the genetic code into amino acid sequences that form proteins.

Metabolism. The group of enzyme-dependent, life-sustaining chemical transformations within living cells. Allows cells to reproduce, grow, maintain structural elements, and respond to their environments.

Metabolites. Molecules, usually small, that are produced during **metabolism**. Can also refer to the molecule that remains after a drug or other substance is broken down (metabolized) by the body.

Methyl group. A stable molecule composed of one carbon atom bonded to three hydrogen atoms.

Methylation. The process by which a methyl group is added to another molecule or another atom or group is replaced by a methyl group.

Methylome. The distribution of methylated sequences on the genome.

MicroRNA (miRNA). A group of small (approx. 22 nucleotides) nonprotein coding RNA molecules that play roles in **transcriptional** and post-**transcription** regulation of **gene expression**.

Misfolding. Misfolded proteins occur when a protein follows the wrong folding pathway directed by the amino acid sequence that makes up the protein. Misfolding can also occur spontaneously as a form of protein **denaturation**. Failure to fold into the correct formation usually produces inactive proteins. However, in some instances misfolded proteins have a modified or toxic functionality, resulting in allergic responses, amyloidosis in the skin, and diseases such as Huntington's and Alzheimer's.

Mismatch repair (MMR). A form of **DNA repair** that detects and repairs the insertion, deletion, or mis-incorporation of **nucleobases**. Examples of mismatched bases include guanine-thymine and adenosine-cytosine.

Mitochondrial biogenesis. The process by which new mitochondria are formed in the cell.

Mitogen activated protein kinase-dependent signaling pathways. Signaling pathways involved in directing cellular responses to a diverse array of stimuli. They regulate cell proliferation and **differentiation**, **gene expression**, cell survival, and **apoptosis**.

Molecular modeling study. A form of research that utilizes techniques to model or mimic molecular behavior.

Monozygotic (MZ). Also known as "identical twins" because they develop from the same fertilized egg and therefore have the identical genome.

Mutation. Unrepaired damage to a DNA **base pair** sequence accumulated over time. If the mutation occurs within a **gene**, the protein it encodes may be produced incorrectly and its function in the body may be altered, sometimes for the worse.

NFkB. A **transcription factor** that controls DNA **transcription**. It is involved in cytokine production and cell survival.

Noncoding DNA. The section of a **genome** that does not code for protein synthesis. In humans, noncoding DNA makes up 98 percent of the genome while in a typical bacterial genome only about 2 percent is noncoding DNA.

Noncoding RNA (ncRNA). A functional RNA molecule that is not translated into a protein. Small RNAs, such as **microRNA**, and **long noncoding RNA**, are noncoding RNAs.

Nrf2. A **transcription factor** that functions as the key controller of the stress response by increasing numerous **endogenous antioxidants** and pollutant-detoxifying **phase II enzymes**.

Nucleic acid. The name for DNA and RNA molecules. The basic component of nucleic acids is the **nucleotide**.

Nucleobase. See **nucleotide**.

Nucleosome Remodeling Deacetylase (NuRD). ATP-dependent **chromatin remodeling** enzyme. May regulate **transcription** of specific genes by interacting with specific **transcription factors**.

Nucleosome. The segment of eight core **histone** proteins that form a "spool" around which the "thread" of DNA is wound.

Nucleotide. The basic component of **nucleic acids**. They consist of a pentose sugar (ribose in RNA, deoxyribose in DNA), a phosphate group and a **nucleobase**. It is the nucleobase that forms the rungs on the DNA "ladder." DNA and RNA each contain four bases. Arginine, guanine, and cytosine are in both nucleic acids. Thymine is in DNA but is substituted during RNA transcription by uricil. Nucleotides pair off, guanine with cytosine and arginine with thymine or uricil. Strings of base pairs strung together in specific sequences are the mechanism for storing and transmitting hereditary information in all life forms. These formations allow for the encoding, transmitting, and expression of genetic information and feature the blueprint for every protein in the body. The human genome is made up of three billion base pairs, or six billion on both chromosomes, although less than 2 percent are known to be protein-coding genes.

Nucleotide excision repair (NER). A form of DNA repair where a small region of DNA, damaged by UV irradiation or chemical exposure, is removed (excised) and replaced by the appropriate DNA sequence.

Oncogenic. The creation of cancer.

P53 gene. See **Tumor suppressor P53 gene**.

Peptidylarginine deiminase 4 (PAD4). A nuclear enzyme in the hydrolase family (enzymes that act on carbon-nitrogen bonds other than peptide bonds) that affects specific **histones**. Also called "protein-arginine deiminase." It is commonly present in inflammation, autoimmune diseases, cancer, severe psoriasis, and other skin diseases.

Phase II enzymes. Cellular detoxification enzymes important in the defense against **xenobiotics** and the transformation and excretion of toxic compounds arising from within the cell. Lack of these enzymes can play a role in several forms of cancer.

Phosphorylation. The addition of a phosphate group to a protein or other organic molecule. Phosphorylation turns many enzymes on and off, thereby altering their function and activity.

Phytoestrogenic. A diverse group of nonsteroidal plant compounds that are structurally similar to the animal-derived estrogen, estradiol. Due to this similarity, they are able to cause estrogenic and anti-estrogenic effects in humans and other animals by sitting in or blocking estrogen receptor sites on cell membranes.

Polycomb-group proteins (PcG). Family of proteins that silence genes by the epigenetic remodeling of chromatin.

Promoter regions. The section of DNA located in front of a gene that initiates gene transcription by providing a secure binding site for RNA polymerase, the enzyme that synthesizes RNA.

Protein denaturation. The process by which a protein unfolds and loses its three-dimensional shape. Denaturation leads to the loss of protein function and can result in cell death.

Protein expression. The process of **transcription** and **translation** that creates a protein.

Protein folding. Protein folding describes the folds, pleats, bulges, coils, and loops that make up a protein's three-dimensional shape, known as the protein's "secondary structure." The secondary structure contributes to the protein's function. Fully folded proteins feature distinct surface traits that govern the molecules with which they can interact.

Protein interaction networks (PINs). A map or graph that depicts the pathways by which proteins interact.

Protein misfolding diseases. Diseases that occur as a result of protein **misfolding**. These include, but are not limited to, cancer, amyloidoses, Huntington's, Alzheimer's, and prion diseases.

Proteostasis. The balance between folded and stabilized proteins and the controlled degradation and removal of proteins. During stress conditions, such as aging, this balance is disturbed when protein damage and clearance is slowed. This results in the accumulation of oxidized, denatured, and dysfunctional proteins.

Random. Having no specific pattern. Unpredictable.

RNA (ribonucleic acid). Group of single-stranded **nuclei acids** found in the cell's nucleus. **mRNA** translates protein-coding **DNA** into amino acid chains that make up proteins. Also see **noncoding RNA**, **small interfering RNA**, **microRNA**, and **long noncoding RNA**.

RNA polymerase. The enzyme that transcribes a DNA sequence to RNA.

S-adenosylmethionine (SAM). A molecule with a chemically reactive **methyl group**, allowing the donation of the methyl group to a number of other molecules during more than 40 different types of metabolic reactions. SAM is the primary universal donor of methyl groups in mammals.

S-adenosylhomocystein (SAH). An amino acid derivative and **SAM** by-product used in several metabolic pathways. SAH is also produced when SAM attaches methyl groups to DNA, RNA, **histones**, and other proteins. SAH is a risk factor for many diseases, including cancer.

SAM-substrated methylation. The transfer of a methyl group from **SAM** to various substrates, including nucleic acids, proteins, and lipids. In biochemistry, a **substrate** is the biomolecule on which an enzyme reacts.

Senescence. The time towards the end of a cell's life when it stops replicating.

Sensor proteins. Intracellular proteins that detect and monitor metabolic signals or messages from outside and within the cell.

Signaling pathways. A molecular-based form of communication between cells and cells and proteins. Also known as "signal transduction pathways."

Single nucleotide polymorphisms (SNPs). The most common type of genetic **variation** in humans, one SNP represents a difference in a single **nucleotide**. For example, an SNP may replace thymine with cytosine in a DNA sequence. SNPs are usually found between genes but when they occur within or near a gene, they may play a role in an individual's response to a certain drug or susceptibility to specific diseases, toxins, and other environmental factors.

SIRT1. An enzyme in the **sirtuin** class that **deacetylates histones** and other proteins that contribute to cellular regulation, reactions to stress, and longevity.

Sirtuins. Class of proteins found in bacteria, archaea, and **eukaryotes** that influence a wide range of cellular events via such processes as protein **deacetylase** activity. Among the processes influenced by sirtuins are aging, **transcription**, **apoptosis**, inflammation, **mitochondrial biogenesis**, and control of circadian rhythms.

Small interfering RNA (siRNA). Also known as "silencing RNA," this short RNA sequence plays many roles but is mostly known for 1) interfering with the expression of genes that mirror, or complement, its **nucleotide** sequence, and 2) shaping the structure of **chromatin**.

Somatic cells. Cells in the body, not used for organism reproduction.

Stem cell. An unspecialized cell that can develop into more tissue-specific cells. A stem cell is a cell that has the ability to divide (self-replicate) for indefinite periods—often throughout the life of the organism. Under the right conditions, or given the right signals, stem cells can give rise (differentiate) to the many different cell types that make up the organism. That is, stem cells have the potential to develop into mature cells that have characteristic shapes and specialized functions, such as heart cells, skin cells, or nerve cells (1). Stem cell differentiation and specificity are governed by epigenetic factors.

Stress response (cellular). A wide range of molecular changes that occur within a cell in response to environmental stress, dehydration, temperature extremes, toxin, and toxicant exposure and mechanical damage.

Substrate. The biomolecule on which an enzyme reacts.

Sumoylation. One of the key regulatory protein modifications performed after amino acids in a protein have been sequenced in **eukaryotic** cells. Utilizes *small ubiquitin-related modifier* (SUMO) proteins to modify **histones** and hundreds of other proteins involved in such processes as **chromatin** organization, **transcription**, and **DNA repair**. Sumoylation is reversible.

Systemic hormonal factors. Hormones transmitted via the circulatory system.

TATA-box binding protein (TAF1). A general transcription factor that binds specifically to the DNA sequence known as the TATA box. TATA-binding proteins help position RNA enzymes over a gene's transcription start site.

Telomere. Region of repetitive nucleotide sequences located at the end of a chromosome. Telomeres appear to protect genes near chromosomal ends from fraying during cell division and prevent a chromosome from fusing with a neighboring chromosome.

Telomeric repeat sequences. The sequence of **nucleotides** that repeat in telomeres. In vertebrates, the sequence is TTAGGG.

Teratogenic. A drug or other chemical capable of interfering with fetal development, usually leading to birth defects.

Terminal cells. See **terminally differentiated cells**.

Terminally differentiated cells. Cells that no longer replicate their genomic DNA. **Keratinocytes** become terminally differentiated when they lose their nuclei and enter the stratum corneum as corneocytes.

Tet proteins. *"Ten-eleven translocation"* proteins may reverse DNA methylation.

Transcription factor (TF). A protein that binds to specific DNA sequences, promoting or blocking the recruitment of **RNA polymerase**.

Transcription protein binding site. The area on DNA where transcription proteins can attach.

Transcription protein. See **transcription factor**.

Transcription. The first step of protein synthesis, in which a segment of DNA is copied into RNA by **RNA polymerase**.

Translation. The process that allows **mRNA** to produce a protein.

Tumor cell invasion. The spread, or metastasis, of cancer cells, either individually or collectively, to surrounding tissues.

Tumor suppressor genes. Genes that stop tumor growth. Also known as the "*Guardians of the Genome*," these genes code for proteins that tell a cell to leave the **cell cycle** and stop the division process until **DNA damage** (mutations) can be repaired. If the damage is irreparable, the cell is directed to undergo **apoptosis** to prevent spreading the mutation via cell division. When tumor suppressor genes are mutated or turned off via epigenetic tags, they can no longer produce tumor suppressor proteins. This results in the spread of cancerous cells.

Tumor suppressor P53 gene. The most studied of all tumor suppressor genes, p53 is also the most frequently mutated gene in human cancers. P53 regulates cell growth, inhibiting the **cell cycle** in certain circumstances, while promoting **apoptosis** in others.

Tumorigenesis. The production or development of tumors.

Ubiquitination. One of the key regulatory protein modifications performed after amino acids in a protein have been sequenced in **eukaryotic** cells. Utilizes *ubiquitin* proteins to modify histones and hundreds of other proteins. Ubiquination is reversible.

Up-regulation. Process by which a gene or cell increases the amount of a cellular component, such as a protein or RNA, in response to external changes.

Variants. Refers to genetic variations. The most common variants are **single-nucleotide polymorphisms (SNPs)** and copy-number variations (CNVs). The latter result in the cell having an abnormal, or for certain genes, a normal variation in the

number of copies of one or more sections of DNA. Gene mutations, another form of variation, are relatively rare.

Xenobiotic. Natural or synthetic antibiotics, hormones, and other chemicals that are 1) not normally produced by or expected to be present within an organism or 2) chemicals that are present at much higher concentrations than normal. Can also refer to pollutants such as dioxin, bisphenol A, vinclozolin, and DEET that are found in biological and/or ecological systems.

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CHRONOBIOLOGY OF THE SKIN SKIN CIRCADIAN RHYTHM AND CLOCK GENES: A NEW APPROACH TO SLOWING DOWN THE AGING PROCESS

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ABSTRACT

It is now well established that our body follows a circadian rhythm, which means that it responds to day-night cycles. This multi-oscillatory network has made human physiology adapt to environmental changes and, importantly, helps us to adjust our internal clocks and achieve proper body function.

The master clock that regulates this 24-hour cycle throughout our body is found in the suprachiasmatic nucleus (SCN), in the part of the brain known as the hypothalamus. This area of the brain responds to light received through the retina of the eye. Recently, mammalian peripheral molecular clocks, also known as clock genes, have been shown to be present in nearly all the cells in our body. The clock genes synchronization is controlled by the suprachiasmatic nuclei (SCN) and is responsible for directing cells to do the right thing at the right time! This mechanism has helped the human species to survive and plays a critical role in genome stability maintenance.

More than 10 percent of our genes are controlled by this circadian cycle and synchronize many of our internal molecular processes. The group of genes under circadian control includes those involved in DNA damage-repair responses, cellular proliferation, cell cycle, cell-cycle arrest, apoptosis, and metabolism. Uncoupling of circadian gene expression from the cell cycle has been shown to induce genome instability, premature aging, and cancer, as well as causing an increased tendency towards diabetes and obesity.

The skin, as the largest organ of our body, also follows a circadian rhythm. Each skin cell contains clock genes that control and synchronize cellular activities for generation of optimal benefits to the skin. This chronobiological phenomenon

shows that during the day most of the cellular energy is used to protect the skin while during the night most of the energy is used to restore, repair, recharge, and regenerate skin cells. Unfortunately, because of different factors (i.e., stress, aging) this well-orchestrated mechanism inevitably goes out of sync. In this chapter we review the benefits of understanding the skin circadian rhythm and the importance of maintaining it in optimal synchronism as long as possible in order to slow down the aging process. As such, this understanding provides opportunities for leads to new breakthroughs in anti-aging technology for the personal care industry

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5.7.1 INTRODUCTION TO CIRCADIAN RHYTHM AND CLOCK GENES

It is now well established that our body follows a circadian rhythm, responding to daylight, which helps us to adjust our internal clocks. Anyone who has traveled long distances by airplane has observed how their body is still working well at the time of their departure but not in the arrival city. This phenomenon is a result of the fact that our internal clocks usually need a few days in order to readjust all of our biological functions so that our cells can function correctly at the right time!

Our circadian clock promotes metabolic efficiency by timing activities with day-night rhythms following the Earth's rotation time over a 24-hour period. This clock gives optimal efficiency to each cellular function, which has helped living species to survive for eons. As a consequence of the Earth's rotation every 24 hours, organisms are subjected to fluctuations in light and temperatures. There is an evolutionary relationship between our internal physiology and the geophysical earth cycle to help our body adapt to the daily cycle of day and night. The fact that we have biological clocks provides us with an adaptive advantage by ensuring that our body functions are optimally adapted to their environment. This pattern results in optimal physiological efficiency. Circadian rhythms and cycles control our sleep pattern, body temperature, heart activity, hormone secretion, blood pressure, oxygen consumption, metabolism, and many other activities. To paraphrase Edmunds (1): "The 'right' substance (molecule) should not only be at the 'right' place in the 'right' amount, but all of this should also happen at the 'right' time." This well-orchestrated organization synchronized by clock genes under SCN control makes sure that all of these functions happen at the right times and repeat every day!

Nowadays considerable research is being directed toward developing an increased understanding of the circadian rhythm and our internal clocks. This intriguing phenomenon has clearly shown, when desynchronized or out-of-sync, to be responsible for certain diseases such as cancer and obesity. Rhythm generation is now understood to be an intrinsic property of single cells that are driven by an intracellular molecular oscillator based on transcriptional negative feedback loops responding to synchronization coming from the suprachiasmatic nucleus (SCN) in the hypothalamus located in the brain. The SCN is the master clock (Figure 1), the SCN that regulates this 24-hour cycle throughout our body and is composed of neurons that can sense external cues such as light and darkness transmitted through the retina. The human circadian rhythm is actually a little longer than exactly 24 hours and will be maintained even when the organism is removed from light. However, as soon as light is received by the retina again, the clock will reset itself to match the earth's rhythm of 24 hours. This is because mammalian molecular clocks are not only located in the SCN but also in nearly all cells in our body.

Figure 1: Circadian rhythm in the body is controlled by the suprachiasmatic nucleus located in the hypothalamus. Environmental cues such as light are transferred to the SCN, which then synchronizes all cellular functions in the different organs. The neuronal and hormonal activities it generates regulate many different body functions in a 24-hour cycle, using around 20,000 neurons.

Most cells and tissues express autonomous clocks. In the past few decades, scientists have discovered the genes responsible for running the internal clocks. Genes hold the information to build and maintain an organism's cells and pass genetic traits to offspring. The human species has more than 20,000 genes encoded by DNA. A part of these genes is the Clock gene family. These include but are not limited to period (per), clock (clk), cycle (cyc), timeless (tim), and others ("Advances in Understanding the Peripheral Circadian Clocks," Richards J, Gumz ML. FASEB J 2012; 26(9): 3602–13). The authors recognize that some readers may not be familiar in detail with genes and their behavior and we direct you to other parts of this book where a more detailed description of these important biological entities and their behavior are described.

A central player in the circadian cycle is the PER gene, which produces the PER protein. PER levels are at their highest during the early evening, and at their lowest early in the day. PER was the first clock gene identified, in 1971 in fruit flies. In 1997, the first mammalian clock gene Clock (Circadian locomotor output cycles kaput) was discovered in mice. Scientists found its location and decoded its DNA sequence. In 2001, Fu et al. (2) discovered the first human clock gene. At the molecular level, circadian rhythms are encoded by an autoregulatory loop composed of a set of clock gene proteins that act as transcriptional factors. The best-characterized of these are CLOCK and BMAL1, which induce expression of its repressors (PER1-3/CRY1-2) that feedback to inhibit the forward mechanism. This is illustrated in Figure 2. The transcription factor dimer CLOCK/BMAL1 drives expression of the target genes periods (PER1-3) and cryptochromes (Cry1-2) by binding to E-box elements in their promoters. The negative feedback comes from the PER/CRY protein complex that moves back to the nucleus, where it blocks CLOCK/BMAL1-mediated transactivation, thereby inhibiting its own transcription. In a second feedback loop, the nuclear receptor REV-ERBα rhythmically represses BMAL1 transcription.

Other genes activated by CLOCK/BMAL1 are referred to as CLOCK-controlled genes (CCGs). These include the D-box binding protein (DBP), REV-ERBα, and RORα, which are responsible for the oscillation of BMAL1 mRNA by repressing and activating BMAL1 transcription, respectively, by binding to ROR response elements (RRE). The time lapse between transcription of PERs and CRYs and nuclear entry of their gene products, as well as regulated degradation of clock components, is responsible for the oscillation of circadian clocks. Casein kinase (CK) phosphorylates PERs, thus regulating both nuclear import and degradation (3).

Figure 2: The mammalian circadian clock. CLOCK and BMAL1 form a heterodimer that binds to E-boxes in regulatory regions to activate transcription of target genes such as PER and CRY. Used with permission, *Journal of Investigative Dermatology*, Geyfman and Andersen (3).

To date, 10 to 20 percent of genes in the body have been shown to follow a circadian oscillation, thereby allowing cellular functions to occur at the right time for optimal results and efficacy. The group of genes under circadian control includes those involved in: DNA damage repair response, cellular proliferation, cell cycle, cell-cycle arrest, apoptosis, and metabolism. In view of the synchronization of cellular activities in different tissues, it is now well accepted that different treatments can have different levels of efficacy based on what time of day they are applied or administered. For example, clinical studies have shown that there is an optimal time of day for the delivery of chemotherapy (4–6).

5.7.2 DESYNCHRONIZATION: CAUSES AND IMPACT

It is now well recognized that the functional integrity of living organisms is closely related to those natural rhythms at the cellular levels, as well as their levels in organs and tissues. In shift workers, for example, the incidence of obesity and other features of metabolic syndrome have been observed to be increased (7). Elevated

cortisol levels and increased BMI (Body Mass Index) were measured in young adults working in shifts. These may also contribute to the increased cardiovascular risk found in shift workers. Cancer rates in long-term shift workers have been measured, and evidence points toward the contribution of circadian disruption as a risk factor (8). Incidence of breast cancer increased significantly in women working nightshifts, being higher among women spending more years and hours per week working at night (9). A study done on Nordic pilots has demonstrated that the relative risk of prostate cancer increases as the number of flight hours in long-distance aircraft increases (10), and a significant association has been made between rotating-shift work and prostate cancer incidence in male Japanese workers (11). Lastly, the link between desynchronization of circadian rhythms and breast cancer has also been discussed by Sahar and Sassone-Corsi (12).

In 2011, Zhu, Stevens et al. (13) reported epigenetic association studies that demonstrated differential promoter methylation in the core circadian genes in breast cancer cases relative to cancer-free controls. These workers reported evidence of cancer related to epigenetic mechanisms in shift workers where altered methylation patterns revealed several cancer-related pathways. One of the top three pathways was associated with DNA repair and replication. Hence, shift working is now recognized as a risk factor for cancer and particularly breast cancer due to the disruption of the natural circadian rhythms (14).

Both PER1 and PER2 have been described as tumor suppressor genes (15, 16).

Further, the relationship between aging and the daily loss of circadian rhythm in humans has also been recognized. Circadian rhythmicity is disrupted and correlates to aging at various levels of biological organization (17–21). The loss of these rhythms, or desynchronization of these clock genes with the earth's cycle, can have dramatic effects on the organism. This relationship is bidirectional! On one hand, dysfunction of circadian clocks promotes and increases age-related diseases; while on the other hand, aging leads to changes and disruptions in circadian behavior and physiology (22). Recently, it was demonstrated that a deficiency in the CLOCK gene reduces life span (23), as well as early aging and age-related pathologies in mice deficient in BMAL1 (24). The relationship between an increase in aging and circadian clocks can now be better understood, since they play an important role in determining cellular division, cellular metabolism, and the strength of cellular responses to DNA damages including repair, checkpoints, and apoptosis (25).

5.7.3 SKIN CIRCADIAN RHYTHM

The skin, as the largest organ in our body, is also directly exposed to the environment and serves as the first barrier of protection against environmental exposure. Therefore, it is understandable that those functions related to the circadian rhythm will increase and optimize protection and repair in order to minimize environmental

impact and damage to our body. In 2000, clock genes were shown to be expressed in human skin cells by Zanello et al. (26). These clock genes control the natural skin circadian rhythms that are related to their different functions over a 24-hour period. As seen in Figure 3, a day-night cycle is illustrated, showing the highest or lowest levels of skin functions during day- or nighttime.

Figure 3: The highest and lowest activities in skin as a function of circadian rhythm.

Under normal and healthy conditions, skin protection is at its highest through changes in skin physiology during the day. In fact, the skin has been reported to be thickest during daytime and skin pH to be much higher when compared to night (27–29). Production of sebum, which contributes to skin moisturization (30, 31), is also at its highest during the day.

At night, skin temperature is higher, as well as increased blood flow and barrier permeability. This, of course, also correlates with a higher moisture loss, which has been measured to be approximately 25 percent higher at night than in the morning or afternoon (32) and correlates well with studies reporting higher efficiency of topical formulations at night than during the day (33). This field is being extensively studied in drug delivery ("chrono-delivery") in order to optimize drug delivery and efficacy in patients (34). Itching is also higher at night (35) and, as shown by Denda and Suchiya (36), the barrier recovery rate in skin is at its lowest between 8 and 11 p.m. Another major phenotype of clock-deficient models has been shown to increase the frequency and severity of dermatitis (23).

At the cellular level, proliferation of skin cells is very low during daytime in order to protect the cells and the DNA as much as possible from the environment and especially from direct UV exposure. DNA repair rates have been measured

to be at their highest levels at night. Alkyl guanine transferase (AGT) activity has been reported to peak at midnight (37). And xeroderma pigmentosum, complementation group A (XPA) expression levels also peak during the night (38). Once DNA damages have been repaired, cells have been shown to evolve and replicate at a higher rate in the dark in order to minimize UV-induced DNA damage. Therefore, it is very understandable that cellular proliferation is much higher at night. Cellular proliferation has been measured to be 30-fold higher at night than at noon (39). These intimate connections existing between circadian rhythm and cell division and DNA damage/repair pathways serve to protect genome integrity. This explains how circadian rhythms support the survival of species and, in this case, skin cell health. These findings provide scientists, ingredient developers, and formulators with new directions that will help us to slow down the aging process, which is, as we know, strongly related to genome instability. Indeed, the uncoupling of circadian gene expression from the cell cycle increases genome instability, premature aging, and ultimately cancer.

In a 2002 study by Kawara et al. (40), clock genes in normal human epidermal keratinocytes (NHEK) were shown to be negatively impacted by ultraviolet B (UVB) radiation. This work monitored clock gene expression in NHEK over time after exposure and found that they are downregulated and continue to be downregulated on average for up to about 20 hours after exposure (Figure 4). During this time, certain cellular functions will be critically affected.

Figure 4: NHEK exposed to 10 mJ/cm^2 UVB have reduced clock gene expression. Used with permission, *Journal of Investigative Dermatology*, Kawara et al. (40).

One of these cellular functions is energy production. As shown by Dong et al. (41), ATP production was shown to follow a rhythm depending on the different cellular needs over time. After a low level of UVB exposure, ATP production in NHEK was measured to decrease (Figure 5) and the natural ATP production rhythm was lost. These data correlate well with the results reported by Kawara et al. and the downregulation of clock genes in NHEK after UVB exposure. After ten hours, the ATP production had not yet recovered its original level.

Figure 5: After release from starvation, NHEK were exposed to 10 mJ/cm^2 UVB and ATP levels compared to unexposed cells over time. Significant decreases in ATP cycling and production were observed.

This phenomenological change will compromise all cellular functions through a lack of energy support and progressively accelerate the aging process. Additionally, sirtuins, which are a family of enzymatic regulatory proteins and described elsewhere in this volume by Pelle and Pernodet (42), have also been found to follow a circadian pattern. After UVB exposure, this rhythm is also affected and as a result, disruption of their cycle can also increase oxidative damage, leading to accelerated aging.

Moreover, a very recent study is now reporting that skin stem cells are also directly related to the circadian rhythm, and this uncovers and reveals the role of circadian rhythms in the regenerative capacity of skin stem cells (43). This research showed that the disruption of natural skin rhythms in the regenerative capacity of skin stem cells results in premature tissue aging and a greater predisposition to the development of skin tumors. By reestablishing the natural biological clock rhythm,

we expect, and see an increase in the long-term regenerative capacity of the tissue. This work also reports that skin stem cells are able to protect themselves from harmful radiation during the peak hours of light exposure, while in the evening and at night, they can divide and regenerate the tissue. In the same way that it has been demonstrated for skin cells, the skin stem cell division rate peaks at night when the skin is no longer exposed to UV radiation. In summary, as stated by Dr. Aznar Benitah (44):

The biological clock enables the *precise* adjustment of the temporal behavior of stem cells in such a way that the system adapts to the needs of the tissue according to the time of day. If this control is lost, stem cells may accumulate DNA damages and the likelihood of cell aging and generation of tumors increase[s] significantly.

It seems that we still have a lot to learn about skin cell activities and functions that are controlled by clock genes. We need to have a better understanding of how the disturbance or desynchronization of these clock genes affects different cellular activities, changing skin physiology, and ultimately, accelerated aging or disease. By understanding these mechanisms and functions related to the natural skin circadian rhythm, we will be, and are already able to, slow down the aging process by keeping skin cell functions synchronized with the circadian rhythm, thereby achieving optimal cellular function. Further, this knowledge can help us obtain optimal efficacy from anti-aging cosmetic products. Penetration will be higher in the evening and night due to higher skin-barrier permeability. As well, more repair and regeneration occur at night. Therefore our anti-aging treatments can be designed to support these cellular functions for achieving optimal results.

Additional research is needed in this area and is underway, with the intention of further unveiling the mechanisms related to skin circadian rhythm. It is expected that this increased mechanistic understanding will be of considerable help in moving towards the future.

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STRESS, SLEEP AND EPIGENETIC ORTHODONTICS: NEW DIRECTIONS FOR NONSURGICAL SKIN ANTI-AGING

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ABSTRACT

"**Beauty Is Only Skin Deep**" is a 1966 hit single recorded by the Temptations on the Motown label. The song's theme refers to one's inner beauty and its value over physical appearance. Inner Beauty in the song refers to one's soul, one's values, and the expression of one's inner being. It is how we present ourselves to the world.

However, in medical terms, one's inner being, that is, one's inner physiologic, metabolic, neurologic, and psychologic well-being also contribute to our physical appearance, particularly our young looking, attractive with vibrant skin.

Part 1 of this chapter provides an overview of what medically occurs in the body relating to how Sleep affects the appearance and quality of the skin. The discussion includes the underlying mechanisms that link sleep deprivation, certain sleep disorders, and Chronic Stress to the process of premature aging. Following this discussion a presentation is made of some ways to combat the process and prevent, or reverse, those undesirable effects on the skin.

Part 2 of this chapter presents a remarkable new technique called **Epigenetic Orthodontics**. The procedure involves stimulating the expression of unexpressed or "sleepy" genes to create structural morphologic changes to the skull and craniofacial region to enhance facial symmetry and create a bony foundation to support smoother skin and a younger more attractive appearance. **Epigenetic Orthodontics has been called Nonsurgical Plastic Surgery**. It is an emerging and exciting field in cosmetic facial dentistry and provides yet another approach and a deeper understanding of how cosmetic anti-aging experts can access the growing need and demand by consumers for looking younger as they age.

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5.8.1 SLEEP, SLEEP DEPRIVATION, SLEEP DISORDERS, AND SKIN AGING

It is not enough to just say that sleep negatively affects the skin and contributes to skin aging, sagging skin, wrinkles, facial unattractiveness, and droopy, tired-looking eyes. Knowing the basics of normal sleep, some sleep physiology, neurology, and endocrinology will prepare you for an understanding that you should, perhaps, readjust your sleep or address a possible sleep disorder. Awareness of sleep hygiene, sleep duration, circadian rhythm, sleep staging, and their neurophysiologic consequences may help one get better sleep, and feel and look better.

The current understanding of the process of sleep and chronic stress only suggests possible future topical therapies for skin anti-aging. They include, in addition to the ubiquitous array of antioxidants (discussed elsewhere in this book), the topical use of beta-blockers such as timolol, angiotensin receptor blockers such as valsartan, glucocorticoid blockers such as mifepristone, and cholinergic modulators including botulinum toxin.[1]

"I need my beauty rest." We have heard the common expression. Well, it's true. Good-quality sleep enhances our physical well-being and promotes a healthy appearance and younger-looking skin. Sleep deprivation affects the skin, especially the facial features of the eyes and mouth. Sleep-deprived individuals have more hanging eyelids, redder, more swollen eyes, dark circles under the eyes, paler skin, increased wrinkles and fines lines, and droopy corners of the mouth.[2]

We make a great effort to take care of ourselves for the 16 hours or so when we are awake, but often, little attention is put on our sleep. Poor-quality sleep, sleep deprivation, and certain sleep disorders can dramatically affect the way we feel and look. Good nutrition, exercise, and a healthy lifestyle are much of our daily routine. Yet, many people lose the benefits of their daytime efforts to stay healthy with poor sleep habits and poor sleep hygiene, not allowing for enough sleep time, disregarding (or not even being aware) of their circadian rhythm, and ignoring the signs and symptoms of sleep disorders.

Sleep physicians agree that 8 hours is the optimal amount of sleep each night. However, Americans, on average, get about 6.8 hours a night. About 10 percent of Americans have trouble falling asleep and then staying asleep. Others say they don't have the time, as the 24/7 society forces them to perform more and do more on less sleep. Sleep deprivation takes both a physical and financial toll. Research shows that sleep is catching up with diet and exercise in terms of influencing overall health. Lack of sleep has been linked to obesity, diabetes, and cardiovascular disease. Not getting enough sleep has led to $16 billion in annual healthcare costs and $50 billion in lost productivity.[3]

During sleep, many brain and bodily functions are recharged, restored, refreshed, energized, and sustained. During the day, the brain uses about 20 percent of the body's available energy and during sleep recharges the energy potential. The brain makes up 2 percent of a person's weight. Despite this, even at rest, the brain consumes 20 percent of the body's energy. The brain consumes energy at ten times the rate of the rest of the body per gram of tissue. The average power consumption of a typical adult is 100 watts; the brain consumes 20 percent of this, making the power of the brain 20 watts.[4, 5, 6] The brain also performs certain functions while sleeping to restore and consolidate memory, and to enhance cognitive function and executive decision-making. Additionally, the body restores its immune system and its ability to fight infection and disease. Growth hormone is released during deep sleep, which in children, promotes general growth of the bones and body, and in adults, aids in tissue repair and healing.

It is important to understand some basics about sleep to appreciate the negative effects that poor sleep or sleep disorders can have on the skin and the aging process.

Normal Sleep

a. Normal Sleep—Sleep Stages and Sleep Cycles:

In the course of an eight-hour sleep period, the brain cycles through several sleep stages. There are about four to five complete cycles throughout the night, each cycle lasting approximately 90 minutes to two hours. The human brain is designed to course through the cycles in a continuous fashion so that at the end of the night there is a certain percent of each sleep stage achieved. When one awakens at the end of the eight hours, one should feel energetic, refreshed, clear minded, and ready to begin the day.

During the day one should not be sleepy. There is a difference between sleepiness and tiredness. We get tired as a result of putting in a full day: using energy, being active, and engaged in life's activities. Excessive daytime sleepiness is not normal. You should not feel sleepy during the day, feeling like you need a nap and being able to sleep at any chance you get. Excessive daytime sleepiness is a sign that you are not getting enough sleep, are sleep deprived, or possibly suffering from a sleep disorder that is causing you to be aroused or awakened throughout the night.

b. Sleep Latency:

There is a period of time from the moment you lie down, place your head on the pillow, and actually fall asleep. In sleep medicine, that time is called sleep onset or sleep latency. It should take you between 5 to 12 minutes to fall asleep. If you fall asleep immediately, that is often an indicator that you are overtired and oversleepy. People who take longer than 30 minutes to fall asleep are considered to have onset insomnia.

c. Sleep Stage N1:

As one transitions from wakefulness to sleep, brainwaves begin to slow down. This is the hallmark of sleep. Lying in bed resting is not the same as sleeping, and one will not get the benefit of sleep. During the day our brainwaves are fast and active (high frequency and low amplitude). The brainwaves during wakefulness are called alpha waves and their frequency, measured on an electroencephalogram (EEG), is about 8–13 Hz (cycles per second). As one falls into sleep the brainwaves slow, and it is only then one acquires the benefits of sleep. During Stage 1 sleep, breathing becomes more regular, the body relaxes, and muscle activity decreases. An individual may experience hypnagogic hallucinations during Stage N1 sleep. These are visual, tactile, and/or auditory sensations sometimes accompanied with muscle twitching or body jerks. Stage 1 sleep is about 5 percent of the total sleep.

d. Sleep Stage N2:

In Stage N2 sleep, the body relaxes further and the heartbeat and respiration continue to slow. We experience Stage N2 sleep several different times during the night. Each time we transition from one stage to another, we pass through Stage 2 sleep. By the end of the night, we would have spent about 50 percent of the total sleep time in Stage 2 sleep.

Stage N1 and Stage N2 sleep combined are referred to as "light sleep" and should comprise about 55 percent of the total sleep time. The first cycle of light sleep should last 35 minutes to one hour.

e. Sleep Stage N3:

Stage N3 sleep is also called "deep sleep." It is the time when the body is very still, the heartbeat and breathing are slow and regular, and the person in deep sleep is difficult to arouse. After a period of light sleep we go back into deep sleep again. During the first four hours of our sleep we enter into about four cycles of deep sleep. After the first three hours it is not common to have any more deep sleep in the night. By the end of the last deep sleep, we should have slept about 20 percent of the total sleep in deep sleep. When we get enough deep sleep, and REM sleep, which will be discussed shortly, we can say we have received "quality" sleep.

In normal healthy adults, the major period of Human Growth Hormone (HGH, or GH) release is in the first period of Stage 3 sleep. The hormone is produced by the pituitary gland located at the base of the brain. It functions to promote bone and tissue growth in children, and it helps maintain healthy bodily tissues in adults.[7] People who lack GH have difficulty healing, especially after surgical procedures and initiating tissue repair.

If a person stays up all night when he or she normally sleeps, there is no surge in growth hormone release. After a period of sleep deprivation, there is extra hormone released when sleep is resumed, and the pattern departs from the normal pulse during slow-wave sleep.

Stages N1, N2, and N3 collectively are referred to as non-REM sleep.

f. Rapid Eye Movement (REM) Sleep

Rapid Eye Movement Sleep (REM) is also referred to as dreaming sleep. The brainwaves are very active, characterized by low amplitude and high frequency. REM sleep, as a percentage of total sleep, is approximately 20 to 25 percent across childhood, adolescence, adulthood, and into old age.[8] During REM sleep our central nervous system "paralyzes" our body so we do not act out our dreams. Sleep study tracings show the eyes create a figure eight movement and the chin lead indicates little to no muscle movement. REM sleep is considered the sleep stage where memory is consolidated, whereby short-term memory is converted into long-term memory in the hippocampus of the brain. Therefore, it is incumbent for us to get good-quality and sufficient REM sleep for learning and memory. College students who "pull all nighters" to study are at a severe disadvantage, as they are robbing themselves of REM sleep and will not be able to remember all that they are studying. Test-takers do better when they get sufficient sleep so as to allow the brain to cycle through all the stages including REM, which occur mostly at the end of our sleeping in the early morning hours.

REM sleep is necessary for clear decision-making and executive function. People who do not get enough REM sleep feel confused, unable to focus, and slow and clouded in their ability to make decisions.

g. Sleep Disorders, Chronic Stress, and the Impact on Aging and Skin

There are many sleep disorders that plague the sleeping person. Of these, two affect aging and skin: Insomnia and Obstructive Sleep Apnea (OSA). It is necessary to understand the pathophysiology of chronic stress as both insomnia and OSA affect the skin as a result of the metabolic impact of stress.

h. The Pathophysiology of Stress—the Hyper-Arousal of the Autonomic Nervous System

There are many functions of the central nervous system that are not in our conscious control, including body metabolism, respiration, heart rate, temperature, emotions, and circadian rhythm, among many others. The division of the central nervous system that controls these physiologic and psychologic functions is the Autonomic Nervous System (ANS). The ANS is a dual system made up of the

Parasympathetic and Sympathetic branches. They work in opposition to each other. With respect to skin, the Sympathetic Nervous System (SNS) is most relevant.

The SNS is often referred to as the "fight or flight" system. It is the system that is activated when we are faced with a real, or perceived, threat. The SNS is an evolutionary mechanism that protects us today, much like our primitive ancestors in the past when they were faced with oncoming danger. When stimulated, the SNS causes our heart rate to increase, blood pressure and respiration increase, and blood is diverted away from the digestive system to the skeletal muscles to allow us to be active with a high metabolic voluntary muscular activity.

When we are confronted with danger, the SNS system that is activated is called the Hypothalamic-Pituitary-Adrenal (HPA) Axis. It is the hypothalamus of the brain that receives the signals of the threat. The HPA axis then releases Corticotrophin Releasing Factor (CRF) and vasopressin, which act synergistically, on the pituitary gland at the base of the brain. The pituitary gland is stimulated to release adrenocorticotropic hormone (ACTH). This, in turn, activates the adrenal glands, sitting on top of the kidneys, to release cortisol, a glucocortcoid, epinephrine (adrenaline), a stimulating hormone, and norepinephrine, a neurotransmitter. In normal function, the cortisol acts in a negative feedback mechanism on the hypothalamus and pituitary (to suppress CRH and ACTH production) to shut the HPA axis off.

The Fight or Flight response is activated so quickly that one reacts without engaging conscious thought. William James, the great American psychologist, once remarked, "It is not that we see a bear, become afraid and run; rather we see a bear, run and then become afraid."[9]

Vasopressin is a hormone that constricts peripheral blood vessels, increases blood pressure and acts to conserve water. It is the body's natural anti-diuretic. Epinephrine and norepinephrine are hormones and neurotransmitters. They increase heart rate, increase respiration, dilate the pupils of the eyes, and in effect, stimulate and awaken the body and mind. They are responsible for our brains and consciousness to become vigilant. Both epinephrine and norepinephrine trigger the release of glucose from energy stores, including the liver, and increase blood flow to skeletal muscle. The net effect is to increase the brain's oxygen and sugar (energy) supply.

Cortisol is a glucocortcoid whose main function is to make glucose available for the brain, generating new energy from stored reserves and diverting energy from low-priority activities, thereby suppressing the immune system. Cortisol also increases blood pressure by increasing the sensitivity to epinephrine and norepinephrine, and it shuts down the reproductive system, decreasing estriol and estrodiol in women and testosterone in men.

In dangerous and stressful situations, the HPA axis protects us as it causes the body, and mind, to spring into action for defense and self-preservation, but it works on a negative feedback system so that as the levels of cortisol, epinephrine, and norepinephrine reach the hypothalamus of the brain, CRF and ACTH are inhibited.

Stress is not altogether bad. Moderate amounts of stress are stimulating and keep us from being bored. An understimulated life leads to poor development, an unwillingness to thrive, depression, and apathy. Stress teaches us mechanisms of coping, as well as responding to our environment and to other people. However, it needs to be in moderate amounts, and should include periods with no stress so that we have time to recover.

i. Sleep and Chronic Stress

Chronic stress, as the name implies, does not refer to the intensity of the stress, but rather, the continued pressure of the stress and the long-term overactivation of the Sympathetic Nervous System (SNS) and the HPA axis.

Stress can cause poor sleep, and poor sleep can cause stress. Chronic stress can lead to weight gain, reduction in sex drive and libido, erectile dysfunction in men, mood disorders, anxiety, hair loss, heart disease, high blood pressure (hypertension), ulcers, and other medical and psychological maladies.[10]

j. Insomnia

There are those who find sleep torture. They may have some form of insomnia. There are two types of insomnia: primary insomnia and secondary insomnia. Primary insomnia means that a person is having sleep problems that are not directly associated with any other health condition or problem. Primary insomnia is often of psychological origin. Secondary insomnia is when a person is having sleep problems because of something else, such as a health condition; arthritis, cancer, or heartburn; sleep disorders such as Obstructive Sleep Apnea, restless leg syndrome; acute or chronic pain; poor sleep environment (noises, intrusive light); medication they are taking; or a substance they are using (like alcohol).[11]

Insomnia also varies in how long it lasts and how often it occurs. It can be short-term (acute insomnia) or can last a long time (chronic insomnia). It can also come and go, with periods of time when a person has no sleep problems (transient insomnia). Acute insomnia can last from one night to a few weeks. Insomnia is called chronic when a person has insomnia at least three nights a week for a month or longer.[12]

A very important distinction must be made between insomnia and sleep deprivation. In the definition of insomnia, it must be understood that the patient is making an effort to sleep, allowing the necessary time to sleep and creating a

favorable environment to sleep. The patient is unable to sleep due to psychological or medical reasons.

Sleep deprivation is when the patient is deprived of sleep, on a voluntary basis, whereby one could sleep if the opportunity was there.

Any insomnia can fall under one of two categories: onset insomnia and maintenance insomnia.

Onset insomnia and is the chronic inability to fall asleep. It is usually defined as the inability to fall asleep in 30 minutes or less. Most often it is chronic-stress induced, but may also be caused by damage to the hypothalamus, the suprachiasmatic nucleus (a small cluster of neuronal cells behind the eyes that are light sensitive and responsible for our circadian rhythm), a decrease in melatonin, or age-related neural dysfunction or atrophy.

People who have onset insomnia cannot get to sleep; pacing the floor, staring at the ceiling, tossing and turning, unable to fall asleep, and then finally falling to sleep in the late night hours of the night only to have to get up and start the day with little sleep. People who have maintenance insomnia are exhausted and fall asleep very quickly (short sleep latency or onset), only to awaken about two hours later and not able to go back to sleep.

k. Obstructive Sleep Apnea

A common sleep disorder that will be discussed in some detail is Obstructive Sleep Apnea (OSA). OSA is defined as a time during sleep whereby people have airway collapse and/or blockage with little to no air inspired, causing the person to arouse out of a deep-sleep stage into lighter stages of sleep, resulting in sleep interruptions and fragmented sleep. Snoring is often the common indicator of OSA and should not be ignored, but rather recognized as a sign of pathology.

Sleep Apnea occurs when there is a disruption of breathing causing a decrease or cessation of airflow into the lungs, a desaturation of oxygen in the blood, resulting in an arousal from a deep sleep stage into a lighter stage or an awakening. There are two main types of sleep apnea, Obstructive and Central.

Central Sleep Apnea (CSA) is of neurologic origin and, although often present in many patients in small amounts, is as rare as the primary sleep apnea condition. During CSA the brain stops sending signals to the muscles of respiration, respiration ceases (the patient make no effort to breathe), and airflow stops, resulting in hypoxemia (a decrease of oxygen in the blood). CSA is usually in the elderly, present in patients with several medical co-morbidities.

The most common type of sleep apnea is Obstructive Sleep Apnea (OSA). This type of sleep apnea is caused by the airway being completely (apnea) or partially (hypopnea) obstructed or collapsed. It usually presents itself when the

person achieves deep or REM sleep and the body is completely relaxed with little to no muscle tone. In this state, the lower jaw (mandible) falls back, the tongue falls back into the throat (pharynx), and the airway is obstructed by the base of the tongue. The person continues to make the effort to breathe, but no air enters the lungs due to the obstruction. This condition of obstruction can occur when one sleeps on their sides or stomach, although it is commonly worse when sleeping on one's back.

As the airway is obstructed and airflow is limited or restricted, the blood oxygen begins to decline. The blood becomes hypoxemic and the tissues in the body become hypoxic. This condition, called a respiratory event, can last 30 seconds, 60 seconds, 90 seconds, or more. The longer the event lasts, the more the oxygen in the blood desaturates. In a normal healthy adult, the oxygen saturation levels are between 94 to 99 percent. However, during an apnea event, especially an event that lasts over a minute, the levels can fall into the 80s, 70s, 60s, or even into the 50 percent levels. This is extremely dangerous, particularly if the person already has restricted blood flow to the heart and/or brain due to high cholesterol, or arteriosclerosis.

OSA triggers the HPA axis due to the decreasing oxygen levels in the blood and is eventually recognized in the brain. As the oxygen levels in the brain decrease due to the apnea event, the hypothalamus of the brain senses the decrease in oxygen. The brain makes no distinction between the event of someone choking a victim and the oxygen loss due to sleep apnea. The brain is registering the oxygen desaturation and therefore, the HPA fight or flight response is stimulated. CRF is released from the hypothalamus, ATCH is released from the pituitary gland, and epinephrine, norepinephrine, and cortisol are released from the adrenal gland. Blood pressure is increased, respiration is increased, motor muscles are stimulated, the brain is awakened, the person is aroused by being bumped out of deep or REM sleep, muscle tone is regained in the muscles of the airway, and a breath is taken to restore breathing.

We need 20 percent deep sleep and 25 percent REM sleep to feel rested and refreshed upon awakening and to get the benefits of deep and REM sleep. As one is aroused and awakened out of these stages, the brain makes an effort to go back into deep and REM sleep. Unfortunately, as the deep and REM sleep is resumed, muscle tone of the head and neck are lost again, and another respiratory event begins. Eventually the oxygen levels drop again and there is another arousal. There is no sustained deep or REM sleep for the OSA patient. Instead there is a bouncing between deep or REM sleep and light sleep.

When reading a sleep study of the sleep apnea patient we can count the number of times the events happen throughout the night. Supposing the number of events of the total sleep time is 80 and the patient slept 8 hours, we can divide the 80 events by the 8 hours and get an average of 10 events per hour. The number of events on an average hourly basis is called the Apnea-Hypopnea Index (AHI). It is the total number of events, both apnea (complete obstructions) and hypopneas (partial obstructions) divided by the number of total sleeping hours. If the AHI is 5 or less, the patient is considered OSA free. If the AHI is 5–15, the classification of OSA is mild; 15–30 AHI is moderate and over 30 AHI is severe.

It is most important to realize that as the AHI increases, the levels of epinephrine, norepinephrine, and cortisol increase due to the HPA axis stimulation. The patient can eventually transition into chronic stress due to the constant levels of these hormones being secreted with no period of rest.

Obstructive sleep apnea is therefore linked to hypertension, rapid heart rate, decreased sex drive, and decreased sex hormones in men and women, decreased immune system function, and decreased human growth hormone.[13]

As cortisol levels rise, they continuously bathe the brain, most particularly the hippocampus, which can atrophy under this condition. The result is memory loss, loss of cognitive function, and early onset of dementia and Alzheimer's disease.[14]

OSA has been shown to impair breathing during sleep, which can lead to a serious brain injury that disrupts memory and thinking. The study focused on structures called mammillary bodies on the underside of the brain.

A UCLA team scanned the brains of 43 sleep apnea patients, using magnetic resonance imaging (MRI) to collect high-resolution images of the entire brain, including slices of the mammillary bodies. When they compared the results to images of 66 control subjects matched for age and gender, the scientists discovered that the sleep apnea patients' mammillary bodies were nearly 20 percent smaller.

The findings are important because patients suffering memory loss from other syndromes, such as alcoholism or Alzheimer disease, also show shrunken mammillary bodies.

It has been hypothesized that repeated drops in oxygen level lead to the brain injury. During an apnea episode there is a state of oxidative stress due to apnea oxygen desaturations starving brain tissue of oxygen and causing cellular death. The process also incites inflammation, which further damages the tissue.[15]

Brain scans reveal that the mammillary bodies (in box) of a sleep apnea patient (right) are smaller than those of a control subject (left). (Credit: UCLA/Harper lab)

5.8.12 SLEEP, AGING, AND AGING SKIN

a. Sleep Quality and Sleep Deprivation

There is a reason it's called "beauty sleep." How much sleep do we need? Seven to eight hours a night is ideal.[16] When we don't get enough sleep the brain does not have time to go through all the sleep stages. If you cut your sleep short, a form of sleep deprivation, you may not be getting enough of the proper amounts of each sleep stage to feel well rested and get the full benefits of a good-quality sleep.

Sleep deprivation is disallowing the amount of sleep needed, yet being able to sleep if given the opportunity. Sleep insomnia is the inability to sleep even though the opportunity to sleep was present.

The percentage of adults who sleep less than 6 hours per night is now greater than at any other time on record.[17] Sleep deprivation affects one's appearance and skin quality. The faces of sleep-deprived individuals are perceived as having more hanging eyelids, redder eyes, more swollen eyes, darker circles under the eyes, paler skin, more wrinkles/fine lines, and more droopy corners of the mouth. In addition, sleep-deprived individuals look sadder than after normal sleep, and sadness is related to looking fatigued.[18] The results show that sleep deprivation affects features relating to the eyes, mouth, and skin, and that these features function as cues of sleep loss to other people. Because these facial regions are important in

communication, facial cues of sleep deprivation and fatigue may carry social consequences for the sleep-deprived individual in everyday life.[19]

Skin aging is affected by insufficient sleep quality. A clinical trial commissioned by Estée Lauder and conducted by physician-scientists at University Hospitals (UH) Case Medical Center found that poor sleepers demonstrated increased signs of skin aging. They also gave a worse assessment of their own skin and facial appearance than people who sleep well.[20] The study was the first to conclusively demonstrate that inadequate sleep is correlated with reduced skin health and accelerates skin aging. Sleep-deprived women showed signs of premature skin aging and a decrease in their skin's ability to recover after sun exposure.[21] Poor-quality sleepers showed increased signs of intrinsic skin aging including fine lines, uneven pigmentation, and slackening of skin and reduced elasticity.[22]

Good-quality sleepers recover more efficiently from stressors to the skin. Recovery from sunburn is more sluggish in poor-quality sleepers and self-perception of attractiveness is significantly better in good-quality sleepers vs. poor-quality sleepers. Poor sleep quality can accelerate signs of skin aging and weaken the skin's ability to repair during the night.[23]

b. Circadian Rhythm

Circadian rhythm is the internal circadian biological clock that regulates the periods of sleepiness and wakefulness throughout the day. The circadian rhythm dips and rises at different times of the day, so adults' strongest sleep drive generally occurs between 2:00 and 4:00 a.m. and in the afternoon between 1:00 and 3:00 p.m., although there is some variation depending on whether you are a "morning person" or "evening person." The sleepiness we experience during these circadian dips will be less intense if we have had sufficient sleep, and more intense when we are sleep deprived.[24]

The circadian biological clock is controlled by a part of the brain called the suprachiasmatic nucleus (SCN), a group of cells in the hypothalamus that respond to light and dark signals. Light travels through the eye to the SCN, signaling the internal clock that it is time to be awake. The SCN signals the brain to raise body temperature and produce hormones like cortisol. The SCN, when exposed to light and activated, also delays the release of the hormone melatonin, which causes sleepiness. Melatonin levels rise in the evening and stay elevated throughout the night, promoting sleep.[25]

Skin harbors an active circadian clock that is under the influence of the central clock. The skin circadian rhythm clock operates in all types of skin cells, and may influence the regulation of several circadian physiological phenomena, including cell proliferation, the time when skin cells proliferate.[[26]] During skin

cell proliferation, their DNA are more susceptible to ultraviolet damage. It is well known that the proliferation process takes place during the day at various times; however, it happens at least 30 times more rapidly at night. Associated with proliferation are such key functions as rate of water loss and blood flow, which are 20 to 30 percent higher at night than in the morning or afternoon. Circadian rhythms in the skin affect the biology of appearance and also have a profound effect on the absorption of applied treatment products.[27, 28]

c. Sleep, Human Growth Hormone: Aging and Skin

HGH in adults is secreted in Stage 3 slow wave delta sleep.[29] It is needed for tissue repair and healing.

HGH is a protein-like substance, produced by the pituitary gland in the base of the brain. It plays a crucial role in many of the body's metabolic processes—too little in childhood leads to stunted growth, and too little in adulthood results in excess body fat, lack of lean muscle tissue, brittle bones and thin, wrinkle-prone skin.[30] The production of natural HGH (human growth hormone, also called the anti-aging hormone) relies on getting a good night's rest.[31]

Decreased amounts of human growth hormone cause many of the symptoms of aging, such as decreased energy, inadequate muscle tone, and wrinkles.[32]

d. Chronic Stress and Sleep; Cortisol, Epinephrine, Aging, and Skin

The adrenal gland releases the stress hormone cortisol during sleep in patients with obstructive sleep apnea, and in patients who suffer from chronic insomnia.

When cortisol levels are too high or too low, rapid-aging processes accelerate.[33]

The ability to enter REM (rapid eye movements) sleep cycles requires normal cortisol levels at night. High nighttime cortisol disrupts REM sleep (restorative and refreshing sleep) and leaves one feeling groggy and fatigued in the morning. This morning fatigue can be easily mistaken by the patient and physician for endogenous depression. If "depression" does not respond to medications directed at serotonin and norepinephrine metabolism, consider evaluation of nighttime cortisol levels.[34]

Skin regenerates mostly during the night. Elevated cortisol reduces skin regeneration. Normal cortisol rhythm is essential for skin health, especially conditions such as "adult acne."

Individuals with skin problems often have low zinc and high copper levels. The connection with cortisol is that excess copper speeds the conversion of dopamine to norepinephrine and adrenalin. The excess adrenalin tends to increase

cortisol. Meanwhile, the lower dopamine increases the risk for Parkinson's and early Alzheimer's.[35]

Increased blood levels of adrenocorticotropic hormone (ACTH), epinephrine, and cortisol were found in people with chronic insomnia, suggesting that there is a round-the-clock activation of the body's system for responding to stress. Average levels of both hormones were significantly higher in the insomniacs than in the non-insomnia patient.[36]

Insomniacs with the highest degree of sleep disturbance secreted the highest amount of cortisol, particularly in the evening and nighttime hours. This means that insomniacs are experiencing hormonal changes in their bodies that prevent them from sleeping.

It has been proposed that the physical mechanism of chronic insomnia differs from that of sleep loss and sleep deprivation, with chronic insomnia being a disorder of hyper-arousal present throughout the 24-hour sleep/wake cycle. Increased production of stress hormones is likely to lead not only to depression, but also to high blood pressure, obesity, and osteoporosis.[37]

In excess amounts, cortisol can break down skin collagen, the protein that keeps skin smooth and elastic. Cortisol-induced collagen loss in the skin is ten times greater than in any other tissue. The administration of cortisol results in a marked and abrupt loss of cutaneous collagen. This collagen loss was associated with the appearance of both collagenolytic and proteolytic activities in the extracellular, extrafibrillar compartment of the skin.[38]

e. Epinephrine and the Skin

The effect of epinephrine on the skin is mainly caused by its binding to alpha-adrenergic receptors, the alpha-2-adrenergic receptor in particular. Restriction of the arteries is caused by the binding of epinephrine to these alpha-adrenergic receptors. This cuts off blood supply to the skin.[39] A signaling cascade is also stimulated. This results in the contraction of the smooth muscle cells in the skin, which cause the raising of the hairs on the surface of the skin.[40]

Stress-induced elevation of epinephrine levels result in delayed healing and the impairment of cell motility and wound re-epithelialization. Since skin injuries such as burns locally generate epinephrine in response to wounding, epinephrine levels are locally, as well as systemically, elevated, and wound healing is impacted by these dual mechanisms. Treatment with beta adrenergic antagonists significantly improves the rate of burn wound re-epithelialization.[[41]]

f. Free Radicals, Sleep and Aging

Your body needs sleep, and depriving yourself of it will undermine your immune system. That means its defenses against infection are lowered. Lack of sleep will also slow down rejuvenation processes in your brain. That is because free radicals, which build up in the brain, are removed while we sleep. Sleep has also been theorized to effectively combat the accumulation of free radicals in the brain, by increasing the efficiency of endogenous antioxidant mechanisms.[42]

Among the various theories proposed to account for the process of aging, the free radical theory is of practical interest since it includes the possibility of retarding this process by administrating natural or synthetic antioxidants and free radical scavengers. Epidemiological studies from several European countries are reported showing correlations between low plasma levels of essential antioxidants and the occurrence of premature aging, coronary heart disease, cancer, and cataract formation.[43]

Sleep apnea events, complete apnea, or partial hypopnea, leads to a decrease in oxygenation and the formation of free radicals in tissues and fluids of the body. [[44]] When oxygenation occurs, the free radicals combine with the oxygen and create more free radicals in an ongoing cascade. Antioxidants can remove free radicals from the system. Reduced antioxidant capacity is an indicator of excessive oxidative stress. Patients with severe OSA have reduced values of antioxidant capacity.[[45]]

OSA therefore increases the levels of oxidative stress that contribute to excess free radical production, soluble adhesion molecules, and decreased levels of nitric oxide (NO), a potent vasodilator. Repeated transient hypopnea/apnea and reoxygenation events resulting from untreated OSA also affect cerebral oxygenation and blood flow velocity. This produces a condition similar to myocardial hypoxia, resulting in increased production of free radicals and adhesion molecules and decreased levels of NO.[[46]]

5.8.13 THERAPY

Therapies for anti-aging and skin rejuvenation are discussed elsewhere in this book. The following section addresses the various sleep disorders and maladies that influence aging and skin appearance, quality, tone, and texture.

Before therapy can begin a diagnosis must be made of the sleep dysfunction being treated. Assessment includes a differential diagnosis to discriminate between similar sleep disorders. Frequently a patient may have several sleep disorders concomitantly with one disorder being of primary significance.

The diagnosis of sleep maladies is made from both subjective and objective data collected. Subjectively, patients will fill out sleepiness forms, keep sleep logs and diaries, and relate symptoms of their complaints. A thorough medical and family history is needed, including current medical co-morbidities and medication being taken.

Objectively, medical data is collected using overnight tests while the patient is sleeping. The most common standard test is a polysomnography. The patient goes to a sleep center in the evening that has beds for the patient to sleep. A technician applies leads to various parts of the patient's body to read tracings on a computer. The test is interpreted and a sleep diagnosis and a recommendation for therapy is offered. It is also possible for the patient to take a sleep test device home and perform a home sleep study. The home studies are accurate, but the parameters of data collected are limited.

Other tests include overnight actigraphy and pulse-oximetry. Actigraphy measures movement, and pulse-oximetry measures oxygen levels and heart rate. Daytime in-office tests for sleep diagnosis are often not valuable, as physiologic and neurologic metabolism changes dramatically when the patient is sleeping.

a. Insomnia

Insomnia therapy can be divided into nonmedical or behavioral approaches and medical treatment. A combination of both the medical and nonmedical modalities work better than one therapy alone, adjusted for changing circumstances of the patient.

Often insomnia is caused or influenced by an overlying medical or psychiatric condition. The insomnia becomes the symptom of the medical or psychological problem and it is necessary to treat that problem and not the insomnia directly.

b. Non-medical Cognitive Behavioral Therapy

The cognitive side of cognitive behavioral therapy for insomnia helps create a cognitive understanding of insomnia and the opportunities the patient has toward addressing the problem. By being cognizant, there is an awareness of the core problem and a confidence in the belief that therapy can work, The behavioral part of cognitive behavioral therapy for insomnia helps develop good sleep habits and avoid behaviors that keep the patient from sleeping well.

c. Sleep Hygiene

Sleep hygiene includes the awareness of one's sleep environment and the sleep patterns that promote good sleep. The benefits of improving sleep hygiene are cost effective and simple, often requiring small adaptations, resulting in a mindfulness that will improve sleep.

Some sleep hygiene suggestions are:

- Sleep as much as possible to feel rested, then get out of bed (do not oversleep). If you sleep more than 8½ hours and are still sleepy, you may be getting too much Stage 1 and 2 light sleeps, and not enough deep and

REM sleep. If you are sleep deprived and have created "sleep debt" by not sleeping enough hours during the week, making up some sleep time is encouraged. You will find that sleep deprivation will lead to increased Stage 3 sleep, which is the brain's way of making up lost sleep. However, if you sleep more than 8½ hours and feel like you can still sleep more, you may want to take a sleep study to determine the percentages of sleep staging you are getting.

- Maintain a regular sleep schedule. Sleep is regulated by our circadian rhythm, influenced by the light/dark cycles of daytime/nighttime and our homeostatic drive for sleep. The homeostatic drive for sleep starts when we wake up and increases during the day. It is regulated by the buildup of adenosine in the brain as the brain metabolizes sugars for energy. Adenosine promotes sleepiness and as adenosine builds up, the drive for sleep increases.
- Do not force yourself to go to sleep. Going to sleep when you are not sleepy creates an anxiety to sleep that can be counterproductive for sleeping. Use the bed only for intimacy and sleeping. Do not read, work, watch TV, eat, or do other mentally stimulating activities in bed.
- Do not drink caffeinated beverages in the afternoon or evening. Caffeine is a stimulant that will delay sleep onset. The half-life of caffeine, the time required for the body to eliminate one-half of the total amount of caffeine, is approximately 4.9 hours.[47] Caffeine is an adenosine antagonist. Caffeine is structurally similar to adenosine, and binds to adenosine receptors on the surface of cells at the neural synapse and blocks the action of adenosine. If adenosine is rendered nonfunctional, the homeostatic drive is diminished.[48]

Be aware of the different foods and drinks that contain caffeine. It is not only coffee, but most teas, chocolate, and sodas.

- Do not drink alcohol prior to going to bed. Alcohol is a central nervous system depressant and will reduce sleep latency, and you will fall asleep faster. However, it will reduce or prevent deep Stage 3 sleep and REM sleep. Alcohol will also cause dehydration, which will cause sleep arousals.
- Do not smoke, especially in the evening. Cigarette smoke contains nicotine and nicotine is a drug that mimics the action of acetylcholine (ACh); it is an acetylcholine agonist. Nicotine enhances attention and vigilance by directly stimulating cholinergic activity for sleep arousal.[49]
- Adjust the bedroom environment to induce sleep. Many people today are disrupting their sleep with intrusive light, noise, and other environmental stimulants. It can be said, that the greatest disruptor of modern-day sleep is Thomas Edison. The light bulb has interfered with our natural light/dark

circadian rhythm and the normal secretion of melatonin in our bodies. Shutting the lights, turning off the TV and computers, blocking out bright LED light from clocks and other sources can be an adjunct for better sleeping. Reducing noise, not sleeping with the children (or the dog) in the bed, and eliminating other distractions are suggested for good sleep.

- Do not go to bed hungry.
- Exercise regularly, but not four to five hours prior to bedtime. Exercise can increase the core body temperature. Sleep is promoted by a cool environment and a slightly cool body temperature. As the body cools from exercise, sleep is enhanced. If the exercise is too close to bedtime, the body will be too warm and sleep is delayed.

d. Obstructive Sleep Apnea (OSA) and Aging

It has been firmly established that there is a strong connection between the stress, fight and flight response, and aging and negative effects on the skin. The primary actions are from the hormones cortisol, epinephrine, and norepinephrine. By treating sleep disorders, and obstructive sleep apnea in particular, many aging and skin maladies may be thwarted. Effective modalities for OSA treatment are available today and they can be considered anti-aging therapies.

e. C-PAP Therapy

C-PAP stands for Continuous Positive Airway Pressure. It is call a pneumatic splint for the airway for OSA. The airway is compromised either when the tongue falls back into the rear of the throat (pharynx) during sleep or the pharynx collapses during respiration while sleeping.

Think of the example of trying to drink a glass of pulp-filled orange juice while sucking through a straw. If some pulp gets clogged in the straw, you can continue to make the effort to suck but no juice comes through. However, if you blow into the straw, at some blowing pressure you will clear the pulp and then can suck the juice through the unobstructed straw. In older individuals, the airway can become weakened as a result of the muscles of the airway aging. Again, if you think of the straw analogy, the weakened airway is now like a wet paper straw and collapses easily even with light sucking pressure. The airway of a teenager can be thought of as a plastic straw and very rigid, not easily collapsible. This partially explains why OSA is more prevalent in an aging population.

The C-PAP machine is like a vacuum cleaner in reverse. It blows air under pressure through the airway to either clear an obstruction or to open the collapsed airway. The pressurized air is administered to the patient through a plastic hose into a mask attached to the patient's face by elastics.

C-PAP is an extremely effective therapy for OSA. Among medical sleep specialists it is considered the "gold standard" of therapy. The pressure of the machine is regulated to the patient.

There are variations of C-PAP to include Bi-level PAP and Auto-PAP. These machines also blow air under pressure to relieve the OSA obstruction but they also self-adjust the pressure based on inspiration and expiration.

Although C-PAP is highly effective, many patients cannot use the treatment for various reasons. The mask is uncomfortable, the pressures are waking them, they are claustrophobic, cannot sleep on their back, and get chronic sinusitis or gastritis from the air forced into the sinus or digestive tract.

f. Oral Appliance Therapy (OAT)

OAT is most often a plastic device worn on the teeth during sleep. They function by gently and minimally guiding the mandible (lower jaw) forward (protrusive) and downward. As the mandible is moving open and forward, the tongue, which is attached to the lower rim of the jaw, is pulled forward and the airway is free from obstruction. Additionally, as the jaw is moved forward, muscles in the chin and neck that are attached to the front (anterior) aspect of the pharynx pull on the airway and prevent the airway from collapsing.

Today, most OATs are custom made by a dentist dedicated to treating patients who suffer from OSA with oral appliances. The appliances are small, light, comfortable, and very kind to the TMJ (Temporal Mandibular Joint) and associated muscles. OAT can be used by patients with mild and moderate OSA, and those patients with severe OSA who are C-PAP noncompliant. OAT is safe, noninvasive, reversible, inexpensive, effective, and easy to wear. The compliance rate is very high and it is tolerated well by most patients. A trained physician or dentist should be consulted on proper selection and use of such devices.

CONCLUSIONS

It is not simply enough to say that poor sleep affects aging and quality of skin. In this chapter we discussed the neurophysiology of sleep and the metabolic consequences of disproportionate sleep staging, disruption of circadian rhythm, the flight-fight hormonal response resulting from intermittent hypoxemia related to obstructive sleep apnea, and the effects of chronic stress and high levels of cortisol.

In the late 1800s the germ theory of disease was popularized by three prominent pioneers; Joseph Lister, a physiologist and surgeon, Robert Koch, a physician and scientist, and Louis Pasteur, a chemist. That led to the notion of the reductionist view of medicine. If one can reduce the pathology, the disease, to a common cause or source, and that cause was treated, eliminated, or rendered inactive, then

the disease process would be reversed and health achieved. Unfortunately, that approach to medicine exists today, albeit operatively sound in many, but not all situations.

In regards to anti-aging and attractive and young-looking skin, the patient and practitioner must take a more global view, a constructionist and not a reductionist methodology. Understanding the value of good quality and quantity of sleep is a key component in warding off premature aging. We take good and conscientious care of our minds and bodies 16 hours of the day, but often neglect the eight hours we need for sleep. Proper sleep is intimate with a rejuvenated immune system, regulated hormonal activity, and balanced metabolic and neurologic function.

Good sleep, coupled with proper nutrition, exercise, and a stimulating and purposeful life allows for a healthy mind and body.

5.8.2 EPIGENETIC ORTHODONTICS AND DENTO-FACIAL ORTHOPEDICS: NONSURGICAL FACIAL ESTHETIC THERAPY

In recent years dentists, particularly orthodontists, have become interested in the effects of orthodontic treatment, not only moving teeth, but also the way in which the treatments often remodel the bones of the face.

It has become popular for adults to undergo orthodontic treatment for improved esthetics and better alignment of teeth. Adult orthodontic therapy has gained broader acceptance due to the invention of the Invisalign technique, which is a tooth movement procedure.

However, in addition to just moving teeth, the dental profession has discovered that by stimulating the sutures of the facial bones, skeletal remodeling can occur, which often results in better symmetry and enhanced esthetic appearance; the anti-aging effect.

For many years, it has been observed by dentists and patients alike, that when a tooth is extracted, other teeth begin to drift and the jawbones change shape; this is called morphologic remodeling. One can consider a tooth extraction to be “disruptive” to the structures of the oral cavity that stimulate the remodeling.

In Dento-Facial Orthopedics, removable dental appliances are worn on the teeth during the evening and nighttime hours that put forces on the teeth and craniofacial bones that cause morphologic remodeling.

Dento-Facial Orthopedics is a practice of dentistry that is concerned with the diagnosis and treatment of malocclusions and concomitant underdeveloped craniofacial skeletal structures, which may be a result of repressed genes that have not expressed their genetic code and remain dormant, causing dental tooth displacement and disproportionate jaw relationships. For the repressed genes to become active, uploaded, they need to be stimulated either chemically or mechanically.

The premise of the skeletal remodeling by the uploading of epigenetically regulated genes influenced by an orthodontic/orthopedic procedure is called Epigenetic Orthodontics. Epigenetics focuses on the changes in gene expression that are *not* caused by changes in the DNA sequence. Our common understanding of genetics is based on the DNA sequence (the genotype). Gene expression (cellular phenotype) from other causes than the DNA sequence is considered epigenetic. Those causes can be from environmental, nutritional, or other biochemical influences.

An epigenetically regulated gene is identified by chemical attachments to the DNA, most commonly a methyl molecule which consists of one carbon atom bonded to three hydrogen atoms (CH^3). A gene with a methyl attachment is called methylated. Generally, the more a gene is methylated, the more inactive it is. A methylated gene can be considered "sleepy," one that does not partially or completely express itself. Once methylated, the DNA tends to stay that way, and will continue to transmit the methylation to descendent cells throughout the lineage of the cell.[50]

The question becomes, how does the mechanism of epigenetic orthodontics turn on the repressed gene, the physiological mechanism of signal transduction?

Practitioners of epigenetic orthodontics use dental appliances referred to as "functional appliances." They create an imbalance of the bite and pressure on the teeth to trigger a specific form of signal called mechano-transduction.

In mechano-transduction the signal that the cell receives is not a cyto-chemical molecule but a mechanical or physical signal, such as stretch, deformation, or a change in proprioceptive information (as registered by the periodontal ligament of a tooth that is receiving orthodontic pressure). This change can be induced using the functional appliance system. For example, in a healthy normal mouth with the teeth in proper alignment, when teeth meet in occlusion (in biting) a certain amount of proprioceptive information is exchanged between the tooth and the surrounding periodontal (gum) tissue, which keeps the teeth in a stable occlusal relationship in the bone.

If a tooth is extracted, the proprioceptive information changes between the now-missing tooth and the tissues it was attached to, and the change in spatial relations is detected by mechanoreceptors in the periodontium (gum tissue) and periosteal membranes (a thin layer of tissue between a tooth and the bone it sits in). This change in spatial relations produces signal transduction, which ultimately leads to a change in the pattern of gene expression, mRNA biosynthesis, until a new position of stability is eventually reached.[51]

Moreover, changes in spatial relations, when induced wearing functional (formational) appliances, stretch or compress soft and hard tissues of the craniofacial system. These changes are detected by mechano-receptors in the periodontium and periosteal membranes, and the ensuing disruptive events can be harnessed for the

correction of the malocclusion through the biosynthesis of new tissues, such as bone formation, i.e., osteo-genesis.[52]

Recent evidence suggests that by changing the spatial relations of the mandible, a change in the pattern of gene expression is induced.[53] This finding provides scientific evidence for the pertinence of the Spatial Matrix Hypothesis.[54] Put simply, a change in jaw relations provides the impetus for a skeletal (foundational) correction, which is one component of the overall correction required to resolve the malocclusion. In other words, a functional mandibular appliance most likely induces a change in form (of the mandible), and not its function. Therefore, functional appliances may be better referred to as "formational" appliances. These formational appliances address the issue of deformity primarily and dysfunction secondarily, in line with the notion of form and function.[55]

Most of our past understanding of the skull and the bony sutures of the face were that the sutures were considered to be closed, or ossified. A suture is a joint formed between two bones that undergo intra-membranous ossification. It is important to understand that human sutures do not synostose (ossify and become rigid) even in adults.[56]

It is well known in Functional Epigenetic Orthodontic therapy that intermittent forces could open a suture, stretch sutural connective tissues, induce new bone deposition, and be subject to homeostasis, which maintained the sutural width.[57]

The important part of the statement is that these appliances and the technique can induce new bone formation and create structural bony morphological changes in the face, improving esthetics and acting as an anti-aging therapy.

As the bone remodels, facial appearance improves. The zygomatic arch (cheekbones) supports the cheeks along with the lateral aspects of the maxilla (upper jaw). There is bone deposition in the cheeks, allowing the skin to be better supported and resulting in smoother, younger-looking skin with higher cheekbones.

Bone is also increased in the mandible, reducing wrinkles and smoothing sagging skin around the lower jaw, and under the chin.

The chin is often enhanced. From the lateral profile prospective, the face becomes more orthognathic, or straight, whereby the forehead, nose, and chin all align without a prominent nose and recessed chin.

The orbits (eye sockets) become more rounded and the supra-orbital ridges (eyebrow area above the eyes) have more definition. There is less pooling of blood under the eyes, and those dark circles disappear. The eyes look brighter and more symmetrical.

Overall, looking at the face of a patient that has completed epigenetic orthodontics, the observer will see a broader, squarer face, one that has greater symmetry. There is more side-to-side width as the maxilla widens. When the upper jaw widens,

there is more space for the teeth and the teeth either align with the orthopedic treatment or are aligned later. However, because the maxilla is wider, the conventional orthodontics to straighten the teeth becomes much easier as space is available.

Some of the benefits of epigenetic orthodontics are not only improved facial appearance but an increase in airway space. The therapy has been promoted as an alternative procedure for treating people with sleep breathing disorders and sleep apnea.

From an evolutionary perspective, as the brain continues to become larger, the cranium becomes larger; causing the middle and lower facial skull to become smaller or the head would be too heavy. We see evidence of this dentally as there are more impacted wisdom teeth (third molars) and more congenitally missing teeth.

When the maxilla becomes smaller, it loses dimension both laterally (side-to-side) and anterior-posterior (front-to-back). The teeth in these cases are very often crowded. Spatially, as the maxilla becomes smaller, the hard palate, which should be only slightly arched upward, becomes vaulted into a V-shape whereby the hard palate has violated the floor of the nose. As this occurs, there is respiratory limitation, which during sleep can cause respiratory arousals. This is very common in young people today, ages 18–40. The respiratory disturbances occur all night and there are many arousals out of deep sleep. These people suffer from a sleep breathing dysfunction called Upper Airway Resistance Syndrome. They are usually very sleepy during the day, have a hard time awakening in the morning and getting out of bed, finally get going about ten a.m., and then crash at three in the afternoon. Psychologically they are very depressed, suffering from mood disorders. They snore but do not have sleep apnea.

Being young, it is common for these patients not to want to wear C-PAP. Oral appliance therapy works on most, but not all of these patients. Surgery is not effective, although helpful if the patient has very large and intrusive tonsils and adenoids.

Epigenetic functional orthopedic orthodontic therapy can be a good treatment for these patients. Younger patients tolerate the appliance very well, the teeth move relatively easily, and morphogenic remodeling of the craniofacial bones occurs more predictably.

Epigenetic orthodontics is a relatively new field in dentistry and not well studied in the medical literature. Most of the evidence is from completed patient cases.

The protocol for treatment can last as short as six months but often as long as two years. After therapy it is not uncommon to need conventional orthodontic braces for fine-tuning for maximum dental alignment.

For photos of before-and-after completed cases, several websites can be accessed. They are:

www.facialdevelopment.com
www.nusmilefacelift.com
www.dnaappliance.com

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NINTH EDITION

Harry's Cosmeticology

Harry's Cosmeticology

9th Edition

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Meyer R. Rosen is President of Interactive Consulting, Inc. (www.chemicalconsult.com). He is a Thought-Leader and expert in the field of Technical Marketing and multi-industry Technology Transfer Applications including, but not limited to: cosmetics and personal care, applied rheology, applied surface and interfacial chemistry, polymers, organosilicones, professional editing and custom preparation of Mind-Maps® for the organization and presentation of complex information.

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Meyer served for six years as the Chief Scientific Advisor and Director of Technical Programming for United Business Media's (UBM) HBA Technical Conferences. He was a former Director of the American Institute of Chemists, past Vice President of the Association of Consulting Chemists and has served on the Scientific Advisory Board of Supply Side West/East: Virgo Publications. Mr. Rosen is also the Founder, Organizer and co-moderator for HBA's Annual Interna-tional Safety, Regulatory and Certification Symposia.

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I am also grateful for the many kind words from my authors and editors who liked my professional editing skills and sharpening them as I went along. Their patience in explaining- in writing- the answers to all of my seemingly endless questions contributed much to my understanding our industry in a way that was far more in depth than I brought to beginning this book. As I said to them, "If you can't explain it to me so I really understand it; then how will our readers understand what you are saying?

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thinking, close, objective, ever-questioning and challenging examination of the "science" associated with cosmetics and personal care. Once upon a time, he told me that while travelling the world, he always stayed in the same type of room of his favorite hotel chain because "it always made him feel like he was at home."

A manager I once had told me that if "I had lemons, I should make lemonade". And so it was with Johann, who turned the enormous amount of time he spent in travelling to producing an incredible volume of questioning, challenging and probing scientific papers for us to read and think about for years to come.

Wherever you are, Johann, I want you to know that your work and critical thinking approach to cosmetic science has impacted us all- and we miss you greatly.

Dedication

This book is dedicated to my wife Selma, my Soulmate, Committed Listener and Partner in the Journey-of-Life.

She who knows, and reminds me, to put the past in the past in order to open the doorway to the creation of new possibilities and generate new directions for growth in areas we do not know that we do not know.

How remarkable it is
When mere words on paper
Grow together
Beyond themselves.

Such words as these
Are tracks in time.

Memories of a mind
Focused
A heart and soul
Ensconced.

—Meyer R. Rosen
July 4, 2014

Preface

Dear Reader:

This book is filled with highly technical and not-so-technical, but critical, information on the current state of the art in the cosmetic and personal care industry.

Before you jump into it, as I hope you will, for years to come, I take the liberty and license of providing some personal thoughts to let you know what motivated me in taking on this project, which has now been about two and a half years in the making.

If you had asked me if there ever would come a time that I would take on another major book project as large as the one I did about ten years ago (*Delivery System Handbook for Personal Care and Cosmetic Products: Technology, Applications, and Formulations*) . . . well, I would have gracefully declined.

However, as with many things in life, "everything has a season"—and there came a day that a soft-spoken, supportive man by the name of Ben Carr, Publisher of Chemical Publishing Company, offered me the opportunity to_be Editor-in-Chief of the widest-selling book in the cosmetic industry over the past sixty years! It was time to say yes, for in my heart, and for over thirty years, I had wanted to deepen my understanding, learn from, and then teach many of the brightest minds in the world about this intriguing area called Cosmetics. The reason it intrigued me is that the concept of creating beauty provides joy for us all in looking good and feeling good as a result.

Ralph Gordon Harry, FRIC, created the first edition of what later became *Harry's Cosmeticology*. At the time (1954) it was called *Cosmetics: Their Principles and Practices*. We believe he also authored the 2nd through the 6th editions as well. The 7th edition was published in 1982 and co-edited by J.B. Wilkinson, MA, BSc, CChem, FRSC, and R.J. Moore, BSc, CChem, MRSC, MIInfSc; followed by the 8th edition in 2000 by Martin M. Rieger, PhD, and now, the 9th edition by myself, Meyer R. Rosen.

The Preface of the first edition describes the evolution of the modern cosmetics industry, which was grounded in the needs of the military in World War II. Some of the areas Harry mentions as stimulants for cosmetic scientific and technological creativity included, but were not limited to, development of safe and efficacious

sun-screening agents for men marooned on liferafts or in the desert who might be subjected to very severe solar exposure without shelter; flashburn creams designed to protect exposed skin surfaces against burns ("commando makeup"); and anti-sunburn lipsticks. Soaps, shaving creams, toothpastes, and fragrances were also developed for the military.

On the home front, special toilet soaps and barrier creams were developed to reduce dermatitis and, at the same time, the value of cosmetics as a morale builder became recognized when it was claimed that they served to combat fatigue and that a dressing room in a factory might improve efficiency by as much as 10 to 15%. All these developments necessarily had repercussions in the cosmetic field and resulted in the synthesis and development of other cosmetic ingredients such as insect repellants, emulsifiers, detergents, antioxidants, preservatives, and more.

As we move forward sixty years to today's cosmetic needs and wants, there have, of course, been major shifts in what sources the development of cosmetics and personal care products. *It is this enormous shift that has motivated me to take on the job of Editor-in-Chief of the 9th edition of* Harry's Cosmeticology.

As I see things, the population of our world has increased dramatically—as has the breadth of its ethnic interchange. This trend is escalating exponentially. It now has spread from a women-only context to men as well. With it, the age of the population is increasing, benefited by the wonders of modern medicine, enormous breakthroughs in understanding the genetic code, and the beginnings of applying that understanding to further improving the health, well-being, and appearance of the old (at any age) yearning to be young(er). In fact, underneath this yearning, I assert, is the wish to live longer and perhaps, unspoken, to live forever.

I don't know if this will be possible someday, but I do know the yearning is there—recall, for example, the age-old search for the Fountain of Youth. What intrigues me is that nowadays, all the research on the skin to make it look younger is merely the surface of a deeper search to make our bodies younger inside as well as out.

If we put aside the regulations that separate cosmetics from drugs; if we put aside the ever-changing regulatory barriers that differ from one geographical area to another, we come, in my opinion, to the heart of the matter.

Whatever you want to call it, be it cosmetics, drugs, the search for medical breakthroughs etc., it is all the same need being expressed. We all want to reverse the "inevitable" impact of gravity and time on ourselves and our loved ones. My intention in becoming Editor-in-Chief of the current 9th edition of *Harry's* is to support that yearning in the best way I know how.

I believe we are at a tipping point in fulfilling this eternal search for youth. With the breakthroughs in understanding genetics, epigenetics, and biochemistry—as well as the enormous growing consumer pull, the need to develop approaches that really fulfill the longing to be young again is the opportunity of our lifetimes.

It is my pleasure to give you, in the best tradition of sixty years of *Harry's Cosmeticology*, the knowledge described and documented in this book. I give it to you for your study and to mentor future generations of beauty/youth-seekers in moving towards this goal.

Meyer R. Rosen

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Editor-in-Chief: *Harry's Cosmeticology*, 9th Edition

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THE SKIN: STRUCTURE, BIOCHEMISTRY, AND FUNCTION

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TOPICAL REDUCTION OF VISIBLE SKIN DETERIORATION DUE TO CELLULITE

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AHAS AND BEYOND: ANTI-AGING INGREDIENTS AND THEIR BENEFITS FOR ALL LAYERS OF THE SKIN

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CYTOKINES, GROWTH FACTORS, AND STEM CELLS: NEWEST APPROACHES TO YOUNGER LOOKING SKIN

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PART 5 ANTI-AGING

PART 5.0

FUNDAMENTALS OF SKIN ANTI-AGING OVERVIEW

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PART 5.1

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PART 6 FORMULATING COSMETICS AND PERSONAL CARE PRODUCTS

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FORMULATING WISDOM CATEGORY BY CATEGORY

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PART 10 NANOCOSMETICS

PART 10.1

NANOCOSMETICS

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PART 11 TESTING

PART 11.1

METHODS TO ASSESS SKIN BARRIER INTEGRITY:
EFFECTS OF CLEANSING PRODUCTS

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BIOPHYSICAL MEASUREMENT AND EVALUATION OF SKIN ELASTICITY AND TOPOGRAPHY

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PART 11.4

A SURVEY OF TEST METHODOLOGY USED IN EVALUATING THE DAMAGE, PROTECTION AND REPAIR OF HAIR

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PART 12 SUSTAINABILITY AND ECO-RESPONSIBILITY

PART 12.0

A GLOBAL APPROACH FOR THE COSMETIC AND PERSONAL CARE INDUSTRY 1990

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PART 12.1

DEFINING SUSTAINABILITY AND HOW IT CHANGES THE INNOVATION PROCESS

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PART 13 COSMETIC MANUFACTURING

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MANUFACTURE OF COSMETICS SECTION OVERVIEW

Meyer R. Rosen

PART 13.1

COSMETIC MANUFACTURING PROCESSES

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PART 13.3

INTELLIGENT SELECTION AND MANUFACTURE OF NATURAL EXTRACTS

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PART 14 PACKAGING

PART 14.1

EMERGING STRATEGIES FOR SUSTAINABLE PACKAGING:
BALANCING MATERIALS, DESIGN, AND APPEARANCE

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ART AND SCIENCE OF FORMULATING COSMETIC PRODUCTS

PART 6

FORMULATING COSMETICS AND PERSONAL CARE PRODUCTS

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ABSTRACT

An overview of the science and art of formulating personal care products follows. Included are: specific product category overviews of hair and skin care products, detailed formulating information on selected product categories (e.g., shampoo) and an overview of the formula/product development process from the formulator's viewpoint.

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SKIN CARE

OVERVIEW

Exactly what is a "formulator"?

First and foremost, a formulator is a good scientist. A formulator has to have a good technical sense and a fundamental understanding of chemistry, biology, physics, statistics, and consumer sciences. The deeper and more complete the technical understanding, the better and more creative the formulating can be.

A formulator needs to understand the chemical relationships among the raw materials being used in whatever category the formulating is being done. Understanding the chemical function of the raw materials and their interactions with other raw materials allows the formulator to choose the best combination(s) for the particular end formula.

Likewise, a formulator has to understand the physics and physical properties of the desired end product – dictated by the category, the manufacturing requirements, the filling and dispensing properties and, most importantly, the physical characteristics desired by the ultimate consumer.

The formulator needs to understand the medium upon which the formulation is to be used—hair or skin and how that medium is impacted (positively and negatively) by the application of the particular formula. In addition, the formulator must understand the potential microbiological perils that can be introduced into the formula and the safest, most effective ways of preventing those contaminations from affecting the formula.

The formulator needs to understand how the formula is put together during manufacture and filling and how those operations impact the properties of the formulation. Additionally, the formulator needs to understand the relationship between the formula and the primary packaging, with special attention to how the formula will be dispensed from the package. Many formulators have had the unpleasant surprise of watching their formula become too thick as it is forced through a small orifice or, worst of all, cannot be dispensed from the package selected.

Consumer science is important for the formulator because the consumer is the ultimate judge of a product and, ultimately, its success in the marketplace. Understanding consumer testing and the statistical analysis of consumer data will always be of great benefit to the formulator. Dealing with consumer expectations is always challenging, but those expectations are extremely important to formulation and the ultimate marketing of a successful product.

Secondly, a good formulator needs to be an artist. Translating the "wish-list" from Marketing or the Consumer into a tangible consumer product is truly an art. Understanding the aesthetic requirements and the particular combinations of raw materials that deliver against those requirements is definitely an artistic endeavor and one that a good formulator must really take the time to pursue.

So, a formulator is a combination of scientist and artist, a good observer of both technical and aesthetic properties, a good listener to all interested partners—Marketing, the Consumer, Manufacturing, etc., and an objective and unbiased evaluator of information.

A formulator needs to understand the category in which the formula is being developed

In skin care, the categories are usually split between hand/body and facial products. In hand/body care there are cleansers, moisturizers, and a group of miscellaneous products, such as sunscreens. In facial care there are cleansers/scrubs, moisturizers, and treatments, such as acne treatments, fine-line and wrinkle treatments, masques, etc. There are other groups of products also, such as perfumes/fragrances, pigmented cosmetics, nail products, and products related to shaving and depilatory products.

In hair care, the categories are: shampoo, conditioner/treatments, styling and reactive products. The formulator also needs to understand the targeted consumer

within the category. A general market shampoo for European hair would have different technical and aesthetic requirements than a shampoo for African/Caribbean hair or a shampoo for Asian hair.

An understanding of the existing environment, individual company, and competitive arena for each category is a good starting point. If the formulator is working for a company, a thorough understanding of the existing formulations within the category is important in understanding the palette of raw materials and familiarity with manufacturing. A review of the competitors in the category will provide insight into what is having success in the category and where there may be formulating opportunities.

This section will be devoted to providing assistance to the new formulator and review and refreshment for the experienced formulator.

FORMULATING WISDOM – CATEGORY BY CATEGORY

Author

Charles Warren

The skin is the largest organ of the human body. Below the outermost layer (stratum corneum) the skin is a living, biologically active organ. Unlike hair, healthy skin can repair itself and every 28 days actually replaces itself. It is the barrier between the internal body (75–80% water) and the external environment (significantly lower water content) and all of the perils it faces from that same external environment.

When formulating for skin care, there are two major categories: Hand and Body Care and Facial Care.

Hand/Body

Cleansers

The general categories of body and hand cleansers are soaps, bodywashes, and liquid hand washes. The function of all of these is to cleanse the body and hands of excess oils, particulates, and other substances adhering to or residing on the skin. Soaps are a special category, while bodywashes and liquid hand cleansers are very similar in form and composition.

The classic definition of a soap is an ionic salt of a long-chain fatty acid. A long-chain fatty acid, such as stearic acid, is neutralized with an alkali material to a stearate. When neutralized with sodium hydroxide, sodium stearate results; with ammonium hydroxide, ammonium stearate, etc. These stearates will emulsify the oils on the skin and adhere to particulates and then allow them to be rinsed away with water. Formulating and manufacturing soaps is a specialized endeavor and is usually handled by suppliers or manufacturers highly skilled in this medium.

Bodywashes and liquid hand cleansers are more prevalent and more reasonably formulated and manufactured by personal care formulators/manufacturers. In general, these are aqueous solutions of primary surfactants (e.g., laureth sulfate), secondary surfactants (e.g., betaines), foam modifiers (e.g., cocamide MEA), conditioning agents (e.g., hydroxypropyl trimonium chloride), aesthetic modifiers

such as sodium chloride (viscosity), color and fragrance, and microbiological preservatives. If the product is to be opaque or pearlescent, materials such as glycol stearate can also be used. Once an acceptable base formulation of surfactants and modifiers is identified, a wide variety of variables can be developed by changing colors, fragrances, and other additives to support positions and claims. These products can be easily formulated to dispense from tubes, bottles, through pumps, etc.

Moisturizers

The largest groups in this category are the hand and body lotions. These formulations are typically oil-in-water emulsions. Their primary function is to develop a light, oily layer on the surface of the skin to prevent water from leaving the lower layers of the skin, causing the surface to dry, flake, and develop a dull appearance. In addition, the other ingredients will help bind and retain moisture in the surface layers of the skin, leave the skin feeling soft and pliable, and impart a pleasing look and feel to the skin for an extended period of time.

A general overview of the ingredients used in these products would include:

- Water – the mobile phase of the emulsion
- Glycerin – a humectant that will reside in the stratum corneum and bind and hold water to itself
- Isopropyl palmitate – a light oily material to lubricate the skin and help occlude for water retention
- Petrolatum – the best occlusive agent to prevent trans-epidermal water loss (TEWL) and provide body and stiffness to the lotion
- Silicones/cyclosilicones – help improve spreadability and feel of the lotion on the skin
- Distearyldimonium chloride – a skin conditioner to help make the skin feel soft and pliable
- Cetyl alcohol/cetearyl alcohol/ceteth-20 – common emulsifiers to hold the oil droplets in the emulsion

In addition, various other fatty esters, cationic polymers, and thickening agents can be used to improve the texture and feel of the lotion.

A word of caution – Certain claims related to protection and "healing" can quickly move a formula from a cosmetic product into the realm of over-the-counter drugs (OTC). OTC drugs have very specific requirements related to the levels of the active ingredient (e.g., dimethicone), the claims and exact wording of the allowable claims, stability and packaging testing, labeling, and manufacturing. A prudent formulator will review formulas and claims with Regulatory and/or Legal groups to ensure compliance.

SKIN LIGHTENING, WHITENING, AND BRIGHTENING: AN OVERVIEW OF APPROACHES, KEY INGREDIENTS, AND FORMULATIONS FOR ENHANCING SKIN APPEARANCE AND CORRECTING/MINIMIZING COMMON SKIN PIGMENTATION DISORDERS

Authors

Eva Patel & Gurpreet (Gogi) Sangha

ABSTRACT:

Skin-lightening products form a major segment in the cosmeceuticals market worldwide; and they carry with them the promise of flawless-appearing skin. This demand for "skin-fairness" products stems from the desire to eliminate hyper-pigmentation, melasma, and post-inflammatory hyperpigmentation as well as a consumer interest to lighten overall skin tone through the use of skin lighteners, whiteners, and brighteners.

The continually growing demand for skin tone corrective treatments by the global beauty market has prompted scientists to develop an array of natural and synthetic raw materials that work on various pathways of skin pigmentation. Most skin lighteners currently in use are of botanical or natural origin and have multi-functional topical benefits. Such materials can also act as free radical scavengers for skin that has been over exposed to UVA and UVB solar radiation. These specialty ingredients can be formulated into topical solutions such as creams, serums, and lotions.

In the U.S., topical compositions containing hydroquinone are the only ones that regulations allow to make a skin-whitening and -lightening claim. Such compositions have been classified by the U.S. FDA as a drug. Many botanical, natural, and synthetic ingredients are available that may produce results similar to those of hydroquinone; however, current regulations covering compositions containing such ingredients are only allowed to make the claim of "skin brightening."

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Skin Lightening, Whitening, Brightening

Skin lightening has become a significant trend attracting the interest of technologists, product developers, and marketing/business leaders. Today, women, and even men, want to alter the tone and appearance of their skin to meet current regional standards of beauty or to address an underlying medical or cosmetic condition. Amazingly, skin whitening has become increasingly popular.

In the USA many people desire flawless and even-toned complexions; however, this is not the case in Asia. In this geographical area, *it is a cultural issue* that one has fair skin as it is a sign of beauty, wealth, and status. An article in *The Economist* titled "The Line of Beauty" (August 27th, 2011) suggests that good-looking people are more likely to succeed, be accepted by their peers, and get higher-paying jobs. In many countries, socioeconomic status depends on your skin color, as for example in India and Mexico. In India even your marriage is based on skin color, and the rising skin-tone consciousness continues to grow rapidly. From an epidemiological point of view, we find this trend-to-whiteness fascinating in view of the well-recognized global shift in the population towards darker-skin peoples.

6.2.1 DEFINITIONS

Skin Lightening & Whitening/Skin Bleaching: A common drug claim for OTC/RX products containing Hydroquinone, as this is the only skin-lightening active ingredient that is recognized by the U.S. FDA.

Skin Brightening: Brightening is a term used throughout the skin care industry to classify formulations that brighten uneven skin tone and contain alternatives to hydroquinone. These alternatives can include botanicals, natural ingredients, peptides, and vitamins. Natural skin lighteners are also considered multifunctional ingredients that provide multiple benefits for the skin.

Hyperpigmentation/Melasma: Overproduction of melanin in the skin that is denoted by dark spots or patches

Hypopigmentation/Vitiligo: Areas of the skin that have lost their pigment completely when there are no longer functioning melanocytes in that area to create pigment, leaving white spots or patches on the skin

Melanogenesis: The process of melanin being formed in the melanocyte located at the dermal/epidermal junction in the skin

Melanin: The substance that gives our skin its color/pigment. This natural pigment is our skin's protective shield against UV aggression. Dark skin tones contain more melanin in their skin and lighter skin tones have less melanin.

6.2.2 COMMON SKIN PIGMENTATION DISORDERS

1. Melasma/Chloasma – A condition that is typically induced from hormones internally. Also known as a pregnancy mask because most cases are seen in women postpartum. This condition can also be induced from menopause and even certain medications. MSH (melanocyte-stimulating hormone) is a natural hormone in our bodies and is linked to pigmentation and hyperpigmentation development in individuals with Melasma/Chloasma.
2. Post-Inflammatory Hyperpigmentation – This condition can be seen in the skin after inflammation has been present. Most commonly found in acneic skin conditions, this term is a broad category and includes "acne prone, reactive, oily skin type," that experiences persistent breakouts and also post–aggressive skin care treatments or medical procedures.
3. Sun Damage – Sun is the number one trigger for hyperpigmentation, causing sun spots from years of excessive exposure to the ultraviolet rays. These are also known as photo damage or age spots.

6.2.3 TRIGGERS FOR HYPERPIGMENTATION

1. Sun (UVA/UVB rays, environmental damages, free radicals)
2. Hormones (internal unbalance, pregnancy, menopause)
3. Inflammation (blemishes, acne, injury, laser treatment, heat, medical procedures, chemical procedures)
4. Oral Medications (diuretics, birth control pills, antibiotics, anti-depressants, etc.)
5. Alcohol (aftershave, colognes, perfumes)

6.2.4 PATHWAY TO HYPERPIGMENTATION:

Formation of melanin is influenced by many different factors including genetics, certain medications, diet, and environmental factors. In today's market there are many different types of skin-brightening ingredients that target the process of pigment formation via multiple modes of action. The term "tyrosinase inhibitor" has been used widely in the market to define a skin-brightening active. This term is

true for certain active ingredients; however, not all skin-brightening ingredients directly interfere with the catalyzing of the tyrosinase enzyme. Tyrosinase is a copper-containing enzyme where copper and oxygen act as catalysts. Kojic acid is a specific ingredient that directly affects tyrosinase by chelating the copper within the enzyme-active site. The antagonist effect of kojic acid results in an inactive form of the tyrosinase enzyme that no longer allows for the production of melanin. As seen in Figure 6.2, the tyrosinase enzyme is a key component for melanin to be produced and should always be targeted in an effective skin-lightening formulation. Another area of focus is to look at other biochemical processes involved in melanogenesis. By looking at the "Pathway to Hyperpigmentation" we can analyze the different stages in which active ingredients can be targeted to more effectively control pigment production and produce the most visible changes in skin tone and appearance.

Figure 6.2. Inhibition of Pigmentation Process

1. One of the first things to occur in the skin along the pathway to hyperpigmentation is the generation of tyrosine, which is an amino acid naturally occurring in our bodies. Tyrosine is then converted to L-dopa by an enzyme called tyrosinase.
2. Tyrosinase catalyzes with L-dopa and converts it into dopaquinone.
3. Melanin is then created and put into our melanosomes.
4. Melanosomes are transferred into the keratinocytes, also known as our skin cells, which then continue on their journey up through the epidermis where they eventually are bio-converted to corneocytes and appear on the surface of the skin.

Besides the aforementioned pathways there are other ways to help suppress melanin formation. **Melanogenesis** is a complex process of oxidative stages in which topical antioxidants are useful to combat hyperpigmentation. Many antioxidants have skin-brightening properties that also provide multifunctional benefits. Antioxidants help to reduce hyperpigmentation by scavenging free radicals that can be a precursor to hyperpigmentation. Another example is MSH, melanin-stimulating hormone (melanotropin), which can be inhibited by certain ingredients. (1)

A variety of other avenues are used to increase cell turnover, which will provide skin-brightening results, as well as remove pigment from the surface for an instantly brighter glow. Alpha Hydroxy Acids (AHA) and kertolytic enzymes, such as papaya and bromalein, help to exfoliate surface stratum corneum cells to promote brighter, more even-tone complexion. Finally, the importance of using a UV absorber is crucial in order to see results and maintain those results from skin-lightening actives. Hyperpigmentation due to UV exposure can be addressed by using preventative measures, which include applying a physical/chemical broad-spectrum sunscreen regularly when exposed to UV radiation. The use of broad-spectrum photo-stable UVA/UVB sunscreens and protective clothing are one of the best ways to enjoy sun exposure while minimizing the deleterious effects of UV aggression on the skin. There are a number of companies who have woven UV absorbent fibers into protective clothing to prevent sun exposure. These items can be wide-brimmed hats, long-sleeved shirts, pants that when coupled with the use of UV-protectant sunglasses all help to protect the skin for extended times outdoors. The best commonsense photo-protective advice is to take cover in the shade when the sun's rays are strongest between 10 a.m. and 4 p.m. Seek cover indoors or at least in a shaded spot during those hours when the UV index is at its most intense. Even though sun protection does not directly interfere with the pigment formation process, it helps to block UV radiation and limit post-sun exposure erythema, which are key triggers for melanocytes to produce melanin. The technological advances described above are quite important for formulators of sun-protection products since they describe the skin hyperpigment formation process and how we can target different pathways to inhibit it.

6.2.5 FORMULATING INGREDIENTS—A PLETHORA OF RAW MATERIALS AND HOW THEY COME INTO PLAY

Actiwhite® PW LS 9860: A synergistic skin-lightening complex based on pea extract and a sugar ester. This innovative mechanism has dual mode of action for optimal skin-lightening effect. The material does not affect the color of the formulation, has excellent compatibility and stability, and can be formulated in a broad pH range, even in combination with AHAs. This active component contains a high capacity to decrease melanogenesis. It works to decrease tyrosinase activity and reduce melanosomes from maturing.

Supplier: BASF Personal Care Ingredients

Arbutin: A potent antioxidant that is an extract from the bearberry plant. Arbutin works by inhibiting the activity of the tyrosinase enzyme to reduce hyperpigmentation and protects the skin against free radical damage. Arbutin suppresses the activity of tyrosinase enzyme and prevents melanosome from forming. Arbutin is chemically similar to hydroquinone.

Bearberry (Uva ursi) Extract: This plant leaf extract contains arbutin and methyl arbutin, both hydroquinone derivatives with skin-whitening properties.

β-White™ (Oligopeptide-68): An encapsulated peptide that mimics the signaling protein of the tyrosinase enzyme to reduce the amount of signaling proteins that activate tyrosinase to produce melanin. According to Unipex Innovations, an *in vitro* comparative study showed that β-White induced significant skin-lightening effects on 23 Asian volunteers with at least one hyperpigmented spot after four weeks.

Supplier: Lucas Meyer Cosmetics

Chromabright™ (Dimethylmethoxy Chromanyl Palmitate): An advanced engineered amino acid that fights against photo-aging. Chromabright reduces dopaquinone so that it cannot convert into DOPA, therefore inhibiting another pathway to melanin formation.

Supplier: Lipotec

Ethyl Ascorbic Acid: A novel vitamin C derivative with excellent whitening, anti-oxidation, radicals-scavenging and collagen-increasing effect. Its stability is superior to other ascorbic acid derivatives. It is widely used in skin care products, especially desirable for whitening and anti-ageing products.

Supplier: EME Ltd.

Glycolic Acid: Known as one of the most-used alpha hydroxy acids in the industry. Glycolic acid is naturally derived from sugar cane. It helps to speed up cell turnover and removes pigmentation from the outer layers of the skin to instantly brighten skin.

Hexylresorcinol: A widely known ingredient that has been used in the food industry for preservation and in oral and topical applications. Hexylresorcinol has antibacterial properties, and inhibits the tyrosinase enzyme to give a brighter, more even skin tone. **(Symwhite: Symrise)**

Hydroquinone: Hydroquinone is a very well-known ingredient to bleach the skin. Working through a variety of mechanisms, this ingredient reduces the formation of melanosomes and even induces melanocyte cytotoxicity. Hydroquinone is the only ingredient approved by the U.S. FDA for skin-whitening claims.

Kojic Acid: Derived from mushrooms and other species of fungus. This form of kojic acid is water soluble and inhibits tyrosinase enzyme by blocking copper

from catalyzing with tyrosinase, which results in an inactive enzyme. This effective brightening agent also provides antibacterial properties.

Kojic Acid Dipalmitate: Derived from mushrooms and other species of fungus. This form of kojic acid is oil soluble and inhibits tyrosinase by chelating the copper contained within the tyrosinase enzyme resulting in a deactivated enzyme. This effective brightening agent also provides antibacterial properties. Compared to kojic acid, it offers improved efficacy and stability, and is easier to make with water-based formulations.

Esterases in the skin hydrolyze the ester bond, cleaving the fatty chain off kojic DP and allowing bioavailable KA to be liberated. (2)

Lactic Acid: A well-known alpha hydroxy acid that contains skin-brightening properties. Mostly derived from sour milk and sugars, this active exfoliates to instantly brighten skin and suppresses tyrosinase.

Licorice Root: This well-known extract inhibits the tyrosinase enzyme without causing cytotoxicity. This material also reduces UV-induced hyperpigmentation and inflammation in the skin, which are precursors to hyperpigmentation.

Lumiskin: Made up of triglycerides, this active ingredient acts as a competitive antagonist to the tyrosinase enzyme and suppresses the tyrosinase activity. Lumiskin inhibits calcium flow into the cells and thus reduces tyrosinase activity (stabilizes it in its inactive form) and melanogenesis.

Supplier: Croda

Lumisphere™: This complex combines Diacetyl Boldine and manganese titanium dioxide (TiO2-Mn) to instantly and gradually brighten the skin. Lumisphere, the Diacetyl Boldine (DAB) stabilizes tyrosinase in its inactive form. It also contains TiO2Mn, which can absorb UVA and UVB and neutralizes free radicals, resulting in a more even skin tone.

Supplier: Croda

Magnesium Ascorbyl Phosphate: Is a stable water-soluble derivative of vitamin C. It is an excellent antioxidant, stimulates collagen production, and is well known for its melanin-inhibiting properties.

Supplier: Caribbean Natural Products Inc.

The bioconversion needed to liberate the active form L-ascorbic acid phosphatase enzymes within the skin change this pro-form into bioavailable ascorbic acid.

References:

Mia Campos et al.: Ascorbic Acid and Its Derivatives in Cosmetic Formulations, Cosmetics and Toiletries, 11,59-62 (2000)

Melanostatine®-5: A skin-brightening peptide that is created in a lab to also inhibit the alpha-melanocyte-stimulating hormone (MSH). This active ingredient

does not allow the hormone to signal the melanocyte to produce melanin, thus preventing and suppressing hyperpigmentation.

Supplier: Lucas Meyer (aka Unipex)

Meiritage: Combination of plant extracts used in Traditional Chinese Medicine: Atractylodes macrocephalae, Bupleurum falcatum, and Astragalus membranaceus roots, to prevent wrinkles and even out the complexion.

Supplier: Croda/Sederma

Mulberry (Morus bombycis): The root and bark extracts of the mulberry plant might also play a role in inhibiting the tyrosinase enzyme that converts tyrosine into melanin's precursors. Mulberry contains arbutin, which inhibits melanin production.

O.D.A. White: Octadecenedioic acid is obtained by a biofermentation process from natural and vegetable oleic acid. O.D.A. White helps to inhibit the entire pathway of melanin synthesis from the nucleus of the melanocyte by reducing levels of tyrosinase mRNA. This active ingredient treats all types of pigmentation, even ethnic skin. The use of antioxidants in combination with ODA White is recommended.

Supplier: Croda

Phytic Acid: Most commonly found in grains, bran, and seeds. Phytic acid has been used as an antioxidant for years in the food industry. It works via a chelation mode of action by blocking the copper contained within the tyrosinase enzyme from catalyzing to form melanin.

Regu®-Fade: A nature identical trans-resveratrol ingredient based on a biofermentation that increases cell longevity and reduces inflammation. Resveratrol is made up of a polyphenol and has strong antioxidant properties to protect skin against free radicals and environmental damages. REGU®-FADE helps reduce the appearance of skin pigmentation as demonstrated in clinical and *in vitro* studies, resulting in noticeably brighter, younger-looking skin.

Supplier: DSM Nutritional Products, Inc.

Sepiwhite MSH (Undecylenoyl Phenylalanine): A highly advanced engineered active ingredient that inhibits the alpha-melanocyte-stimulating hormone (MSH).

Supplier: Seppic

Tetrahexyldecyl Ascorbate: Most commonly derived from citrus components. Advancements of vitamin C have been engineered to contain multiple benefits with greater efficacy and stability than L-Ascorbic Acid alone. Tetrahexyldecyl Ascorbate works as an antioxidant in the skin that also encourages brighter skin tone. This engineered form allows for better penetration into the skin and even suppresses melanogenesis up to 80% by inhibiting tyrosinase enzyme.

Tyrostat™ (rurnex occidentalis extract): Derived from plants native to Canada. Extracts of this plant have strong effect to inhibit the tyrosinase enzyme, resulting in a more even skin tone. Unipex screens the plant material for tyrosinase activity before it enters the extraction process to guarantee optimal activity. *In vitro* and clinical data have demonstrated that Tyrostat™ safely reduces skin pigmentation and erythema for a more uniform complexion. It also has outstanding results in the reduction of the appearance of age spots.

Supplier: Lucas Meyer Cosmetics

WONDERLIGHT™: An active ingredient of plant origin (hop cone), a regulator of the pigment disorders by inhibiting the cytokine GM-CSF (activator of melanogenesis).

Supplier: Croda/Sederma

Vitamin A: Also known as Retinol, is a multifunctional active ingredient that promotes youthful and even-toned skin. Vitamin A inhibits tyrosinase activity and reduces the amounts of melanosomes that are produced. This ingredient will also help to increase cell turnover and increase penetration of skin-brightening actives into the skin. When using a product containing Vitamin A and or its derivatives, it is mandatory to use in the evening only and accompany it with a broad-spectrum UVA/UVB SPF 30 during the day.

It is clear from the extensive array of the skin-whitening ingredients described above that formulators have great flexibility in selecting optimal agents and combinations of those agents to formulate products that address the global interest in skin whitening and brightening.

Figure 6.2.1

6.2.6 FORMULATIONS FOR INDIVIDUAL SKIN CONDITIONS

Whitening Cream

Ingredient Listing: Aqua (Water), Glycerin, Isopropyl Palmitate, Cetyl Alcohol, Stearyl Alcohol, Cetearyl Alcohol, Alpha Arbutin, Butylene Glycol, Kojic Dipalmitate, Dicetyl Phosphate, Ceteth-10 Phosphate, Hydrogenated Lecithin, Sodium Oleate, Oligopeptide-68, Glycyrrhiza Glabra (Licorice) Root Extract, Dimethylmethoxy Chromanyl Palmitate (Chromabright Proposed INCI), Sodium Metabisulfite, Sodium Sulfite, Phenoxyethanol, Sodium Ascorbyl Phosphate, Hexylresorcinol, Potassium Sorbate, Sodium Benzoate, Xanthan gum, Disodium EDTA

PHASE INGREDIENT/SUPPLIER

Phase A	**wt/%**
Cetearyl Alcohol (and) Ceteareth 18 Symrise (2/014200 Dragowax S.E.)	8.00
Cetearyl Octanoate (and) Isopropyl Myristate Symrise (2/066210 PCL Liquid)	3.00
Mineral Oil Exxon Mobil (Marcol 82)	5.00
Octyl Octanoate Symrise (2/044115 Dragoxat EH)	1.00
Dimethicone Dow corning (200/350 cs)	1.00
Phase B	
Deionized Water	75.45
Xanthan Gum (Keltrol F)	0.10
Pentylene Glycol Symrise (2/016020 Hydrolite-5)	3.00
Glycerin BP	1.20
Phenoxyethanol (and) Methylparaben (and) Ethylparaben (and) Isobutylparaben (and) Propylparaben (and) Butylparaben Symrise (2/060140 Dragocide Liquid)	0.80
Sodium Hydroxide 1% Solution	0.10
C Tyrostat™-11 Unipex innovations	1.00
D Fragrance O/H 64583 The Vert Symrise	0.30

PROCEDURE:

1. Disperse the Keltrol F in water.
2. Heat Phase A and B separately to 80°C.
3. Add Phase B into A under vigorous stirring and homogenize.
4. Allow batch to cool to 45°C and add Tyrostat-11 and homogenize.
5. Add the fragrance at 35°C.

Skin-Brightening & Anti-Aging Moisturizing Lotion with Resveratrol

Phase A	**wt/%**
Water (aqua) (deionized)	70.98
Versene 220 Crystals (Dow Chemical) (Tetrasodium EDTA)	0.02
Rhodicare XC (Rhodia) (Xanthan gum)	0.20
Propylene Glycol USP (Lyondell)	3.00
CoSept PEP (HallStar) (Phenoxyethanol, methylparaben, ethylparaben, butylparaben, propylparaben, isobutylparaben)	0.60
Phase B	
HallStar GC (HallStar) (Caprylic/capric triglycerides)	5.00
BioChemica Jojoba Oil — Ultra Refined (HallStar) (Simmondsia chinensis [jojoba] seed oil)	3.00
BioChemica Sunflower Seed Oil (HallStar) (Helianthus annuus [sunflower] seed oil)	2.00
BioChemica Shea Butter — Ultra Refined (HallStar) (Butyrospermum parkii [shea butter])	3.00
HallStar GMS Pure (HallStar) (Glyceryl stearate)	0.32
HallStar GMS SE/AS (HallStar) (Glyceryl stearate, PEG-100 stearate)	3.00
HallStar TA-1618 Cetearyl Alcohol (HallStar) (Cetearyl alcohol)	0.70
SolaStay S1 (HallStar) (Ethylhexyl methoxycrylene)	4.00
RTD HC-40 (HallStar) (PEG-40 Hydrogenated castor oil)	1.00
Phase C	
BioChemica Vitamin E Natural (HallStar) (Tocopherol)	1.00
BHT (Merisol Antioxidants) (BHT)	0.08
Regu-Fade (DSM Nutritional) (Resveratrol)	0.10
Phase D	
MSS-500W (Kobo Products) (Silica)	2.00

PROCEDURE:

To a suitable vessel equipped with mixing, heating, and cooling capabilities, add phase A and start mixing. To a second-phase vessel equipped with heating and cooling capabilities, charge phase B. Mix phases A/B while heating to 70°–75°C. When phases A/B are at 70°–75°C, slowly add phase B to phase A while maintaining temperature and adequate mixing. Homogenize for 10 minutes and then resume mixing and start cooling. At 122°F (50°C) or lower, add phase C and D; mix well while recirculating through a pump/mill combination. Cool to ambient temperature and then add water as needed to bring to full batch mass. When again uniform, stop mixing, perform final quality-assurance checks, and package product.

PROPERTIES:

(25°C): Appearance—Light yellow lotion; viscosity (RV, T-D, 5, 20 & 100 rpm, cP)—10800, 3500 & 1120; pH—6.0.

Reference: The HallStar Company

Skin-Lightening Cream Gel with Alpha-Arbutin and DISMUTIN®-PF

Phase A	**wt/%**
Ethylhexyl Isononanoate (Pelemol 89/Phoenix Chemicals)	5.00
Isohexadecane (Arlamol HD/Uniqema)	2.00
Diethylhexyl Carbonate (Tegosoft DEC/Evonik)	5.00
Dimethicone (DC200fluid/200cts/Dow Corning)	1.00
Phase B	
Ammonium Acryloyldimethyltaurate & VP Copolymer (Arisoflex AVC/Clariant)	1.20
Phase C	
Deionized Water	68.90
Glycerin (Rita Glycerin USP/Rita)	5.00
DISMUTIN-PF	0.20
DIOCIDE	0.70
Phase D	
Deionized Water	10.00
ALPHA-ARBUTIN	1.00
Phase E	
Citric Acid (10% solution)	q.s. to pH 5

PROCEDURE:

1. Mix phase A together. Add phase B to phase A, mix until homogeneous.
2. Mix together phase C.
3. Premix phase D ingredients and add to phase C.
4. Add phase C/D to phase A/B. Homogenize to obtain a homogenous cream gel.

Reference: Centerchem, Inc.

High Protection SPF 30 Sunscreen with Skin-Lightening Properties

Phase A	**wt/%**
Parsol HMS (DSM) (Homosalate; USAN)	10.00
Parsol 1789 (DSM) (Butyl methoxydibenzoylmethane) (Avobenzone; USAN)	3.00
Parsol 340 (DSM) (Octocrylene) (octocrilene; USAN)	2.70
Parsol EHS (DSM) (Ethylhexyl salicylate Octisalate; USAN)	5.00

Parsol SLX (DSM) (Polysilicone-15)	0.99
Amphisol K (DSM) (Potassium cetyl phosphate)	2.00
Eusolex 4360 (Merck) (Benzophenone-3) (Oxybenzone; USAN)	6.00
Lanette O (Cognis Deutschland) (Cetearyl alcohol)	2.50
dl-alpha-Tocopherol (DSM) (Tocopherol)	0.20
Euxyl PE 9010 (Schülke & Mayr) (Phenoxyethanol & ethylhexylglycerin)	1.00
Cetiol CC (Cognis) (Dicaprylyl carbonate)	3.00
Finsolv TN (Innospec) (C12-15 alkyl benzoate)	3.00
Lipocire Na 10 Pastilles (Gattefossé) (Hydrogenated coco-glycerides)	1.00
Antaron V-216 (International Specialty Products) (VP/hexadecene copolymer)	1.00
Phase B	
1,3-Butylenglykol (Brenntag AG) (Butylene glycol)	3.00
Keltrol CG SF (CP Kelco) (Xanthan gum)	0.20
Edeta BD (BASF) (Disodium EDTA)	0.10
Water dem. (Aqua)	51.31
Phase C	
Orgasol Caresse (Atofina) (Polyamide-5)	2.00
Phase D	
Alpha-Arbutin (DSM)	2.00

PROCEDURE:

Heat phase A to 85°C while stirring. Heat phase B to 80°C and add to phase A while stirring and homogenizing the emulsion. Cool down the emulsion to 40°C, add phase C, and homogenize again.

PROPERTIES:

pH—5.56 ;Viscosity—14600 cps (Brookfield, RV4,10 rpm); SPF *in vivo*—34.0 *In vitro* UVAPF (Colipa 2007)—1.7; PA—UVAPF/SPF (30 labeled); >0.33—0.39; PFA: Critical Wavelength—376.0.

Reference: DSM Nutritional Products

Skin-Brightening Cream with Chromabright MFF

Phase A	**wt/%**
Deionized water	65.95
Ultrez 10 (Lubrizol)	0.15

1,3-butylene glycol	6.00
Hispagel 200 NS (Cognis Iberia)	10.00
Phase B	
Promulgen D (Lubrizol) (Cetearyl alcohol and ceteareth-20)	1.50
Cutina GMS-V NA (Cognis) (Glyceryl stearate)	1.25
Myrj 59 (Uniqema) (PEG-100 Stearate)	1.00
Fitoderm (Lipotec)	4.00
Tegosoft CT (Evonik) (Caprylic/capric triglyceride)	3.00
Lameform TGI (Cognis) (Polyglyceryl-3 diisostearate)	0.50
Phase C	
Tealan 99% (RITA) (Triethanolamine)	0.15
Deionized water	1.00
Phase D	
Chromabright MFF (Lipotec)	5.00
Diocide (Centerchem)	0.50

PRODEDURE:

Heat phase B to 75°C. Slowly add carbomer to phase A water and mix until uniform. Begin heating to 75°C. Add remaining phase A ingredients and mix until uniform. When both phases are at 75°C, add phase B to phase A under moderate mixing. Mix for 10 minutes. Begin cool down. At 50°C, add phase C as a solution. Mix until uniform. At 35°C, add phase D ingredients. Mix until uniform.

Reference: Centerchem, Inc.,

Age Spot Treatment Cream

Phase A	**wt/%**
Estol 3650 (Uniqema) (Glyceryl myristate)	2.50
Lanette 16 (Cognis) (Cetyl Alcohol)	2.50
Parsol EHS (DSM) (Ethylhexyl salicylate) (octisalate; USAN)	5.00
Parsol 1789 (DSM) (Butyl Methoxydibenzoylmethane) (Avobenzone; USAN)	2.00
Parsol 340 (DSM) (Octocrylene ; USAN)	1.70
Sweet almond oil (Gustav Heess) (Prunus amygdalus dulcis [sweet almond] oil)	2.00
Finsolv TN (Innospec) (C12-15 alkyl benzoate)	8.00
Butylated hydroxytoluene (Merck) (BHT)	0.05
dl-alpha Tocopheryl acetate (DSM) (Tocopheryl acetate)	1.00
Phenonip (Clariant) (Phenoxyethanol &	

methylparaben & ethylparaben & butylparaben & propylparaben & isobutylparaben)	0.80
Brij 72 (Uniqema) (Steareth-2)	2.00
Brij 721 (Uniqmea) (Steareth-21)	2.00
Dow Corning 345 Fluid (Dow Corning) (Cyclopentasiloxane & cyclohexasiloxane)	4.00
Phase B	
Water demineralized	45.35
(Merck) (Butylene glycol)	2.00
Glycerin (Cognis)	3.00
Edeta BD (BASF) (Disodium EDTA)	0.10
Keltrol CG-T (CP Kelco) (Xanthan gum)	0.20
Carbopol Ultrez 21 (Noveon) (Acrylates/ C10-30 alkyl acrylate crosspolymer)	0.25
Phase C	
Water demineralized	10.00
Stay-C 50 (DSM) (Sodium ascorbyl phosphate)	2.00
Niacinamide PC (DSM) (Niacinamide)	3.00
(Merck) (Sodium metabisulfite)	0.05

PROCEDURE:

Heat phase A up to 85°C; and also heat phase B up to 85°C. When both have the same temperature, add phase B to phase A while homogenizing intensively. Cool down the product to 35°C while stirring. Now add phase C and homogenize intensively again. It is generally recommended to use vacuum while producing the emulsion.

PROPERTIES:

pH—7.19; Viscosity—52800 cps (Brookfield, RV 6, 10 rpm).

Reference: DSM Nutritional Products

Brightening Lemon Under-Eye Crème

Phase A	**wt/%**
Water	q.s.
ABS White Willow Bark Extract Powder	2.00
(Salix alba [willow] bark extract) (Active Concepts) Glycerin	2.00
Dermofeel PA-3 (Kinetik Technologies) (Sodium phytate)	0.20
Ultrez 10 (Protameen) (Carbomer)	0.50

Phase B	
Procol CS-20-D (Protameen) (Cetearyl alcohol [and] ceteareth-20)	3.00
Olive oil (Arista) (Olea europaea [olive] fruit oil)	1.00
Stearic Acid (RITA Corp.) (Stearic acid)	2.00
Jojoba Oil Clear (Arista) (Simmondsia chinensis [jojoba] seed oil)	1.00
RITA SA (RITA Corp.) (Stearyl alcohol)	1.50
Dermofeel BGC (Kinetik Technologies) (Butylene glycol dicaprylate/dicaprate)	1.00
Phase C	
ACB Bamboo Bioferment (Active Concepts) (Lactobacillus/ arundinaria gigantea ferment extract)	1.00
Phase D	
ACB Lemon Peel Extract (Active Concepts) (Lactobacillus/citrus medica limonum [lemon] peel ferment extract [and] butylene glycol)	2.00
AC DermaPeptide Micro C (Active Concepts) (Saccharomyces/capsicum annum fruit ferment filtrate)	0.50
ACB Yogurt Dermal Respiratory Factor (Active Concepts) (Lactobacillus bulgaricus ferment filtrate)	5.00
Stabilized Vitamin C (Active Concepts) (Ascorbic acid)	0.50
Phase E	
Glycerin	5.00
AC Leucidal (Active Concepts) (Leconostoc kimchii [radish] root ferment filtrate)	0.50
Phase F	
Lavender and Vanilla Sage 302669 (American Flavors and Fragrances) (Fragrance)	0.25

PROCEDURE:

Phase A: Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 75°C. Sift in ABS white willow bark powder extract. Charge glycerin and dermofeel PA-3. Sift in Ultrez 10.

Phase B: In a separate container, blend ingredients and heat to 80°C. Once temperatures have been reached, add to main. Maintain temperature of 78°C and continue mixing for 15 minutes.

Phase C: Remove heat. Add at 60°C.

Phase D: Add each at 45°C. Slowly sift in stabilized vitamin C. Pre-blend and add to main.

Reference: Active Concepts

Skin-Lightening Gel

Phase A	**wt%**
Water	77.30
Natrosol 250 HHR (Hercules F) (Hydroxyethylcellulose)	1.50
Abil B 88183 (Degussa/Goldschmidt) (PEG/PPG-20/6 dimethicone)	2.00
Phase B	
Glycerin	3.00
Preservative	q.s.
Phase C	
NaOH 10% (Sodium hydroxide)	q.s. to pH 8.5
Citric acid	q.s. to pH 4-5
Phase D	
EDG Plus (Moellhausen S.p.A.) (Ethoxydiglycol)	5.0
Phase E	
Water	10.00
Alpha-Arbutin (Pentapharm)	1.00
Uvinul DS 49 (BASF) (Benzophenone-9)	0.20

PROCEDURE:

Disperse Natrosol 250 HHR in water, then add Abil B 88183. Mix phase B, then add it to phase A. Adjust the pH to approx. 8.5 with NaOH 10% to obtain a clear gel. Then adjust the pH to approx. 4.0–5.0 with citric acid. Add phase D under stirring. Add phase E and mix to obtain a homogeneous gel.

Reference: Centerchem, Inc.

Skin-Lightening Cream for Age Spots

Phase A	**wt/%**
Cremophor A6 (Ceteareth 6, stearyl alchohol)	2.50
Cremophor A25 (Ceteareth 25)	2.50

Cutina GMS V (Glyceryl stearate)	4.00
Lanette O (Cetearyl alcohol)	3.00
Stearic acid	1.00
Paraffin oil (Mineral oil)	10.00
Cetiol SN (Cetearyl isononanoate)	5.00
Vaseline white (Petrolatum white)	3.00
Abil-350 (Dimethicone)	4.00
Phase B	
Deionized water	51.10
Pentavitin (Centerchem, Pentapharm) (Saccharide isomerate)	5.00
Phase C	
Glycerin	3.00
Phenonip	0.50
Phase D	
Melfade J (Centerchem, Pentapharm) (Water, arctostaphylos uva ursi leaf extract, glycerin, magnesium ascorbyl phosphate)	5.00
Sodium metabisulfite	0.10
Phase E	
Fragrance (Chiara 0/238927)	0.30

PROCEDURE:

Heat the ingredients of fatty phase A to 70°C. Heat the ingredients of water phase B to 75°C. While stirring add phase B to phase A, cool to 50°C, homogenize and cool to 30°C. Then add phase C and stir cold. Finally, incorporate phases D and E one after the other and adjust the pH to 4.5.

Reference: Centerchem, Inc.

Age Spot Defense	**wt/%**
Deionized water	86.5
Hydrolite-5 (pentylene glycol)	5.00
Tego Cosmo C 250 (Degussa Goldschmidt PC) (1-methylhydantoine-2-imide)	0.10
Aerosil R 812 S VV 60 (Degussa AG Aerosils & Silanes) (silica silylate)	5.00
Dimethicone 20 cps	2.00

Abil B 8843 (Degussa Goldschmidt PC) (PEG-14 dimethicone)	0.50
Liquipar Oil (isobutylparaben [and] isopropylparaben [and] butylparaben)	0.15
Covagel (sodium carboxymethyl starch)	0.75

PROCEDURE:

Place water and Hydrolite-5 in a mixer; add Sericite FSE and Tego Cosmo C 250 while stirring. Disperse by homogenizing briefly. Add Aerosil R 812 S VV 60 while homogenizing and homogenize for another 10 minutes. Check for complete dispersion. Add dimethicone 20cps, Abil B 8843 and Liquipar Oil while stirring and stir for another two to three minutes. Add Covagel while stirring and stir for another 15 minutes.

Reference: Degussa Corp. Business Line Aerosil

6.2.7 CLAIMS/REGULATIONS IN USA

Skin-Lightening Claims for USA:

Definition of a skin-bleaching active ingredient, according to the U.S. Food & Drug Association (FDA), is an agent designed to bleach or otherwise lighten limited areas of hyperpigmented skin through the suppression of melanin pigment formation within skin cells.

Hydroquinone is the only skin-bleaching active ingredient recognized by the FDA. The active ingredient and its concentration in the product are as follows for OTC: Hydroquinone 1.5 to 2.0 percent. Any increased concentration over 2 percent is considered a prescription that needs to be prescribed by a doctor.

Label Claims:

Hydroquinone may be combined with any generally recognized safe and effective sunscreen active ingredient, provided that the product is labeled accordingly.

The labeling of the product must contain the established name of the drug. It should identify the product as a skin-bleaching agent, skin lightener, or skin bleaching, e.g., lotion, cream, gel, etc. The labeling of the product contains a statement of the indications that is limited to the following phrases: “For the gradual fading or lightening of dark discolorations/spots/areas/freckles/age spots.” The label should also contain the appropriate warnings identified by the FDA.

For skin-lightening products that *do not* contain Hydroquinone, which are considered natural alternatives to hydroquinone, refrain from making any of the above label claims. These products contain other skin-brightening actives like kojic acid, brightening peptides, and licorice root extract. A common term used to describe

these alternatives is "skin brightening," or you can say it helps to promote a more even skin tone. Only skin-lightening products that contain Hydroquinone can make skin-lightening claims because Hydroquinone is the only active ingredient that is actually recognized by the FDA as a skin-bleaching agent.

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2. Sarmad Al-Edresi et al., In-vitro and in-vivo evaluation of a photo-protective kojic dipalmitate loaded into nano-creams. **Asian Journal of Pharmaceutical Sciences 2010, 5 (6): 251–265.** Supplier: Caribbean Natural Products Inc.

SUNSCREENS

Author

Charles Warren

Sunscreens (for body)

Sunshine contains both UV A and UV B rays, which are harmful to the living cells in the skin. UV B radiation is the cause of sunburn ranging from cellular damage to painful skin reddening and in some cases blistering and peeling. UV A radiation is related to the degradation of the cells and destruction of collagen and elastin cells leading to premature aging of the skin with all of the attendant negative effects—wrinkling, leathery skin appearance, etc. Sunscreens are chemicals that absorb the UV radiation and prevent the radiation from reaching the cells within the skin.

In the United States, sunscreens are regulated under an OTC monograph that dictates allowable sunscreens and levels, testing requirements, claims, labeling requirements, etc. Other countries throughout the world have their own regulations related to allowable sunscreens, levels, claims, label requirements, etc., and all of these requirements are not consistent. Sunscreens, levels, and claims allowed in one country may not be allowed in another. Similarly, countries may have different required testing for claims, stability, safety, etc. Formulators should consult the appropriate regulatory/legal groups prior to initiating formulation to determine what is and is not allowed in the market(s) of interest, what testing (functionality, analytical, stability, safety, etc.) may be required and any special registrations or preapprovals needed.

Sunscreens for the body are usually found in the following forms: cream/lotion, nonpressurized pump spray, or pressurized aerosol spray. The sunscreen ingredients for these products are chosen to: a) deliver the desired SPF rating; and b) be stable in the base formula for the desired form. The combination of and concentration of the individual sunscreens are what provide the desired SPF rating. Commonly employed sunscreens are: avobenzone, octisalate, oxybenzone, homosalate, octocrylene, titanium dioxide, and zinc oxide (the latter two actually blocking the UV radiation). The allowable levels of each sunscreen are indicated in the

sunscreen monograph. Additional sunscreens are also included in the monograph. Suppliers of the various sunscreens can be very helpful in suggesting appropriate combinations and levels to achieve a particular SPF rating.

The creams and lotions are generally oil-in-water emulsions, composed of a water phase including water, glycerin, a thickening polymer (e.g., carbomer or the copolymers, such as acrylates/octylacrylamide copolymer), glycerin, $C_{12–15}$ alcohol benzoate, and so on. The oil phase can include fatty alcohols (e.g., cetyl alcohol), fatty acids (e.g., stearic acid), dimethicone, distearyldimonium chloride, cyclopentasiloxane, and so on. The emulsification system, usually incorporated in the oil phase, is commonly a mixture of ethoxylated fatty alcohols (e.g., ceteth-20), and so on. The emulsion must be aesthetically acceptable both from visual and tactile perspective and must be stable, ensuring the stability of the entire formula, sunscreens included, to pass the requirements for expiration-date testing and functional delivery of the claimed SPF. It is not unusual to try multiple emulsions and blends to develop an acceptable formula that remains stable with the sunscreens incorporated.

Both pump sprays and pressurized aerosol sprays have similar base formulas, the difference being the use of the propellants in the pressurized aerosol forms. These forms are usually hydro-alcoholic solutions of silicones (e.g., dimethicone, cyclopentasiloxane), solvents/conditioners (e.g., glycerin), and other materials to assist in the dispensing of the sunscreens and provide aesthetically pleasing skin feel and uniform spreading of the sunscreen when applied. Propellants employed in the pressurized aerosol sprays include butane, isobutane, propane, and hydrofluorocarbon 152a, for VOC regulation.

Self-Tanning Lotions

During the late 90s and after, self-tanning lotion/sprays became popular. These formulas all rely on the chemical dihydroxy acetone (1,3 –hydrox-2-propanone). DHA reacts with the proteins in the skin to form a brown color approximately 1–2 hours after application. The concentration of the DHA (2–12%) dictates the darkness of the tan. The resultant “tan” is a purely visual, cosmetic effect, providing no UV protection to the skin as does the natural formation of melanin in an unaided exposure to sun. However, the development of a “tan” is accomplished without exposure to the other harmful effects of UVA and UVB radiation.

DHA can be formulated into creams/lotions (oil-in-water) emulsions and sprays (nonpressurized and pressurized). Formulas for these forms are similar to formulas already discussed in Hand and Body Lotions and Sunscreens. The aesthetics of the cream/lotion, the skin feel during and after application, and the development of the desired shade are the issues of importance to the formulator. Incorporation of erythrulose in the formula can impart depth and richness to the final shade obtained.

ANTIPERSPIRANTS / DEODORANTS

Author

Charles Warren

The most significant difference between these two subcategories is that an A/P is an over-the-counter drug while a deodorant is purely a cosmetic product. While the formulations may be very similar in nature, the OTC drug requirements are significantly different.

Both of these product subcategories are found in similar forms. The most common forms are: stick, pressurized aerosol spray, liquid/lotion/cream, roll-on, and powder spray. These forms also come under the regulation of maximum allowable VOCs, which can vary from state to state. Prior to initiating formulation, regulatory or legal groups should be consulted regarding the applicable regulations that will impact the final formula.

Perspiration is the body's natural mechanism for cooling. Sweat, particularly in the armpit area, pools and allows bacteria to grow (warm, moist, undisturbed area). This accumulation of bacteria leads to the characteristic negative odor associated with the sweat. Deodorants are applied to allow the body to sweat but mask the unpleasant odor. Some deodorants contain materials to actually kill the bacteria while making the malodor. Antiperspirants contain materials that actually stop the sweat from exuding from the pores by creating small, gel-like plugs. These products also provide masking for any malodors that may develop from low levels of sweat that manage to exude. Because the function of the antiperspirant is to interfere or interact with a bodily function, it falls under the purveyance of an over-the-counter drug (OTC) and is bound by the specific monograph related to antiperspirants. The monograph indicates which active ingredients are allowed, what levels they may be used at, claims that may be made, etc. General OTC requirements govern stability testing required, manufacturing site registration, labeling requirements, etc. Again, of formulating OTC products, consultation with regulatory/legal groups is highly recommended.

The most common active ingredients found for the OTC products are aluminum zirconium tetrachlorohydrate (commonly used in sticks) and aluminum chlorohydrate (commonly used in sprays and roll-ons).

The base formulations for sticks generally include ingredients such as cyclopentasiloxane, stearyl alcohol, dimethicone, talc, and hydrogenated castor oil. There are a large number of other ingredients that can be used to provide different variations of stick composition. Sprays generally contain ingredients such as cyclopentasiloxane, isopropyl myristate, dimethicones, and propellants such as propane/butane/isoubutane blends and hydrofluorocarbon 152a (for VOC compliance). Lotions and creams are generally oil-in-water emulsions that contain lipoidal materials such as stearyl alcohol and ethoxylated fatty alcohols as emulsifiers.

ACNE, OILY, AND AGING SKIN PRODUCT FORMULATION ESTHETIC MANAGEMENT OF ACNE-PRONE AND CLOG-PRONE SKIN

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ABSTRACT

Acne and acne-prone skin conditions are frequently seen by estheticians in the salon, spa, and clinical settings. Clients who have acne-prone skin have often experienced conditions due to the use of some skin care products or cosmetics, which may contain ingredients that worsen or aggravate acne conditions or cause the development of comedones, which are follicular plugs of keratin and sebum. Comedogenic ingredients are agents that can cause or exacerbate follicular hyperkeratosis, a thickening of the walls of the follicle due to retention of keratinocytes, resulting in comedo formation. Still other ingredients can be inflammatory, causing irritation or inflammation in the follicles, which can result in sudden flares of papules or pustules. These are known as acnegenic reactions.

Successful management of acne-prone and clog-prone skin involves a number of approaches. These include: proper management of excess sebum and follicular exfoliation to loosen plugged follicles and prevent further plugging of the follicles, and the use of products that do not contain comedogenic or acnegenic ingredients, and have been documented non-comedogenic and non-acnegenic by independent testing laboratories using scientifically accepted protocols. Use-testing the products on individuals with acne-prone skin to ensure that the products meet the beauty needs of these individuals while not causing comedogenic or acnegenic reactions will lead marketers and formulators to new and effective products. This chapter includes some of these approaches with the intention of supporting formulators to create new and enhanced performing products in this category.

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6.5.1 INTRODUCTION

a. The Acne-Prone and Clog-Prone Skin: A Client Profile

The American Academy of Dermatology estimates that 40 to 50 million Americans are affected by acne and that 85% of the population has problems with acne at some time in their lives. While acne is often thought to primarily affect teenagers, it also affects many people in their adult years as well.

Estheticians frequently treat these consumers who have routine issues with acne breakouts, clogged pores, and frequent blemishes. The age of these acne-prone clients begins in the early teens and can easily reach into the 50s and sometimes even into the 60s. While acne as a condition is largely genetic and hormonal in nature, it is also influenced by the environment, including use of skin care and cosmetic products. Some skin care and cosmetic ingredients and products can increase the development of clogged pores as well as flares of acne blemishes.

Acne-prone and clog-prone skin can also be affected by aging skin conditions. The onset of wrinkles, discolorations, and elastosis associated with aging or photo-aging (aging symptoms associated with cumulative sun exposure) are a real concern for these consumers.

Many skin care products intended to help diminish the appearance of aging are also designed for helping dry skin that needs additional emollient to protect against dehydration and trans-epidermal water loss. Such materials also reduce the appearance of wrinkles and the rougher skin texture associated with dehydrated skin. These moisturizing products can be very helpful for dry and aging

skin. However, because of their high emollient content, they can potentially cause problems for those clients with oily and acne-prone aging skin.

Product systems for acne-prone aging skin should contain performance ingredients that address the appearance of aging, but should be carefully formulated—avoiding ingredients known to cause clogged follicles (pores), as well as flares of acne blemishes. Professional skin care practitioners tend to attract these acne-prone aging clients because they seek professional help in treating their skin, which is both acne-prone and aging. Clinical estheticians are often charged with designing specific home care and in-clinic programs to help these clients clear their acne problems while still addressing the appearance of aging skin.

6.5.2 REVIEW OF FACTORS IN ACNE DEVELOPMENT

a. Genetics

Acne is a disorder of the skin in which the sebaceous follicle becomes obstructed by a buildup of keratinized cells mixed with excessive sebum. There are several different types of acne lesions. These include noninflammatory open comedones (blackheads) and closed comedones (whiteheads), as well as inflammatory papules and pustules. In some individuals, infected follicles can eventually develop into nodules or cysts, which can result in damage to tissue causing scarring.

The tendency to develop acne and clogged pores is largely genetic. In fact, it has been estimated that causation factors in acne is 80% genetically based. Careful management of conditions caused by these genetic factors can result in clearer skin for these acne-prone individuals.

People with acne-prone skin have two specific genetic traits. The first trait is the tendency for keratinized cells with sebaceous follicles not to shed as normal keratinocytes do; rather, they "build up" on the walls inside the follicle. This condition is known as retention hyperkeratosis.

The second genetic trait that exists in acne-prone individuals is the overproduction of sebum by the sebaceous glands. The sebum "coats over" the keratinized cell buildup on the follicle walls and adds further to the thickening of the follicle wall. Sebum also can fill the follicle and harden, thereby forming a sebaceous plug. The amount of sebum produced, the size of the sebaceous glands, and the numbers of sebaceous glands are all genetically sourced factors. It has been shown that an increased amount of sebum in the skin is directly correlated with the severity of an individual's acne condition. Therefore, it is apparent that controlling excessive sebum plays a major role in managing acne-prone skin.

Propionibacterium acnes (p. acnes) are anaerobic bacteria that exist within the flora of the sebaceous follicle. When the follicles become blocked with these plugs of hyperkeratinized cells and solidified sebum, an anaerobic environment is the

result. This environment is an ideal setting for the undesirable proliferation of the p. acnes bacteria.

The sebum within the sebaceous follicles serves as the source of nourishment for p. acnes bacteria. The p. acnes bacteria produce an enzyme called lipase, which breaks triglycerides in the sebum into glycerol and fatty acids. The bacteria ingest the glycerol, and the fatty acids become a source of inflammation inside the follicle. This inflammation causes swelling in the already-occluded follicle, and can eventually cause a rupture of the follicle wall.

b. The Development of Acne Lesions

As retention hyperkeratosis occurs within the sebaceous follicle and thickens the follicle walls, the abundance of sebum coats the buildup and fills the follicle. Under these conditions, one of several types of acne lesions can develop:

A microcomedo (comedones-plural) is characterized by a "clumping" of dead cells and sebum occurring deep in a sebaceous follicle. This lesion is not visible to the naked eye and is detectable only through skin biopsies. A microcomedo is the beginning of an acne lesion. In clients with acne, there are always large numbers of unseen microcomedones for every visible lesion. It is imperative to treat these pre-lesions in order to prevent them from evolving into larger visible lesions. In the next section, we will discuss this type of treatment.

An open comedo, better known as a blackhead, is a follicle filled with dead keratinocytes and hardened sebum. The black top of an open comedo is caused by melanin pigment, likely produced as an inflammatory response. The ostium (opening; plural-ostia) of the follicle is stretched out by the debris in the follicle, hence the term open comedo. The open comedo seldom evolves into any other type of acne lesion, as the follicle is enlarged enough that oxygen is able to reach the bottom of the follicle, thereby keeping p. acnes bacteria from proliferating.

A closed comedo, sometimes called a whitehead, appears as a small bump just under the skin surface and may be viewed as a "buried" clogged follicle. Close inspection of the closed comedo reveals a very small follicle opening. In closed comedones, the follicles have filled with sebum very quickly, and the ostia are not distended.

These structures have been referred to as "time bombs" because very little oxygen can penetrate these follicles, thereby allowing p. acnes bacteria to go unchecked. The p. acnes bacteria break down the abundant sebum trapped in the follicle and produce inflammatory fatty acids. The pressure within the follicle builds until a rupture occurs in the lower walls of the follicle. This rupture causes an inflammatory immune response, with leukocytes (white blood cells) arriving at the rupture via the blood stream. Blood engulfs the follicle to allow the

leukocytes to fight the bacteria. When the blood engulfs the follicle, the lesion turns red from the blood. Red lesions are indicative of immune system involvement and are known as inflammatory acne lesions. Inflammatory acne lesions include papules—red, headless, elevated lesions, often sore to the touch due to pressure within the lesions. Papules often become pustules, which are red lesions with a pustular head. Pus is comprised primarily of dead white blood cells. Open and closed comedones do not have ruptures in the follicles and are called noninflammatory acne lesions.

c. Hormonal Factors

Hormones play an extremely strong role in acne development. Male hormones known as androgens are responsible for stimulation of the sebaceous glands, which in turn produce sebum. Androgens are produced in the testes of men, and primarily in the adrenal glands in women. Androgen hormones include testosterone, androstanedione, and dihydroepiandrosterone (DHEA). The latter two androgens can bio-convert to testosterone. Testosterone is converted to the more potent dihydrotestosterone in the skin by the enzyme 5-alpha reductase.

It is dihydrotestosterone that stimulates the sebocytes, the cells within the sebaceous gland that produce sebum. As the sebocytes produce sebum, they fill with the sebum, and then rupture, spilling the sebum into the follicle.

With the onset of puberty, androgens begin being produced in both sexes. This is the time when acne first appears, in those young individuals who are genetically predisposed. Males are more likely to have acne than females in their pubescent years. Women are much more likely to have acne as adults, often never having acne flares until they are in their early 20s, or even later. Birth control, hormonal disorders, fluctuations during the menstrual cycle, pregnancy, and other hormonal issues can cause surges of androgens that can flare acne.

At menopause, estrogen levels decrease substantially. This change can result in larger concentrations of blood androgen, which can flare acne, as well as cause hirsutism (unwanted hair growth). Because estrogen is involved in the synthesis of collagen, these hormonal changes can also result in hormonally related aging skin symptoms, characterized by worsening in skin wrinkling, increased elastosis, and also may be the cause of hyperpigmentation issues or melasma.

The body responds to stress by producing cortisol in the adrenal glands. In women, most androgen is also produced in the adrenal gland. When stress is present in women, androgens may be also overproduced along with the production of cortisol. In short, stress causes hormonal fluctuations that can potentially worsen or cause acne flares.

d. Topical and Environmental Factors

We have already discussed the genetic and hormonal factors affecting the development of acne. There are also external environmental factors that can affect acne. These may include heat and UV light (sun) exposure, skin exposure to greasy or inflammatory materials, and mechanical factors such as friction from headgear or headbands.

Decades ago, dermatologists observed that a significant percentage of acne patients who wore makeup regularly seemed to have more problems with comedones and acne flares. In the 1970s, research began on comedogenicity, the tendency of cosmetic products or ingredients to cause or worsen the development of comedones, which can lead to inflammatory acne lesions. The well-known dermatologist and researcher Albert Kligman, M.D., Ph.D. coined the term "acne cosmetica," to describe this condition.

Researchers developed testing techniques to determine the severity of potential comedogenicity in both raw ingredients and finished products.

Skin care products or cosmetics that are not specifically designed for acne-prone skin can potentially cause or worsen development of comedones and cause acne flares. Skin creams, foundations, and other products that are formulated with fatty materials such as certain oils, fatty acids, or fatty esters can cause problems in acne-prone and clog-prone clients. These fatty materials can increase hyperkeratosis and plugging of the follicles and noninflammatory comedones, which may evolve into flares of inflammatory acne lesions.

Topical agents that cause or worsen the development of comedones are referred to as comedogenic. Comedones that develop from use of comedogenic products can take weeks, if not months, to appear. Non-comedogenic products are developed avoiding known comedogenic ingredients, and then tested by an independent laboratory to confirm the product's non-comedogenic status.

Sometimes products or ingredients can cause sudden flares of acne papules and pustules, but may not necessarily cause comedones to form. These materials are known as acnegenic. In acnegenic reactions, the follicle walls become irritated and inflamed from exposure to the material, resulting in sudden flares of inflammatory acne blemishes. Acnegenic reactions occur within a few days of beginning to use a new product. Acnegenic reactions have been associated with some surfactants/emulsifiers, some fragrances or essential oils, and extreme pH levels, as well as physical/mechanical contact.

Tests to determine comedogenicity are performed using both animal (rabbit ear) and human testing. Either neat ingredients or finished products can be tested. After routine exposure to the material being tested, skin is carefully checked

for clinically visible comedo development, and follicular biopsies are taken to measure increases in thickness of the keratinized cell buildup on the follicle walls. These results are then compared to measurements taken in untreated skin in the same model. The measurements are then scored on a 0–3 or a 0–5 scale based on the increase of thickness measured. A score of 0 indicates no potential for comedogenicity, and as the score increases, so does the potential for comedogenicity. (See Table 1.)

Common Comedogenic Ingredients			
Highly Comedogenic (4-5/5 or 5/3)	**Moderately Comedogenic (3-4/5 or 2/3)**	**Mildly Comedogenic (2-3/5 or 1/3)**	**Noncomedogenic**
Acetylated Lanolin Coal Tar Cocoa Butter Coconut Oil Isopropyl Isostearate Isopropyl Linoleate Isopropyl Myristate Isopropyl Palmitate Isostearic Acid Lanolic Acid Linseed Oil Myreth 3 Myristate Myristyl Myristate Oleic Acid Oleth-3 Oleyl Alcohol Squalene	Butyl Stearate Decyl Oleate Grape Seed Oil Isostearyl Neopentanoate Lauric Acid Mink Oil Most D & C Red Pigments Myristyl Lactate Octyl Palmitate Octyldodecanol Sorbitan Oleate Soybean Oil Tocopherol	Avocado Oil Caprylic/Capric Triglycerides Corn Oil Evening Primrose Oil Glyceryl Stearate Hexylene Glycol Lanolin Lanolin Alcohol Lauryl Alcohol Mineral Oil Olive Oil Peanut Oil Sesame Oil Safflower Oil Stearic Acid Sunflower Oil (Please note that mildly comedogenic	Allantoin Behenic Acid Butylene Glycol Carbomer Castor Oil Cetyl Palmitate Cholesterol Cyclomethicone Cyclopentasiloxane Dimethicone Glycerin Iron Oxides Isopropyl Alcohol Jojoba Oil Kaolin Lecithin Octyldodecyl Stearate Octyldodecyl Stearoyl Stearate Octinoxate Octisalate Oxybenzone Panthenol

Common Comedogenic Ingredients			
Highly Comedogenic (4-5/5 or 5/3)	**Moderately Comedogenic (3-4/5 or 2/3)**	**Mildly Comedogenic (2-3/5 or 1/3)**	**Noncomedogenic**
		ingredients are generally not a problem when used in diluted concentrations. Check to see their ranking of concentration on the ingredient label.)	Petrolatum Phenyl Trimethicone Polysorbates Propylene Glycol Propylene Glycol Dicaprate/Dicaprylate SD Alcohol Sodium Hyaluronate Sodium PCA Sorbitol Soya Sterol Squalane Tridecyl Stearate Tridecyl Trimellitate Water Zinc Oxide Zinc Stearate

References 3,4,5,6,7

Tests are also conducted for follicle irritancy to determine acnegenicity potential. These tests may detect inflammation through follicle biopsies, or observation of flares of acne in actual product use studies.

In 1989, the American Academy of Dermatology held an invitational symposium on comedogenicity. This meeting resulted in a published report defining comedogenicity and acnegenicity, and reviewing accepted testing methods for determining comedogenic or acnegenic status of ingredients or products. One of the primary points of this report was the importance of testing the finished product. (1)

Because oils, waxes, and emollient fatty materials are primarily used in the formulation of a product vehicle, the ingredients of the product vehicle are most often the reason products are comedogenic. Performance-enhancing actives such

as peptides, alpha hydroxy acids, sunscreen actives, and antioxidants are rarely comedogenic. Because the aforementioned fatty materials are often used as the vehicle in skin care products, they are used in larger concentrations and make up a significant portion of the product.

Since estheticians treat the skin solely from a topical perspective, helping consumers choose appropriate non-comedogenic and non-acnegenic products, especially for use at home, the above-stated knowledge is of great value in achieving a successful outcome.

6.5.3 MANAGEMENT OF ACNE-PRONE SKIN

There are three basic concepts in topical esthetic management of acne-prone and clog-prone skin:

a. Sebum/Oiliness Management

There is a correlation between excessive sebum and the severity of acne conditions. (2) Programs for managing acne-prone skin should include appropriate cleansers to rid the skin of excessive sebum, and help control oiliness and shininess. This is primarily achieved through surfactant cleansers that help to remove excess oil. Surfactant cleansers are user-friendly and should be developed to rinse easily. Estheticians carefully select cleansers with the appropriate amount of surfactant so they effectively cleanse the skin without overdrying it, or causing irritation from barrier function disruption. Cleansing milks are often used for makeup removal, but should be designed to not leave residues of potentially comedogenic fats on the skin. Toners for acne-prone skin sometimes contain alcohols, but overuse of these can cause dryness and potential irritation that consumers find objectionable, especially when they may be using drying agents or keratolytics to clear the acne. The main functions of toners are to remove residue from the cleanser, and to adjust the pH of the skin surface to 5.5–6.2.

Consumers with oily, clogged, or acne-prone skin generally prefer extremely lightweight moisturizers and other products such as sunscreens, foundation, or serums. Avoidance of oily materials in all wearable products helps to decrease physical oiliness, as well as likely reducing potential comedogenicity.

b. Follicular Keratolytics

Follicular keratolytic nonprescription products such as those containing alpha hydroxy acids, salicylic acid, or benzoyl peroxide help to flush follicles of debris, drying up acne lesions, breaking loose existing comedones, and chemically sloughing the follicles to help prevent development of new comedones.

Choice of active agent/ingredients and product type such as gel, liquid, or lotion, will vary with the severity of the acne condition. Aging changes and sensitive skin conditions are also a consideration. Stronger concentrations of keratolytics and antibacterials such as benzoyl peroxide or salicylic acid may be needed for more severe cases. Likewise, a vehicle containing a significant amount of SD alcohol may be helpful for a younger oily skin, but a silicone vehicle would be a better choice for an older, aging, acne-prone skin because it is less likely to dehydrate skin and accentuate wrinkles or other aging distortions.

Alpha and beta hydroxy acids are a great choice for acne-prone aging skin because they not only help to clear follicles, but they also exfoliate the surface and can significantly improve the appearance of wrinkles, hyperpigmentation, and texture issues associated with photoaging.

Benzoyl peroxide gel is often used in teenagers as well as clients of any age with more inflamed and pustular acne due to its keratolytic and antibacterial properties.

c. Avoidance of Acnegenic and Comedogenic Products

As discussed previously, treating the acne-prone and clog-prone skin with products that have been tested and determined to be non-comedogenic and acnegenic is a mainstay of esthetic management of this skin type. Moisturizers, makeup products, sunscreens, and other wearable products should all be non-comedogenic and non-acnegenic.

Vehicle and emollient ingredients are the most likely to cause comedogenicity issues and must be carefully selected. The concentration of these ingredients should also be carefully determined since the skin type is already oily. Most wearable products for this skin type are in lotion or fluid form due to their low emollient or fatty material content.

Finished products for acne-prone skin should be lightweight, as clients with oilier skin generally do not like wearing heavier or oily products.

Products must be efficacious and meet the beauty needs of the client. They must be practical and of high quality and cosmetic elegance. For example, a moisturizer that is documented non-comedogenic is of no value if it is sticky, hard to apply, or pills under makeup.

A product that is easy to use will result in better compliance with the skin care program. This will include feel and practicality, ease of application, rinsability of cleansers, and the proper selection of a container that facilitates easy application of the product. Compliance with the program will result in better results and consistency in product use.

All skin care and cosmetic products in a regimen designed for acne-prone and clog-prone skin must be documented non-comedogenic in independent testing.

6.5.4 A PROGRAM APPROACH

Education of the client with acne or clogged-pore tendency is highly important to achieve success in improving this skin condition. Estheticians will thoroughly consult with clients with acne on the client's first visit to the esthetics salon or clinic. The client should be informed as to the probable causes of the condition, the reasons for using the selected product system at home, and the need to be consistent in treating the skin to achieve positive results.

It should also be emphasized that treating any skin condition, especially acne-prone skin, involves numerous factors in both causation and treatment, and that a "program approach" is the best way to successfully improve the skin's condition. This includes using the step-by-step program as designed by esthetician, avoiding the use of potentially comedogenic products outside the program, and complying with the system instructions.

a. Case Studies

Case#1 (See Figures 1 a & l b.)

Figure 1a. Before: This skin is both clogged and dehydrated. Note the clogged follicles and obvious rough textures and uneven pigmentation.

Figure b. After: After treating the skin for six weeks with a custom-designed home care program, note improvements in the clogged follicles, skin texture, hydration, clarity, and evenness of color.

(Morning)

Step 1. Skin is cleansed with a foaming rinse-off cleanser.

Step 2. A nonalcoholic toner is sprayed onto the skin and the skin is patted dry.

Step 3. Application of a 10% blended alpha and beta hydroxy acid gel-liquid

Step 4. Application of a non-comedogenic hydrating broad-spectrum sunscreen SPF-15

(Evening)

Step 1. The skin is thoroughly cleansed with non-comedogenic cleansing milk, removing all makeup.

Step 2. A nonalcoholic toner is sprayed onto the skin and the skin is patted dry.

Step 3. Application of a 10% blended alpha and beta hydroxy acid gel-liquid

Step 4. Application of a non-comedogenic hydration fluid

Cases 2–4 (See photo #2a & 2b, 3a &3b, 4a & 4b.)

Figure 2a.

Figure 2b.

Figure 3a.

Figure 3b.

Figure 4a.

Figure 4b.

All of these participants had both acne-prone and clog-prone skin with both non-inflammatory and inflammatory acne lesions. After four months using an esthetician-designed home care system, tremendous improvement was seen in skin clarity, tone, texture, and frequency and severity of all types of acne lesions.

Program for these case studies included:

(Morning)

Step 1. Skin is cleansed with a rinse-off foaming cleansing wash with 2.5% benzoyl peroxide (OTC).

Step 2. A toner is applied to the skin with a dampened cotton pledget and allowed to dry.

Step 3. Application of a 10% blended alpha and beta hydroxy acid gel-liquid

Step 4. Application of a non-comedogenic broad-spectrum sunscreen SPF-30

(Evening)

Step 1. The skin is thoroughly cleansed with non-comedogenic cleansing milk, removing all makeup.

Step 2. A toner is applied to the skin with a dampened cotton pledget and allowed to dry.

Step 3. Application of a 2.5% benzoyl peroxide gel medication (OTC) lightly to all areas (except eyes). Individual blemishes also treated with small additional drops of medication.

Step 4. Application of a non-comedogenic hydration fluid

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FACE AND BODY - MASKS / SCRUBS

Author

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As the face is an area of the body that is visible virtually all of the time, it requires formulations that function effectively and safely, leaving the consumer satisfied that the appearance and texture of the facial skin is acceptable to her/him and to the external world. Products should not create or exacerbate problems for the consumer. As facial skin is usually more sensitive than other skin on the body, formulations that would effectively clean/moisturize body skin would not be applicable to the face.

The facial skin care category is usually broken down into subcategories based on the skin type: normal, oily, sensitive, blemish control, etc. Each of these subcategories has specifically targeted products and forms. The formulator has to have a clear definition of the skin type and claims for the particular product in order to develop an effective, functional, and safe product. The products intended for blemish control or other nonstandard skin conditions are covered by OTC drug monographs and require specific active ingredients and levels, testing, manufacturing, and claims.

6.6.1 CLEANSERS/SCRUBS

As with other cleansing products, the function of facial cleansers and scrubs is to emulsify and remove excess oils and particulate from the skin. Residual makeup, flaky dry skin cells, surface bacteria, and so on must also be removed from the face. Formulas exist for normal skin, oily skin, sensitive skin, and problem (e.g., blemishes) skin.

In general, facial cleansers are an aqueous solution of surfactants and modifiers. The primary surfactants vary from soaps (e.g., ammonium stearate) to standard detergents (e.g., lauryl/laureth sulfates) to milder surfactants (e.g., taurates, sultaines). Additionally, these formulas are usually blends of surfactants such as disodium lauroamphodiacetate, sodium lauroyl (or myristoyl or cocoyl) sarcosinates, decyl or lauryl glucosides or betaines as secondary surfactants. The

formulas can also contain foam boosters and modifiers to provide pleasant skin feel while washing and pleasant feel after completion (e.g., glycerin). Formulas must contain a microbiological preservative system and usually contain claims ingredients (e.g., vitamins). Low levels of fragrance are often incorporated, but fragrance-free facial cleansers are common and often desired by consumers with self-diagnosed sensitive skin. Oil-free cleansers are also available that require selections of ingredients not chemically defined as oils.

Scrubs are cleanser formulations that also include a solid particulate to assist in the mechanical abrasion and removal of the dead skin cells at the surface. These abrasive materials include: finely ground nut shells, fatty ester beads (e.g., jojoba waxes), finely ground polyethylene, etc. The choice of the abrasive material is important to ensure that the abrasion is only at the surface of the skin and does not remove the fresher skin cells below the surface. The formulations must keep the nonsoluble abrasive particulate suspended uniformly through the product for the duration of the shelf-life of the product. Suspension of the particulate can be obtained by increasing the viscosity of the system or incorporating specific polymers to aid in suspension. The surfactant systems and auxiliary ingredients are similar to those found in the cleanser formulations previously discussed.

Cleansers/scrubs for "deep cleansing" or used in "blemish" or acne control are OTC products and regulated by the monograph related to acne control. The monograph provides the allowable materials and levels that can be used as well as the claims, testing requirements, etc. for these products. In cleansers/scrubs/wipes, the most commonly used of the allowable materials is salicylic acid. This material can be incorporated in solutions of the surfactants described above for cleansers and scrubs. The formulations must be tested to ensure that the salicylic acid is stable throughout the expiration date timing indicated on the package.

6.6.2 WIPES

Facial cleansing wipes can be formulated in a dry form (i.e., wet with water prior to use) or in pre-moistened form. Pre-moistened wipes are either surfactant free, containing glycerin, propylene glycol, or other water-soluble materials or saturated with a surfactant solution. If a surfactant solution is used it would contain surfactants similar to those used in the cleansers above (i.e., disodium lauroamphodiacetate, sodium lauroyl (or myristoyl or cocoyl) sarcosinates, decyl or lauryl glucosides or betaines) used at low levels to allow ease of saturation of the nonwoven fabric and ease of use in washing the face. Wipes for acne treatment are similar solutions with the addition of one or more of the OTC-approved acne treatment ingredients (e.g., salicylic acid).

6.6.3 MOISTURIZERS

The very general subcategories for facial moisturizers are: day creams and night creams. The main difference in these two subcategories is the type and concentration of the lipoidal materials employed in the formula—lower levels of lighter moisturizers used in day creams and higher levels of heavier moisturizers used overnight.

Day creams are generally oil-in-water emulsions composed of an aqueous phase containing humectants (e.g., glycerin), lubricants/conditioners (e.g., $C_{12–15}$ Alkyl Benzoates), polymers related to emulsion stability (e.g., carbomer), and so on, and an oil phase containing the lipoidal materials such as fatty alcohols (e.g., cetyl alcohol), mineral oil, silicones (e.g., dimethicone), lighter weight oils (e.g., isopropyl myristate), and so on. The oil phase also contains the emulsification system usually comprised of ethoxylated fatty acids (e.g., ceteth-20), higher-level PEG moieties (e.g., PEG-100 Stearate) and so on. These emulsions are formulated to be very light to the touch, spread quickly and evenly over the skin, and absorb quickly. Day creams are also available in "oil-free" formulas. These formulas usually contain lipoidal materials that are not technically oils in the lipid phase. An example would be ethylhexyl palmitate.

Many day creams also contain sunscreens to attain an SPF rating. The addition of sunscreens makes the formula an OTC drug, and all of the issues indicated in the section on sunscreens for the body are applicable. The monograph provides the allowed sunscreens and levels, testing, registrations, claims, etc.

Night creams typically contain higher levels of heavier, more efficacious occlusive agents. Applied before going to bed, the "greasiness" of these creams while sleeping is not an issue and the better occlusive materials used allow the surface skin to be hydrated from beneath. These formulations are also water-in-oil emulsions containing similar materials to the day creams but also containing higher levels of materials such as mineral oil and petrolatum. Sunscreens are rarely contained in night creams because there is no exposure to UV radiation overnight. Night creams would not be acceptable for daytime use due to the increased oiliness.

6.6.4 TREATMENTS

The area of skin treatment is very complicated. Some treatments (e.g., acne) are an OTC drug, covered by a specific monograph. Some treatments (e.g., masques) are purely cosmetic and are used on an occasional basis. In formulation in this category, the formulator needs to be careful that the desired claims do no move a purely cosmetic product into the realm of a new drug, subject to a very different set of requirements. For example, "improving the appearance of fine lines and

wrinkles" is a purely cosmetic claim, while "eliminating fine lines and wrinkles" is a drug claim. The former can be accomplished by the use of alpha-hydroxy acid treatments; the latter has been reserved for medical treatments such as dermabrasion or injection. If formulating in this category, close consultation with marketing, regulatory, and legal groups is highly advisable.

Blackhead/acne treatment masks usually fall under the OTC acne treatment monograph and are bound by all of the provisions therein. Benzoyl peroxide (2–10%) and salicylic acid are the most commonly used, allowed ingredients in this product grouping. These treatments are usually paired with a salicylic acid containing cleanser or scrub as a first step in a regimen. Benzoyl peroxide or salicylic acid is then applied to the area to be treated. All of these products work by killing the acne bacteria and loosening the upper layers or agglomerations of dead skin cells in the pores and inflamed areas.

Surface fine lines and wrinkles result from the aging process and accumulated exposure to UV radiation. Cosmetic treatment of fine lines and wrinkles involves only the uppermost layer of the stratum corneum. Deep wrinkles in the skin can only be treated medically. Retinol or retinal and retynyl derivatives are the most common active ingredients in this grouping. This grouping would also include the α- and β-hydroxyl acids (e.g., glycolic acid). These active ingredients work by removing the top layers of the stratum corneum, exposing the fresher, more compact lower layers. As these uppermost layers are removed, the appearance of fine lines and wrinkles is reduced, leaving the skin looking fresher and more uniform. Because these materials work chemically to removes the skin cells, they are often referred to as "chemical peels," differentiated from the abrasive action of the scrubs containing fine particulate materials.

Masks are usually very thick water-in-oil emulsions that are intended to be applied on the face and neck, left on for a prolonged period, and then rinsed off. Many of these formulas contain kaolin, bentonite, or other clays to provide bulk and thickness and support the position of a "deep treatment."

Another grouping in facial treatments is astringents/toners. These products are usually alcoholic or hydroalcoholic solutions meant to dry and slightly tighten the skin after cleansing. "Witch Hazel" extract is a commonly found ingredient in these type formulations.

Another category of skin treatments are the skin-lightening or skin-brightening products, used to correct darker portions of the skin (lighteners) or provide a fresher, brighter appearance to the skin. Skin lighteners are covered under an OTC monograph. The only currently approved active ingredient in the U.S. monograph is hydroquinone. Other ingredients (e.g., kojic acid) are allowed in other countries. Rules and regulations around skin lightening are in a constant

state of flux and require consultation with Regulatory/Legal groups prior to initiating formulation. Incorporation of a sunscreen ingredient is also required for these products in the U.S.

6.6.5 PERFUMES/FRAGRANCES

Actual perfume oils are created from individual fragrance ingredients by highly skilled and extensively trained formulators. Most perfumers work at fragrance suppliers and work with internal customer service/fragrance evaluators to develop fragrances specifically for client/customer groups. The cosmetic formulator will select and use these fragrances to formulate the consumer fragrance products that actually go to market.

Perfumes/colognes/eau de toilettes/etc. all have specific definitions related to the concentration of the fragrance oil in an alcoholic system. Body splashes and sprays are a lower level of fragrance oils in a hydroalcoholic system.

Fragrance ingredients can be skin sensitizers, and different fragrance oil ingredients become eliminated on a fairly regular basis. Perfumers, fragrance suppliers, and their internal safety and regulatory groups maintain current status on the regulatory and safety environment and are of great assistance to formulators who do not deal with this ever-changing environment on a regular basis.

SHAVING PREPARATIONS: PRE AND POST

Author
Charles Warren

The removal of hair from undesired areas is another subgroup off specialty skin care products. The unwanted hair can be removed mechanically (e.g., shaving) or chemically (e.g., depilation). All of the processes are aided by various products, and all of the processes leave the skin in a state that usually requires some degree of post-process conditioning.

Oftentimes, men's and women's products in the subgroups are differentiated solely by fragrance, packaging, and advertising claims. Other products are gender focused.

6.7.1 MEN'S PRODUCTS

a. Shave Creams

Men's shave creams are available in a multitude of forms: pressurized foams, pressurized self-foaming gels, creams, soaps, liquids, etc. The pressurized foams and self-foaming gels are the largest segments. The purpose of all of these products is to help hydrate the individual hairs, raising them from the surface of the skin and making them easier to cut with a razor.

Pressurized foams are generally an aqueous solution of fatty acid soaps, formed *in situ* by combining a long-chain fatty acid (usually palmitic or stearic) with an alkali (such as triethanolamine or sodium hydroxide). In addition there will be secondary surfactants (e.g., sodium lauryl or laureth sulfate), emulsifiers, such as ethoxylated fatty alcohols (e.g., laureth-23), fragrance and, if needed, skin moisturizers (e.g., glycerin). Some foams incorporate menthol for a cooling sensation during the actual shaving process and after completion.

The soap-base is then placed in a can with a propellant system (propane, butane, isobutane, etc.). By shaking immediately before use, propellant is incorporated into the aqueous soap base and, when dispensed, expands to create the thick creamy foam.

Self-foaming gels have become extremely popular with both men and women. These products are both a result of formulation and mechanical dispensing. The

gel itself is composed of a soap base, not unrelated to the based used in the creams above. Usually the gel base will include a material such as hydroxyethylcellulose to provide the gel-like form when dispensed. The gel base will also include secondary surfactants, humectants (e.g., glycerin or sorbitol), fragrances, colors, etc. The gel base is placed in a piston can and the propellant system is placed in the lower chamber to push the gel through the dispenser as the gel is dispensed. Isopentane is injected into the gel base after filling, and it is this material that causes the gel to blossom into a foam when dispensed onto the hand. This blossoming is slower than the shave cream foam generation and requires some mechanical action with the hands to develop.

As the shave creams and gels employ hydrocarbons as propellants or dispensing media, they too fall under VOC regulations. Formulating in these arenas requires consult with appropriate regulatory and legal groups to ensure necessary compliance.

Shaving lotions (nonpressurized) are generally oil-in-water emulsions that contain blends of surfactants (anionic and or nonionic), emulsifiers (e.g., ethoxylated fatty alcohols), humectants (e.g., glycerin or sorbitol), fragrances, etc. These are applied as a lotion and serve the same general purpose of hair hydration and lift for razor removal.

Simple shaving liquids can be formulated from aqueous solutions of humectants (e.g., propylene glycol or sorbitol) or a blend of lightweight oils such as isopropyl palmitate. While effective, these liquids do not enjoy the widespread popularity of the shaving creams or gels.

b. After-Shave Lotions

Both the process of shaving with a razor and the materials used (e.g., shaving cream) can leave the skin mildly abraded and/or dry. After-shave treatments (liquids and lotions) can be developed to ameliorate these effects.

The simplest after-shave is a hydroalcoholic solution of fragrance. These can also incorporate hydoalcoholic-soluble ingredients such as glycerin, sorbitol, menthol, etc., depending on the desired end product.

After-shave lotions have also become popular. In general, these are thin, oil-in-water emulsions containing an aqueous phase with water, humectants, nonionic surfactants, and other water-soluble ingredients and an oil phase containing fatty alcohols (e.g., cetyl alcohol), fatty esters (e.g., isopropypalmitate), emulsifiers (e.g., ethoxylated fatty alcohols), etc. These formulas are similar to facial moisturizers and hand and body lotions, but are balanced to be much thinner and less occlusive. Again, their purpose is to ameliorate the negative effects of shaving, immediately after the process.

6.7.2 WOMEN'S PRODUCTS

a. Shaving Products

The largest category of shaving products for women are the self-foaming shave gels, which, in base formula, are identical to the same products described for men above. They are differentiated by fragrance, concentration of conditioning agents (e.g., sorbitol), and packaging graphics. In form and function they are identical.

b. Depilatories

Depilatories are formulas that actually shear the hair off (at the skin level), chemically. They employ an alkaline solution of calcium or potassium thioglycolate—the same thioglycolate ion that is employed in permanent waving. The formulations are made alkaline (pH 9–10) with a strong alkali, such as sodium hydroxide. The base cream formulation is usually an oil-in-water emulsion consisting of a water phase containing the thioglycolate, alkali, and any other water-soluble ingredients desired, and an oil phase consisting of fatty alcohols (e.g., cetyl alcohol), oils (e.g., mineral oil, fatty esters), and the emulsification system (usually ethoxylated fatty alcohols or other fatty esters). Emulsions may also contain polymeric materials (e.g., cross-linked polymers) that are compatible with the reducing agent and the highly alkaline water phase.

Due to the reactive nature of depilatory formulations, skin irritation can be expected and, therefore, the formulator needs to include ingredients in the formula to mitigate the dryness and irritation that may result. Materials such as mineral oil, isopropyl myristate, and other fatty ester oils can be used to keep the skin soft and moisturized and reduce contact with the active ingredients.

While not chemical depilatories, other forms of depilation are available for women. These include purely mechanical hair removal (e.g., depilation mitts) to waxes, which involve placing liquefied wax on the area for depilation and removal of hardened wax (and imbedded hair) via cloth strips while affixed while wax is still in molten phase. These waxes are usually blends of rosinates (e.g., hydrogenated rosinate) and beeswax, modified with materials to control melting points and pliability.

c. Bleaches

While not technically hair-removal formulations, light bleach formulations are available for hair on the upper lip and around the mouth (e.g., chin). These products bleach the melanin in the darker hair, leaving it white or almost transparent. Because these hairs are usually fine (e.g., small diameter) the bleaching makes them less visible to the eye. The base formulas are usually oil-in-water emulsions or simple polymeric (e.g., carbomer) solutions that contain low levels (2–5%) hydrogen peroxide, as the active bleaching agent.

PIGMENTED COSMETICS

PART 6.8

COLOR COSMETICS: AN INTRODUCTION TO FORMULATION AND APPROACHES FOR MASCARAS, FOUNDATIONS AND LIPSTICKS

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ABSTRACT

The chapter will first cover the basic formulation types and important considerations in each of the categories and then take an in-depth look at the role and benefits of ingredients within these types of products and their influence on the finished formulation performance. In addition, advanced benefits such as water and wear resistances will be addressed in terms of ingredients and their influence on the formulation's behavior. Finally, the use of skin care ingredients in color cosmetics will be presented and their benefits in the formulations discussed from a formulator and from a consumer perspective.

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6.8.1 COLOR COSMETICS AND THE CONSUMER PERSPECTIVE

The use of cosmetics for beautification has been known in multiple cultures such as Egypt and China since antiquity. Color cosmetics represent a subcategory of all cosmetics, and they are defined as bringing color changes to the appearance of various parts of the consumer, whether face, neck or arms, hands, legs, and feet. This class of cosmetic products can affect the physical appearance such as eyelash shape and length, in addition to the visual perception such as darkening eyelashes and giving a more pleasing visual contrast.

Color cosmetics encompass a wide range in products targeting different physical aspects: the eye area (mascaras, eyeliners, eye shadows), facial skin (foundations, concealers, blushes), the lip area (lipsticks, lip-gloss, lip-liners), finger- and toenails (nail lacquers).

In all these areas, products often combine optical benefit (color, sparkle, diffusion) with skin appearance improvements (even toner, concealers, wrinkle fillers, and active ingredients such as firming or healthy nail growth).

6.8.2 FOUNDATIONS

Three main types of foundations can be differentiated: water based, oil based, and silicone. Foundations are intended to provide desired coverage of skin inhomogeneities as well as color shades while maintaining the skin's natural breathing.

Color shade changes can readily be adapted by slight changes in the blend of color pigments used, and consumers have a generally wide range in shades at their disposal to either maintain, enhance, or strengthen their natural skin tone. Foundations are one class of products used to counter the perceived age of a person by matching pigment composition to the type of skin imperfections of a consumer [1–3].

Skin inhomogeneities are of two types: brown/age spots and rednesses. Both need to be addressed by a foundation to provide a maximum impact on the skin's visual appearance. An example is given in Figure 26.1 below, where a sheer foundation application can be separated into both color components corresponding to respective inhomogeneities. One can clearly see that this foundation is more efficient at covering rednesses than brown spots.

Figure 26.1. Effect of a sheer foundation on the coverage of brown/age spot and redness imperfections. Natural light images of a panelist are shown on the top: before (left) and after (right) foundation application. Both images have been filtered for their brown and red color channels to visualize respective skin imperfections and the efficiency of the formulation at covering each of these.

Figure 26.2. Examples of complementary color approaches used to counter skin local imperfections, e.g., insect bites, redness spots

It is therefore to add pigments countering both types of imperfections. This can be performed by two approaches: (a) adding pigments of solid mass tone or neutral fillers such as talc or titanium dioxide to give more color-neutral coverage, or (b) countering the imperfection color by means of a complementary color pigment. The last approach is commonly used in spot concealers to hide local rednesses, in which case a greenish pigment is used. Examples are shown in Figure 26.2 below.

Complementary color concealers bring selective color corrections to local areas and are more appropriate for local corrections. The final aspect should be as close as possible to the desired skin natural tone. For larger areas of skin imperfections or inducing a skin shade change, pure high-coverage cosmetic products with the intended skin color shade are more adapted.

Thirdly, foundation layer breathability is very important when applied to skin to avoid sebum and water buildup near the interface between foundation and the skin. The natural membrane property of skin needs to be maintained and disturbed as little as possible to avoid rash and more severe side effects. Film-forming ingredients such as polymers can help here while maintaining film integrity and limit rub-off. Different regions of the world such as Europe or some Asian countries will place more importance on skin compatibility and therefore avoid "heavy" (oil-based) or high coverage foundations containing high pigment loads and thus more prone to skin occlusion effects. The following foundation illustrates the approach of a more sheer "breathable" liquid foundation in which film integrity and wear resistance were brought by an acrylate/C_{12}–C_{22} alkyl methacrylate copolymer. This polymer also ensures good adhesion to the skin.

a. Formulas

Formula 26.1. Transfer resistant liquid foundation with an SPF of 23 (*in vitro* test)

Phase	Ingredients	% W/W
A	Water	50.95
	Glycerin	2
	disodium edta	0.1
	triethanolamine	0.1
	Acrylic Acid/VP crosspolymer	0.1
B	SB700 Silica Beads	1
	BTD-401 ITT Treated TIO2	5.08
	BYO-12 ITT Treated Yellow Iron Oxide	0.48
	BRO-12 ITT Treated Red Iron Oxide	0.18
	BBO-12 ITT Treated Black Iron Oxide	0.1
	O-13 ITT Treated Sericite	1.56
C	GLYCERYL Stearate (and) Laureth-23	2.3
	Ceteareth-20	1.3
	Decyl Oleate	1.5
	Isocetyl Stearate	0.75
	Octocrylene	3
	Ethylhexyl salycilate	3
	Avobenzone	2
	Dimethicone	3
	Trimethylsiloxyphenyl Dimethicone	7
	Triacontanyl PVP	3
	VP/Eicosene Copolymer	2
D	Cyclopentasiloxane	6
E	Acrylates/C12–22 Alkylmethacrylate Copolymer	2
	Caprylyl Glycol, Phenoxyethanol	1.5
	Total:	100

PROCEDURE

1. Pre-weigh water, glycerin, and disodium EDTA and mix until clear; add TEA and mix until uniform. Sprinkle ultrathix slowly until all is in. Let mix for one hour until completely hydrated.
2. Weigh ingredients of phase B and pulverize for about ten minutes.
3. In a separate beaker, add ingredients of phase C and heat to 75–80°C while mixing.
4. Switch phase C to homomixer and add phase B. Homomix phase B+C, maintaining temperature 75–80°C
5. Heat phase A to 75–80°C and add phase B+C to A while homogenizing. Homomix for about ten minutes.
6. Begin cooling the batch to 55°C. Add phase D and continue homomixing.
7. Switch to sweep agitation and cool to 35°C. Add phase D and continue mixing to R.T.

Initial viscosity: LVT-TB @ 5rpm = 7X800 = 5,600 cps PH = 6.53

24-hour viscosity: LVT-TB @ 5rpm @ 25c: 10 × 800 = 8,000 cps PH = 6.54

Formula 26.2. Silicone Foundation

Phase	INCI	% W/W
A	Ethylhexyl Palmitate	3.00
	Isodecyl Neopentanoate	4.00
B	Cyclopentasiloxane, PEG/PPG-18/18 Dimethicone	5.00
	Lauryl PEG-9, Polydimethyl Siloxyethyl Dimethicone	2.30
	Cyclopentasiloxane, Dimethicone	5.00
	Vinyltrimethylsiloxysilicate crosspolymer Cyclopentasiloxane	6.00
C	Silica	1.00
	Titanium Dioxide (and) Isopropyl Titanium Triisostearate	6.70
	Iron Oxides (and) Isopropyl Titanium Triisostearate	0.87
	Iron Oxides (and) Isopropyl Titanium Triisostearate	0.33

Phase	INCI	% W/W
C	Iron Oxides (and) Isopropyl Titanium Triisostearate	0.14
	Mica (and) Isopropyl Titanium Triisostearate	1.10
D	Water	60.31
	Sodium Chloride	0.50
E	Propylene Glycol	3.00
	Phenoxyethanol, MethylParaben, Butyl Paraben, EthylParaben, Propylparaben	0.75
	Total:	100

PROCEDURE

1. Heat phase A to 72°C until melted and uniform. Cool to RT.
2. Add phase A to phase B at RT. Mix until uniform. *No heat*.
3. Pulverize phase C ingredients and add to phase AB mixture using high-speed mixing.
4. In a separate vessel, premix phase D ingredients homogenizing for ten minutes.
5. Premix phase E ingredients until uniform.
6. Add phase E to D (premix) and mix until uniform.
7. Add phase DE to phase ABC very slowly with paddle mixing.
8. When completely mixed, switch to homogenizer and homomix for five minutes.

Viscosity: with 0.1% oatmeal: 42 × 2000 = 84,000 cps, PH = 4.94

Formula 26.3. Radiance Skin Illuminating Foundation

Phase	Ingredients	% W/W
A	Deionized Water	36.46
	Glycerin	1.00
	Disodium EDTA	0.10
	Magnesium Aluminum Silicate	1.00
	Xanthan Gum	0.20
	Butylene Glycol	4.00
B	SB700 Silica Beads	1.00
	BTD-401 ITT Treated TIO2	6.78
	BYO-12 ITT Treated	0.87

Phase	Ingredients	% W/W
B	Yellow Iron Oxide	
	BRO-12 ITT Treated	0.33
	Red Iron Oxide	
	BBO-12 ITT Treated	0.20
	Black Iron Oxide	
	O-13 ITT Treated Sericite	1.82
C	GLYCERYL Stearate (and) Behenyl Alcohol (and) Palmitic Acid (and) Stearic Acid (and) Lecithin (and) Lauryl Alcohol (and) Myristyl Alcohol (and) Cetyl Alcohol	5.00
	C12–15 Alkyl Lactate	3.00
	Decyl Oleate	1.50
	Isocetyl Stearate	0.75
	Ethylhexyl Methoxycinnamate	7.00
	Ethylhexyl Salycilate	3.00
	Dimethicone	1.00
D	Acrylates/C12–22 Alkylmethacrylate Copolymer	1.00
E	Cyclopentasiloxane	7.50
F	Diazolidinyl Urea (and) Iodopropynyl Butylcarbamate	0.50
G	Styrene/Acrylates Copolymer	10.99
H	Mica (and) Titanium Dioxide	3.00
	Total:	100

PROCEDURE

1. Heat water to 75°C, add remaining ingredients of phase A (pre-wet gums with Butylene Glycol).
2. Mix well until gums are completely hydrated.
3. Pre-blend phase B (pulverize). Add phase B to phase C at 75°C using homomixer.
4. Add phases B & C (70–75°C) to phase A (75°C) using homomixing. Homogenize for ten minutes.
5. Add phase D with homomixing; mix until uniform.
6. Cool to 55°C with slow homomixing; add phases E & F (pre-mixed). Mix until uniform. Add phase G. Mix until uniform.

7. Switch to sweep-mixing; cool batch to room temperature with sweep-mixing. Add phase H to batch; mix until uniform.

Common ingredients of a liquid foundation:

b. Pigments

Pigments impart color to the final formulation. They can be of two types: absorption pigments of interference pigments. Absorption pigments give color by absorbing part of the visible light spectrum. Their observed color results from reflection of the nonabsorbed light spectrum. Combining absorption pigments needs to consider the reflected light colors in order to avoid overlap of reflected light wavelengths and absorbed wavelengths. This is termed "subtraction color composition." It is illustrated by a trichromatic schematic showing that blue is the result of light absorption in the yellow or that red is the resulting color of light absorption at red wavelengths. Combining red and blue absorption pigments will yield a green shade instead of magenta. A good example of this color composition system is the CMY (cyan/magenta/yellow) system widespread in the printing industry. In a subtractive system, one starts from a neutral white light spectrum and each pigment removes a specific wavelength region from the white spectrum, leaving its remaining colors [4].

Figure 26.3. RGB and CMY trichromic color composition systems.

On the contrary, additive colors are obtained when pigments are the actual source of the color shade. Interference pigments act by selective reflection of a wavelength from the visible spectrum, and as such, pigments act similar to a light source. In this system, using a red and blue light sources will give a magenta light. In the additive composition system, one starts here with an empty light spectrum (dark). Each interference pigment adds a source of light to this dark background, which color relates to the reflective interference wavelength.

An illustration of the differences is given in Figure 26.4.

Figure 26.4. Additive (top) and subtractive (bottom) color composition systems

Fillers

The purpose of fillers is to opacify formulations to adjust the degree of transparency from a high-coverage foundation to a sheer foundation without affecting the color shade of a formulation. Fillers can lighten a formulation (higher concentrations above 5%) and they can induce a thickening of formulations. The most common fillers are titanium dioxides (white), talc (used less now), and silica particles. The particle size of fillers also influences the optical appearance of the product on skin.

Finer particles will give a softer appearance than larger particles. Finally, silicon dioxide particles are sometimes used to bring a slight light-scattering effect in foundations applied to skin, resulting in a soft-focus effect. They are often termed "light diffusion particles" and are generally coated for secondary purposes such as more hydrophilic/hydrophobic characteristics. The coating can also affect the optical refractive index and enhance the light-diffusion effect. In addition, the coating generally prevents any sensitization risks when applied to sensitive skin.

Surfactants

These ingredients are often dependent on the type and concentrations of pigments as well as fillers. Their role is to disperse and maintain a homogenous dispersion in the liquid form. Common surfactants include stearates, dimethicones, oleates, and polyoxyethylene ethers (nonionic).

Thickeners

Thickeners serve to bring more texture to the formulation while also reducing the risk of pigment sedimentation inside a bottle. They are used in low amounts when needed, since the pigments and fillers will bring a formulation quite high in viscosity.

Film formers

Oils and silicones can be used to enhance spreadability on skin. Dimethicones are typical for spreadability while giving a dryer skin feel post-application. These compounds will also help skin adhesion of the formulation during its first minute following application. A good compromise needs to be reached in terms of time of setting of the foundation on the skin and time allowed for a consumer to evenly spread a formulation over the desired area.

Polymers

Polymers are widely used in foundations for multiple reasons. They will enhance film adhesion on skin (e.g., acrylate/C_{12}–C_{22} alkylmethacrylate copolymer). Polymers also bring stability to the foundation film by maintaining pigments evenly dispersed while providing a degree of porosity and breathability (e.g., triacontanyl PVP). As mentioned before, film breathability is very important for skin to avoid occlusion effects. Several polymers are often combined to ensure adhesion on skin and good retention of pigments over time of wear. This last benefit is responsible for wear resistance of a foundation to avoid any rub-off or staining of consumer clothes during the daily wear time of a foundation. Lastly, polymers can also bring a mattifying effect via sebum absorption/trapping and thus prevent the development of skin shine and oily appearance.

Additional ingredients

In addition to water, pH adjusters, preservatives, and antioxidants fall in this category to address the stability of the final formulation and its good compatibility with skin. Alcohols can also be used to give a slightly faster drytime.

6.8.3 LIPSTICKS AND LIP-GLOSSES

The lip area of a person has been one of the attraction features of beauty in many cultures around the world [5]. Properties such as fullness, shape, definition, and volume have all been associated with desire to enhance beauty and attractiveness. An example of extreme contrast enhancement can be seen for geishas in the Japanese culture, in which case bright red lip color is commonly used on a near-white skin background makeup. It is therefore natural for consumers to have a high interest in products enhancing the lips. While in the past, lipsticks have mainly focused on bringing decorative benefits (color shade, gloss), they now also bring functional (sun protection) and skincare (moisturizing, plumping) benefits to the lip skin.

The importance of lipsticks and lip-gloss in modern culture can be seen by the wide selection in color shades available in commerce. An interesting point is the large proportion of color shade names associated with fruits or senses such as berry, pomegranate, plum, luscious, passion, sensual, all of which use the mentally associated image of fresh/richness with an enhanced feel of beauty.

a. Formulas

Formula 26.4. Example of transfer-resistant lipstick formulation

Phase	Ingredients	% W/W
A	Ozokerite	10.50
	Polyethylene	5.00
	Octyldodecyl Stearate	13.00
	Diisopropyl Adipate	2.00
	Isocetyl Stearoyl Stearate	14.70
	Shea Butter	4.00
	$C_{12–15}$ Alkyl Lactate	11.00
	Hydrogenated Polyisobutene	5.00
	VP/Eicosene Copolymer	5.00
	VP/Hexadecene Copolymer	5.00
	Tocopheryl Acetate	0.20

Phase	Ingredients	% W/W
A	Retynyl Palmitate	0.10
B	Isopropylparaben (and) Isobutylparaben (and) Butylparaben	0.40
C	Red 7 Lake (C6507 D&C Red #7 Calcium Lake)	1.00
	Iron Oxides (C33-8073 Yellow Iron Oxide)	1.00
	Titanium Dioxide (White)	1.20
	Iron Oxides (C33-5198 Black Iron Oxide)	0.35
	Isocetyl Stearoyl Stearate	3.45
	Tricontanyl PVP	0.10
D	Mica	13.00
	Mica (and) Iron Oxides (and) Titanium Dioxide	4.00
	Total:	100

Procedure

1. Prepare phase C color grind using roller mill (Melt Ganex® WP-660; add to color mix before milling)
2. Melt phase A ingredients. Heat to 90–95°C; mix until uniform.
3. Cool batch to 82–85°C.
4. Add phase B; mix until smooth.
5. Add phase C color grind; mix approximately 30 min.
6. Add phase D, avoid aerating batch; mix until uniform.
7. Pour samples at 82–85°C.

Formula 26.5. Example of lip-gloss formulation.

Phase	Ingredients	% W/W
A	VP/Hexadecene Copolymer	20.30
	Polybutene	19.00
	Isostearyl Neopentanoate	8.20
	Ethylhexyl Palmitate	8.00
	Octyldodecyl Stearate	8.00

Phase	Ingredients	% W/W
A	Myristyl Lactate	9.00
	Tribehenin	3.00
	Beeswax	3.00
	Tocopheryl Acetate	0.10
B	Isopropylparaben (and) Isobutylparaben (and) Butylparaben	0.40
	Mica	11.00
	Mica (and) Titanium Dioxide (and) Red 7 Lake (and) Hydrogenated Polyisobutene (and) Palmitic Acid (Cellini Red)	2.00
C	Mica (and) Iron Oxides (and) Titanium Dioxide (Gemtone Tan Opal)	8.00
	Total:	100

PROCEDURE

1. Melt phase A ingredients; heat to 85–90°C until melted and uniform.
2. Cool batch to 80°C.
3. Add phase B; mix until uniform.
4. Add phase C; avoid aerating batch; mix until uniform.
5. Pour samples at 80°C.

b. Color

First of all, lipsticks are intended to enhance or bring color to lips. This is generally achieved by adding colorants to the product, which is applied to lips as a thin coating. Most shades found nowadays contain some red dye and range from pale pink/orange to bright red and to darker tones of red, brown, or purple. Some unusual lipstick colors such as green, blue, or black can also be found. The color imparts a higher visibility to lips and shades are also considered to reflect the occasional environment of the consumer. Color has also been found in the form of skin dyes such as eosin and bromofluoresceins. These dyes penetrate slightly into the first layer of lip skin, which makes them more wear resistant. As a consequence, they also can induce some sensitization, even irritation or allergic reactions in consumers.

The color of lipsticks can be modulated by a degree of transparency. Lipsticks can thus range from “natural” near colorless or slight coloring to high coverage

in their applied layer on lips. Titanium dioxides are sometimes used to increase coverage, but they also introduce a whitening effect on the resulting lip shade due to their white initial color. These particles also need special attention due to their mattifying effect on a formulation, which can counter an expected gloss or high shine expectation. Color is imparted by absorbing dye pigments such as D&C dyes. Additional optical effects can be introduced by means of interference pigments (based on silica, mica, borosilicates) reflecting selective color wavelengths. The size of these pigments plays an important role, since larger particles give a glitter effect but finer particles can bring a silky appearance to a lip product. Interference pigments (positive color component system) can often contrast with darker base colors of a lipstick. For example, fine golden interference pigments, bring a warm tone to a darker red/ochre lipstick. Typical colorant concentrations range from 4 to 20%.

c. Gloss ingredients

Lipsticks and lip-glosses are often intended to bring high gloss and shine when applied on lips. This is generally obtained by use of oils, esters, and silicones helping to form a homogeneous coating on lips and enhancing the light reflection by increasing the refractive index of the lipstick film. These ingredients also help the cohesion of a formulation, especially when using fillers and larger pigments that disrupt the cohesion of a lipstick during molding.

d. Oils

Many oils can be used in lipsticks, such as castor oil, mineral oils, and hydrogenated vegetable oils. Their viscosity ranges from liquid to near wax-like, and they play a role of dispersant for colorants as well as cohesion enhancer in lipsticks. Excessive amounts can lead to heavy feel, rancidity, or too much payoff when lipsticks are being applied by a consumer. Many oils need a co-solvent such as fatty alcohols to ensure their good dissolution in a formulation. Typical oil concentrations range from 6 to 10%.

e. Waxes

The most commonly used waxes in lipsticks are beeswax and Carnauba wax. Generally, waxes are used to increase the viscosity of a lipstick and balance the effects of oils and esters. Waxes are harder ingredients and they raise the melting point of a formulation. This control in the melting temperature of the lipstick also controls the payoff of a lipstick, which is the amount of product transferred from the lipstick to the lips of a consumer. Payoff needs to be adjusted based on the amount of colorants and the expected degree of coverage. Excessive use of waxes can lead to tackiness, graininess, and unpleasant application feel. Typical wax concentrations range from 8 to 18%.

f. Solvents

Alcohols and esters are generally used as solvents to disperse color pigments and waxes. Many esters are available for this: linear and branched alkyl esters, and from stearates (e.g., glyceryl-, iso-, hydroxyl-stearates) to palmitates, lanolin alcohols, caprylates, and others. Typical solvent concentrations range from 3 to 10%.

g. Silicones

These ingredients can range from liquids to wax-like consistency. They bring a lighter feel to the lipstick and decrease the greasy/heavy perception for consumers. Polar esters are often used as co-solvent with silicones. Typical concentrations range from 1 to 5%.

h. Polymers

Polymers are used to impart film-forming properties to lipsticks as well as to ensure the global film cohesion once applied onto lips. Another critical benefit of polymers is wear resistance. Usually, a large-scale polymer is used for film adhesion and flexibility to follow the movements of the lips while a finer-branched polymer serves to create the three-dimensional local network inside the film and traps colorant dyes, preventing their release on textiles or drinking containers (glass, ceramics). Polymers such as an acrylate/$C_{12–22}$ alkylmethacrylate copolymers also provide good adhesion of the lipstick during application on lips. Finally, polymers can contribute to gloss in a lipstick by improving the quality of the film on lips and/or by increasing the refractive index of the lipstick [6].

Typical polymer concentrations range from 0.2 to 2%.

i. Additional ingredients

Sunscreens are often included in lipstick products to bring a protection against ultraviolet light. Sun-protection factors range typically from 8 to 15. These ingredients are generally oils, so higher amounts of waxes are used to counter their lowering of viscosity. A good dispersion of sunscreen filters can be obtained by alcohol co-solvents.

Moisturizing ingredients such as glycerol are sometimes used in lipsticks. Adding a moisturizer helps maintain the skin condition but also the fullness of lips and thus more attractiveness.

Antioxidants are sometimes used in lipsticks at lower concentrations (0.1–1%) and intended to remove ultraviolet-induced radicals inside the skin of lips.

Numerous fragrances can be used in lipsticks to give a fruity smell/taste to products or sometimes to mask heavier greasy ingredients of less appealing perception.

6.8.4 MASCARAS

The formulation of mascaras has been a very active field over the last 20 years as formulations are requested to improve very different aspects of eye lashes: color, physical characteristics such as curling and thickening, and daily wear resistance such as water and sweat resistance [7]. These effects can be almost instantly experienced and judged by the consumer, since mascara has a direct impact on visual perception. One must also keep in mind when formulating, that mascara formulations need to be adjusted to the applicator used in a final product, especially in terms of viscosity and application fluidity versus solvent evaporation.

Mascara formulations are found of two main types: water based and organic-solvent based. Both types can give smooth and homogenous applications on eyelashes. Also, a very important aspect of mascaras is that formulations need to be matched with applicator brushes. This point is illustrated by the numerous patents existing on mascara applicator brushes [8–11].

a. Basic formulation

A general composition breakdown of mascaras is given in Table 26.1 as a base of discussion to follow.

Table 26.1 Typical mascara ingredients and ranges of commonly used concentrations

Ingredient	Concentration (% wt.)	Examples
Color	1–12%	iron oxide, mica pigments
Emulsifier	3–10%	myristoyl penta/hexapeptides
Emollients	25–50%	oils (jojoba, wheat, argan)
Texture modifiers	5–20%	hydroxycellulose, waxes
Preservative	0–1.5%	phenoxyethanol, propylparaben
Film formers	1–8%	silicones, dimethicone
Solvent	Q.S.	water, petroleum distillates

The primary purpose of a mascara is to give visual impression of long dark eyelashes to enhance the beauty of the eye area. Therefore, a first component will be the color pigments. For a best contrast of the visibility of eyelashes against the background skin and eye, black is the most common primary pigment and is given by black iron oxide particles. Titanium dioxide is often added to disperse black pigments and give a deeper black perception The main color can be modified as a mass-tone color by addition of light-absorption pigments

such as browns, reds, and blue pigments (e.g., mica-based pigments). Two types of light-effect pigments can be used: light-absorbing (dye pigments) and light-reflecting/interfering (multicoated interference pigments). The second type is generally used in mascaras for its better perception due to selective color of light being reflected but also due to the lower toxicity of mica and its outer coating when in contact with eyelid skin and the humid mucosal membrane hosting lash roots.

Stronger mascara colors are also present on the market and represent a smaller fraction of consumer uses.

Emulsifiers are then added to create a stable dispersion of these pigments.

Thickeners such as hydroxyethylcellulose serve to thicken this suspension and can add volume to the mascara coating applied to the eyelash. They have a wide compatibility with most formulation bases, and while being commonly used, they also increase viscosity so that the system needs to be a little more fluidized (e.g., oils or emollients) for a smooth flow of mascara application.

Oils are commonly present to bring both better adhesion on the keratin of the lash keratin surface but also to fluidize the formulation to regularize flow and homogeneity during application. Oils also bring nourishment to the lash fiber by slightly penetrating into its cortex. Thickeners, such as waxes, have an opposite effect on the formulation by increasing viscosity but also induce a slight solidification of the mascara film during drying on eyelashes as the solvent evaporates. For this reason, it is generally recommended to move the applicator brush up and down in mascara tubes prior to use. The effect is to coat the locality, refluidize the formulation, and have a fresh layer of formulation on the brush when used. The same is recommended when using mascara consecutively.

b. Advanced ingredients

Specialty ingredients can be included to give a better texture or regularity when applied with a brush as well as additional benefits: adhesion promoters, formulation stabilizers, thickeners, polymers, color pigments, active ingredients, and fragrance. These ingredients are more and more frequently added to enhance the mascara film formation and stability on the eyelash and to bring new benefits to the final formula, such as water and wear resistance.

A detailed water-based mascara formulation is shown in Formula 26.6 below. This formulation has been shown to bring lash curling and water resistance but also a modest increase in lash thickness (volumizing effect).

Formula 26.6. Volume curling mascara with high water resistance

Phase	Ingredients	% (wt)
A	D.I. WATER	45.42
	Disodium EDTA (Versene Na2 Crystal)	0.1
	Triethanolamine (TEA 99%)	0.38
	Acrylic acid/VP Crosspolymer (Ultrathix p-100)	0.5
	Xantham gum (Keltrol T)	0.3
B	Water	10
	Polyninyl pyrrolidone (PVP-K30)	1
C	Iron oxide (Unipure black LC989)	12
D	Glyceyl stearate and Laureth-23 (CERASYNT 945)	3
	$C_{18–36}$ acid triglyceride (Synchrowax HGLC)	5
	White beeswax	5
	Stearic acid	2
	Copernicia cerifera (Carnauba) wax SP63	3
	Euphorbia cerifera (Candelilla) wax	1
	PEG-20 stearate (Lipo PEG10-S)	2
	Tricontanyl PVP (Ganex WP-660)	0.5
E	Triethanolamine (TEA 99%)	1
F	Water	5
	Allantoin	0.1
	Vitamin E (Tocopheryl acetate)	0.2
	Acrylate/alkylmethacrylate copolymer (Allianz OPT)	1
G	Phenoxyethanol, octanediol (optiphen)	1.5
	Total:	100

Procedure

1. Pre-weigh water and disodium EDTA and mix until clear; add TEA and mix until clear. Sprinkle Ultrathix slowly until all in.
2. Transfer to homomixer and slowly add xantham gum.
3. Premix ingredients of phase B and add to phase A; begin heating to 75–80°C

4. Weigh ingredients of phase C and pulverize using blender for about five minutes. Then, add to previous mixture and continue homogenizing.
5. Separately, add ingredients of phase D and heat to 75–80°C while mixing.
6. Add phase D to phases ABC while homogenizing for about ten minutes.
7. Add TEA (phase E) and continue homogenizing for ten minutes. Begin cooling the mixture to 55°C.
8. Switch to sweep agitation. Add phases F and G and cool to 25–30°C.

Adhesion promoters such as silicones improve film compatibility of the mascara with the keratin of the eyelash cuticles. Adhesives help to prevent mascara flaking under daily stresses such as finger contact or bending or inter-eyelash friction. Stabilizers are used as homogenizers in the mascara. Thickeners such as hydroxyl celluloses provide richer coating applications on the lash and can thus bring volumizing properties to the finished product. Mechanical benefits such as anti-flaking or water resistance can be given to a formulation via polymers such as Allianz OPT or Ganex line. They provide two benefits to the mascara film applied on the eyelash: trapping of dyes and global film integrity. They give hold to the mascara dye particles by forming a three-dimensional network around them and throughout the film and thus prevent their release when lashes come in contact with waterous media such as tears. In addition, polymers give film integrity to the mascara while still adapting to the bending stress of eyelashes during daily activities. This prevents mascara breakage with risk of dye leakage or potential flaking of the film.

Figure 26.5. Effect of 1% acrylate/C_{12}–C_{22} alkyl methacrylate copolymer on water resistance: Test 1 (Top): Bending under wet conditions and Test 2 (Bottom):

particles released after 60 seconds of eyelash immersion in deionized water. (1) Mascara base without polymer; (2) mascara with 1% acrylate/C_{12}–C_{22} alkyl methacrylate copolymer. In all cases, mascara was applied on eyelashes and given 20 minutes drytime at air prior to tests.

Much work is devoted by finished product manufacturers to prescreen and test polymers and polymer mixtures for they glassy transition temperature, i.e., the temperature at which the material transitions from liquid to an amorphous glass-like state. A polymer mixture with a transition temperature near 68°F (20°C) would provide stability and luster to the applied mascara. The same polymers can also bring curling to a mascara (Figure 26.6)

Figure 26.6. Side view of eyelashes before and after two-stroke application of formulation 1. Artificial eyelashes made of natural untreated Caucasian hair have been used here, mounted in a linear holder to control base hair alignment. The increase in curvature can be clearly seen on the right picture.

A multidimensional approach to polymer benefits in mascaras can serve the formulator to choose appropriate ingredients faster. A large number of commercial polymers have been evaluated in the mascara base at 1% active concentration (Formula 26.5). Four properties of mascaras have been evaluated as described above: volume, curling, water resistance, and shine. Results are graphed together on a spider chart to show best combinations of polymers providing multiple benefits to consumers. For each property, scales were normalized with the top performer in each category. For comparison, the six top-selling commercial mascaras were also tested. While most commercial products show very good performance in one property, only two of them showed two benefits at a high level. In comparison, 1% triacontanyl PVP showed high water resistance as well as curling and some degree of shine. In a 0.5% combination of triacontanyl PVP with 0.5% AOPT, all three properties were brought up to 80% of the highest performances.

Figure 26.7. Global overview of mascara performances in each of four properties: volume, curling, water resistance, and shine (calculated from ten line profiles obtained by macrophotography followed by shine calculated by means of the Reich-Robbins formula)

Color pigments, such as micas, can bring highlights and color shades to the applied mascara. Finally, active ingredients can be incorporated inside mascaras or mascara pretreatments for various purposes such as reinforcing thin lashes or stimulating lash growth. Growth-stimulating actives are intended to penetrate into the lash follicle and act on the cell keratinization and differentiation.

6.8.5 SKINCARE ACTIVES IN FOUNDATIONS AND LIPSTICKS

The most common skin-active ingredients found in foundations and lipsticks are sunscreens. Numerous examples can be found where both types of products also claim sunscreen protection. Daily wear products now generally incorporate sunscreen filters against UVB and UVA. Very often, filters will be minerals such as titanium dioxide and zinc oxide, since organic filters tend to bind and react with oxide pigments. Another concern of organic filters is their absorption by pigments, which potentially leads to a decrease in SPF value. Mineral sunscreens have the advantage of dispersing well within the formulation and the application on skin while behaving neutral towards pigments. They are often used to achieve SPF values of 15–20 with concentrations of 10% w.

An additional skin care benefit brought to those products is moisturization. Commonly, glycerol is used in those cases but it also brings a slight drop in

viscosity of the bulk formula as well as an oilier appearance when applied onto a person. This oily perception can be countered in foundations by either a slight increase in pigment fillers or by use of a polymer to give the product a finer visual texture instead of a local shine. However, it is less of a problem in lipsticks, since shine is an expected attribute. Lipsticks use a number of additional ingredients for moisturization and conditioning from oils (macademia, olive, castor seed, lavender, shea butter) to more complex natural ingredients (silk complex).

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ABBREVIATIONS

AOPT, Acrylates/C12-22 Alkylmethacrylate copolymer; ADS, Vinyl Caprolactam/VP/Dimethylaminoethyl Methacrylate Copolymer; DC79, acrylates/acrylamide copolymer; A4R-450, PPG-17/IPDI/DMPA copolymer.

HAI R CARE

PART 6.9

FORMULATING WISDOM CATEGORY BY CATEGORY

Hair's original purpose was to provide body coverage and warmth—the first line of the body's defense against the environment. Hair has evolved to provide minimal protective function but becomes highly important in the areas of appearance and hygiene.

Composed of nonbiologically active protein, the hair has many unique and important properties—flexibility, extendability, insolubility (to normally experienced solvents), and adaptability (to changes in length, style, color, etc.). Hair grows at approximately 0.25–0.33 inches per month. Unlike living skin, hair cannot repair itself—whatever damage occurs remains until the hair is lost naturally by shedding, is cut, or breaks off.

The products developed for hair care serve multiple purposes—cleaning, conditioning, treating, coloring, etc. The following is a description of the categories involved in hair care.

SHAMPOOS
INGREDIENTS, FORMULATION AND EFFICACY EVALUATION

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ABSTRACT

When formulating a successful shampoo, there are many components and interactions to take into consideration. This chapter will begin with Section I by introducing some of the basic ingredients used in the formulation of shampoos as well as what differentiates some of the types of shampoos on the market. The subsequent section will discuss the actual cleansing mechanism of shampoos and the effects that shampoos and surfactants have on the hair. Section III introduces a few of the factors used to evaluate a shampoo formulation as well as some of the key attributes that need to be taken into consideration when evaluating a shampoo's performance. The final section wraps up with a brief look into the future trends of the shampoo industry.

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INTRODUCTION

It would seem that modern day shampoos have come a long way from the first commercial shampoos that were introduced in the early 20th century, and in many respects, they have. Modern formulations have become more personalized, less harsh, and tend to follow certain consumer-driven trends. On the other hand, shampoos cannot stray far from their origination because design designates them to do one primary function: clean the hair and scalp.

When formulating a successful shampoo, there are many components and interactions to take into consideration. The surfactant system may seem to be the most important part of the formulation due to the primary functionality of a shampoo. It is very important to use a carefully chosen blend of surfactants that will deliver the appropriate detergency, lather, and wetability to a formula, but this should not delineate from the importance of the other ingredients in a shampoo for-

mulation. Consumers have certain expectations from their shampoos. They expect a certain viscosity and rheology as well as other benefits such as good combability, shine, and manageability.

In the following sections, this chapter will outline some of the basic ingredients used to formulate different types of shampoos as well as examples of starting formulas. A detailed explanation of how these ingredients clean the hair and scalp as well as the effect that they can have on hair will be discussed. The final sections focus on evaluation components for shampoo formulas as well as for performance attributes and end with a brief look into the future of the shampoo industry.

SECTION I: TYPICAL SHAMPOO INGREDIENTS

6.10.1 SURFACTANTS

The surfactant component of a formula provides many basic functions to a shampoo. Not only do these ingredients provide the cleansing mechanism for the shampoo, but surfactants also provide the foaming, contribute to the formulation rheology, and also play a large part in the safety of the shampoo. Foaming properties have to be adequate, both in quantity and quality, for the consumer to consider a shampoo to be adequate. Foam does not equal quality to a formulator, but to a consumer, copious amounts of rich foam are a basic expectation for a good shampoo.

Shampoo formulations usually consist of primary as well as secondary surfactants. The primary surfactants are responsible for the main cleansing mechanism in the formula as well as the foaming and some of the viscosity control. Secondary surfactants are usually gentler than the primary surfactants but by themselves do not have the best foaming or cleansing properties. These surfactants are usually added to help boost the foaming and detergency as well as reduce irritation caused by many primary surfactants. Most primary surfactants in shampoos (not including baby shampoos or special formulations) are anionic or negatively charged molecules, while most secondary surfactants are nonionic (have no charge), amphoteric (can be positive or negative depending on pH), or cationic (positively charged).

Anionic Surfactants

Alkyl and alkyl either sulfates are the most commonly used primary surfactants in the majority of shampoos on the market. These surfactants are excellent cleansers and foamers. They are cost effective and can easily be thickened with sodium chloride, which makes them a popular choice for many shampoo formulations. Most mainstream formulations contain some combination of alkyl and/or alkyl ether sulfate with sodium lauryl sulfate (SLS), sodium laureth sulfate (SLES), ammonium lauryl sulfate (ALS), and ammonium laureth sulfate (ALES) being the most prevalent.

The lauryl and laureth sulfates are produced by a sulfation reaction using mostly C_{12} fatty alcohols. This chain length is chosen to maximize the foaming and wetting traits of the final surfactant. Various mixes of C_{12}–C_{14} chain lengths are commonly used but will vary depending on the manufacturer [1].

As stated above, SLS and ALS are excellent foamers and cleansers, but many of the 12 carbon chain-length fatty alcohols can cause irritation to the scalp and eyes and have some solubility issues. This is reduced by the reaction of the fatty alcohols with ethylene oxide (EO) prior to the sulfation process, producing SLES as well as ALES. The higher the degree of ethoxylation, the more soluble the compound as well as the higher the reduction of irritation is seen. SLES is usually reacted with an average of two moles of EO while ALES is commonly ethoxylated to an average three moles of EO.

The lauryl and laureth sulfates are sometimes used by themselves but are most commonly used in combination with each other. Lauryl sulfates by themselves produce a creamier foam with smaller bubbles while laureth sulfates produce better flash foam. When used in combination, they complement each other. When deciding between the sodium and ammonium salts, ALS/ALES produce a slightly higher amount of foam and can be easier to thicken, but care has to be taken when using these surfactants because they can release free ammonia at higher pH values.

Although most mainstream shampoos will have some sort of alkyl or alkyl ether sulfate combination as their primary surfactant base, alpha olefin sulfonates (AOS) rank second in use [2]. The alpha olefin sulfonates are usually 14 to16 carbons in length and are comparable to SLS and SLES in foaming capacity (in the presence of sebum). AOS is easily solubilized and contains very little color or odor. It is stable over a wide pH range including lower pH systems, which is a benefit over the alkyl and alkyl ether sulfates. In lower pH formulations, alkyl and alkyl ether sulfates can be hydrolyzed. AOS is usually not used in premium shampoos because it has been reported to leave a harsher feel than the lauryl or lauryl sulfates and is also harder to thicken. Due to AOS surfactants containing a sulfonate group instead of a sulfate group, these surfactants can be formulated into sulfate-free formulas.

Other Anionic Surfactants

Sulfosuccinates. Sulfosuccinates contain both a carboxylate and a sulfonate group and are often used in combination with alkyl and alkyl ether sulfates in mild shampoo formulations or as part of a surfactant combination in baby shampoo formulas. The addition of a sulfosuccinate surfactant helps reduce eye and skin irritation, improves the lather, and contributes some conditioning benefits.

N-Acyl Methyltaurates. N-acyl methyltaurates (AMT) are the fatty amides of methyltaurines. They are noted for their low irritancy and are claimed to protect hair against cuticle damage. This class of surfactants finds limited usage in shampoos because of poor solubility and foaming performance, especially in hard water [3].

Sarcosinates. N-acyl sarcosinates are produced by reacting a fatty acyl chloride with N-methylglycine. These secondary surfactants do not lather well and tend to form precipitates in hard water, but are extremely mild. They can be used in combination with other anionic surfactants to reduce eye and skin irritation as well as provide some conditioning properties. The two most common forms of this surfactant that are used in shampoos is cocoyl sarcosinate and sodium lauryl sarcosinate [4].

Isethionates. Acyl Isethionates are condensation products of fatty acid chlorides and the sodium salt of 2-hydroxyethanesulfonic acid. They are very mild to the scalp and hair and produce a creamy, soap-like lather in both soft and hard water. They hydrolyze easily at low and high pHs. They have limited solubility at room temperature and so are not used in clear formulations [3].

N-acyl polypeptide condensates (protein derivatives). This class of surfactants is produced by the condensation of fatty acid chlorides and low-molecular-weight protein hydrolysates. Most often the cation is either potassium or triethanolamine, and the acyl group is a cocoyl moiety [3]. These surfactants have poor flash-foaming properties but produce a creamy, tight lather. They are stable in hard water, very mild to the eyes and skin, and have some conditioning attributes, but can be susceptible to bacteria [5].

Nonionic Surfactants

Although the detergency of nonionic surfactants is equal to, and in many instances superior to, that of anionic surfactants, nonionics are generally not used as primary surfactants in shampoos. This is due to inferior foaming characteristics, which result from their large surface area per molecule and the lack of charge on the surface films in nonionic foams [1]. However, nonionics are used extensively in a secondary capacity as foam modifiers, viscosity builders, emulsifiers, solubilizing aids, and in some case, conditioning agents.

Alkanolamides

One of the most common classes of nonionic surfactants is the fatty alkanolamides. They are produced by the condensation of a mixture of a primary or secondary alkanolamine with a fatty acid or derivative. The ratio of the amine to fatty acid yields differing products. The 1:1 condensates, referred to as “superamides,” are

much more prevalent in shampoo formulations. Products derived from different fatty derivatives and different condensation ratios carry the same name, and wide variations in the chemical composition and performance of Alkanolamides from different manufacturers is not unexpected [3].

Alkanolamides are used for foam stabilization as well as viscosity enhancement, but care has to be used when choosing the alkanolamide to formulate with. Many formulators shy away from formulating with cocamide DEA or diethanolamine due to the potential for free amine production, which can form carcinogenic N-nitosamines. Many formulators will use Cocamide MEA (monoethanolamine), which is the monomer form. The foam stability is comparable to that of the DEA form, but the MEA form will build slightly higher viscosity.

Amine Oxides

Another widely used class of nonionic surfactant, the amine oxides are prepared by the oxidation of tertiary amines with hydrogen peroxide. The N-O bond is highly polarized, and at below-neutral pHs, amine oxides become protonated, which allows them to take on cationic characteristics such as conditioning and anti-static benefits. At pH 7 and above, the molecule is nonionic. The performance of an amine oxide can be compared to that of an alkanolamide. Amine oxides are best known for their ability to enhance foam characteristics and stabilize lather as secondary surfactants [3].

Alkyl Polyglucosides

One of the newer nonionic classes of nonionic surfactants are the alkyl polyglucosides. These can be considered hybrids combining properties from nonionic and anionic surfactants. They are prepared by reacting corn starch glucose with a fatty alcohol. The result is group of surfactants that are good foamers, have good detergency and wetting properties, and are toxicologically safe and biodegradable [1]. They are mostly used as secondary surfactants to help reduce irritation from the primary surfactant.

Polyethoxylated Surfactants

These surfactants represent the largest group of nonionics and include fatty alcohols, the ethoxylated derivatives of alkylphenols, fatty esters, and diglycerides. They have poor foaming power but are excellent cleansers, which makes them unusable in many shampoos as a primary surfactant. When used as secondary surfactants, both polysorbate-20 and PEG-80 sorbitan laurate can greatly reduce eye irritation caused by anionic surfactants and will not affect the foaming attributes

[1]. It is because of these reasons that these nonionic surfactants are usually formulated into baby shampoos.

Amphoterics

Amphoterics can be divided into two groups based on their pH response. The first group contains the carboxylated imidzoles and N-alkyl betaines. This group is zwitterionic at pHs at and above their isoelectric points and cationic at lower pHs. The second group contains the sulfobetaines and phosphobetaines, which are zwitterionic as the anionic portion is dissociated at all pHs [1]. The amphoterics have a very low irritation potential and are almost completely nonstinging to the eyes. These surfactants are compatible with a wide range of other surfactants and can be formulated into many different types of shampoos including baby shampoos. For instance, they are often found in conditioning shampoos due to their compatibility with quaternary compounds.

It should be noted that true amphoterics actually contain dual functional groups in the same molecule that allows them to exist in nonionic, cationic, as well as anionic states depending on pH. Betaines exist only in their nonionic and cationic states, depending on pH [1]. Betaines function as excellent foam boosters and viscosity enhancers when utilized as secondary surfactants, having good water solubility over a wide pH range [3]. Cocamidopropyl betaine is the most widely used example of this type of amphoteric surfactant and is often used in amide-free formulas as well as baby shampoos.

Cationic Surfactants

For several reasons, the use of cationic (positively charged) surfactants in shampoo formulations is more limited than that of other surfactant classes. They are generally not as effective detergents due to their ability to strongly bind to the hair's negatively charged surface. Their foaming properties are inferior to those of anionics. Furthermore, because they are not efficiently removed during rinsing, the hair is left more hydrophobic with the cationic's hydrophobic tail extending from the surface. This results in increased attraction of oily soils to the hair. Therefore, their use (at low levels) has been limited to their hair conditioning, lubricating, and antistatic benefits [3].

6.10.2 RHEOLOGY AND VISCOSITY MODIFIERS

Surfactants are fundamental to the formulation of a shampoo, but the rest of the ingredients should not be overlooked. Many of these ingredients play an important role in how a shampoo is viewed by the consumer. A surfactant blend diluted in water cleans just fine, but the average person won't be convinced of this. The

consumer is conditioned to look for certain attributes in a shampoo, and much of the initial perception of a shampoo's cleaning ability is dependent upon the rheology and viscosity of the formula.

Shampoo rheology and viscosity are difficult to discuss independently. Rheology and viscosity are not interchangeable terms, but rheology, or the flow properties of a product, cannot be determined without taking into consideration viscosity or thickness, of non-Newtonian fluids such as shampoos. Viscosity modifiers have their own rheology, which must be taken into consideration when used to thicken because they are going to lend some of these rheological characteristics to the final formula. Some of the common viscosity modifiers are going to be discussed as well as the rheology they contribute to the final shampoo formula.

Salts

The most common way to add viscosity to a surfactant system is by adding a salt. While salt is not necessarily a viscosifier in the same means as the other reagents that will be discussed, salts are one of the most common substances often used in conjunction with other agents (salt-responsive thickeners), to control viscosity and modify rheology in anionic and nonionic surfactant systems. Sodium chloride is a commonly used monovalent salt in this process, but multivalent salts can be used also and are more effective since they contribute more ionic strength to the solution. Divalent salts such as calcium and magnesium chloride are not used as often because they tend to precipitate out and raise the cloud point of a shampoo formula. Ammonium chloride is another commonly used multivalent salt and is very effective, but pH and concentration have to be taken into consideration, especially if using an ammonium-based surfactant. Free ammonia can be given off if formulated above a neutral pH.

Salts themselves do not provide the thickening mechanism but in conjunction with surfactants, they promote a thickening of the surfactant system. Surfactants contain molecules with hydrophilic as well as hydrophobic portions. When specific surfactant concentrations are introduced to water, micellular structures are formed. These structures have hydrophilic or polar heads that orient themselves together in the water, while the hydrophobic tail regions sequester themselves in the center of the structure away from the water. These micelles are constantly moving and rearranging themselves. When salt is added to an anionic, nonionic, or some anionic-amphoteric surfactant systems, the charge density of the solution is lowered; this allows the micelles to pack more closely together, forming micellular agglomerates. These large agglomerates are what increase the viscosity of the shampoo. This mechanism does not work exactly the same in every formula. The salt-thickening response depends on the type and concentration of surfactants as

well as the type of electrolyte used. Solution temperature and initial charge density of the formula also has to be considered [9]. Most surfactants already contain a small amount of free organic salts, so there is already some electrolyte present in the system, which can affect the amount of salt needed.

Salt addition is not always a simple way to thicken a surfactant system. Too much salt can create a phenomenon called "salting out." A small amount of electrolyte (usually between 0.1 and 3%) is needed to thicken most systems. Salting out happens when the maximum electrolyte balance has been reached and the amount of free electrolyte starts to interfere with the micelle structure. The charge density is then lowered and the micelles begin to repel each other, which dramatically decreases viscosity. The proper amount of electrolyte can be determined by plotting a salt curve using increments of 0.1% electrolyte and then taking a viscosity measurement [10]. It is important to stay on the upside of the curve in order to achieve maximum results. The ideal amount of salt will provide a Newtonian rheology to a shampoo formula. Too much salt can also create an undesirable rheology, which can be stringy and pituitous.

Alkanolamides

Foam-stabilizing agents such as alkanolamides (i.e., cocamide DEA, MEA) are discussed earlier in this chapter under the surfactant section. These agents are often added to shampoo formulas not only to create longer-lasting foam and a tighter structure, but also to build viscosity and modify the rheology of a shampoo formulation. These are salt-responsive thickeners. They will add a pseudoplastic rheology and provide thickening by interacting with the surfactant micelle [11]. This interaction shifts the salt curve, allowing less salt to be used to achieve maximum viscosity.

Acrylic Acid Polymers

Another group of commonly used viscosity builders and rheology modifiers used in shampoos are the acrylic acid polymers. This group includes, but is not limited to, carbomers, and acrylate crosspolymers and copolymers. Acrylic acid polymers are high-molecular-weight synthetic polymers that have the ability to swell in aqueous solutions. This swelling of the high-molecular-weight polymer is what provides the thickening mechanism. They cannot swell, however, without being neutralized by a base that converts them to their salt form. This can be done by the addition of NaOH, NH_4OH, or KOH. Acrylic acid polymers are very efficient thickeners that impart a highly pseudoplastic rheology. These polymers are normally compatible with most surfactants including cationics, but most have some amount of electrolyte and pH sensitivity [11].

Gums

Gums are frequently used to modify viscosity and rheology in shampoos. There are many gums available, but only a few that are commonly used in shampoo formulations. One of the most common, nonmodified gums used is Xanthan. Xanthan is enzymatically produced from the bacteria Xanthomonas campestis and is considered a natural thickener/rheology modifier. It is compatible with anionic and nonionic surfactants and also with most mono- and divalent salts. When used in very small amounts (0.1–0.5%), it provides sufficient thickening and a pseudoplastic flow [11]. When used in excess, Xanthan can have a very negative impact on rheology. The solution can turn to a soft gel upon standing and the flow becomes very elastic.

Gums can be modified to create certain characteristics, enhance solubility, or create a specific rheology. Cationic and hydroxypropyl guar (HP guar) are examples of modified guar gum. Cationic guar is substituted with a quaternary ammonia compound. The cationic portion of the molecule is substantive, so it creates a conditioning feel while the guar portion provides the thickening and rheological attributes. The HP guar is hydroxypropylated, which provides better pH and electrolyte stability.

Corn Starches

Using corn starch is another way to thicken a shampoo while controlling the rheology. Unfortunately, unmodified corn starch is rarely used as a viscosity control agent in personal care products due to its unmodified characteristics. Unmodified dent corn will retrograde and form opaque gels, and it also imparts a high viscosity during processing, which makes it hard to work with. Waxy corn, which is also a base commonly used to make corn starch, won't retrograde to the same extent as a dent corn–based starch due to its high amylopectin content, but it has a very stringy, uneven flow that is not favored for a shampoo. Most corn starch that is used for rheology modification is chemically and or physically modified. These modifications (primarily hydroxypropylation) help create starches with increased clarity, less retrogradation, lower gelatinization temperatures, greater pH toleration, and the ability to help with foam stability due to increased film-forming capabilities. The addition of modified starch to a shampoo formula can add a short, thixotropic rheology to a shampoo and will enhance spreadability.

Corn starches are made up of small granules that swell when hydrated. This mechanism is what causes an increase in viscosity. When added into a solution containing a high amount of surfactant such as a shampoo, the surfactant can interfere with the swelling of the granules by grabbing up the available water, causing

a decrease in normal viscosity development as well as an upward shift in the gelatinization temperature. The situation is dependent on the starch base as well as the type of surfactant used. This may be linked to the amount of amylose contained in the starch granule [12]. Waxy maize starches with very little if any amylose tend to see less of this phenomenon, whereas dent-based starches which contain roughly 24% amylose will see this decrease in viscosity. For the shampoo formulator using corn starch as a viscosity/rheology modifier, it is important to know this when formulating. As a rule of thumb, waxy-based corn starches will contribute higher viscosity with less usage but can have a slightly longer texture, dent-based corn starches require a higher usage but will contribute a shorter, smoother texture. Corn starches can also be used to thicken most cationic-based surfactants where some of the other viscosity modifiers fail.

Cellulose Derivatives

Cellulose derivatives are often used for viscosity control and rheology modification, but much like corn starch, are used in their modified form. Cellulose derivatives can be modified to be more or less hydrophilic as well as pH and electrolyte tolerant. Some of the most frequently used modified cellulosics are methylcellulose, hydroxypropyl methylcellulose, and hydroxyethylcellulose. They provide a pseudoplastic rheology and are typically used at 0.5–1.5% [11].

Modified Polyethylene Glycols

Modified polyethylene glycols (PEG) are nonionic thickeners that provide different rheologies depending on the R-group that is attached to the basic chemical structure. The R-groups can consist of either a fatty alcohol, a glyceryl ester, or a propylene glycol. Of these polymers, the most commonly used PEGs for shampoos are the stearic acid esters of polyethylene glycol, typically PEG-150 distearate. The number associated with the PEG denotes the number of PEG moles in the polymer. PEG-150 distearate is a high-molecular-weight polymer that is compatible with most surfactants as well as pH, and is temperature stable, which imparts a shear-thinning rheology to a shampoo formulation [11].

Others

Some of the other viscosity modifiers used in shampoos are clays such as bentonite, hectorite, and magnesium aluminum silicate. They impart a thixotropic rheology to a formula by swelling to produce viscosity. This group of thickeners can also be used to stabilize colloidal or pearl systems in shampoos as well as for stability in anti-dandruff shampoos.

6.10.3 OTHER SHAMPOO INGREDIENTS

Opacifiers and Pearlizing Agents

Opacity or pearlescence can be imparted to a shampoo by several different types of raw materials. Ethylene glycol stearate, glyceryl stearate, and cetyl or stearyl alcohols are frequently used with alkyl sulfates. These materials are incorporated into the surfactant solution at temperatures above their melting points and then crystallize upon cooling, producing a pearlescent appearance. The opacifying effect is dependent on the crystal size, distribution, and reflectance [3].

Antioxidants/Sequestrants/UV absorbers

Traditionally, antioxidants are included in shampoo formulations to avoid oxidation of unsaturated components such as vegetable oils and oleic acid derivatives. Typical antioxidants used are BHT, BHA, and tocopherol [3]. In some newer formulations, antioxidants have taken on a more modern role and have been marketed as helping to provide healthier hair by fighting free radicals. The antioxidants used in these shampoos are more often of the "superfruit" type.

Sequestering agents are included in shampoos to prevent the formation of insoluble metal ions, which can be formed in the presence of hard water. These ions can create a film on the hair, making it appear dull. The sequestering agent binds with the metals ions to form soluble complexes. Typically, EDTA and its salts, citric acid, or polyphosphates are used.

UV absorbers are not used in shampoo to the same degree and traditional sense as they once were. UV absorbers such as benzophenone are still used to protect formulations in clear packages against color fading or discoloration upon prolonged light exposure, but most shampoos manufactured today are contained in opaque packaging. When discussing UV absorbers in the present sense, most shampoo manufacturers market them as a protectant for the fading of hair color from light exposure. In order for a UV absorber to work in this way, it has to be water soluble as well as substantive to the hair. There are newer compounds available that combine quaternary ammonium compounds with UV filters to create this type of protection (multifunctional ingredients). One such ingredient is cinnamidopropyltrimonium chloride, which can be found on the ingredient statement of some shampoos and conditioners claiming color protection [6].

6.10.4 FRAGRANCE

Fragrance has become an increasingly important aspect of a shampoo formulation. The ability of a fragrance to influence consumers' perception of a shampoo should not be underestimated. The fragrance also serves the purpose of

covering any base odor in the formula, particularly as the product ages. The level of fragrance incorporated into a shampoo formula above that necessary to cover base odor depends primarily on the intended shampoo market. The fragrance must be compatible with the shampoo formula, it should not adversely affect viscosity or stability, nor should it induce irritation. Factors affecting fragrance selection include evaluations of the residual fragrance left on the hair following shampooing in addition to assessments of the fragrance in the package and during use [3].

6.10.5 PRESERVATIVES

Preservatives are essential components of a shampoo formulation to protect against microbial growth. Among the potential sources of microbial contamination are water supplies, improperly cleaned manufacturing equipment, and raw materials [3].

There are generally three types of commonly used preservatives: parabens, formaldehyde donors, and MIT (methylisothiazolinone) systems. All have been under public fire for one reason or another, but all usage is highly regulated by each country's government.

Parabens include the esters such as methyl, ethyl, and propylparaben. These are very active against fungi and are weaker against gram-negative bacteria. They are also only active in the water phase of a formulation and can be inactivated, either partially or fully, by strong hydrogen bonders such as polysorbates, cellulose derivatives, and proteins [7].

Formaldehyde was once a common preservative in cosmetic and personal care formulations, but is rarely used in its raw form due to its carcinogenic link. It is banned in the EU as well as in Japan. The formaldehyde donors (DMDM hydantoin, quaternium 15, imidazolidinyl urea, diazolidinyl urea) tend to give off a small amount of free formaldehyde, but it is in a much lower quantity and since they are effective in such small percentages, these preservatives are used in place of formaldehyde. The formaldehyde donors all have their different strengths and weaknesses, but most perform the strongest against bacteria and the weakest against fungi. Formaldehyde-releasing preservatives should not be used with protein derivatives as they are inactivated during condensation with the free amino groups.

MIT can be used alone or in combination with other isothiazolinones such as chloromethyl isothiazolinone. These are broad-spectrum biocides that perform well against both bacteria and fungi [7].

Other Additives

Many modern shampoos have additives that take them beyond the basic cleansing shampoo and suggest additional cosmetic benefits. Some of these additives include proteins such as hydrolyzed wheat protein, hydrolyzed silk or collagen, vitamins such as panthenol and biotin, moisturizers and humectants, oils and botanicals. All have been marketed as an added benefit to the hair and scalp. These are normally added at such small percentages that there is some question of their actual benefits.

6.10.6 TYPES OF SHAMPOOS

Basic Cleansing

The basic cleansing or clarifying shampoo formula is formulated to remove substantive residues from styling products as well as provide a deeper cleaning than some of the other formulas. They are usually formulated with higher levels of anionic surfactants to boost the cleaning potential. The following is a typical basic formula for a cleansing shampoo:

Cleansing Shampoo

Ingredient	% wt/wt
Sodium laureth sulfate	25.0
Cocamidopropyl betaine	7.0
Cocamide MEA	2.0
Fragrance	0.7
Preservative	0.5
Citric acid	q.s. pH 5.5–6.5
Sodium chloride	q.s. viscosity
Water	q.s. to 100

Mild/Baby

Baby shampoos and other mild formulas (children's shampoos) are formulated for gentleness, especially to the eyes. To achieve this, these products are formulated with higher levels of nonionic and amphoteric surfactants, which are less irritating than the alkyl and alkyl ether sulfates. As mentioned before, the presence of ethylene oxide reduces the irritation of the anionic surfactants, so Polysorbate 20 and PEG-80 (20 and 80 moles of ethylene oxide respectively) are often used as anti-irritants in baby shampoos.

Baby Shampoo

Ingredient	% wt/wt
PEG-80 sorbitan laurate	12.0
Sodium trideceth sulfate	5.0
Sodium lauroamphoacetate	5.0
PEG-120 methyl glucose dioleate	2.0
Cocamidopropyl hydroxysultaine	1.0
Fragrance	0.7
Preservative	0.5
Water	q.s. to 100

Source: Reference [3]

Conditioning/Two-in-One

Most shampoos on the market today have some type of conditioning agent in them even if they are not labeled as an actual two-in-one shampoo. This helps to improve the wet combability, and adds softness and shine to the hair. These formulas are more complex than a typical basic or cleansing shampoo since most of the conditioning agents are not soluble in water, and an emulsion has to be made (usually oil in water), which will require the addition of some type of emulsifying agent.

Silicones such as dimethicone, dimethiconol, amodimethicone, and dimethicone copolyol are frequently used in these types of formulas. Silicones provide light conditioning by forming a thin film on the surface of the hair. This type of conditioning is less than that seen by a separate conditioner, but is sufficient to improve aesthetics over that of a shampoo without a conditioner.

A number of non-silicone conditioning agents have been used in shampoos, either alone or as secondary conditioners in two-in-one formulations. Because of their compatibility with anionic surfactants, cationic polymers such as polyquaternium-10 and polyquaternium-7 are sometimes used in these types of formulas. Normally, these two cationic polymers can be very substantive to the hair and can form deposits, but due to complexes that are formed with the cationic polymer and anionic surfactant, this deposition is greatly reduced [1].

Below is an example of a two-in-one formula from U.S. Patent 6,007,802 [13] containing polyquaternium-10 and an anionic surfactant as well as a dimethicone.

Conditioning (Two-in-One) Shampoo

Ingredient	% wt/wt
Ammonium laureth sulfate (3 EO)	14.00
Cocamidopropyl betaine	2.70

Ingredient	% wt/wt
Polyquaternium-10	0.15
B8/C10 diester of adipic acid	0.30
Cocamide MEA	0.80
Cetyl alcohol	0.42
Stearyl alcohol	0.18
Carbapol 981	0.50
Dimethicone	1.00
Fragrance	0.37
DMDM hydantoin	0.37
Color solution (ppm)	64
Water	q.s. to 100

Source: Reference [2]

Anti-Dandruff

In the United States shampoos and non-shampoos containing an anti-dandruff agent are considered drugs. Similar controls over these preparations exist in other legislative areas. Active anti-dandruff ingredients that can be used in the United States, as well as claims that can be made, are governed by the FDA under 21CFR358 subpart H. There are only a few active ingredients approved for use in the U.S. See Table 29.1 for the list of ingredients as well as the approved percentages.

Table 29.1. Anti-Dandruff Ingredients

Active Ingredient	Allowable Usage (%)
Coal tar	0.5–5.0
Salicylic acid	1.8–3.0
Sulfur	2.0–5.0
Selenium sulfide	1.0
Zinc pyrithione	0.3–2.0
Ketoconozole	1.0
Climbazole	1.0–2.0

Source: 21CFR358.D.2013

Most anti-dandruff agents are water-insoluble so that when the hair is rinsed after shampooing, the insoluble particulates remain on the hair and scalp for further treatment. This insolubility makes the actives harder to formulate with. Anti-dandruff agents can be difficult to suspend due to high specific densities, but much

work has been done to improve formulations so that many now contain milder surfactants and conditioning agents.

The Sulfate/Amide-Free Shampoo

Trends come and go in the personal care world, but shampoo manufacturers must succumb to the consumer. Sulfate- and sometimes amide-free shampoos have become more mainstream in the last few years. Once only seen in specialty salons, they are now seen on the common store shelves. As consumers begin to pay more attention to ingredients in the products they use, certain ingredients come under scrutiny; surfactants containing sulfates are one of them. Sulfate-free shampoos are not always easy to formulate because the surfactants can be harder to thicken and may not foam as well as other conventional surfactants. ALS/ALES and SLS/SLES are used because of their excellent foaming and cleansing properties as well as their low cost. They are easy to thicken with electrolytes or other conventional thickeners and are readily available. Surfactant companies are constantly coming out with new surfactants to meet the needs of the formulator. The following is an example of a sulfate- and amide-free shampoo formula.

Amide- and Sulfate-Free Shampoo

Ingredient	% wt/wt
A. Water	31.72
Disodium laureth sufocuccinate (40%)	27.68
Cocamidopropyl betaine	17.89
Ammonium cocoyl isethionate (30%)	13.92
Lauramine oxide	4.83
PEG-30 glyceryl cocoate	1.00
Polyquaternium-7	0.60
B. PEG-120 methyl glucose dioleate	1.91
C. Preservative	0.05
D. Citric acid (adjust final pH 6.0–6.5)	0.40

Source: Reference [13]

Shampoos with Cosmetic Benefits

Most mainstream shampoos sold on the market today have some sort of cosmetic benefit tied to them. Cosmetic benefits can range from the simple addition of a humectant for moisturization to the claim of added volume, increased shine, strengthening, or revitalizing. These shampoos have become very popular as the consumer

has grown to expect more from a shampoo than just basic cleansing. Below is an example of a volumizing shampoo.

Volumizing Shampoo

Ingredient	% wt/wt
A. Water	24.50
B. Polyquaternium-4	0.25
C. Sodium chloride	2.50
D. Water	26.27
E. Sodium laureth sulfate (28% active)	42.90
F. Cocamidopropyl betaine (30% active)	3.34
G. DMDM hydantoin	0.24
H. Citric acid	q.s. to pH 6.0–6.5

Source: Reference [13]

SECTION II: HAIR-CLEANSING MECHANISM

This chapter has discussed the basic ingredients that are involved in the formulation of a shampoo as well as the different types of shampoos that are commonly formulated. This next section is an excerpt from chapter 29 of *Harry's Cosmeticology 8th Edition* that does an excellent job explaining how shampoos carry out the cleansing mechanism as well as their effects on hair.

The surfactants play the leading role in cleansing mechanism, so an understanding of how surfactants carry out their hair-cleansing function is necessary in order to assess the relative cleaning efficiencies of different surfactants and surfactant combinations. It is also important for determining how best to balance, in a formulation, the often-conflicting aims of optimum cleaning, foam, viscosity, actives deposition, mildness, and so forth.

The epicutical of hair is substituted and coated with material responsible in large part for the observed hydrophobicity of untreated hair surfaces. Because of their protein composition, however, these surfaces can also contain charged hydrophilic sites. For virgin hair, the observed isoelectric point is near 3.67, which ensures that the hydrophilic sites on this hair will carry a negative charge at the ordinary pH level of shampoos. The combination of negative charge and the hydrophobicity affects not only the type of soils and actives that bind to the hair but also the ease with which different soils are removed from the fiber surface.

Most consumer-bought shampoos are not going to be used on virgin hair. Most modern hair succumbs to numerous cycles of chemical treatment as well as exposure to sunlight. This will affect the cleaning mechanism of a shampoo due to the fact that the distribution of negatively charged sites on untreated hair is uneven, increasing from root to tip. This is a result of exposure to sunlight, which oxidizes cystine in the hair to cystine S-sulfonate and cysteic acid. In addition to oxidation by sunlight, hair can also be chemically oxidized as a result of perming, bleaching, or permanent dyeing. These treatments, all of which include oxidative steps, convert cystine to cysteic acid. The degree of resultant negative charge is generally greater than that from sunlight oxidation. In many cases after sufficient treatment, the entire hair surface can be converted from a hydrophobic to a hydrophilic character. This, of course, also affects deposition and removal of materials on the hair surface.

6.10.7 CLEANING OF SOLID PARTICULATES

For studies of hair-surface cleaning, it is convenient to divide possible soils into two types: solid particulates and oily or liquid deposits. Particulate soils can come from the environment or from hair care products. Examples of the latter include many anti-dandruff agents, while the former include carbon particles in the form of soot, clays, or rubber abraded from automobile tires.

In general, solids soils adhere to the hair surface through ionic or van der Waals forces. The ease of removing these soils from a surface in water depends on the relative affinities for each other of the water, soil, and substrate. A hydrophobic particle, for example, would be much easier to remove from a hydrophilic substrate than from a hydrophobic surface. This can be seen in the equation for the work of adhesion, Wa, which is defined as the free-energy change per unit area involved in removing a solid particle from a surface to which it is adhered:

$$Wa = \gamma PW + \gamma HW - \gamma PH$$

In the above equation, γ is the interfacial tension between any two surfaces, P represents the particle, H represents the hair surface, while W represents water. For hydrophobic particles, γHW would be larger and γPH would be smaller for hydrophobic surfaces than for hydrophilic substrates. The resultant larger work of adhesion indicates again that hydrophobic particles are more difficult to remove from hydrophobic than from hydrophilic surfaces.

Anionic and nonionic detergents can effect removal of particles from hair surfaces by adsorbing to these substrates with their hydrophobic portions in contact with surface and their hydrophilic heads oriented toward the water. This reduces γHW and, therefore, Wa. Similarly, binding of surfactant to a hydrophobic soil reduces γPW, effecting an additional decrease in the work of adhesion.

A more effective cleaning mechanism than the preceding results when anionic surfactants adsorb to solid particles and the surfaces to which they are adhered. Such adsorption effectively deposits negative charge on both soil and substrate, facilitating soil removal as a result of mutual charge repulsion. Since nonionics cannot impart a charge potential on surfaces, they are not in general as effective as anionic surfactants in cleaning soils.

6.10.8 CLEANING OF OILY SOIL

The second major class of soils found on hair is hydrophobic, or oily, soil that is liquid, at least at cleaning temperatures. Examples include sebum from the scalp, which is mostly liquid at body temperature; silicones, oils and waxes from hair care products; and lipids from skin cells. There are a number of possible detergency mechanisms for these types of soils, including rollback, emulsification, solubilization, and mesophase formation. These are discussed in the following sections.

Rollback Mechanism

The expression for work of adhesion in the previous section applies to oily soils as well as particulates. As with particulates, the more hydrophobic the liquid soil, the more difficult it is to remove from a hydrophobic substrate. Also as with particulates, detergents can effect soil removal of oils by adsorbing to the hair surface. In this case, the increased affinity of the surface for water permits the water to displace the oil droplet and simply roll it up. This process is termed the rollback mechanism. This mechanism is described quantitatively by Young's equation, which for aqueous systems is written as:

$$\gamma HW = \gamma HO + \gamma OW \cos \theta$$

where γ is the interfacial tension between two phases, H represents hair, O represents the oil phase, and W represents water. θ in the above equation is the contact angle between the soil and the hair surface; the lower this angle, the greater is the contact between the two phases.

It is implicit in Young's equation that adsorption of a surfactant to the hair surface, which lowers γHW, will increase the contact angle of the oil droplet. For sufficiently large increases in γHW, θ will increase to 180°, and the oil droplet will spontaneously separate from the hair surface. In practice, application of mechanical work during shampooing—for example, flexing and rubbing of hair—will help to completely remove those soils. Increased temperature also aids in soil removal, facilitating droplet roll-up by reducing soil viscosity and also by increasing rates of surfactant adsorption. These temperature effects are particularly important in view of the short cleaning times involved in the shampooing process.

Solubilization of Soils

The rollback process is the most important soil-removal mechanism for products that are highly diluted during cleaning, so that surfactants are present primarily in the form of monomers.

If surfactant concentration is sufficiently high during use, then soil removal can also proceed via solubilization. This is accomplished by surfactant aggregates called micelles, which form above a certain concentration termed the critical micelle concentration, or CMC. The surfactants in micelles are arranged so that the surfactant heads form a hydrophilic surface in contact with the water, while the tails are lined up in the micelle interior, forming a hydrophobic core.

Micelles solubilize soils by incorporation into the micellular structure. More polar soils are incorporated near the hydrophilic heads, while hydrophobic soils end up deep in the micelle interior. Kinetic investigations on fatty acid solubilization indicate that solubilization begins by diffusion of micelles to the soil surface, followed by surfactant adsorption onto the soil and incorporation of soil into the surfactant aggregate. The process ends by desorption of the soil containing micelle and diffusion away from the surface. The latter two steps were found to control the rate of the solubilization process. The critical micelle concentration for most anionic shampoo is about 5×10^{-3} M surfactants. It can be seen that in a shampoo containing 10–20% sodium lauryl sulfate, most of the detergent (98–99%) is present in the form of micelles. Even if the shampoo is diluted tenfold upon application to wet hair, as much as 88% of the surfactant is still in the form of micellular aggregates. The possibility exists, therefore, that solubilization is a major factor in shampoo cleaning.

As with rollback, soil removal by solubilization is greatly increased through rubbing and flexing of hair (mechanical work) and increasing temperature. More work needs to be done to precisely determine the contribution of solubilization in the hair-cleaning process.

Emulsification, Penetration, and Mesophase Formation

Emulsification and mesophase, or liquid crystal, formation are two important mechanisms that can be involved in removal of soil from hair. Emulsification involves the breaking down of an oily soil into smaller particles that can form a stable suspension. It requires a low interfacial tension between the oily soil and the bath medium, which may be accomplished by adsorption of shampoo surfactant onto the soil surface. Mechanical work also helps in breaking up of the soil.

Amphiphilic compounds in soils, such as fatty alcohols or fatty acids, can greatly aid the emulsification process by interacting with the shampoo detergent to

spontaneously emulsify the soil. Since the shampooing process is short, emulsified soils need be suspended for only a few moments.

Phase diagrams of detergents, water, and certain polar salts such as fatty acids and alcohols can contain large regions that flow easily and are composed of a liquid crystalline phase. Such mesophase formation with amphiphiles, or oily soils containing amphiphiles, constitutes another important mechanism for soil removal from hair.

6.10.9 EFFICACY OF SOIL REMOVAL BY SHAMPOOS

The soils most commonly found on hair originate from three sources: secretions from the body, residues from hair care products, and deposits from the environment. The first type of soil is composed mostly of sebum from the sebaceous glands. Residues from hair care products include hairspray resins and various conditioning materials, while environmental soils include particulate matter from soot, clays, rubber from automobile tires, and so forth.

The efficiency of shampoos in removing these soils and the types of mechanisms by which such removal is carried out depend on a number of factors, including the nature of the soil, the state and condition of the hair surface, and the particular surfactant or combination of surfactants employed in the shampoo. In the following sections the principles of cleaning of different soils by shampoos will be discussed.

6.10.10 CLEANING OF SEBUM

Sebum is probably the single most important soil found on human hair. It is composed of a mixture of lipid materials that are secreted into the follicular duct by the sebaceous glands. The emerging terminal scalp hair is coated with sebum, and mechanical actions such as combing and rubbing against pillows ensure that the sebum becomes distributed more or less evenly over the entire hair surface.

Sebum, which is almost completely molten at body temperature, lubricates the hair, giving it (when not present in excess) a smooth, moisturized feel. Hair with too much sebum on the surface, however, becomes limp and clumped together and is perceived by consumers as dull, dirty, and greasy. Moreover, because it is sticky, the presence of sebum leads to further soiling as a result of adhesion of airborne particulates and other material with which it comes in contact. In addition, sebum can act as a binder, cementing many soil particles together.

Detergents do not penetrate hair appreciably during the relatively short times involved in shampooing. As a result, cleaning of soils such as sebum by shampoos is confined primarily to the hair surface. Internal lipids, however, do not appear to contribute to consumer-perceivable soiling effects. Thus Robbins found that

the same quantity of internal lipid (as much as 9% of the total hair weight) could be extracted from both oily and dry hair, indicating that the oily feel of the hair was entirely due to surface sebum. Because of its composition and physical state, sebum can potentially be cleaned from hair by any of the cleaning mechanisms presented in the previous sections. Sebum can be removed from hair by a rollback mechanism. Sebum is also subject to removal by emulsification and mesophase formation. Finally, since the concentrations of surfactants during cleaning are generally well above the surfactant CMCs, sebum can also be cleaned from hair by solubilization.

The relative importance of these mechanisms in cleaning sebum from hair is not identical and more than one cleaning mechanism can certainly operate simultaneously. In any case, the multiplicity of possible cleaning mechanisms for sebum might lead one to expect that shampoos would be very effective in removing sebum from hair. This expectation is supported by much of the literature: a number of studies have demonstrated that anionic surfactants at normal shampoo concentrations can clean surface lipids effectively. Effective cleaning of lipids by anionic surfactants was also reported, and gas-chromatographic techniques were used to measure percent removal from hair of the various components of an artificial sebum. Table 29.2 lists some of the results from this work. It can be seen that the more polar fractions of the sebum, such as free fatty acids, were removed from hair to a greater extent than the less polar fractions, such as paraffin. In other words, hydrophobic soils, like paraffin, have a greater affinity for the hydrophobic hair surface than do more polar deposits and are therefore more difficult to remove.

Sodium laureth-2 sulfate (SLES-2) is seen in Table 29.2 to remove sebum fractions from hair more effectively than ammonium lauryl sulfate (ALS). One reason for this may be related to higher adsorption of SLES-2 than of SLS, which would favor increased removal of sebum by the rollback mechanism.

Table 29.2. Sebum Cleaning by Surfactants

Sebum Component	% Removal by SLES-2	% Removal by ALS
Triglycerides	94.7	94.6
Free fatty acids	96.2	97.1
Spermaceti wax	96.2	84.6
Squalene	98.4	87.6
Paraffin	95.2	80.8
Average % removal	**95.9**	**85.9**

Source: Reference [3]

6.10.11 CLEANING OF QUATERNARY AMMONIUM COMPOUNDS

Conditioners are used to increase ease of hair combing, reduce flyaway, and improve the feel of the hair. The most widely used conditioning agents in commercial products are quaternary ammonium compounds. These compounds are generally used in combination with lipid conditioners such as long-chain alcohols.

Two of the most widely used quaternary conditioners are stearalkonium chloride (SAC) and cetrimonium chloride (CTAC). Other important quats include steartrimonium chloride, dicetydimonium chloride, and tricetylmonium chloride. The most important lipid conditioners include cetyl and stearyl alcohols. Concentrations of cationic surfactants in commercial conditioners are generally on the order of 1–2%, while lipid concentrations are equal to or greater than those of the quats.

Conditioners are generally used at pH levels above the isoelectric point of hair, that is, on negatively charged fiber surfaces. Quaternary ammonium compounds, by virtue of their positive charge, are therefore substantive to the hair surface. Treating hair with these compounds results in a hydrophobic coating that is soft and easy to comb. The binding to hair of quaternary compounds has been found to increase with increases in the hydrophobic chain length and number of chains. This hydrophobic dependence indicates that van der Waals forces play an important role in the deposition of quats on hair.

Deposition of quaternary conditioners on hair is also a function of the degree of negative charge on the hair surface. Compared to virgin hair, bleached hair, which has a more negatively charged surface, retains more than twice the amount of stearalkonium chloride.

Table 29.3. Detergent Cleaning of Stearalkonium Chloride

Treatment	SAC (mg)/g Wool	Detergent (mg)/g Wool
1% SAC	6.68	--
5% ALS	--	1.94
1% SAC/5% ALS	4.58	4.09
5% SDES-2	--	1.94
1% SAC/5% SDES-2	2.52	2.12

Source: Reference [3]

Many quats deposited from conditioning products have been reported to build up over time, indicating that removal of these materials from hair may be more difficult than is the case for sebum. One reason for this is simply the strong

electrostatic attraction between the positively charged quats and the negatively charged hair surface. Another reason is that, since quaternary ammonium conditioners are solids, they are not subject to removal by the rollback mechanism. In addition, the positive charge on quats interferes with the mechanism for particulate soil removal, that is, the introduction of negative potentials on soil and substrate as a result of adsorption of anionic surfactant.

Solubilization is a possible mechanism for cleaning of quaternary conditioners. Reich and coworkers have shown, however, that, at least for SAC and CTAC, solubilization by lauryl and laureth sulfates is ineffective. Instead, cleaning with these surfactants results in the formation of insoluble surfactant:quat complexes that are dulling and difficult to remove from hair.

Reducing the hydrophobic chain length of the cleaning surfactant to ten carbon atoms results in formation of more soluble surfactant:quat complexes, which can be more readily cleaned from the hair. Thus washing deposited SAC with 5% sodium deceth-2 sulfate (SDES-2) resulted in higher removal of the deposited conditioner.

The above findings apply to cleaning of the pure quaternary conditioners; cleaning of deposits from fully formulated conditioners lead to improved removal by ALS.

Despite increased quat removal from a fully formulated conditioner, insoluble complex formation between SAC and ALS still occurs. This is evidenced by the increase in deposited ALS measured after cleaning the residue with this surfactant.

6.10.12 CLEANING OF POLYMERIC RESIDUE

Several types of polymers can be found on hair as a result of use of hair care products, including hairspray resins, silicone conditioners, and cationic conditioning polymers. The ease of removal of these polymers from hair depends on several factors, including charge, molecular weight, structure, nature of side chains, and so forth. In the following sections, the ease of cleaning of several important examples of hair care polymers will be discussed.

Cationic Conditioning Polymers

Several cationic polymers are available commercially that provide conditioning benefits, especially increased ease of wet combing. Important examples include polyquaternium 10, a quaternized hydroxyethylcellulose polymer; polyquaternium-11, a copolymer of vinylpyrrolidone and dimethylaminoethyl methacrylate quaternized with dimethyl sulfate; polyquaternium-16, a copolymer of vinylpyrrolidone and quaternized vinylimidazole; polyquaternium-7, a copolymer of diallyldimethylammonium chloride and acrylamide; and polyquaternium-6, a homopolymer of

diallyldimethylammonium chloride. As a result of their cationic nature, these conditioning polymers are substantive to hair.

Deposition on hair fibers has been claimed to be an inverse function of charge density, an effect that has been explained by noting that the greater the charge density, the lower the weight of polymer needed to neutralize the total negative charge on the hair.

Polyquaternium-10 is quite substantive to hair, resisting complete removal by SLS even after exposure to detergent for as long as 30 minutes. Similar results were obtained in cleaning experiments with radio-tagged polyquaternium-10. First, as with monofunctional quats, deposition of polyquaternium-10 was found to increase with increasing negative charge on the hair. Thus bleached hair was found to bind more than 2.3 times the amount of polyquat as did untreated hair. In addition, bound polyquaternium-10 was found to be difficult to remove: only 43% of the polyquat could be removed from wool swatches in a single washing with SLS.

Reducing the charge density on the polyquaternium-10 led to more complete cleaning. Thus in a single SLS wash, 75% of this polyquaternium could be removed from wool swatches. Results similar to the above were obtained with polyquaternium-7, which was found to be about as resistant as polyquaternium-10 to removal from wool substrates. The difficulty in cleaning these polymers does not appear to be related to formation of insoluble conditioner:detergent complexes, as was the case with SAC and CTAC. This is evidenced by the fact that cleaning polymer-treated wool with SLS was not observed to result in detergent buildup, even after several cycles of polymer/detergent treatment. It is concluded, therefore, that the difficulty in cleaning polyquats is most likely related to the multiple points of attachment between the polymer and the keratin surface. In order to clean these polymers, it is necessary to break all the points of attachment at the same time, a more difficult proposition than the elimination of the single point of attachment between a small molecule and the hair surface.

Fixative Residue

The holding properties of styling products such as hairsprays, mousses, gels, and setting lotions are provided by various polymeric resins. These materials are generally neutral or negatively charged in order to facilitate removal from hair. Typical examples include the copolymer of vinyl acetate and crotonic acid, the ethyl ester of the copolymer of polyvinyl methyl ether and maleic anhydride (PVM/MA), the copolymer of polyvinyl pyrrolidone and vinyl acetate (PVP/VA), and the copolymer of octylacrylamide/acrylates/butylaminoethyl methacrylate.

The ease of cleaning of hairspray resins was measured using the radio-tagged ethyl ester of PVM/MA. A single washing with 10% SLS resulted in removal of 89%

of the deposited resin. This is also consistent with the expectation that noncationic fixatives would be easier to remove from hair than positively charged polymers.

Dimethicone Residue

As was stated in the introduction, the active ingredient in most two-in-one shampoos is dimethicone, a hydrophobic polymer (polydimethylsiloxane), which is commonly found in many conditioners.

As was the case with fixative resins, little has been published in the literature on the ease of removal of dimethicone. One quantitative study, however, was performed by Rushton and coworkers, who used electronic spectrum for chemical analysis (ESCA) and atomic absorption measurements to study buildup and cleaning of dimethicone. ESCA measurements by Rushton indicated a dimethicone buildup of roughly 35% after five washings of virgin hair with a commercial two-in-one shampoo. After five washings, however, no further buildup was observed. In addition, Rushton found that more than 90% of deposited dimethicone could be removed by a single wash with a commercial shampoo.

6.10.13 EFFECT OF SHAMPOOS ON HAIR

The immediately preceding sections dealt with cleaning of soils by shampoos. Shampoos are also involved in damage of hair, either directly, through removal of structural components of the hair fiber, or indirectly, through removal of protective deposits on the hair. These processes are discussed in the following sections.

Direct Damage by Shampoos

Studies in the literature have indicated that the nonkeratinous regions of the hair, which include the endocuticle, or inner portion of the cuticle, and the cell membrane complex are susceptible to damage by surfactant molecules.

These and additional experiments indicate that exposure to shampoos can have a deleterious effect on hair structure over time. The extent and consequences of these effects are unclear, especially since preexisting damage may render the hair more susceptible to surfactant damage.

Indirect Damage by Shampoos

The use of shampoos can damage hair indirectly as a result of fiber abrasion occurring when hairs are rubbed against each other during cleaning. More important to the damage process, however, is the removal of sebum from the fiber surface during shampooing. This is because sebum acts as a natural lubricant for hair; removal of this material increases damage from grooming, due to chipping, fragmenting, and tearing away of cuticle cells, and it should be attributed to combing and brushing.

Figure 29.1 shows an example of the damage than can occur from grooming. This rather extreme example was induced by washing a tress of virgin hair with a cleaning shampoo and then combing it 700 times while wet. It can be seen that cuticle damage is widespread, with noticeable loss of some cuticle cells and extensive lifting of others from the hair surface. Repeated grooming of the hair gradually erodes the cuticle cells, with the greatest loss occurring at the tips of the hair. Eventually all of the cuticle cells can be lost, exposing the cortex and resulting in a split end. An example of such splitting is shown in Figure 29.2.

Damage to Chemically Treated Hair

Permanent waving, bleaching, and oxidative dyeing can result in significant damage to the hair. In addition to causing tensile damage, all of these treatments oxidize the surface of the hair, resulting in a considerable increase in surface friction. This subjects the hair to increased grooming damage because sebum is easier to clean from an oxidized hair surface, while combing forces are increased as a result of increased friction.

Chemically treated hair is also subject to increased water swelling and penetration by different materials as a result of reduced disulfide cross-linking and damage to nonkeratinous regions of the hair, including the cell membrane complex. Increased uptake of surfactants can, of course, lead to direct damage as a result of increased extraction of structural components of the hair fiber.

Figure 29.1. Combing damage resulting from washing a hair tress with a cleaning shampoo and then combing 700 times while wet. The scanning electron micrograph (SEM) is typical of hair taken from the combed tress. Note raised and chipped cuticle cells, and areas where cells have been completely torn away.

Figure 29.2. SEM photograph of a split end. Note the structure in the exposed cortex.

SECTION III: SHAMPOO EVALUATION

Formula Evaluation

Along with testing on actual hair, the shampoo formula itself needs to be tested for certain attributes. One of the most important tests a formula must make it through is shelf stability. Accelerated testing in different temperature/humidity extremes is usually used to evaluate the stability of a formula. Foam height and density is another very important attribute, but before it is tested on tresses, the actual foam height needs to be measured. The last formula attribute that needs to be taken into consideration is the ease of manufacturing. If the formula is not scalable, it is not marketable.

Stability

Products going on the store shelves should have at least two years of shelf life. During this time period, the shampoo should not see appreciable changes in viscosity, color, or fragrance. Multiphase products should not separate and there should be no precipitation seen.

Accelerated aging tests are run to test product stability. These tests are usually run at temperature extremes to simulate warehouse storage or shipping conditions. There are no standard accelerated aging tests for hair care products, so most manufacturers develop their own. A typical shampoo accelerated aging test suggests oven storage at 40°C and 50°C for three to six months as well as 25°C for a year.

Samples should be packaged in glass as well as the packaging that the shampoo would be sold in. The products should also go through one or more freeze/thaw cycles to evaluate the effects of lower temperatures [2].

Foam Height/Density

Foam is a very important attribute of a shampoo. Consumers will evaluate a shampoo based on the quality and quantity of the foam produced even though most shampoos have much more surfactant in them than actually needed to clean the hair. Strong abundant foam is directly related to the cleaning mechanism of a shampoo according to the average consumer.

There are various tests to measure the foaming potential of shampoos in the absence and presence of lipids. One very simple test is by shaking a known concentration of the shampoo formula in a graduated cylinder for a set amount of time. The foam height is then measured after shaking.

Manufacturing Ease

When formulating a shampoo, the ease in which it is manufactured or scaled up must be taken into consideration. A complex formula opens up more opportunity for production error. Order of addition, viscosity adjustments, mixing equipment, as well as heating capacity all need to be thought out carefully when transferring a formula from lab or pilot scale to manufacturing scale.

Evaluation of Shampoo Performance

A stable formulation is just the beginning for a shampoo formulator; performance of the shampoo ties together the entire gamete of consumer-driven traits that a formula must exhibit. These can be tested in a laboratory on tresses and if all goes well, the formulas can be tested by trained cosmetologists using half-head test methodology. This type of testing does not involve consumer evaluation, but is useful because it eliminates the differences seen in various hair types. Finally, once the shampoo passes all laboratory and half-head testing, it can move on to consumer testing. This is usually carried out in salons or at cosmetology schools with salons. Only after consumer approval for efficacy and safety, should the formulas go to market.

The following sections introduce some of the important attributes that are looked at when evaluating a shampoo's performance.

Ease of Application

This attribute is related to shampoo viscosity. A shampoo should be viscous enough to remain in the palm of the hand during pouring, but should easily disperse over the hair during application.

Lather

It is important for the foam height of the shampoo to be checked before it is added to hair, but the lather needs to be evaluated on tresses also. This should take into consideration the speed and amount of lather generated, the quality as well as the stability on hair. An extension of lather generation is lather rinsability. Water hardness, temperature, and rinsing rate all need to be taken into account when rinsing is evaluated, as well as any residuals left on the hair once rinsing is complete.

Wet/Dry Comb

Combing ease can be defined as the ease of aligning hair fibers in a parallel arrangement with a comb. Evaluations of combing ease, especially on wet hair, are often used as the primary assessment for the broader consumer attribute of hair conditioning, which also includes hair softness and lubricity. Hair fiber properties that improve combing ease are increasing stiffness, diameter, and cohesion; and decreasing curvature, friction, length, and static charge. Studies have indicated how different types of hair treatments can affect combing ease. Shampoos can affect several of these properties depending on the particular formulation. For instance, high-cleaning shampoos can make the hair more difficult to comb by removing sebum and oily soils that can lubricate the hair, while conditioning shampoos can deposit materials on the hair that decrease friction and thereby make the hair easier to comb [3].

The ease of combing attribute includes ease of snag removal and ease of comb slip on both wet and dry hair. Methods to evaluate combing ease include both qualitative combing of tresses combined with statistical evaluation of the results and quantitative instrumental methods. Instrumental methods involve the measurement of combing forces on tresses following treatment using an Instron or Diastron tensile tester [3].

Luster/Shine

Luster or shine is a very important aesthetic property to the consumer. Aging hair, chemical treatments, buildup of styling products, use of heated styling tools, as well as certain shampoo ingredients can all add to the dulling process. Shampoos that claim to add shine have a great market advantage as consumers associate this added shine or luster to creating healthy strong hair.

There are many instrumental methods as well as subjective evaluations that can be used to evaluate shine or luster on hair. No matter which method is employed, there are several factors that need to be taken into consideration when substantiating a claim for luster or shine enhancement: fiber straightness, degree of alignment of the hair, as well as hair color.

Volume/Body

The consumer attribute of body is both a visual and a tactile hair property indicative of fullness and volume combined with springiness and bounce. The effects shampoos have on hair body are formula dependent. For instance, deep-cleaning shampoos containing higher amounts of anionic surfactants can provide an increase in body by removing surface oils that weigh down hair. These types of shampoos can be marketed as volumizing or shampoos for fine/thin hair. Alternatively, shampoos containing materials than can build up on the hair can reduce hair body [3].

Several instrumental methods have been developed to evaluate parameters associated with hair body. These approaches measure changes in fiber friction, stiffness, curvature, diameter, weight, cohesion, and length. Treatments that increase the hair's curvature or diameter increase the frictional forces between fibers, or make the hair stiffer will increase body; those that increase the cohesion between fibers or weigh them down will decrease hair body [3].

Manageability

Manageability is a measure of styling ease and style retention. It is a complex consumer attribute that is difficult to assess by any one hair parameter or method. It has been suggested that evaluators consider three types of manageability: style arrangement manageability, style retention manageability, and flyaway manageability (static control). These properties can be assessed best by using a control treatment. As with other attributes, the effects that a particular shampoo has on manageability can vary. Any assessment of manageability is further complicated by factors involving the type of hair, humidity, and style [3].

Fragrance

Fragrance may not be a performance attribute for a shampoo formulation, but its importance cannot be overlooked. The effects that fragrance can have on a shampoo go far beyond that of formulation compatibility. The lasting power and acceptability of a fragrance needs to be taken into consideration before a formulation goes to market.

SECTION IV: FUTURE TRENDS IN SHAMPOOS

Consumer demand directs the mainstream shampoo market, which is why it is important for the formulator to stay abreast of any upcoming or lasting trends.

Natural and Biodegradable Ingredients

As stated before, consumers are becoming more aware of what they are putting on their bodies, as well as what is being done to the environment. Consumers perceive natural or naturally derived ingredients to be milder and safer than their synthetic

counterparts as well as more eco-friendly. Ingredients that have chemical-sounding names are usually not deemed natural by the average consumer, so cosmetics and personal care items are trending more toward naturally derived ingredients and simplicity on the label. The biggest problem plaguing the formulator is that natural ingredients are sometimes hard to work with and don't necessarily function the same as the synthetic or chemically modified ingredients. Yet the consumer expects the natural shampoo to have the same attributes as a regular shampoo.

Many shampoo manufacturers have come out with "natural" product lines. These formulations may not be all-natural, but many contain biodegradable surfactants as well as botanicals and oils to replace some of the synthetic ingredients used in their regular formulas.

Anti-Aging Benefits

It is no secret that the anti-aging trend has taken over the cosmetic world. Staying young, or at least the perception of staying young, has spilled over into the hair care industry. As hair gets older, it gets coarser, the color fades; it becomes thinner and loses some of its natural luster. Shampoos are being manufactured that have anti-aging benefits that claim to help combat some of the issues hair goes through as it ages.

The "Free" Shampoo

Whether it be sulfate-free, amide-free, paraben-free, or silicone-free, there will always be the consumer demand for some type of "free" shampoo. These trends cycle and depend highly upon which ingredient is the hot topic of the moment. Even though the type of "free" shampoo can have a short life span and are of the specialty type, these shampoos have made it from being seen only in salons, to the mainstream store shelves.

Dry Shampoos

Although not necessarily a shampoo in conventional terms, the dry shampoo, once popular in the 1970s, is making a strong comeback, especially in European countries. This type of shampoo is not only brought back for its convenience factor, but also to satisfy the growing number of people who worry about the consequences of shampooing every day.

Dry shampoos are simple formulas consisting primarily of a corn or rice starch, which is used to absorb oils from the scalp and hair. Most modern dry shampoos also contain some sort of conditioning agent so that they don't leave the hair stiff like the very first formulas did. The problem facing this type of "shampoo" is that the starch used for these shampoos is white, so it is not very adequate for use in very dark hair.

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GLOSSARY

viscosity – thickness of a liquid or resistance to flow.

rheology – the study of the flow of liquids.

anionic – negatively charged ion.

nonionic – does not have a positive or negative charge.

cationic – positively charged ion.

amphoteric – can act as a positive or negative ion depending upon pH.

zwitterionic – ion with both positive and negative charge attached to the same molecule with an overall neutral charge but can act as either a positive or a negative depending on pH.

flash foam – foam that develops immediately, may not have lasting power.

emulsifier – substance that stabilizes an emulsion by keeping both the water and oil phases mixed.

cloud point – point at which dissolved solids are no longer completely soluble, causing a solution to appear cloudy.

hydrophilic – has an affinity for water.

hydrophobic – repels water.

micelle – an aggregate of molecules that form in colloidal solutions.

pseudoplastic rheology – also known as sheer thinning. Viscosity will thin upon movement.

substantive – will bind to a substrate (hair) and not wash off with normal washing.

dent corn – a common variety of corn that has a ratio of about 76% amylopectin and 24% amylose. Used for making corn starches.

waxy corn – a variant of corn that contains 100% amylopectin. Used for making corn starches, not as common as dent corn.

amylopectin – a branched polysaccharide with α-1,4 and α-1,6 linkages. Makes up 76% of dent corn starch and 100% of waxy corn starch.

amylose – a linear polysaccharide with α-1,4 linkages. Makes up 24% of dent corn starch.

gelatinization temperature – the temperature at which a starch begins to swell.

antioxidant – substance that counteracts the damaging effects of oxidation.

sequestrant – form chelate compounds with metal ions often found in hard water. This allows for a shampoo to be rinsed easily from the hair without forming a layer of "scum" on the hair.

humectant – a substance that retains moisture.

critical micelle concentration (CMC) – concentration of surfactant above which micelles form and all additional surfactants added will spontaneously form micelles.

sebum – an oily secretion of the sebaceous gland that can be found on the scalp.

HAIR STYLING

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a. Nonpressurized Styling Products

b. Pressurized Styling Products

The art of styling hair requires a broad spectrum of products to help achieve and maintain the desired coiffure. These products vary from those intended to simply hold hair in place (finishing products) to those intended to actually build a desired style (working products). A professional stylist would say that all styling is related to either lifting hair up or holding hair down. Styles can be achieved chemically by permanent waves or straighteners, mechanically by rollers, or thermally by irons or any combination of the former. In the development and maintenance of the desired configuration, a wide range of styling products may be used.

The styling product category is the largest and most confusing of all of the categories to the consumer. The formulator needs to understand what the product is to be used for in order to develop the best formula for the intended product. Once the targeted consumer and the desired functionality is understood, the formulator can develop the required formula and form to deliver the intended result in an aesthetically pleasing form.

In general, styling products can be separated into two categories: non-aerosolized styling products and pressurized aerosol styling products. Non-aerosolized styling products can be categorized into liquids, gels, nonpressurized sprays, and specialized (e.g., waxes, hairdressings) styling products. Pressurized aerosolized products can be categorized as fixatives (e.g., hairsprays/mousses) or specialty (e.g., shine sprays) products.

Because of the nature of styling products—the basic formulations and usage platforms—they are the most highly regulated category in hair care. The formulator needs to be aware of all of the regulatory requirements by category in order to develop functional, compliant formulations. The Volatile Organic Compound (VOC) regulations on styling products exist at the federal level (EPA) and at individual state levels, California being one of the strictest. Because the regulatory environment is dynamic and new interpretations arise on an almost daily basis, great

diligence is required in formulating and even naming products in this category. The formulator must work closely with legal and regulatory advisors to avoid inadvertently developing a formula that will later be declared noncompliant and subject to punitive legal issues.

a. Nonpressurized Styling Products

Liquids and Gels

In general, both of these subcategories fulfill similar functions—to develop a style and then, once dry, maintain that desired configuration. Styling liquids are water-thin, aqueous, or hydro-alcoholic solutions of a holding, film-forming polymer (e.g., PVP and/or derivatives), modifiers to increase plasticity (i.e., flexibility) of dried film, and aesthetic modifiers (e.g., fragrance). They are generally applied to damp hair, worked through the hair, then dried after the desired configuration is achieved. They can be used in combination with brushing while blow-drying to achieve volume/fullness, which will be maintained as the film dries on the individual hairs.

Styling gels serve a similar function, but applied in a gel form. The gel itself is formed by the use of a polymeric material (e.g., carbomer) and provides a thicker coating to the individual hairs. Gels may be used to hold hair in a flat configuration or in conjunction with brushing and blow-drying to achieve volume or raise hair from the scalp.

When liquids or gels dry they form a film on the hairs and cause the hairs to hold together in a relatively rigid configuration. If combed or manipulated after drying, this film coating and the resultant "welding" of hairs together is disrupted and will no longer hold. This disruption can be negative if the desired configuration was achieved or positive if the desired configuration is less rigid and will be supplemented with other fixative products. Regardless, as the film is disrupted it breaks into small particles that can manifest themselves as flakes or dust. The formulator can incorporate materials to ameliorate this negative appearance, but must certainly be aware of the potential for this occurring.

Nonpressurized Aerosol Sprays

These sprays are hydro-alcoholic solutions of a hair-fixative polymer or polymers, film modifiers, and aesthetic materials. They are sprayed directly onto damp (working sprays) or dry (fixative) hair. They provide a modicum of coating to the individual hair fibers (used as working spray) or "spot welds" at the junction of individual hairs (used as fixative spray).

When used as a working product, these function in a similar fashion to liquids and gels, but allow lower levels of product to be applied. As a finishing product,

these sprays dispense a fine coating of fixative polymer on the final coiffure to help maintain the desired configuration. Dispensing water or hydro-alcoholic solution onto dry hair will disrupt the hydrogen bonds of the hair and can cause the desired configuration to collapse or be disrupted. The formulator needs to be aware of how the product can disrupt a coiffure and can select a sprayer that, in conjunction with the degree of hold required (i.e., the amount of fixative polymer delivered to the hair), will ameliorate this collapse or disruption.

b. Pressurized Styling Products

Aerosol Hairsprays

Pressurized styling products (e.g., hairsprays and mousse) have been and are the largest section of the styling products universe and, combined with gels, comprise between 80–90% of the entire styling category.

Formulating pressurized hairsprays requires technical understanding of the basic formulas and mechanical understanding of the way that pressurized aerosols work and, specifically, how the formula, propellant, and spray button/valve system interact to deliver the desired outcome. In this particular subcategory, all of the parts make up the "formula," and changing any of the parts will have s significant impact on the final product. In addition, this category is highly impacted by the VOC regulations mentioned earlier.

The base formula for hairsprays is essentially an alcoholic or hydroalcoholic solution containing a polymeric resin or resins (fixatives), a neutralizing agent (if required by resin type), plasticizing agents to impact the stiffness/brittleness of the dried film, fragrance, and claims ingredients. The propellants used for hairsprays are propane, butane, isobutane, and in the world of VOC regulation, hydrofluorocarbon 152a.

The ratio of base formula to propellant (base-prop ratio) is important for product performance. In general, the lower the base-prop ratio, the finer and dryer the resulting spray.

In general, the volatile organic compounds in a pressurized aerosol hairspray are the alcohol (solvent for the base) and the hydrocarbon propellants. To achieve the requisite VOC maximum, the formulator would commonly use: a) a combination of alcohol, water, dimethylether (DME—a solvent/propellant that ameliorates propellant/base miscibility but is a VOC), and hydrocarbon; or, b) a combination of alcohol, hydrocarbon, and hydrofluorocarbon 152a. In the DME/water approach, the water, resins, neutralizing agents, and other miscellaneous ingredients are the non-VOC portion; the propellant, DME, and alcohol are the VOCs, which have a maximum level allowable. In the 152a approach, the resins, neutralizing agents, and other miscellaneous ingredients are the non-VOC portion; the propellant, 152a, and alcohol are the VOCs, which have a maximum level allowable. There

are some special exemption formulations that have been used for compliance, but the two approaches indicated are the most common.

Water-containing hairsprays tend to be "wetter" when applied, and water can negatively impact coiffures, can cause corrosion in electro-tinplated steel cans and valves (commonly used for hairsprays), and can require special gaskets not negatively impacted by DME. The use of water in the hairspray can make the formula less expensive than the 152a approach. The 152a approach yields a finer, drier spray, does not impact the final coiffure, and is not corrosive to valves and cans. However, the 152a approach is significantly more expensive.

Mousse

Mousse styling products are used for a combination of styling and hair holding. In general, mousse formulations are an aqueous solution of holding resins (e.g., VP/VA copolymer), conditioners (e.g., polyquaternium-4, dimethicone copolyol), surfactant materials (e.g., polysorbate 60), aesthetic/claims ingredients (e.g., fragrance), and propellant (e.g., butane, propane, and isoubutane). When properly formulated, the propellant phase becomes dispersed in the liquid base phase such that when dispensed, the product immediately forms a thick foam in the hand, which can then be applied to wet or dry hair for styling. Some mousse formulations contain alcohol to assist the mixing of the water phase and the propellant phase.

Mousse formulations are generally used for a conditioning hold, with the ratio of holding resin and conditioners being varied to provide more or less of the desired property. These products are generally used as the preliminary styling product to create volume or create desired coiffure. They are then followed by a finishing spray to hold the configuration in place. They can be used alone to build and maintain style depending on the desired configuration or in conjunction with other styling forms such as gels or gums.

As are all pressurized styling products, mousse formulations are governed by VOC regulations. The levels of VOC allowed for mousse are substantially lower than that for aerosol hairsprays. The VOCs in mousse formulations are the propellant and alcohol, if included in the formula.

Pressurized Shine Sprays

Generally, pressurized shine sprays are an anhydrous solution of lightweight oils and lipoidal materials, fragrance, and propellant. These products are primarily used by consumers with relaxed, African-Caribbean hair to impart shine. Shine sprays are also regulated for VOC content, and the formulator must be aware of the categorization of the oils used before starting. In addition, the ultimate particle size of the dispensed spray must be closely monitored. Too fine a particle size can lead to inhalation of the oily particles, which can result in significant health issues. The formulator needs to check the current regulations.

SPECIALTY STYLING PRODUCTS

Author
Charles Warren

The category of specialty styling products is huge and grows on an almost daily basis. Products are developed to achieve styles and effects. These products usually come from the professional stylist arena and are the result of a creative stylist using an new or alternate material to achieve a particular "look" or style on a particular hair type.

Included in this category are waxes, hairdressings, silicone shine products, gums, etc. These products can all be used to impart a targeted effect on the hair. Waxes and gums are blends of waxes and other higher-molecular-weight fatty materials to impart stiffness to short hair or stiffness to small sections of a larger coiffure—small braids or sideburns for example. Hair dressings are blends of fatty materials or oil/water emulsions or microemulsions used to provide shine, immediate lubrication, and a modicum of control, especially on dry hair. Silicone shine treatments are blends of silicones used to lightly coat the hair and provide a glossy shine.

In general, the formulator needs to know the targeted consumer/hair type, the effect desired, the claims to be made, and the way the product will be used by the consumer to begin formulation work. A survey of the competitive products used for the same purpose will be helpful as a starting point.

PERMANENT WAVING

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Permanent waves are two- or three-component systems applied to hair rolled on appropriate rods to achieve a specific curl or wave pattern that is durable ("permanent"). The first solution is either the waving lotion alone or a two-component system for creating the wave lotion. The waving lotion alone (cold wave) is an aqueous mixture of ammonium hydroxide (1–2% ammonia), thioglycolate (8–10% as thioglycolic acid), surfactant, and other water-soluble ingredients desired. (The pH of a traditional cold wave is 9–9.5.) Ammonium thioglycolate and monoethanolamine thioglycolate are the common forms of thioglycolate employed. It is the thioglycolate ion that is the reactive species. The two-component waving lotion involves an aqueous solution of ammonium hydroxide (1–3% ammonia) to which is added glyceryl monothioglycolate (GMT) immediately prior to application. Again, it is the thioglycolate that is the reactive species. In either case, the waving lotion is applied to the hair wrapped around rods of the appropriate size for the desired curl pattern. The waving lotion is allowed to process under a plastic cap (for the cold wave or some GMT waves) or with a cap under a hot dryer for acid GMT waves) until the desired curl pattern is achieved (determined by the operator unrolling a rod and reviewing a "test curl"). Once the pattern is acceptable, the waving solution is rinsed from the hair and the second component (neutralizer), an aqueous solution of hydrogen peroxide (2–2.5%) is applied to reform the cystine bonds in the new configuration. After five minutes, the neutralizer is rinsed and the rods are removed.

Permanent waves using bisulfate (ammonium or sodium) are also used. These function in a similar fashion to the thioglycolate waves, but have lower negative odor. They are slightly less aggressive on the hair than thioglycolate waves and are subject to a greater degree of reversion to the original hair configuration. Nonetheless, they can be effective on the right hair and with the right expectations.

Permanent waving is moderately damaging to hair due to the alkaline nature of the products and the fact that not all of the cystine bonds that are broken re-form, leaving the hair weaker than prior to the treatment.

CONDITIONERS/TREATMENTS

Author
Charles Warren

Hair is continually being damaged by mechanical stresses (e.g., combing/brushing), thermal stresses (e.g., blow dryers), and chemical stresses (e.g., coloring/bleaching). As hair has no biological means of repairing itself, the damage is cumulative. Hair grows at a rate of 0.25 to 0.33 inches per month, so hair that is eight inches long (medium length) has undergone 2–2.5 years of cumulative damage. The additional mechanical stress of hair fibers rubbing back and forth across each other (fiber-fiber) interaction is an additional source of damage that is not usually even thought of.

Damaged hair becomes weaker, leads to breakage and fiber splitting, loses cuticle (the flattened outer protective layer of hair), and develops an overall negative surface charge. This negative charge results in "fly-away" hair, appearing frizzy and dull. Damage on the hairs' surface also interferes with the wicking of the natural oils (sebum) secreted at the hair roots, further exacerbating the negative appearance and lubrication of the individual fibers.

Conditioners and treatments serve to neutralize the negative charge on the hairs' surface, restore oil to the fibers' and leave the hair softer and shinier, at least on a temporary basis. Rinses, conditioners, and treatments generally serve the same functions, the difference being the amount and types of materials used. They are all usually oil-in-water emulsions (lipoidal ingredients suspended in a continuous phase of water, held together with emulsifiers). They usually contain a cationic (positively charged moiety) such as stearalkonium or cetrimonium chloride (quaternary ammonium compounds) and a blend of fatty alcohols, such as cetyl or stearyl alcohols. In addition, there are aesthetic ingredients such as fragrance and colors, microbiological preservatives and other conditioning/claims ingredients such as proteins, and vitamins.

Conditioners can be divided into the general categories of leave-in conditioners, rinses, moisturizing conditioners, volumizing conditioners, conditioning treatments, and specialty conditioners, such as oil or protein treatments. Again, there may be multiple names and types of conditioners, but they will usually fit into one of these categories.

The formulator needs to balance the ingredients to maintain a stable emulsion and deliver the end result claimed. Understanding the emulsion and the desired end result is usually a significant challenge and requires technical understanding as well as the ability to interpret all of the aesthetic requirements for the emulsion itself, the manufacture and dispensing of the emulsion, the feel of the product on the hands and hair as applied, and, most importantly, the final appearance and feel of the hair. Not enough conditioning will provide the hair with little benefit and too much conditioning can leave the hair looking/feeling greasy and weighed down. A poor emulsion will result in separation and instability, or if overemulsified, an unpleasant greasy and ineffective formula.

Leave-in conditioners are either water-thin aqueous solutions containing a cationic moiety (usually a cationic polymeric material) to neutralize charge and provide some conditioning to the hair fibers. They are low in lipoidal materials and usually dispensed via a spray directly on the hair or onto the hands and then worked through the hair.

Rinses are thin, rinse-out emulsions applied to the hair post-shampoo and contain lower percentages of cationic materials (quaternary ammonium salts), fatty alcohols, and ancillary or aesthetic ingredients. They provide a lower level of conditioning, desired by some consumers and effective in ameliorating the negative effects of relatively minor damage. As these are relatively thin emulsions, they can be problematic in maintaining stability over time and through extremes of heat and cold.

Moisturizing conditioners are emulsions containing higher levels of both cationic moieties (quaternary ammonium salts, cationic polymers) and lipoidal materials (fatty alcohols and oils). They are rinse-out, post-shampoo treatments and are dispensed into the hand and applied to the hair, left on for an interval, and then rinsed from the hair. They are generally thicker emulsions and have a creamy feel that is aesthetically pleasing to the consumer while being applied and worked through the hair, signaling efficacy while doing the actual conditioning of the hair. These conditioners are effective in neutralizing the negative charge, leaving residual oils on the hair surface and softening the hair fibers—the greater the degree of damage to the hair (and, thus, the porosity or ability for hair to absorb), the higher the degree of tolerance to lipoidal materials. For example, chemically relaxed (straightened) hair will tolerate and require higher levels of oils and conditioning materials than hair that is damaged by simple combing/blow drying. The formulator needs to be aware of the targeted consumer/hair-type when developing a formula. What delivers an effective and aesthetically pleasing level of conditioning and hair-feel to one consumer will be viewed as oily/greasy to another.

Volumizing conditioners will provide conditioning benefits to finer/thinner hair at low levels and will have the added benefit of leaving behind materials (usually polymeric materials) that will attach to the hair electrostatically and thicken the hair, resulting in a fuller-appearing coiffure. These conditioners are generally trickier to formulate, as consumers with fine/thin hair are highly sensitive to materials perceived as weighing the hair down, leaving it looking flatter and appearing oily or greasy. This category of conditioner is probably the smallest of all of the categories.

Specialty conditioner treatments (e.g., oil or protein treatments) are rinse-out, post-shampoo treatments intended to be applied on an infrequent basis and are generally for a specific problem (e.g., severely damaged hair). In general, the formulas contain higher levels of specific ingredients (e.g., fatty materials), are applied and left on for extended periods (1–10 minutes), and may include the use of a plastic covering (cap) or a moist towel. Some specialty treatments require a shampoo step after the treatment to remove any excess oils or conditioning ingredients. Relaxed (straightened), bleached, or extremely fragile or dry hair are the targeted types for these products.

HAIR COLORANTS AND PROTECTION

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ABSTRACT

Most permanent hair-coloring technology is based on the 100+ year-old approach of oxidation of *p*-diamines and *p*-aminophenols by hydrogen peroxide at alkaline pH to form an active intermediate. These active intermediates then react inside the hair fiber with couplers that produce colored molecules of varying size depending on the nature of the primary intermediates and couplers. Being bigger, these color molecules are trapped inside the hair fiber, thus providing resistance to removal. Permanent hair color, however, is far from "permanent." Factors such as frequent washing, UV exposure, and heat styling are generally known to cause hair-color fading.

Additionally, hair-coloring process imparts oxidative damage to hair lipids and keratin and disrupts hair cuticle. When the cuticle is disrupted, it becomes fragmented and rough, resulting in less light reflection from the hair surface. The dramatic increase in the use of hair-coloring products and other chemical treatments requires specific products for the care and protection of color as well as the hair that underwent damage due to oxidation of lipids and proteins. Enhancing the protection of color, shine, vibrancy, and overall appearance and conditioning of color-treated hair requires products that are formulated differently from regular hair care products. Color- and shine-protecting products contain substances that smooth down the cuticle and increase light reflection.

This chapter describes the fundamentals of permanent hair coloring, the change in hair properties associated with hair coloring, and the factors that influence color fading, vibrancy, and shine. Further, it also covers formulation techniques to deliver long-lasting color, color vibrancy, and shine and remedies for protection of color-treated hair to preserved color vibrancy and shine. Beyond these approaches, the author has also provided a unique and critical review of selected ingredients and products in the market offering various benefits for color-treated hair.

The underlying reasons and remedies for hair-color protection described in this chapter provide important leads to ingredient-seekers and product formulators for color care and presents an opportunity for a myriad of novel products that consumers long for. The chapter is also unique in that it provides thoughtful, practical recommendations for developing better hair color and hair care products to address the needs of color-treated hair. This wisdom goes beyond the traditional approach of finding new ingredients or formulations to achieve the goals of beautiful hair that is shiny and vibrant. It is recommended that these approaches be incorporated in literature by product manufacturers as important information to consumers.

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6.15.1 INTRODUCTION

Hair-coloring products are a multibillion-dollar international industry composed of three types of products: temporary, semi-permanent, and permanent hair-color products. The majority of its growth (~70%) is derived from permanent hair-coloring products, which owe their popularity to their long-lasting effect, ease of application, natural look, versatility, and allowing virtually any color to be achieved.[1] This industry is poised for even greater growth in the future due to an increasingly aging, and thus graying, global population longing for a youthful appearance. Permanent coloring technology, which is also called lift and deposit technology, has the ability to lift the natural color of hair and deposit new color that lasts until the hair is replaced by new growth. Most permanent hair-coloring technology is based on the 100+ year-old approach of oxidation of colorless p-diamines and p-aminophenols by hydrogen peroxide at alkaline pH to form active intermediates. Permanent hair colorants are normally marketed in the form of a two-component kit comprising an alkaline composition of oxidation hair dyes in a liquid, gel, or cream vehicle and a developer composition that utilizes an oxidizing agent, usually hydrogen peroxide. The two compositions are mixed immediately prior to application to the hair. The

alkaline pH of the resultant mixture causes the hair shaft to swell, allowing the dye precursors to penetrate into the cortex of the hair. These dye precursors are then oxidized and combine to form larger molecules. These larger molecules contain a significant level of conjugated double bonds, hence producing a colored product that is visible from the exterior of the hair. After an appropriate development time during which the composition resides on the hair, the mixture is rinsed from the hair. The color of the hair is then permanently altered.

Permanent hair color, however, is far from "permanent" due to color fading by factors such as frequent washing, light exposure, and other daily grooming habits such as heat styling. Permanent hair coloring changes hair structure due to removal of soluble lipids and oxidation of keratin protein and lipids. These changes in hair structure make colored hair to be more porous than virgin hair. As hair becomes more porous, it absorbs more color and it also removes more color on washing. The fading of permanent hair color due to frequent washing, daily grooming, and by environmental factors has become a common problem of color-treated hair and a source of frequent complaints by consumers.

Photo damage to the hair shaft contributes to dullness by altering the chemistry of the dyed hair through oxidative damage. UV exposure affects hair strength and elasticity and cause photo-oxidation, which triggers discoloration and fading in both natural and color-treated hair. Work by Ph. Maillan reports the use of a leave-on formulation with a polymeric organosiloxane sunscreen to protect the artificially colored hair. This polymeric filter was reported to be more effective than classic sunscreen ethylhexyl methoxycinnamate.

Luster or shine is another important feature of hair's appearance, and this visual effect is a key objective in the consumer hair care market. Thus, shine-protecting hair care products are worthwhile for the consumer with color-treated hair demanding youthful hair appearance. Color strength or color vibrancy is another important attribute for colored hair, but no parameter or test method has previously been developed to combine the two and enable quantified vibrancy claims. In relation, based on work by Lefaudeux et al., the authors define a new parameter for such claims, the hair-color vibrance factor (HCVF).

6.15.2 FUNDAMENTALS OF HAIR COLORING

Hair dyes can be divided mainly into three categories, each with a specific composition and action mechanism: temporary dyes (water-soluble dyes that withstand only one-time shampooing), semi-permanent dyes (which can withstand four to five shampooings), and permanent or oxidative dyes that needs reapplication only when new roots are shown. Temporary hair color works on the surface of hair to add sheer color and it typically only lasts one to two shampoos. When applied to prelightened or extremely porous hair, temporary hair color may penetrate further into the cortex and stain the hair shaft.

Semi-permanent hair color works on the surface of hair to deposit color, yet lasts longer than temporary hair color. Semi-permanent contains large and small color molecules that adhere to the outside of the hair, while some penetrate the cuticle layer, depending on the hair's porosity. Some color is removed with each shampoo, but color typically lasts from four to eight shampoos.

Oxidative hair dyes are the most popular hair-dye products. They may be further divided into three subcategories: permanent, demi-permanent, and auto-oxidative hair dyes. Permanent hair color contains ammonia and is mixed with developer, typically hydrogen peroxide, in various volumes in order to permanently change hair color. Depending upon the pH of the mixture and the strength of the developer, these systems have the capability to simultaneously lighten the hair's natural pigment and deposit color. The ammonia is an alkalizing agent that swells the hair fiber and opens the cuticle layer, allowing the color molecules and developer to penetrate into the cortex. Once inside, the developer lightens natural pigment and develops the color molecules so they remain permanently in the cortex. Permanent hair-color technologies' common uses include gray coverage, change of natural hair color, and lightening natural hair color. L'Oreal Excellence Crème hair color, Garnier Color Naturals Nourishing Permanent Hair Color Cream, Revlon ColorSilk, and Clairol Nice'n Easy are some examples of product in this category.

Demi-permanent formulas are ideal for enhancing, refreshing, or darkening hair color (either natural or previously colored). These formulas do not contain ammonia and use low-volume peroxide. Generally, they last up to six weeks, so they offer less commitment than permanent hair color. Because the developer is low strength, it is mild and the hair remains in optimal condition. Demi-permanents are also called "deposit-only permanent colors" as they do not lighten the hair, but only deposit color. Majority of the men's hair color in the market belongs to this class of dyes. Demi-permanent hair color is the fastest-growing color category in the industry because of its versatility. It is excellent for glazing or for refreshing previously tinted hair for added vibrancy and shine, and is essential for color correction. L'Oreal Healthy look crème gloss, Bigen Powder Hair Color, Just for Men Natural Hair Color, and Clairol Natural Instincts Hair Color are some examples of product in this category.

Auto-oxidative hair dyes involve the oxidation of dye precursors by oxygen in air without the use of an oxidant. This technology is based on precursors such as 1, 2, 4-trihydroxybenzene, 2,4-diaminophenol, 2-methoxy-p-phenylene diamine. Unlike, classic oxidative dyeing, hair is not lightened, so such systems are used to color gray hair or to change color from lighter to darker shades. Formulas containing these dyes involve no mixing, they are sold in one component and free of ammonia and hydrogen peroxide, and hence are milder than both demi-permanent and permanent hair-color products. Just for Men AutoStop hair color is an example of product in this category.

Concern over the safety of PPD and other hair-dye ingredients, the damage of hair due to oxidative coloring, and the demand for more convenient hair dyeing

methods has fostered an increase in research in this area on new dyes and alternative hair-coloring technologies. Manufacturers have in recent decades embarked on intense research toward new dyes and precursors, as well as into alternative technologies for permanent hair dyeing. This research has been aimed not only at addressing potential toxicological issues but also technical weaknesses such as to mitigate hair damage, long-lasting color, no ammonia, no peroxide hair color, ease of application, and convenience.

6.15.3 FACTORS INFLUENCING COLOR FADING AND COLOR REMOVAL

Permanent hair-coloring products are formulated to provide excellent gray coverage, change existing hair color through removal of current hair color (lift) and deposit of new colors. Additionally, permanent hair-color products are formulated to give long-lasting and vibrant hair colors that provide overall youthful appearance. Several factors starting from formulation, product application, frequent washing, daily grooming habits, and environmental factors such as sun and pollution can accelerate color changes, color fading, vibrancy, and shine.

- **Color formulation and color application.**

When formulating hair-color products, several factors need to be taken into account in designing the appropriate vehicle. If color has not been given enough time to penetrate the hair shaft, color molecules will stay inside the cuticle layers and on the surface, allowing them to be washed away quickly. Most of fading appears after the first two washes, indicating that the color molecules settled on the outside of the hair shaft and had not penetrated deeply enough to be retained through washes. U.S. patent by Padmaja et al. teaches a two-step hair-color method, where the color precursors are applied in a first step that provides adequate time for effective penetration inside the hair fiber. In a second step, the hair is treated with hydrogen peroxide, so the reaction takes place inside the fiber and the dyes are trapped in the cortex, making them resistance to washing. The authors have shown more vibrant and longer-lasting color effect from this two-step system compared to conventional one-step hair coloring. In traditional permanent hair-coloring products as soon as the dye precursors are mixed with the developer, two competing factors come into play, the diffusion of dye precursors and peroxide into the fiber, the reaction of color precursor with peroxide outside the fiber. An optimal balance between the two factors contributes to overall color quality, long-lasting color, and color vibrancy.

- **Cuticle damage.**

The cuticle is the primary protection for the hair cortex where the majority of color molecules are housed. If friction or excessive chemical treatments damage the cuticle and strip away its protective fatty layer, the exposed cortex will chip away, and color

molecules will escape during shampooing. Damaged hair is more porous and hydrophilic and hence absorbs more color during coloring and loses more color during washing. Protection of the cuticle with shampoos and conditioners that contain friction-reducing silicones helps reduce this type of color loss. Repair of hair damage using daily shampoos and conditioners containing protective polymers improves the state of the fiber and minimizes color loss due to the damage aspects of hair.

- **Rinsing with water.**

Fading due to water exposure is often related to a damaged cuticle. When the cuticle is overly compromised, water can more easily enter the cortex and some of the color molecules can be pulled out and washed away. Shampoos do not strip color. Studies show that water alone is responsible for the majority of color lost during shampooing.

- **Exposure to sunlight (UV and visible light).**

UV light is known to fade color in many substrates such as wood, cloth and paint. Hair is no different. UV radiation penetrates the hair and breaks down color molecules. The process happens with both natural and artificial pigments in the cortex and is the reason people develop highlights after spending long periods of time in the sun. UV protectants are added to some shampoos and styling products to help prevent hair color fade due to sunlight, although their efficacy is debated.

- **Some hair-color shades fade more quickly than others.**

Red shades tend to fade more than brown or black shades due to their relatively small molecule size, which can diffuse from the hair and wash away more quickly than other shades. UV exposure also breaks down the red tone more easily, while pure browns and blacks resist fading because their color molecules tend to be larger. Blonde shades have little color, so fading is less of a concern.

6.15.4 COLOR PROTECTION

Consumer demand for high-performance, vibrant, and fashion-oriented, yet natural-looking hair colorants continues to grow. To meet this trend, formulators must create high-impact products that provide lasting, natural-looking color as well as improved hair shine and conditioning effects. In recent years, the number of hair care products specifically designed to address such needs of color-treated hair is steadily increasing. The number of products claiming color protection continues to increase, and consumer product makers employ a variety of formulation strategies to address the needs of people who have color-treated hair. Rinse-off products such as shampoos and conditioners dominate the market and typically contain ingredients that emphasize the benefits of moisture retention, deposition of protective

films on hair surface, and protection of color from sun fading using UV filters. Leave-on products, on the other hand, emphasize ingredients that serve to seal hair through deposition of polymers. Both formulation strategies address consumers who want to maintain the vitality of natural-looking hair following hair-color treatment. Naturally, consumers have a strong interest in adopting a hair care regimen that can extend the color vibrancy of hair, yet color fading and the loss of color vibrancy typically comes with everyday cleansing.

Studies by X et al. showed that color fading primarily occurs through physical removal of dyes by rinsing with water. These authors have investigated the physical and chemical factors that influence hair-color removal during the washing process by testing hair dye dissolution in water from dyed hair samples with variation of surfactant type, pH, and hair type. They have investigated a series of polymers with various functional groups and demonstrated that polymers with hydrophobically modified and cationic functionalities are the most effective in preventing hair-dye dissolution in water. A primary example of a polymer within this category is a cationic terpolymer of vinylpyrrolidone, dimethylaminopropyl methacrylamide, and methacryloylaminopropyl lauryldimonium chloride (INCI: Polyquaternium-55). Figure 1 shows the anti-fading test results of the four polymers in a leave-in treatment at 2% level using bleached hair tresses. The results demonstrate that Polyquaternium 55 (VP/DMAPA/C12-MAPTAC copolymer) leave-in treatment provides the highest color protection with 38% higher color improvement at the end of 10× washes over the control, and the effect is well perceivable by eye as shown in the picture.

Figure 1. Pictures and percentages of color protection for dyed hair samples after 10× SLES washes and leave-in treatment of 2% of various polymers.

Both visual and instrumental measurement results indicate that Polyquaternium-55 provides a high level of color protection when formulated in a hair-color protection regimen with up to 50% color protection. Conditioning shampoos and conditioners with cationic surfactants and water proofing amino silicones can help combat the effects of water exposure.

Croda Inc. has commercialized a quaternized diester (ChromAveil) claimed to provide broad-spectrum sun protection to hair in rinse-off systems. It is a broad-spectrum UV filter that protected hair from color fading and hair integrity damage. Figure 2 below shows that hair treated with the diester shampoo protects the color from UV fading as evidenced by the lower value of Delta E, the total color change.

Figure 2. Total color change of medium brown-colored hair treated with different shampoos after 15 days of UV exposure

Silicones can help protect hair treated with permanent and nonpermanent colorants from discoloration and fading. Some color stays on the upper layers of cuticle and can be removed during washing. With its spreading behavior, silicone may help colorant further penetrate the hair cuticle for longer-lasting retention. By forming a water-insoluble film, the silicone may also help prevent color from washing out due to the hydrophobic nature of the silicone deposits on hair surface. Based on tests with several silicones, Figure 3 shows color retention properties for two silicones at 2% active level in a rinse-off conditioner. In this series of results, expressed by ΔE value, the silicones significantly reduced color loss compared to the control (the same rinse-off conditioner without silicone). The lower ΔE value for hair treated with the silicones indicates they help maintain color intensity and depth.

Figure 3. ΔE through seven cycles (each cycle = 7 hours of UV exposure + shampoo rinse + conditioner rinse at 2% active silicone)

A siloxane copolymer from Evonik grafted with methoxycinnamic acid ester and cationic alkoylamidopropyl dimethyl ammonium groups called Polysilicone-19 (trade name ABIL UV Quat 50) was investigated for color protection from UV. An active level (0.5–1%) of this silicone was incorporated in a shampoo and conditioner formulation. Colored hair tresses were treated with the shampoo and conditioner and irradiated by UV for a period of time. Color fading was compared to an untreated hair tress and other competitor ingredients. In a shampoo formulation at 0.5%, the silicone offered 50% reduced color fading and at 1.0% level from a conditioner application it offered 40% reduced color fading. The new silicone, Silicone Quaternium-22 microemulsion (trade name: ABIL® ME 45), offers significant color protection against washing. As a result, the combination of Silicone Quaternium-22 microemulsion and Polysilicone-19 in shampoos and conditioners can provide comprehensive color protection from UV and washing and premium hair-conditioning benefits with both visible and tangible benefits for consumers.

New color protection products include leave-on systems. Leave-on systems allow formulators to include functional ingredients like cationic film-forming polymers that bind with negatively charged amino acids on the surface of hair. These types of film-forming polymers can be found primarily in hair-styling gel and mousse formulations designed for hair hold, but they can also

be used in personal care applications where a need exists to form a protective barrier on the hair cuticle. This protective barrier phenomenon can be strongly applied to products claiming hair-color protection. The film formed on the hair is believed to protect hair color from losing its vibrancy as a result of excess washing.

6.15.5 COLOR VIBRANCY AND SHINE

Human perception of color depends on the ability of our eyes and brains to detect and interpret the reflection of light on objects. When light shines on hair, part of it penetrates and is absorbed by the dye molecules, and part is reflected back. Shine-inducing shampoos and conditioners can be used on almost all hair types, creating the illusion of healthier hair. Darker hair tends to be shinier than lighter hair because it reflects light better, but as long as the cuticle and shaft are smooth, light is properly reflected off each individual strand. Hair shine is an important feature of hair appearance and this attractive visual effect is a key consumer objective in the hair care market. Hair-color intensity is another important attribute for colored hair.

Certain products and ingredients can enhance this effect. Conditioners are especially important when it comes to achieving shine because they replace natural oils and sebum that is lost further down the hair shaft. They penetrate, adding back lost moisture and giving the hair the ability to reflect light and therefore look shinier.

Consumers always want to have their colored hair appear bright and striking, or vibrant. Until now, neither a parameter nor a test method has been defined and developed to make such quantified "vibrancy" claims. Lefaudeus et al. have published principles for measuring hair luster (shine) using a Color Hair Visual Appearance Study System (SAMBA Hair system). SAMBA Hair System based on an innovative polarization camera SAMBA. They have observed that there are two bands on captured colored hair images: the "Shine" band (first reflection, no color) and the "Chroma" band (second reflection with color). The authors have defined an "Overlapping Coefficient" to describe overlapping degrees between "Shine" and "Chroma," the scalar product of the shine distribution and the chroma distribution. They have observed that the overlapping coefficient increases after specific cosmetic treatments and the treated hair demonstrated correspondingly shiner and richer color. Based on these observations and the established interaction mechanism between hair fiber and the light, a new parameter called "Hair Vibrancy Factor (HCVF)" is defined to describe shine and color intensity. Figure 4 shows the hair vibrancy factor measured on auburn-colored hair swatches after treatment with two hair-shine sprays using SAMBA.

Figure 4. HCVF values of various hair samples measured using SAMBA

6.15.6 REMEDIES FOR COLOR PROTECTION, VIBRANCY, AND SHINE

The color-treated hair structure has a unique set of needs and characteristics. Dry hair that feels like straw and a dull, drab appearance are common complaints among those with color-treated hair. During oxidative hair coloring, the chemicals can also penetrate deep into color-treated hair and change the internal structure. Hair-strengthening proteins are attacked and porosity also increases, affecting hair's ability to hold the color molecules within the fiber. This leads to weaker hair that is more susceptible to hair-color fade. Red hair is especially susceptible to color fade, as the intense red pigments are the smallest and easily escape from a porous hair structure. Color-treated hair needs specialized care, so shampoos with gentle cleansing that protects the integrity of the color while still removing dirt, styling aids, and excess oils should be used. Damaged hair lacks moisture, so color-treated hair shampoos also infuse fresh hydration to help rejuvenate the hair's natural moisture. This grouping of products is further broken down into specialized formulas that provide additional capabilities, such as adding volume, protecting against UV damage, preserving color, enhancing color, augmenting highlights, and correcting tone.

Many color-safe shampoos and conditioners, particularly those that are geared toward a certain shade of hair color, will contain small bits of color and color enhancers to give your hair color a refreshed look after washing. They are wonderful for slowing the color-fading process. In addition to a good, color-safe shampoo and conditioner, you should select supporting daily products that contain sunscreens and light oils. Keep your hair thoroughly moisturized using conditioners that are specifically designed to address the needs of color-treated hair. Regular conditioning goes a long way toward preserving hair color and keeping the hair radiant.

Finally, use finishing products like hairsprays, spritzes, mousses, and gels sparingly. The alcohols used in these style-setting products are used to help your hair dry faster in your style, but can fade your hair color prematurely. Some have also reported that volumizing product lines, or those for fine hair, also fade new hair colors quickly because they allow a slight lifting of the cuticle layers to provide the illusion of increased hair-shaft thickness.

6.15.7 INGREDIENTS AND PRODUCTS FOR COLOR-TREATED HAIR

Ingredients for color protection, shine, and vibrancy include the well-established ones such as: silicones for color protection and shine, quaternary ammonium compounds for ease of combing, and conditioning and vegetable oils for shine. Formulating shampoos and conditioners with just 2% of Dow Corning's CE-8411 nonionic silicone emulsion provide color protection benefits, enhance hair shine, and help prevent hair breakage. Many ingredients are recently launched to address hair and scalp aging at the cellular level, improve follicle health, reduce hair loss, and offer antioxidant properties.

Natural vegetable oils provide nourishing conditioning and shine to hair. Coconut oil, being a triglyceride of lauric acid (principal fatty acid), has a high affinity for hair proteins and, because of its low molecular weight and straight linear chain, is able to penetrate inside the hair shaft.[21] Avocado and Jojoba oil are other oils for nourishing, conditioning, and shine benefits. Sunscreens are another category of ingredients now found in a variety of hair care products, including shampoos, conditioners, and hairsprays. They prevent breakdown of keratin due to sun exposure and also keep hair dye from fading in ultraviolet light. Ingredients like Crodasorb™ UV-HPP from Croda and PARSOL® SLX provide multiple benefits like prevention of color fading, strengthening hair, and enhancing gloss when delivered through hair color, or hair care products, especially leave-on sprays. Table 1 below lists some advanced functional hair care ingredients for color protection and shine popular in today's hair care market.

Table 1. Advanced hair care ingredients used for color protection and shine

Hair Care Ingredients	Benefits	Supplier
ABIL® UV Quat 50	Efficient protection of hair color against fading in sun light. Protection of fiber integrity against damage by V irradiation.	Evonik
Linseed Oil™	Linseed Oil provides hair-color protection and radiant shine to color-treated hair.	Textron
ChromAveil ®	Protects the hair from the damaging effects of sunlight. Gives color protection benefits to dyed hair from UVA and protects the mechanical properties of the hair from UVB.	Croda Inc.
Crodasorb™ UV-HPP	Polyester polyquaternized ingredient that is substantive to hair and protects against the damaging effects of UV-B radiation	Croda Inc.
Lustreplex®	Creates lustrous, healthy looking hair from anionic systems. Provides frizz control, shine, detangling, and conditioning benefits.	Croda Inc.
Dow Corning® CE-8411 Smooth Plus Emulsion	Repair colored, gray, and heat-damaged hair by restoring moisturized feel, shine, & alignment. Protects hair from further damage and color loss.	Dow Corning
Dow Corning® 5-7113 Silicone Quat Microemulsion	Restore smoothness, shine and Alignment. Prolong hair color. Protects hair from breakage.	Dow Corning
Coconut Oil	Provides conditioning, smoothness, and vibrant shine to color-treated hair.	Natural Sourcing
PARSOL® SLX	PARSOL® SLX is a silicone-based UV-B absorber delivers multiple benefits including prevention of color fading, gloss enhancement, and conditioning.	DSM

The hair care market is overloaded with products specifically designed to care for color-treated hair and protect the color from fading, so color can last longer. Redken has launched Color Extend, a collection of four products—shampoo, conditioner, treatment, and color protection—for complete care of color-treated hair. Shampoo for color-treated hair protects hair color from fading and provides UVA/UVB ray protection. Conditioner resurfaces the cuticle for a smooth, vibrant finish and helps with color retention; anti-fade protection maximizes color vibrancy and strengthens hair.

Protective treatment provides deep conditioning to leave hair manageable and vibrant. Specially formulated to provide stronger protection to extend life of hair color, this treatment for color-treated hair uses Fade Resist Complex with UVA & UVB filters to help lock in color and block out aggressors. Hair-color protection spray delivers anti-fade color protection and cuticle reinforcement to maintain color vibrancy and shine.

Pantene has recently launched a range of seven products for color-treated hair, Smooth Shampoo & Conditioner, Volume Shampoo & Conditioner, Color Seal Concentrate, two-minute Damage Rescue Treatment, and Instant Nourishing Shine Spray. The active formula in this shampoo helps to preserve the color for long-lasting brilliance. The active formula in this shampoo helps to preserve the color for long-lasting brilliance. This hair-moisturizing shampoo cleanses your hair while also infusing it with hydration. It features special moisturizers that build a layer around the hair fibers to protect hair from damage and color loss. Shampooing with the Pantene shampoo will add vibrancy and volume to color-treated hair.

6.15.8 CONCLUSIONS

Consumer demand for high-performance, vibrant and fashion-oriented, yet natural-looking hair colorants continues to grow. The global increase in hair-color products raises the need for color care products in the market. The number of hair care products specifically designed to address such needs of color-treated hair is steadily increasing and products claiming long-lasting color, shine, and color enhancement continue to increase. Rinse-off products such as shampoos and conditioners dominate the market and typically contain ingredients that emphasize the benefits of moisture retention, deposition of protective films on hair surface, and protection of color from sun fading using UV filters. Leave-on products, on the other hand, emphasize ingredients that serve to seal hair through deposition of polymers. Both formulation strategies address consumers who want to maintain the vitality of natural-looking hair following hair color. Despite the improvements made on color protection using specialized hair care products containing silicones, vegetable oils, and cationic polymers, a complete color protection is still far from reality. This is due to the fact that color loss, and loss in shine and vibrancy, are

linked to hair damage and the majority of hair-color products in the market induce hair damage during the hair-coloring process. Over the years, hair-color product manufacturers have continually improved hair-color formulations to minimize hair damage. A complete damage-free hair color remains as a dream despite of the technological advancements. Regular use of color-treated hair care products significantly improves the appearance of color-treated hair, providing shine and vibrancy, and it prolongs the life color by 40–50%. This chapter provides an overview of the fundamentals of hair coloring, the factors influencing hair fading, and finally a compilation of some hair ingredients and color-protection products currently available in the market.

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REACTIVE HAIR PRODUCTS

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Reactive hair care products are those products that chemically react with or in the hair to provide a desired effect. These products are hair colors, bleaches, relaxers/straighteners, and permanent waves.

6.16.1 COLORS

Temporary Hair Colors

There are generally two types of products in this category: hair cosmetics and temporary stains.

Hair cosmetics are generally suspensions of colored pigments (e.g., iron oxides) and/or tinted glitter particles in an aqueous or hydroalcoholic solution containing holding resins (e.g., VP/VA copolymer). These formulas are applied directly to the hair and upon drying provide visual effects—"color" or "sparkle," depending on the formulation. The pigments/glitter are actually incorporated in the film on the hair and, therefore, can be easily removed by breaking the film and brushing the particulate out or by simply washing the hair to remove the film and the particulate.

"6 Wash" Colors

Temporary stains are actually mixtures of direct colors (i.e., the color molecules are the color they will remain) applied to the hair to stain the exterior (cuticle) with minimal penetration to the cortex. These "direct dyes" are usually applied in a shampoo or shampoo-like solution and can be simply washed in (color-enhancing

shampoos) or applied and left on the hair for a period of time to achieve the depth and color desired. Color-enhancing shampoos provide minimal "gray" coverage and are used primarily to refresh existing color. Temporary colors (referred to as "6 Wash Colors") provide coverage to gray that is less than 10% of the whole hair volume and will remain on the hair for approximately six washes. Each washing or wetting of the hair will remove some of the stain, so care must be taken that the dyes are removed in a similar fashion so that the color remains as true as possible.

"Demi"-Permanent (non-ammoniacal, 12 wash colors)

Demi-permanent hair colors are two component colors. The first component, the color solution, is an aqueous mixtures of traditional hair-dye intermediates (e.g., paraphenylenediamine or PPD), monethanolamine (MEA), surfactants, and additives. The mixture of the dye intermediates is critical to the development of the desired colors and is very similar to the standard permanent hair-color mixtures. The MEA makes the solution slightly alkaline to assist with the color development when mixed with the developer.

The second component, the developer, is an aqueous solution of hydrogen peroxide (generally, 3–6%).

When mixed together, the MEA helps make the hydrogen peroxide more reactive with the dye intermediates to develop the highly colored polymerized color molecules that ultimately are the desired color. The MEA also serves to swell the hair and allow permeation of the developing color polymers into the subcuticular layers of the hair. Because the hydrogen peroxide is at a relatively low concentration and the MEA only slightly raises the pH (7–8), this coloring process results in little, if any, bleaching of the native hair color—thus, the "tone-on-tone" nomenclature for these type colors. Demi-permanent colors will not allow for a shade lighter than the starting shade. Demi-permanent colors will last for 12–20 shampoos and will provide coverage of gray hair that is less than 25–30% of the total hair volume.

Because demi-permanent hair colors function at lower pH and lower peroxide levels, there is relatively little damage to the hair. This category of hair color has seen the largest share of growth in the consumer market over the past 20 years.

Permanent Colors

Similar to the demi-permanent colors, the permanent color products are two component systems: the color solution and the developer solution.

The color solution is an aqueous mixture of reactive dye intermediates (e.g., PPD), ammonia, and additives. As in the demi-permanent colors, it is the mixture (quantities and types of intermediates) that yields the ultimate final color. The pH of this color solution is usually around 9, one of the significant differences between demi- and permanent colors.

The developer solution is an aqueous solution of hydrogen peroxide. The peroxide content of the developer solution can vary from 6% to 12%. There are some higher peroxide level developers, but those are predominantly only in the professional salon market and relatively few in number. Peroxides are also referred to by "volume" of oxygen delivered. For practical purposes, 6% = 20 volume, 9% = 30 volume, and 12% = 40 volume. In the retail market, 6% is the most common peroxide level.

When the two components are combined, the peroxide becomes extremely reactive and quickly initiates polymerization of the dye intermediates and, simultaneously, the bleaching of the native color. The high pH of the mixture swells the hair and allows the dye intermediates and peroxide to quickly penetrate throughout the hair shaft. The highly colored, polymerized dyes become too large to leave the hair and therefore replace the color of the hair. The higher the level of peroxide used will dictate the degree of bleaching that will be accomplished. Permanent hair coloring can lighten the hair significantly and can result in significant color change. These colors are durable and will usually last six weeks or through 24–30 wash cycles. By this time in the process, root growth has become the factor that usually requires the re-colorization process.

Permanent hair coloring is the most damaging of all of the additive coloring processes. This damage can be ameliorated by using a "conditioning" base, or a special conditioning treatment for post-coloring application.

6.16.2 BLEACHES

Bleaching is the process by which the native melanin in the hair is partially or completely destroyed, leaving the hair slightly to radically lighter than the starting point. Highlighting is the bleaching of selected portions of the hair to create a mixture of light and darker hair throughout the entire coiffure. Multiple techniques of application are used to create multiple effects, but the fundamental destruction of native melanin remains the same.

Bleaching can be accomplished by mixing an aqueous, ammonia (ph = 9–10), surfactant solution with 6%–12% hydrogen peroxide and monitoring the color removal until the desired shade is attained. Bleach powders, blends of highly reactive peroxydisulfates (sodium, potassium, and ammonium salts), can be mixed with peroxide to provide faster and more complete melanin destruction. Bleach oils, mixtures of lipoidal materials, and surfactants can also be employed to provide easier spreading throughout the hair and easier removal once the desired shade is attained.

Due to the high pH and high levels of peroxide (which not only destroys melanin, but can oxidize the inherent protein comprising hair), bleaching is one of the

most damaging processes done on the hair. The hair is left in a weaker state and is therefore subject to more dryness and breakage than before the bleaching operation. Overbleaching, resulting from too high a level of bleaching or repeated bleach processing to the same hair, can and will result in breakage.

6.16.3 STRAIGHTENERS

Hair straightening has existed since the time of the ancient Egyptians. Extremely kinky, curly hair (e.g., Afro-Caribbean hair) or highly curly hair (e.g., European hair) can be very difficult to manage and style. Straighteners have been developed to release the natural curl of the hair and leave it more amenable to traditional styling.

Relaxers

The use of alkaline material to straighten curly/kinky hair has been documented from the time of the ancient Egyptian dynasties when women at court applied mud mixed with ash to the hair and left in place until the desired straightness was achieved. Today's relaxers are technically superior, but apply the same basic chemical principles.

The backbone of all human hair, the reason that hair has its configuration, tensile strength, elasticity, etc. is attributed primarily to the cystine (-S-S-) cross-linked bonds throughout the hair. Clearly to change the configuration of the hair, these bonds need to be altered. Highly alkaline conditions can convert the cystine linkages (-S-S-) to lanthionine linkages (-S-) while the hair is being mechanically placed into a new configuration.

As indicated, relaxing hair is both a chemical and mechanical process. The cystine bonds must be broken (chemical) and the hair must be placed and held in a new configuration (mechanical) until the new lanthionine bonds (chemical) are reformed, keeping the hair in its new configuration.

Sodium hydroxide (also known as lye) is one of the oldest alkaline agents used to straighten hair. At a concentration between 1.8% and 2.1%, sodium hydroxide–containing relaxers can effectively straighten hair to a level of manageability 15–20 minutes. The earliest versions of relaxers were simply lye (NaOH) mixed in with a thick animal fat (e.g., lard). Subsequent versions became more sophisticated, but still required a step called "basing," wherein a layer of non-lye-containing grease was placed as a protective barrier on the scalp and on the skin around the hairline. Modern relaxers do not require this "basing" step and are therefore referred to as "No-Base" relaxers.

The modern alkali relaxer is a mixture of an aqueous phase containing the alkali ingredient (sodium hydroxide, potassium hydroxide, or lithium hydroxide), propylene glycol, and other water-soluble ingredients and an oil-phase-containing

petrolatum, mineral oil, and fatty alcohols held together in a slightly stable emulsion by an emulsifier. The oil phase is mixed and then the aqueous phase is added. The mixture is mixed hot until semi-emulsified and then cooled. As it cools the non-emulsified petrolatum thickens along with the emulsion and serves to physically hold the entire mixture together. The resultant mixture can best be described as a dual emulsion (part oil-in-water and part water-in-oil). This quasi-emulsion is by design so that when applied and worked through the hair, both oil and water containing the alkali will be released onto the hair to accomplish the chemical breakage of the cystine bonds. The thickness of the petrolatum helps hold the hair straight while the lanthionization process is completed and hair is chemically held in a new straighter configuration. When the desired straightness is attained the mixture is rinsed out and washed out with an acidic shampoo to neutralize any remaining alkali.

Alkali relaxation is damaging to the hair and is corrosive to skin if allowed to remain in contact for too long a period. Overrelaxation can result in immediate hair breakage or significant hair breakage over a short period after the process. Skin damage can result in mild edema or significant burning if contact time is prolonged. Once successfully completed, the hair requires conditioning and addition of significant levels of oil to remain soft, pliable, and have a pleasing appearance. Regrowth will still be kinky/curly and is usually the driving force for a new application (new growth only) in four to six weeks.

In the 1970s the modern consumer "no lye" relaxer was developed. These relaxers are two component relaxers. The relaxer crème is simply the oil phase (quasi-emulsified), similar to the alkali relaxer in which is suspended relatively insoluble calcium hydroxide particles. The activator is an aqueous solution of guanidine carbonate. When the two components are mixed immediately before application, guanidine hydroxide is formed, which chemically performs exactly like the alkali relaxers, creating the new lanthionine linkage in the new configuration. This process is slower than a straight alkali relaxer, as it takes time for the guanidine hydroxide to form and react with the cystine in the hair. This lower reactivity makes the relaxer less potentially damaging to the hair and scalp and is the main reason that most relaxers sold at the retail, use-at-home level are of this type. However, the potential safety issues are still there and hair breakage and skin damage are still possible. The opportunity to prevent these negative occurrences is greater with this type of relaxer. Thorough rinsing and shampoo with an acidic shampoo is still required. Guanidine hydroxide is unstable and will decompose in a matter of hours, so the mixed relaxer must be used immediately and disposed when process is complete.

Relaxers based on conventional reducing agents (thioglycolates and bisulfites) have been marketed. These relaxers are based on the reduction of the cystine bonds

by the reducing substance, mechanical reconfiguration of hair, and subsequent reformation of the cystine bonds by the use of hydrogen peroxide (with thioglycolate) or alkaline shampoo (with bisulfate). The reducing agent is generally in an aqueous gel form, applied to the hair, combed straight and then placed under moderate heat. The reducing solution is then rinsed and followed with the neutralization step, an aqueous solution of hydrogen peroxide 2–3% or an alkaline shampoo (pH 8). These reducing relaxers are not as effective on kinky, curly hair and moderately effective on curly wavy hair.

The "Japanese" straightening system employs a reducing solution followed immediately by pressing the hair in a hot flat iron in thin sections. Peroxide neutralization follows. This system is highly effective but requires significant time and operator expertise.

The most recent relaxer/straightening system is the "Brazilian" or "Keratin" straightening system. This system is an aqueous solution of hydrolyzed keratin and a significant quantity of formaldehyde or formaldehyde precursor chemicals. When heat is applied via hot flat iron, the resultant formaldehyde cross-links the protein and holds it in the new, straighter configuration. While highly effective in straightening and retaining hair in straight configuration, formaldehyde has significant safety issues, especially in the gaseous form, created when the hot flat irons are used.

Blow Dry Lotions

Straightening lotions, creams, gels used with blow dryers are generally mixtures of high-molecular-weight silicones that allow the hair to be continually brushed while heat is applied via a blow dryer. This approach will straighten moderately curly hair, but the effect is temporary and highly susceptible to reversion of the hydrogen bonds from applied water or environmental humidity.

FORMULA/PRODUCT DEVELOPMENT FROM THE FORMULATOR'S VIEWPOINT

(Expectations, Initial Prototypes, Final Prototypes)

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ABSTRACT

Formulators must always be aware of what the consumer requires and expects from the formula being developed. Each category and subcategory has its own particular set of expectations and requirements. The best way to understand these is to listen and observe consumers talking about the particular products/formulas and to observe those same consumers as they talk about them. Marketing groups usually conduct focus groups where consumers will talk about their issues or their experiences with products. These discussions can be invaluable to a formulator who wants to create a successful product.

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6.17.1 FUNCTIONALITY/PERFORMANCE

Every product must deliver against its primary function—shampoos must clean, conditioners must provide a conditioning benefit, hand and body lotions must leave the skin moisturized, etc. Unfortunately, not every consumer will love every product because no two consumers are exactly alike. Formulators will develop functional products that will meet the performance requirements of a large number of consumers—that's the best they can hope for.

Form

Consumers will always gravitate to products that deliver on their promises. They are more likely to work with a product that is in a form that they are familiar with and is easy to use. The formulator must consider all of the aspects of consumer use while developing a particular formula. A cream or a lotion that is difficult to dispense or leaves a large amount of product unused in a package will quickly be abandoned by the consumer, and that consumer will look to one of the other hundreds of similar products available.

A good formulator will survey the competitive product arena in the specific area where they are formulating to determine what consumers are interested in and how those products are used and received. New and novel approaches are always of interest, but need to be evaluated thoroughly to ensure that the new form will be accepted by the desired number and category of consumers.

Beliefs

Learning, working with, and formulating against consumer beliefs can be a challenge for the formulator. These beliefs vary in individual markets and can vary wildly across cultural barriers. For example, in the United States most consumers believe that a shampoo or bodywash should deliver copious amounts of foam to deliver the cleaning they require. In other countries or cultures, excessive foam is viewed as a distinct negative. The formulator needs to be aware of the beliefs surrounding the formulating area of interest. Again, a survey of the successful products in a given market can provide insight into what the consumers may believe about the category.

A product that is sold as a moisturizing or conditioning will probably have to have a creamy texture and be opaque or translucent to have the consumer believe that it will actually perform. A clear conditioner or moisturizing lotion may provide excellent conditioning and moisturization, but would have a fairly high belief hurdle to conquer with the consumer. Clear products can be easily believed to deliver good cleaning properties, and expectations of conditioning or moisturization are low from the onset.

In addition to visual beliefs, are the olfactive beliefs. In some cultures lemon-scented cleanser will be thought of as cleaning while a vanilla-scented cleanser will be thought of as conditioning/moisturizing. A formulator must be careful to not unconsciously set up belief contradictions in the consumer's mind. Fragrance suppliers can be of great assistance in understanding the role that fragrance plays in the perception of product performance.

Post-usage beliefs are another issue for the formulator. If the hair or skin is left with a perceived coating or residue after usage, the consumer can believe that the hair or skin is coated and not aesthetically acceptable even though the residue may play an important role in delivering the functional benefit. Hairsprays or styling products that do not create a stiff film (e.g., "crust") will be perceived as not holding strongly enough even though consumers may say that they dislike the "crust" and would prefer products that do not impart that degree of stiffness.

Listening to the consumer, observing the consumer, and reading consumer comments are the best way to understand beliefs and find a way to use those beliefs to create more acceptable formulas.

6.17.2 MARKETING REQUIREMENTS/EXPECTATIONS

The Marketing groups, with whom formulators work, also have a large and varied set of requirements and expectations. In order for a formulator to develop formulas that address these, there must be constant communication and clarification with their Marketing partners in order to avoid unpleasant reactions or surprises related to the formula developed.

Functionality/Performance

Marketing will always expect the formula to deliver against the basic premise of the product—a fair expectation. They will always hope that the formula will perform better than the competition, support all of the claims that they would desire to make, and be less expensive to allow for lower price points and higher profits—more complicated expectations.

Communication between the formulator and the Marketing group early on and continuously throughout the development and finalization process can help manage expectations and can often lead to better formulas that fulfill most of the requirements (technical and marketplace) of the product. Upfront definition of product requirements, competitive performance requirements, advertising claims performance, and cost requirements are of utmost importance. If the organization does not have a "Product Description" process, a formulator will need to create one that provides information and standards that must be considered and used while formulation is in process.

Form/Package

Differentiation between products in the market is always desired. A new product form or a revolutionary new package are methods of differentiation in a crowded marketplace that can make a formula/product stand out. However, these forms and packages can create significant issues in development, testing, usage, and stability for the formulator. Part of the early definition of the product needs to identify all of the issues for a new form or package and, then, throughout the development process all of these issues (plus new ones discovered) must be communicated and resolved for both the formulator and the Marketing group.

Cost

Formula cost and total product cost (formula, packaging—primary and secondary, manufacturing, etc.) are a significant issue for the formulator. Again, early identification of formula cost allowable will dictate what the formulator can choose from the formulation toolbox. Rarely is total cost freedom provided to a formulator, so an eye must always be kept on the cost/benefit equation during the development of formulas.

Formula costs are generally thought of as the cost of the raw materials used to assemble the formula, and those costs are usually what appear in the financial analysis of a new product. However, other costs need to be identified early on to keep the entire process cost-effective. Testing costs (consumer use testing, claims testing, specialized analytical testing, competitive products, required efficacy testing, safety testing, packaging for testing, etc.) can all be hidden costs that need to be identified, discussed, and approved prior to and during formula development.

Stability/Shelf-Life

Expectations that a formula and entire product will remain stable and consumer acceptable throughout the shelf-life of a product are reasonable and required. A formulator must create formulas that will remain stable (i.e., not separate, not change color, not develop malodor, not negatively interact with packaging, continue to dispense and perform properly, etc.) from manufacture through warehousing through shipping, through shelf storage to consumer purchase and usage. In the case of OTC drugs, this is beyond an expectation; it is a legal requirement. The formulator needs to be aware of these requirements and test the formula (as reasonably as possible) to ensure that appropriate stability has been attained.

There are many references available for the formulator to review regarding stability and shelf-life testing. Most organizations have their own requirements and protocols for stability testing and, in the case of OTC drugs, there are specific stability-testing requirements.

Claims

Advertising claims (on package, in print, in media) are another important way of differentiating products in the same category. The fundamental regulation related to claims for a product is that there must be support for every product claim. The type, quantity and quality of the support is usually only defined when there is a challenge to a particular claim and that usually occurs after the fact. Some claims can be supported simply by product category—a bodywash formula containing surfactants will support general cleaning claims. Other "contains" claims (e.g., "with jojoba oil") can be supported by simply incorporating the claimed ingredient(s) in the formula. Performance claims or competitive claims are usually more difficult to support, but support must be developed nonetheless.

Desired claims are another area that must be discussed early on in the formulation process and the verbiage and method of support should be reviewed and agreed to among the formulating, marketing, regulatory and legal groups. Often times, the discussions around claims and support become discussions of risk and consequences.

Claims regarding product performance can be challenged in multiple ways. Legal departments from one company can challenge another company's claims via a simple letter, using the National Advertising Division (NAD) of the Better Business Bureau's self-regulating challenge process or by legal action. The NAD can challenge claims independently. Various countries and advertising regulating agencies have their own procedures for requiring claim support for claims used in advertising. Formulators can learn a great deal about support principles and claim support by reading the findings from these challenges.

6.17.3 MANUFACTURING REQUIREMENTS/EXPECTATIONS

Materials

The Manufacturing or Supply Chain groups within an organization reasonably require/expect that the materials selected for a formula are readily available and in quantity sufficient to support manufacturing of the formula. When a formula selects new or novel materials or works with a new, unfamiliar supplier, communication with the various Supply Chain groups will be necessary to ensure that the formula can be successfully manufactured on a continual basis without significant issues.

Equipment

The Supply Chain organizations will determine if a formula can be manufactured internally, using existing equipment, internally with new equipment, or externally at a contract manufacturer that has the appropriate equipment or registrations (e.g.,

OTC drug manufacture registration). The formulator will need to work with all of these groups to ensure proper and smooth manufacturing of their formulas.

Warehousing/Shipping

The final product (formula and packaging) will undoubtedly need to be stored at some point between manufacture and consumer purchase. The Supply Chain organization will expect that the formula will be stable throughout the storage under conditions that are normally experienced during this storage (i.e., unheated or non-cooled warehouse).

Regulatory/Legal Requirements

Throughout prior sections, the role of the Regulatory and Legal groups has been referenced. These groups are responsible for the compliance of the organization in all aspects related to the formula and the ultimate finished product. Formulators should engage these groups early on in the formulating process to ensure that everything possible is done to keep the product on track and compliant with any and all regulations.

OTC vs. Non-OTC

As indicated previously, cosmetic formulas and OTC formulas exist in close proximity. Formulators should be aware of those products that automatically fall under the governance of the OTC monographs and all of the formula requirements (e.g., active ingredients) that those monographs indicate. Consultation with Regulatory and Legal groups is imperative when operating in or close to this arena.

Safety

The manufacturer/distributor of a product is responsible for assuring the safety of the products it markets. The formulator is responsible for developing and testing formulas that support this responsibility. Most organizations have protocols that dictate the degree of safety evaluation and/or testing that is required for each formula prior to consumer exposure, either in testing or in actual product marketing. Communication with the groups within the organization responsible for product safety is essential for the formulator.

Intellectual Property

Intellectual property protection (e.g., patents) is essential to ensure an organization's rights to formulas/products developed by that company. The formulator has two responsibilities related to this protection: 1) to ensure that formulas developed do not infringe on the intellectual properties of individuals or organizations; and

2) to ensure that proprietary development can be and is protected for the formulas developed.

The first responsibility can be exercised by obtaining a Freedom to Practice approval from the appropriate body within the organization or via a consultant skilled in this determination. This will involve patent searches (domestic and foreign) conducted by skilled groups in consultation and discussion with the formulator.

The second responsibility is exercised by maintaining adequate and thorough documentation of the formulation from the earliest point in the process and continuing throughout the development and testing. Most organizations have processes for submitting and processing ideas that may or may not ultimately result in patents, and the prudent formulator will become familiar with these processes early on in the development process.

Initial Prototypes

Once the formulator has direction on the type, form, etc. of the formula to be developed, the actual formulation work and development of initial prototypes can be initiated.

The best places to start when developing initial prototypes within a given category are:

- Existing formulas in the organization's current or historical formulae portfolio
- Supplier formularies (available from supplier representative or through the supplier website) from suppliers supplying materials to the specific category (e.g., surfactant suppliers for shampoos, bodywashes, cleansers)

These formulas can be modified with additional materials to increase various aspects or used to identify materials and ratios of materials to use in new prototype development. The formulator can also look at competitive formulas in the category of interest to determine what materials are currently being used to achieve desired benefits.

Existing Bases/Forms

Existing organizations usually have or have had a portfolio of formulas in the categories of interest. Often a formulator can use one of these existing formulas as a starting point in the development of a new formula. The positives of this approach are related to the entire organizational familiarity with the materials, manufacturing, safety, etc. of the formula. The negatives are that the formula will probably not exhibit the novelty that will differentiate it from the products in the marketplace. These negatives can be assuaged by slight changes (e.g., fragrance, claims ingredients) to the formulas or new and novel claims that can be developed for the existing base formula.

Stocked/Currently Used Raw Materials

A formulator should start the formulation process by looking at the raw materials currently used by the organization. There is no need to source a new version of a material that is essentially identical to a material already sourced and used. Most organizations have many examples of multiple versions of the same raw material, differing only in source of supply. Differences in raw materials that make significant formulation differences are the cause for selecting one raw material over another even if they would apparently have little difference. The responsible formulator will identify these issues and make sure that the differences actually require the selection of new or different sources.

6.17.4 NEW RAW MATERIALS, BASES, FORMS

Raw Material Availability (quantity and geography)

Once a formulator has selected a new or novel raw material to use in a formula, the availability of the material in quantity sufficient to support manufacturing requirements in all of the markets where manufacturing will occur should be determined. Transporting materials internationally can impact the timing and ability for organizations to provide product to the marketplaces desired. The formulator can work with the supplier of the raw materials and the appropriate supply chain groups to ensure that all parties are comfortable with the ability to support the marketing of the formula.

Storage Requirements

Specialized storage requirements (e.g., refrigeration) for new raw materials or final formulas will need to be communicated to the organization early on in the development process.

Cost vs. Performance

New raw materials, bases, or forms are generally more expensive than existing versions. The formulator needs to evaluate the benefits (performance, manufacturability, claims, safety, etc.) versus the cost. This evaluation process is often ignored or conducted too late in the development process to be of value.

Regulatory Requirements/Issues

Using a new raw material, base, or form can result in an entirely new set of regulatory requirements (e.g., VOC maximums). Early on in the development process, a formulator should review these issues with the appropriate regulatory groups and then keep those groups advised throughout the developmental process.

Ease of Use in Manufacturing

New raw materials or formula bases can often create problems for the manufacturing groups. These problems can result from the material/base/form itself or, in the case of the raw material, how it is packaged or delivered (i.e., pails, drums, or bulk containers). All of these problems need to be discussed with the manufacturing groups early on to ensure the smooth transition from initial prototypes to production.

Claims Ingredients

Different ingredients can deliver different claims for a product, which can actually be important to performance or which simply allow advertisable claims. The formulator needs to know the differences and select the best way of proceeding.

Delivering Perceivable, Differentiated Claims in Formula

Raw materials in this category are the "gold standard" of claims ingredients. If the material can deliver a consumer-perceivable difference that can be measured (technically or through consumer testing) and used to support meaningful claims, the formulator has a significant win. This win will allow differentiation in the marketplace and consumer satisfaction versus the advertised claims.

Delivering Technical Claims in Formula

Raw materials in this category are important, as they allow product differentiation via technical claim support. The formula base itself may deliver the consumer-acceptable performance required and the inclusion of the technical support directly attributable to an ingredient will be of great value.

"Contains"

Raw materials in this category are the most ubiquitous in the marketplace. The materials may or may not deliver any benefit at the level used in the formula, but they are included for label or advertising purposes. Trendy new extracts, oils, etc. are widely used in products simply so that the "with X" claim can appear on the label or in advertising.

"Contains" ingredients and claims should require certain levels of use in the formula, depending on the placement on product. If the "claim" ingredient is an integral part of the product name, it should be used at a more significant level than if it is only included as a "with" claim. Any performance claims made for the use of these raw materials must be carefully evaluated to ensure

that there are no false or misleading claims that could be subject to challenge or litigation.

6.17.5 FINAL PACKAGING

Material of Construction

Starting with initial prototypes, the formulator needs to be aware of the composition of the final package into which the formula will be placed. Transparent versus translucent versus opaque packaging could have significant impact on the stability of a formula. Determining what testing should be done during the early phases of prototype development is important for the formulator as the development process is initiated.

Dispensing Mechanism

Of equal importance is the means by which the formula is dispensed for use by the consumer. The formula needs to be easily and completely dispensed from the primary package. A thick cream may not be able to be pumped; a thin solution might not be appropriate for a wide mouth; etc. Once prototype formulas are developed, dispensing tests should be conducted with dispensing devices similar to those for the final products to make sure that there are no issues.

Special Manufacturing Considerations

The formulator needs to be aware of where the formula will ultimately be manufactured. Internal versus contract manufacturing, multiple plants with differing manufacturing equipment, and sources of raw material supply and specialized testing equipment are all requirements that need to be considered by the formulator in order to provide counsel to the supply-chain groups involved with the decision.

6.17.6 STABILITY

Formula

Preliminary formulas that may become candidates for finalization should undergo some degree of accelerated temperature stability testing in order for the formulator to determine if formulation or processing changes will be made to ensure formula stability.

These "informal" stabilities are usually conducted in glass bottles or jars and have a condensed stability protocol. Typically, samples will be placed at 45°C, Ambient (25°C), 5°C, and perhaps Freeze/Thaw cycling and exposure to sunlight, if product is clear or packaging is transparent. Observations are usually made weekly for approximately four weeks to assess overall formula stability. At this

point, the formulator is looking for separation, discoloration, excessive changes in viscosity (that can be seen by observing product movement in package), malodor formation, etc. These stability evaluations are simply tools to help the formulator determine what may need to be done through the formula finalization process and are not used to ensure ultimate formula stability.

Alcohol-containing products or formula bases for pressurized packaging have specialized stability test packaging and may require special ovens or containers for accelerated temperature testing.

Formula in Package

If the final packaging or a similar package (material of construction) is available, samples should be placed on the same informal stability evaluation as the glass samples above. This will provide the formulator with information regarding gross interactions between the product and primary package.

Microbiology

Initial prototype development should include the preservative system identified for the formula/product of interest. Initial prototypes should undergo microbiological testing (initial total plate count and efficacy of preservation testing) to ensure that the preservative system is adequate to be carried through the remainder of the development process. Most organizations have a protocol for microbiological testing, and there are other protocols available through professional and regulatory groups (e.g., Personal Care Products Council).

Cost

Formula costs should be determined on preliminary prototypes to ensure compliance with the targets established at the onset of a project. Some organizations have protocols established for this early costing; otherwise, the formulator should independently calculate the approximate cost of prototypes using the standard costs or supplier costs provided for the raw materials contained in the prototype formulas.

Patent Status

The legal group should be provided key initial prototype formulations in order to obtain Freedom to Practice clearance or to determine if a new invention process should be initiated.

Selecting Final Prototype(s)

During the initial prototype development phase, a primary prototype(s) will be identified. These prototypes will successfully meet all of the evaluations conducted

in the preliminary phase or result from modifications made at that time. It is at this point that the formulator needs to be very objective in evaluations. Forgiving prototype failures early on will eventually cause significant problems in finalizing the formula.

6.17.7 PERSONAL TRIAL

Dispensing from Package

The formulator should spend time simply dispensing the product from the package and dispensing mechanism established for the product. Observing product being dispensed into the hand or on a substrate similar to the substrate for which it is intended will provide the formulator with the information required to determine if formula should remain a viable candidate.

Aesthetics

The formulator should critically evaluate the aesthetic properties of the leading candidate formulas before, during, and after stability testing. Color, clarity, emulsion appearance, etc. should all be reviewed. If the formulator has any doubts regarding objectivity, other formulators or colleagues can be asked to provide an unbiased opinion.

Performance

If at all possible, the formulator should personally try the leading prototype(s) to develop a sense of the ultimate consumer experience. Other formulators and colleagues can also be enlisted to try the prototypes and offer their opinions/comments regarding the performance.

If there are safety issues related to using the products, or if the products are of a reactive nature (e.g., color), the formulator can use the product on artificial substrates (e.g., human hair) to determine how the prototype applies, is worked through, rinses out, and the final feel and/or appearance of the substrate.

6.17.8 PRELIMINARY STABILITY

Glass and Package of Interest

Prior to selecting the primary prototype(s), the formulator should critically review all of the stability information from the informal stabilities in glass and, if available, in packaging approximating the final product primary package. Again, it is critically important for the formulator to remain as objective as possible regarding the formula stability. If necessary, small batches of the primary prototype(s) should be prepared and the stability repeated, at least in glass. Any changes in processing parameters on these small batches should be diligently recorded to be used in developing the final

manufacturing process. Again, having other formulators or colleagues offer their opinions on the stabilities can help the formulator remain unbiased.

Microbiology

All results related to the microbiological integrity of the primary prototype(s) and the efficacy of the selected preservative system in any of the prototypes tested should be reviewed. Special attention should be paid to the adequacy of preservation testing.

Pilot Scale Manufacturing/Filling

The primary prototype(s) should now be manufactured at a pilot scale. Some organizations have process engineering groups to perform this function, other organizations leave this to the manufacturing group, and still others leave this responsibility with the formulation group. Regardless, it is important to test the manufacture of the formula at a larger scale. Mixing, heating, and cooling rates, etc. are all different with scale, and those differences can have significant impact on the type and stability of final product, especially in the cases of emulsions. Filling can also impact the final aesthetics of the formula, so final prototype(s) should be filled by a method that approximates final filling operations as closely as possible.

Product manufactured at this small-scale level can and should be used for a complete stability test (glass and package) at all of the organizationally required conditions. These should include 45°C, 35°C, Ambient (25°C), 5°C, Freeze/Thaw (at least three and preferably five cycles), and exposure to sunlight. Stability should include sensory evaluation (appearance, odor, color), physical measurement (e.g., viscosity), chemical measurements (pH and active ingredients, if applicable), and microbiological testing (TPC and Challenge testing) at least at three and six months during the stability evaluation. Organizational stability protocols should be followed but should include evaluations at two weeks, one month, three months, six months, and one year at a minimum, with most protocols requiring an additional evaluation at two years, at least at ambient conditions.

Cost Verification

The formula cost of the final prototype(s) should be verified at this point. New raw material costs should be confirmed and whatever costs related to the formula should be included. Small-scale pilot manufacture may provide manufacturing cost estimates. The formulator should be aware of all of the costs, but is most concerned with the actual formulation costs.

Small-Scale Consumer Testing

Product from the pilot-scale manufacturing can be used for small-scale consumer testing if possible. Some degree of safety testing or review and approval is usually

required prior to exposing formulas to consumers. Various organizations have different ways of conducting small-scale consumer testing—some have dedicated groups to recruit consumers, place products, and gather and analyze data, some place this testing through consumer research organizations that handle all of the administration and reporting, and some organizations use employee panels for this testing. This testing should be done to ensure acceptable product performance prior to more expensive and complex, large-scale consumer testing or actual marketing of product.

Claims Review/Testing

With the selection of final prototypes, claims testing should begin in earnest. All serious primary prototypes should be tested, as well as controls. This testing may involve the use of existing or published claims methods (e.g., diastron tensile strength testing), require the development of new testing methodologies, or use expert consumers or large-scale consumer testing to support claims.

No matter what the testing methodology is, a plan should be developed that specifically lists all of the claims to be made and the methodology to be used to provide the support. Some claims may be supported by a simple written document (e.g., "contains" claims). Other claims will require significant testing. It has been said that "extraordinary claims require extraordinary support." All parties involved with the claim development, support, and approval (Formulation, Marketing, Legal/Regulatory) should review and approve the claims support plan.

6.17.9 FINAL FORMULATION

Large-Scale Manufacturing and Filling

Once the final formulation has been identified, a large-scale production batch should be manufactured and filled to finalize manufacturing procedure and to provide "real world" samples for consumer testing, final stability testing, final microbiological testing, final claims testing, and if required, final safety testing.

This large-scale batch should use all of the equipment that is expected to be used in routine manufacture. This will provide the formulator with information to finalize finished formula specifications and provide confidence that the formula that will ultimately move into the consumer's hands exhibits all of the properties desired by the formulator.

Cost Verification

The large-scale manufacturing/filling trial will provide information related to the final overall cost of the product. While the formulator is really most concerned with the formula cost, all of the costs that go into a product can impact the total formula cost permitted.

6.17.10 FINAL STABILITY

Formula

Accelerated stability testing should be conducted on samples from the large-scale manufacturing trial. Most organizations have a stability protocol, but this should include 45°C, 35°C, Ambient (25°C), 5°C, F/T (at least three cycles), and exposure to sunlight. The formula should test samples taken from the batch prior to filling (in glass) along with the filled samples. This will identify any stability issues created by the pumping and filling process. Samples used in the final stability evaluation should be in glass (for observation) and in the final package, to be used for other testing (e.g., viscosity measurement).

Packaging

If the package has been labeled or decorated, the formulator may want to test the effect of product on the label or decoration. This particular testing may be conducted by a separate group (Packaging), but it should be completed on these samples.

Microbiological Integrity

Total plate count on adequacy of preservation testing should be conducted on samples obtained before the transfer/filling operation and also on samples that have gone through the entire filling operation. This will provide the formulator with confidence that the formula can withstand the rigors of normal production, from a microbiological integrity perspective.

Safety Testing

If required, safety testing or evaluation can be conducted on samples obtained from the large-scale manufacturing/filling trial.

Large-Scale Consumer Testing

Samples from the large-scale manufacturing/filling test can be used for large-scale consumer testing, if required. Once organizational product safety requirements have been met, samples may be placed with a large number of consumers (n = 300) to obtain final information related to consumer satisfaction. These tests are usually conducted by organizations whose specific function is to conduct consumer testing. This testing may require undecorated packaging, so the formulator needs to be aware of all requirements for this testing.

ORAL CARE: FORMULATING PRODUCTS AND PRACTICES FOR HEALTH AND BEAUTY

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ABSTRACT

The desire to have a beautiful smile with glistening white teeth is a universal aspiration, as important to the aesthetic sense as having a youthful, wrinkle-free skin or a beautifully sculpted body. This article highlights the importance of this area to both the dental profession and the consumer, and discusses the growing demand for aesthetic dental procedures.

Having a beautiful smile involves more than brushing one's teeth twice a day. For generations, the mouth was not considered to have an effect on total body health, nor was the mouth considered to reflect total body health. The relationship of total body health and oral health is explained in this chapter. However, it is clear that total body health includes having a state of oral health. Current issues around oral health are also discussed, such as the effects of aging on oral health.

This chapter contains scientific, evidence-based information on daily oral health regimes that are thorough and effective, including proper brushing, cleaning between teeth, and techniques for resolving halitosis. The key to a healthy mouth is the thorough, daily removal of dental plaque biofilm that must be performed mechanically, whether with a manual or powered toothbrush and with some method of removing the dental plaque biofilm from the surfaces of the tongue and between the teeth.

The chapter contains detailed information (i.e., ingredients, design) about oral hygiene products necessary for achieving and maintaining oral health. The oral hygiene products encompassed are toothpastes, mouthrinses, manual and powered toothbrushes, and waterflossers (Waterpik) as well as products for cleaning surfaces between the teeth and the ingredients that make them effective for specific oral problems, such as inflamed gums (gingivitis), cavities (dental caries), removal of dental stains and/or whitening teeth, and treatment of hypersensitivity.

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6.18 INTRODUCTION

A. Important Issues in Oral Health

Dental Plaque Biofilm

The role of dental plaque biofilm has been recognized for decades as being the agent responsible for dental caries and periodontal disease. Through ongoing research it is now recognized that dental plaque biofilm has a potential, if not definitive role, in the link between systemic and oral diseases. Dental plaque biofilm consists of over 500 species of bacteria that live in a well-organized bacterial community and are embedded in an extracellular slime layer that adheres to the surface of teeth and soft oral tissues. The links between dental plaque biofilm and systemic disease are related to the magnitude of the number of bacteria that reside in dental plaque biofilm as well as the inflammatory conditions they set up in the oral cavity and become involved with other parts of the body and thus, systemic disease. Research on the links between systemic and oral diseases have been and continue to be heavily researched.

Dental Caries

The past 50 years have seen a shift in what has previously been considered "oral" health. The shift has been from a focus on teeth and gingiva to the realization, by multiple healthcare professions, that the mouth is a mirror of the state of health or disease in individuals.[1] Great strides have been made in the past

fifty years in the reduction of the prevalence of dental caries, although this is clearly still a significant public health issue. Clearly, fluoride is effective when delivered from toothpaste and incorporated in drinking water. By this means dental caries rates have decreased 30–50%.[2] Fluoridated water supplies in the U.S. have saved more than $4.6 billion annually in dental costs.[2] On a community level, the Centers for Disease Control and Prevention have reported that for every dollar spent on water fluoridation, $7.00–$42.00 is saved in oral health treatment costs, depending on the size of the community.[2] Assessments to determine risk for dental caries are being utilized (Caries Assessment and Management By Risk Assessment: CAMBRA), and notably there have been advancements in dental caries detection devices, particularly with the use of digital radiographs, quantitative light fluorescence, and fiber optic trans-illumination along with other new caries detection devices.[3] For over a decade we have had the scientific evidence that dental sealants do work, particularly those applied to children in school programs.[4] In addition, fluoride varnishes are playing an ever-increasing major role in the fight against cavities due to their proven efficacy and ease of application at the dentist office.

Periodontal Disease

The success for preventing periodontal disease has not been as effective as it has for dental caries. The results of a major study, Prevalence of Periodontitis in Adults in the United States: 2009 and 2010 estimates that 47.2%, or 64.7 million American adults, have some form of periodontal disease. Gingivitis, an inflammation of the gingiva, can be reversed; periodontitis cannot. If gingivitis is left untreated, inflammation can progress from the gingiva and will begin to affect the supporting structures of the teeth. The bone that supports the tooth in the affected socket(s) will be destroyed as well as the fibers that connect the root of the teeth to the bone.

Periodontal disease can be stopped most times with appropriate professional treatment and diligent homecare by the patient but the destruction, for the most part, cannot be reversed. Periodontal disease can be mild, moderate or severe, is a cumulative disease and, in adults 65 and older, its prevalence rate (evidence of having had period at some point in their lives) increases to 70.1 percent as described in a study published in the *Journal of Dental Research*, the official publication of the International and American Associations for Dental Research.

Currently there is ongoing research on oral changes that occur due to systemic diseases, access to oral care initiatives, and studies of the effects of aging on the oral cavity. There is no doubt at this time that a focus will remain on prevention and treatment of dental caries and periodontal disease.[5] It is also strongly believed there will continue to be research on oral cancer and the links between systemic diseases such as diabetes and the state of health or disease of the oral cavity as well

as the effect that they have upon each other. The Centers for Disease Control and Prevention's Oral Health Program is currently developing new Strategic Planning for 2015 and several years beyond. Notably, some of their priority areas in their strategic planning include, but are not limited to study of: dental caries, periodontal diseases, oral and pharyngeal (mouth and throat) cancers, infection control, and elimination of health disparities.

In addition to dental caries and periodontal disease there are some other topics that are of paramount interest to both patients and dental health care providers. These topics include:

- Aesthetic dentistry,
- Halitosis (bad breath), and
- Oral issues related to aging.

Since this is a chapter in a book on Beauty and Health, we call particular attention to these latter subjects, for they are the quintessential focus of the aging population's search to look good and feel good in their advancing years. We are all concerned, no matter our age, with how we look, and healthy white teeth that can be used to chew what we like is a fundamental quest for all.

B. Importance of Aesthetics in Dentistry

The importance of an individual's appearance for aesthetic reasons is easy to assess, even if one did nothing but look at the sales of tooth-whitening agents. Americans spent approximately $1.4 billion on over-the-counter whitening agents in 2013.[6] Every four years the American Academy of Cosmetic Dentistry (AACD) conducts a survey of its membership. The most widely read one, the Cosmetic Dentistry State of the Industry 2011 Survey,[7] was distributed to AACD members in 2011 and had a response rate of 76% (only AACD members received the survey). Table 1 contains a list of the percentage of aesthetic procedures performed by AACD members, which these dentists have reported as distinctly not essential restorative procedures. As seen in Table 1, 85 to 95% of the procedures performed by these dentists are very expensive and procedures performed strictly for aesthetic reasons are few and far between since they are generally not covered by dental insurance.

Another interesting factor that resulted from analysis of the AACD 2011 survey responses is that the results indicated what the patients in these cosmetic practices really cared about most in terms of the cosmetic dentistry they received. The factor that was listed as the most important one, by 97% of the respondents, was appearance.

Table 1. Percent of esthetic procedures performed by members of the American Academy of Cosmetic Dentistry in 2010.[8]

COSMETIC PROCEDURES	PERCENT OF PROCEDURES PERFORMED IN 2010 BY AACD MEMBER SURVEY RESPONDENTS
Crown and bridge	95
Bleaching/ whitening	91
Direct bonding: Anterior	90
Veneers	85
Removable Prosthetics	84
Implants	83
Direct bonding: Posterior	83
Inlays or Onlays	67
Other cosmetic procedures	54
Orthodontics	54

C. Halitosis (oral malodor)

Along with appearance, having halitosis or bad breath is of great concern by the public. According to a popular Internet dating source[9] it is estimated that in the U.S., people spent approximately $10 billion dollars per year on mouthwashes, breath mints, rinses, gum, toothpastes and other products to treat or mask halitosis. Specifically, in 2011–2012, people in the U.S. spend approximately $1.6 billion per year on toothpaste and $1.43 billion on toothbrushes and dental accessories such as interdental cleaners and dental floss. *These statistics provide insight as to how important appearance and alleviation of bad breath (and oral health) is to individuals and how much money they are willing to spend on their appearance and to have fresh breath.*

Before halitosis can be alleviated, the source of the bad breath needs to be detected. The source may be related to a systemic disease or problem—or, it can have origins in the oral cavity. If the oral cavity is not suspected as the source for the halitosis, the patient should be referred to a physician for evaluation of body systems. Non-oral sources for halitosis[10, 11] include:

- Nasal cavity
- Nasopharyngeal areas
- Sinuses
- Oropharyngeal areas
- Lung

- Lower respiratory tract
- Systemic diseases
- Gastrointestinal diseases and disorders
- Foods, fluids, and medications

However, 80–90% of halitosis originates in the oral cavity.[12–14] Halitosis is classified into categories of genuine halitosis, pseudo-halitosis, and halitophobia. Genuine halitosis is further classified as pathogenic halitosis or physiologic halitosis. Pathogenic halitosis is further classified as oral and extra-oral halitosis, indicating that the halitosis has its origin in the oral cavity or outside the oral cavity. Patients with pseudo-halitosis and halitophobia complain about oral halitosis that does not exist. Dental healthcare providers are equipped to work with individuals who have pseudo-halitosis, but halite-phobic patients may need psychiatric treatment.

Halitosis is best measured by gas chromatography analysis. Gas chromatography is specific for identifying the concentration of volatile sulfur compounds, which is the cause of most oral malodors.[10] Individuals with periodontal disease and halitosis would have their halitosis classified as pathogenic in that it results from the bacteria involved in periodontal disease. When patients have periodontal pockets of 4 mm or more, volatile sulfuric compounds are released and responsible for halitosis.

Patients with xerostomia, or dry mouth, may have halitosis, since bacteria and oral debris are not physiologically washed away as they normally would be during routine cleaning of the mouth. Giving patients with xerostomia salivary substitutes can help the halitosis if the teeth are kept as free as possible from dental plaque and the tongue is cleaned on a regular basis. Dental hygienists and dentists are poised to address the reasons for halitosis that are oral related. If a patient mentions halitosis, the dental hygienist needs to include the halitosis as something that needs to be addressed in the treatment plan. The dental hygienist can work individually with each patient to show them how to best remove the dental plaque and debris from their mouth and what products to use. This approach can be effective in helping patients prevent halitosis; if the patient does practice thorough oral hygiene and still has halitosis, he may need to be referred to medical personnel for further evaluation.

Mouthrinses and breath mints can be effective in masking some oral malodors, but usually these products have to be used repeatedly every few hours unless they have antimicrobial properties. Halitosis can be resolved in some patients with through regular dental plaque removal utilizing a toothbrush and a method for cleaning between the teeth. If dental floss is too narrow to touch the surfaces of both teeth when placed between them, then dental floss needs to be discarded and a new method of interdental cleaning should be utilized. One alternative in such

cases is the use of interdental brushes. These come in several sizes and shapes and the brushes that can fill the interdental space will most likely remove the most dental plaque and biofilm.[15]

Patients will use a variety of products to fight oral malodor, in addition to using a toothbrush and interdental cleaning device. Mouthrinses that are antimicrobial such as chlorhexidine may be useful for neutralizing volatile sulfur-containing compounds. Table 2 contains a list of ingredients used to fight oral malodor and can be found in mouthrinses, toothpastes, breath mints, and chewing gum.

Table 2. List of active ingredients used to fight oral malodor and how they work to alleviate oral malodor

ACTIVE INGREDIENTS USED TO FIGHT ORAL MALODOR	ACTION AGAINST ORAL MALODOR
Chlorhexidine	Antimicrobial; neutralizes volatile sulfuric compounds
Cetylpridinium chloride Triclosan Essential oils	Mild antimicrobial Agent affecting volatile sulfuric compound producing microorganisms
Chlorine dioxide Zinc	Volatile sulfuric compound neutralizing agent

D. Oral Issues Related to Aging

1. Demographics of Aging: What to Expect

There is a burgeoning population of people aged 65 years and over in the United States. They are generally described as the "Baby Boomers." These individuals represent the post-World War II generation born between the years of 1946 and 1964. In the year 2010, the "Baby Boomers" began turning age 65 and with this tipping point a new market emerged focused on the needs of these aging citizens. The growth of the population of those 65 years and over has many societal effects as the needs of these aging individuals have to be met. This growth of numbers of the aging population will have an increasingly significant effect on healthcare providers, businesses, families, and policymakers at all levels of government. It is important to be familiar with some of the "facts" related to this expanding group of aging citizens.[16]

- In 2010, there were 40 million people age 65 and over who represented 13 percent of the total U.S. population; by the year 2030, 20 percent of all Americans will be age 65 or older.
- After 2030, the growth rate of the group of individuals 65 and over is projected to remain at a steady 20 percent.

- After 2030, the "Baby Boomers" will enter the "oldest-old population" (those 85 and over), causing this group to grow rapidly.
- In 2010, the population of those aged 85 years and over was 5.5 million. By 2050, the U.S. Census Bureau projects that the "oldest-old population" will grow to 19 million. Further, there are some research projections that predict that the death rates of the "oldest-old population" may decline at a more rapid rate than is predicted by the U.S. Census Bureau.

2. Oral Health and the Aging

The current U.S. population of aging seniors has enjoyed unprecedented advances in oral hygiene and greater access to dental treatment than previous generations. Some of the advances they have benefited from include:

- Availability of over-the-counter fluoridated toothpaste (1955) and water fluoridation
- Availability of dental insurance in the (1970s)
- *U.S. dental healthcare providers embraced the concept of preventing dental disease* rather than treating the consequences of common dental diseases. Increasingly an expanded focus has been generated in the areas of dental caries and emphasis has been placed on plaque-control programs with individualized oral hygiene instructions for patients (late 1960s and early 1970s)
- Dental sealants (1974), fluoride varnishes, and high-fluoride toothpastes (1980s)

As a result of these advances, the majority of current seniors have all or most of their teeth.[17] This presents a unique problem when these seniors enter hospitals, nursing homes, and long-term care facilities. Nursing care staff are presented with having to provide or assist these seniors with brushing and related oral hygiene procedures. Nursing staff are now required to do much more than "scrub" dentures in a sink.

Certainly not all senior citizens reside in nursing homes or long-term care facilities. There is a distinct difference between the type of oral health issues senior citizens face, depending on whether they are living independently or live in a facility where they must depend on assistance with oral hygiene procedures. There are some oral health issues that seniors face no matter where they live and are congruent with the normal aging process. Some of the normal signs of aging that can be observed in the oral cavity of those over 65 years of age can be seen in Table 3.

Dental examinations and professional cleaning of the teeth are as important during the senior years of life as they have been through the previous stages of life. Dentists and dental hygienists can find early signs of problems and take measures to help the patient halt damage and prevent greater damage. There are

Table 3. Clinical Signs Of Aging And The Associated Cause[18]

CLINICAL SIGN OF AGING IN THE ORAL CAVITY	APPEARANCE IN THE ORAL CAVITY	CAUSE
Dry lips, tongue and inside of the oral cavity; rapidly progressing tooth decay	The smooth surfaces inside the oral cavity will appear dry and normal elasticity is not present; smooth tissue surfaces as well as gingiva will be red and inflamed; large amounts of dental plaque will accumulate at the gum line of the teeth. Teeth may experience rapid tooth decay due to the loss of the cleansing effect of saliva. Swallowing may be difficult.	Diminished salivary gland flow; can be caused as a result of normal aging or as a result of the effects of medication. This condition is known as xerostomia. There are over 800 medications that can cause xerostomia or dry mouth.
Recession of the gingiva	Teeth will appear to have a "longer appearance" as the gingiva recedes	Gingiva may recede as a normal part of aging; can be a sign of periodontal disease; 75% of seniors have some form of periodontal disease
Erosion, attrition of the teeth, dental decay	Flattening of the chewing surfaces of the teeth; notches in the enamel at the gum line of the tooth; teeth may be hypersensitive and the patient may react to pain when ingesting sugary foods or food that is hot or cold	Wearing of tooth structure due to chewing. Can occur due to abnormal alignment of teeth, repeated exposure to acid from foods or acidic drinks; lack of removal of dental plaque (bacteria) on a daily basis and/or lack of professional cleaning and fluoride treatments.
Mobile teeth	May appear normal but during chewing the tooth or teeth are loose.	Bone loss and loss of fibers holding the tooth in the socket due to periodontal disease
Stained teeth	Teeth may have a yellow, gray or brown discoloration of the teeth.	Smoking, coffee and tea stains, natural process of aging; dentinal tubules are filled with minerals and the teeth take on a yellowed or gray appearance

medications and products that are especially formulated to alleviate some of the symptoms of dry mouth. Professional fluoride applications in the form of fluoride treatments with trays or fluoride varnish can provide relief from hypersensitivity and the softening of enamel due to the acidic destruction process that occurs with the dental decay process.

Dental healthcare professionals can recommend dentifrices that are specially formulated, for example to prevent or stop decay, stop hypersensitivity, or whiten teeth. Therefore, it is important for dental hygienists and dentists to stay abreast of advancements made with various oral care products, new benefits, and the ingredients they are formulated with. In this way they can guide their patients to the most appropriate dentifrice to meet their patient's needs. Table 4 contains a list of popular ingredients found in dentifrices and their functions.

Table 4. Basic Ingredients in Dentifrices[21]

INGREDIENT	AMOUNT	PURPOSE	SPECIFIC INGREDIENTS
Abrasives	20-40%	Clean and polish	Calcium carbonate, silicone oxides, aluminum oxide, calcium pyrophosphate
Binders	2%	Prevents separation of ingredients	Alginate, synthetic cellulose, gums
Coloring agents	2%	Attractive appearance	Vegetable dyes
Detergents	1-2%	Foaming action; helps loose debris	Sodium lauryl sulfate, sodium lauryl sarcosinate
Flavoring agents		Pleasant taste that is a lingering aftertaste	Essential oils: peppermint, spearmint, wintergreen, cinnamon, menthol
Preservatives	<1%	Prevent growth of bacteria and mold	Sodium benzoate, alcohols, dichlorinated phenols
Sweeteners	2%	Pleasant flavor	Saccharine, sorbitol, mannitol, xylitol, glycerin
Water and humectants	20-40%	Maintain moisture and consistency	Sorbitol, glycerin, propylene glycol, mannitol

6.18.1 PERSONAL ORAL CARE

The number and type of oral care products available for personal oral care is unprecedented. Notably, there is a vast range of prices, making some of these oral care products affordable for most everyone. In 2013, $1.8 billion was spent on dentifrices and spent $775 million on toothbrushes.[18, 19] There are many additional products that consumers use for oral care. These include but are not limited to products such as dental floss, interdental products for cleaning between teeth, mouthrinses, tongue cleaners (tongue cleaning is critically important to controlling halitosis), fluoride-containing products, antibacterial mouthrinses, waterflossers such as the Water-Pik, sonic devices that pulse water between teeth, and whitening products.

It is important for dental hygienists and dentists to be familiar with the various products so that when recommending a product to a patient, they can recommend the one that best fits the patient's needs. In this Internet age, it is also important for consumers to be knowledgeable about personal oral care products as well. Otherwise, they may make purchases of products that are ineffective or not appropriate for meeting their needs. A review of dental products is important so that patients and dental healthcare providers are knowledgeable about new developments in oral care products and especially the results of research findings about the efficacy of personal oral care products. Clinical research and systematic reviews have played a crucial role in revealing which products and ingredients are the most effective.

A. Dentifrices

One of the most common questions that dental hygienists and dentists are asked by patients is, "What brand of toothpaste should I use?" Most often, the response from the dental healthcare professional will be based on the patient's needs. The conditions that are considered when professionally recommending a dentifrice are prevention of dental caries and/or the demineralization/erosion of enamel, gingivitis and/or periodontal disease, sensitivity, or stained teeth. Additionally, dental healthcare professionals most often recommend dentifrices that have the Seal of Acceptance from the American Dental Association: http://www.ada.org/en/public-programs/ada-seal-of-acceptance-program/.

1. Regulation (Therapeutic vs. Cosmetic Benefits)

The American Dental Association awards its Seal of Acceptance to dental products based on a review of: (1) the data supporting a product's efficacy and safety, and (2) the claims made for the product, to ensure that all claims are supported by the science. Application for the American Dental Association's Seal of Acceptance is strictly voluntary and manufacturers submit their dentifrices to the American Dental Association for testing and review. Dentists and dental hygienists need to be familiar with the wide array of dentifrices so they can guide their patients to the most appropriate dentifrice that is safe and effective and meet their needs.

It is important to note that the U.S. Food and Drug Administration regulates dentifrices under the Federal Food, Drug and Cosmetic Act. The FDA sets standards on dentifrice effectiveness and safety and regulates the amount and type of ingredients that can be in a dentifrice formulation. Table 4 contains information about the ingredients common to most dentifrice formulations regulated by the FDA.

Dentifrices are considered by the FDA to be a drug, as most dentifrices contain fluoride to prevent tooth decay or specific ingredients to treat sensitivity and gingivitis, among others. Therapeutic agents are by definition concerned with the treatment of disease. Cosmetics, as defined by the U.S. Food and Drug Administration, are "intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions."

2. Stain Removal

Dentifrices, along with toothbrushes, are the most widely used and recommended oral products. Dentifrices have been used to freshen breath since the time of the Greeks and Romans, and have usually included abrasive agents to help remove stain. Dentifrices alone, however, will not remove dental plaque and biofilm. At this time, dental plaque must be mechanically removed. Use of dentifrices adds the benefit of containing abrasive agents that help with the mechanical removal of dental plaque and stain (thus also providing whitening efficacy) and most are flavored with the intent to freshen breath.

3. Abrasion

Another important fact that dental hygienists and dentists need to be aware of is the abrasiveness of dentifrices. There are several issues that need to be addressed when a dental hygienist or dentist recommends a dentifrice to a patient: whether the patient needs a dentifrice 1) with fluoride for dental caries prevention and/or control, 2) with antimicrobial medications for the prevention and control of gingivitis, 3) sensitivity ingredients to control dentinal hypersensitivity, and 4) with odor-controlling ingredients to manage halitosis. Another issue is the type of restorations and restorative materials that are in the patient's mouth—especially aesthetic-restorative materials. Aesthetic-restorative materials, which include several types of tooth-colored materials, can be easily damaged with highly abrasive dentifrices.

Dentifrice abrasiveness has traditionally been determined by the Relative Dentin Abrasivity Index (RDA), a standardized test developed by the American Dental Association. One should note there are many other factors that affect the impact toothbrushing can have on hard and soft tissues, such as type of brush used (soft, medium, hard bristles), consumer habits, etc., and one should not put too much

emphasis on any single factor. The maximum recommended RDA value for toothpastes is set at 250.

The abrasives used in dentifrices help remove dental plaque and stains from the tooth surface. An ideal abrasive level of a dentifrice would provide the ability to clean the tooth surfaces and remove stains without causing damage to the tooth surface or to the surface of dental restorations. The Council on Dental Therapeutics of the American Dental Association states that "a dentifrice should be no more abrasive than is necessary to keep the teeth clean—that is, free of accessible plaque, debris, and superficial stain. The degree of abrasivity needed to accomplish this purpose may vary from one individual to another."

The abrasivity and cleaning action of abrasives are related to abrasive particle size, shape, brittleness, and hardness. The most commonly used abrasives are: hydrated silica, calcium carbonate, calcium pyrophosphate, and dicalcium phosphate dihydrate. Other materials include sodium bicarbonate, tricalcium phosphate, sodium metaphosphate, and alumina or aluminum hydroxide.

4. Ingredients

Dentists and dental hygienists need to be familiar with the wide array of dentifrices and their ingredients so they can guide their patients to the most appropriate dentifrice to meet their needs. For example, a patient may want to know what the best whitening dentifrice to buy is, but the dental hygienist has taken note that this patient has dentinal hypersensitivity. A whitening dentifrice containing hydrogen peroxide or carbamide peroxide or a dentifrice that is highly abrasive would all be contraindicated for this patient as these types of dentifrices could worsen the dentinal hypersensitivity.

Table 5 contains a list of popular active ingredients found in dentifrices and the conditions they are intended to target. Adding active ingredients is not easily accomplished, as many potential actives can interact or react with the other ingredients. Interactions between potential therapeutic agents and inactive ingredients are well covered in a separate review.[20]

Abrasives. *Hydrated silica* (or silica as it is sometimes called) has become the most widely used abrasive in the United States and many other countries over the last 25 years. Hydrated silica used as an abrasive is manufactured by either gelation or precipitation. A xerogel is manufactured by drying the gel formed by gelation with subsequent milling to obtain the desired particle size. The precipitates are collected and dried. Use of these hydrated silica abrasives is the basis for clear gel toothpastes; their use is practical in opacified gel toothpastes as well. The advantages in using hydrated silicas as abrasives include: a high degree of compatibility with fluoride salts, flavors, and other materials, as well as higher efficiency in cleaning tooth surfaces as compared to most other toothpaste abrasives.

Table 5. List of popular ingredients in dentifrices and the functions they serve.

ACTIVE INGREDIENT	PURPOSE
Calcium carbonate	Remineralization
Carbamide peroxide	Whitening agent for bleaching teeth
Humectants, lubricants, enzymes, polymers	Alleviate symptoms of xerostomia (dry mouth); helps to retain moisture in oral soft tissues such as the inside of the cheeks and lips
Hydrogen peroxide	Antibacterial; whitening agent for bleaching teeth
Potassium nitrate 5% (some dentifrices contain 5% potassium nitrate and fluoride	Desensitization; relieve hypersensitivity caused by exposed dentin; helps block sensations of pain
Pyrophosphates: sodium hexametaphosphate, tetrapotassium pyrophosphate; gantrez, zinc chloride, zinc citrate	Anticalculus capabilities; softens calculus making removal easier
Sodium bicarbonate	Neutralizes acids from dental plaque; very low abrasion, effectively removes stains, inhibits volatile sulfuric compounds to reduce oral malodor
Sodium fluoride	Works to strengthen enamel and helps prevent dental caries
Sodium monofluorophosphate	Works to strengthen enamel and helps prevent dental caries
Stannous fluoride	Works to remineralize enamel after dental caries attack helps to prevent root caries; anticaries, antiplaque and antigingivitis effects
Stannous fluoride (0454%) and sodium hexametaphosphate	Anticaries, antigingivitis, antiplaque, reduction of sensitivity and stain
Strontium chloride	Desensitization; relieve hypersensitivity caused by exposed dentin; helps block sensations of pain
Triclosan	Broad-spectrum antibacterial agent; antiplaque, antigingivitis and anti-inflammatory properties
Xylitol	Naturally occurring sugar substitute that has anticariogenic properties

Hydrated silica is available in many grades from various suppliers worldwide and ranges in abrasivity from fairly low to quite high. Common Particle Size Distributions used for abrasives range from about 5 to 35 microns. There also are dual-function hydrated silica grades available that also serve as toothpaste thickeners.

Calcium carbonate or chalk is available in a number of grades varying in crystalline form, particle size, and surface area. Precipitated calcium carbonate (PCC) is one of the earliest abrasives used and is still in use today, mainly due to its inherently low cost. Natural calcium carbonate is also available commercially and offers an even lower-cost option. Precipitated and natural calcium carbonate is sometimes used in combination to achieve optimal formulations in terms of cost and cleaning efficacy. All grades of calcium carbonate contain a finite amount of water-soluble calcium salts and will require compatible fluoride sources such as sodium monofluorophosphate. All calcium carbonate toothpastes are alkaline, and therefore they may also require flavors compatible at this higher pH.

Calcium phosphates include diverse chemicals, such as dicalcium phosphate dihydrate, dicalcium phosphate, tricalcium phosphate, calcium pyrophosphate, and synthetic apatites, all of which are used as toothpaste abrasives. *Dicalcium phosphate dihydrate* (DCP-D) is the calcium phosphate most commonly used in dentifrices. The pH of toothpaste made with DCP-D normally ranges from about 6 to 8. The taste of DCP-D-based toothpastes tends to be better than that of calcium carbonate–based products (not chalky), and flavor stability is usually less of a problem. DCP-D is in a metastable state and reverts to the anhydrous form with consequent hardening of the paste. This hardening is accelerated in the presence of fluoride ions and therefore DCP-D may be supplied with a stabilizer to delay or prevent this change. Trimagnesium phosphate, tetrasodium pyrophosphate, and other pyrophosphates are common stabilizers.

Calcium pyrophosphate (CPP) was originally developed as the abrasive of choice for products containing sodium or stannous fluoride. It is claimed that the low availability of soluble calcium ions contributes to the stability of the fluoride and this abrasive is experiencing a revival in whitening toothpastes such as Colgate Optic White™ (from Colgate-Palmolive) due to its inertness and excellent cleaning and polishing efficacy without corresponding higher dentine abrasivity.

Sodium bicarbonate or baking soda has been used as an abrasive in the United States since the early 1960s. Its drawbacks can be saltiness and high product pH, which may lead to some incompatibilities. It serves a second function because it can neutralize some odors in the mouth. In the United States, it has a mystique of being an odor reducer and a mild/gentle cleanser.

When recommending a dentifrice based on abrasivity, dental hygienists and dentists need to be certain that a patient received brushing instructions and does

not brush too hard. A standard toothbrushing pressure is 150 grams of force (mass). If a patient is brushing in such a way that the bristles of the toothbrush are splayed outward, they likely are brushing much too hard. Figure 1 illustrates a toothbrush that has been used with too much force during brushing.

Figure 1. Toothbrush with splayed bristles caused from excessive brushing force. (Photo copyright by 123RF).

Humectants

It is necessary to incorporate a component with humectant properties to prevent toothpastes from drying out. This is most likely to happen if the cap is not replaced on the tube. Alternatively, if toothpaste is allowed to build up on the tube nozzle threads, the cap can become locked in place. An additional important factor is the enhanced and more pleasing "mouth-feel" achieved using the humectants listed below. *Glycerin* is the perfect humectant in that it is stable and nontoxic, has some solubilizing properties, contributes some sweetness and improves dentifrice mouth-feel. Today glycerin is available mostly in synthetic grades at 99% or higher levels. *Sorbitol* syrup 70% has been found useful since it has some properties similar to those of glycerin.

Propylene glycol has also been used as a component of the humectant system. The drawback here is that at high-usage levels it has a tendency to be somewhat

bitter and is more costly than Sorbitol or Glycerin. *Xylitol* is manufactured from xylose and is noted for its pleasant coolness in the mouth when used at high levels. There are published studies supporting anti-caries properties, although U.S. regulations do not permit it to be claimed as an actual active ingredient in dentifrice products. While it is noticeably sweeter than sorbitol, it is also more expensive.

Polyethylene glycol or PEG is one of the dual-functional materials that can be used in toothpaste. The lower-molecular-weight materials, such as PEG-8, exhibit some humectant-like effects and act as lubricants to some degree and as processing aids to disperse some of the thickening agents that require hydration. In hydrated silica-based products even the low-molecular-weight PEGs act as thickening agents, with a more pronounced effect resulting from higher-molecular-weight PEGs up to PEG-20M. Problems related to their usage include the fact that the low-molecular-weight PEGs may be bitter and those higher in MW than PEG-18 require heat during processing in order to facilitate incorporation.

Water, which is used to provide semi-solid consistency, is a key ingredient and a critical component in dentifrice compositions, but also acts as a solubilizer for active agents such as the various forms of fluoride (neutral sodium fluoride, monosodium fluoride, stannous fluoride). Water is also included in most toothpaste intentionally due to its low cost and indirectly in other ingredients (some humectants contain 30% water). Keeping the overall water level low enough so as to prevent microbial growth is important. Measurements of water activity have been developed to help in formulating a microbiologically safe product without the need for using strong preservatives.

Surfactants

Tooth cleaning is partially a detergent-type process, and almost all toothpastes incorporate a surface-active agent. The ideal detergent should be tasteless, nontoxic, and nonirritant to the oral mucosa, while producing a large volume of dense but low expansion, foam with non-gagging qualities. Foam quality is important since it has a significant influence on the subjective assessment of toothpaste performance.

Sodium lauryl sulfate (SLS) is the most widely used detergent for oral products and satisfies almost all the requirements of the ideal detergent. In addition to sodium lauryl sulfate, various other detergents are also used.

Although not widely used, some other anionic detergents, i.e., *sodium methyl cocoyl taurate (tauranol)*, have found limited application in toothpaste. In addition, *poloxamer 407* and similar nonionic block copolymers have also been included in toothpaste formulations. Other types of foam boosters, such as cocoamidopropyl betaine, are zwitterionic.

Thickening and Binding Agents

A thickening or gelling agent is necessary in order to maintain the stability of a high-solids suspension. The thickening agent also modifies the dispersibility, foam character, and mouth-feel of dentifrices. Thickening agents used in toothpastes can be natural or synthetic gums that thicken when hydrated or inorganic materials such as hydrated silica or magnesium aluminum silicate.

Carrageenan is the generic name given to gums derived from the seaweed *Chondrus crispus* or Irish moss. Commercial carrageenans are standardized products of uniform and reproducible quality. This type of gum is rarely used by itself and is usually used in combination with others listed below.

Cellulose gum or sodium carboxymethyl cellulose (SCMC) comes in a variety of grades exhibiting different physical properties that may be controlled by adjusting the degree of breakdown of the cellulose before substitution, and by the degree of substitution. SCMC is reasonably stable in the presence of electrolytes and calcium ions and in general are suitable for most toothpaste formulations. Cellulose gum is the most commonly used gum for toothpastes. It imparts little color and is nontoxic and relatively tasteless. Due to its anionic nature, SCMC is not suitable for toothpastes containing cationic ingredients, especially certain antibacterials. For these pastes, nonionic cellulose derivatives such as *hydroxyethylcellulose* or *hydroxypropyl methylcellulose* may be used.

Xanthan gum is a bioengineered fermentation product that also finds application as a thickener in toothpaste and is widely used in the United States and elsewhere in toothpaste products. It might be the ideal thickener for toothpaste except for its relatively high cost.

Carbomer is available in various grades and has been used in toothpastes as a thickening agent. Thickening efficacy and utility depend on the pH and electrolyte content of the toothpaste, and therefore have limited utility and must usually be combined with other thickening agents to provide the necessary rheological properties necessary for consumer acceptable formulations, such as its stand-up.[20] The ideal toothpaste, from a rheological view, is one that is pseudoplastic and has a yield stress. This combination of properties allows the toothpaste to stay in the tube when the cap is off (high yield stress) but extrudable when the tube is squeezed (i.e., viscosity decreases with increasing stress; pseudoplastic).

Clays, in particular *magnesium aluminum silicate* and *sodium magnesium silicate*, continue to find usage as thickening agents in toothpaste.

Hydrated silica, discussed previously as an abrasive, exists in both gel and precipitated forms that are widely used as toothpaste thickeners. The average particle sizes used range from submicron to about 5 microns. Although both abrasive and thickening silicas are classified as hydrated silica, they differ in the degree

to which these ingredients impart viscosity, which is dependent on porosity and structure properties of these raw materials.

Fumed or pyrogenic silica is rarely used, but offers significant advantages under conditions where high purity is required, such as in presence of reactive ingredients, i.e., oxidizing agents that can readily decompose in the presence of impurities often found in hydrated silicas. All forms of silica are highly compatible with fluorides and can yield a rapidly dispersing and highly consumer-acceptable toothpaste or gel. This type of silica is also useful as a thickener in toothpastes, especially in low-water or anhydrous compositions, where there is insufficient amount of water to properly hydrate the rheological additives listed above. It can be effective for this purpose, especially in conjunction with other polymers that are also effective in low-water systems.

Flavors. The perceived flavor of toothpaste is one of the most important characteristics influencing consumer acceptance. Consumers expect a clean mouth-feel after brushing and this is delivered in part by the flavor. Flavor also plays a large role in delivering fresh breath either by masking or modifying the perception of malodors. Flavor oils are usually the most expensive ingredients in toothpaste and can have the greatest impact on consumer acceptance, and are therefore created by experienced flavorists who tailor these for different regions of the globe.

In the United States, flavors have usually been based on spearmint, peppermint, and, to a smaller degree, wintergreen. Menthol is sometimes added, as are other cooling materials, to increase the cooling effect. Flavors can be modified with clove, eucalyptus, aniseed, cinnamon, and others. Other flavor modifiers such as woody, herbal, medicinal, and floral notes are used in different countries, and some are quite successful.

Various other ingredients of the toothpaste formulation can contribute to the perceived taste; for example, dicalcium phosphate dihydrate–based pastes usually have a taste preferred over those based on calcium carbonate. The hydrated silica–based toothpastes or gels are also noted for not having a significant taste impact. The flavor may also be modified by the presence of an active ingredient and even by the pH or electrolyte content of the product. The nature of the foam and the dispersibility of the paste can also modify flavor perception in the mouth. For these reasons, consumer acceptance of a given flavor should only be judged in the intended full base formulation and should be assessed in large consumer tests to ensure market success prior to launching of the product.

Colorants and Preservatives. Colors are often added to toothpastes, sometimes to mask an off-color from the base formulation either initially or upon storage. The range of colors available is restricted somewhat by food or drug additive regulations. In the United States, the predominantly used colorants are FD&C and D&C approved dyes and lakes, as well as a few allowable natural colorants.

Titanium dioxide is widely used in hydrated silica–based toothpastes as an opacifier and in other abrasive-system toothpastes to impart a bright white color to the products.

The range of toothpastes available today is quite varied. These can be opaque, white, or colored pastes, "water white" or colored clear gels, striped products, different-colored toothpastes, and gels delivered from two-compartment pumps or tubes. There are even products containing colored speckles composed of agglomerates of abrasive or speckles encapsulated by polymers and waxes. It is apparent that consumers have an interest in new visual effects in toothpaste products as well as by the mouth-feel delivered by the toothpaste, including particles that deliver a "dentist clean feel." There are also novel polymer systems to enhance the smoothness and thickness of foam generated by the surfactant systems such as those used in food industries as fat substitutes (i.e., microcrystalline cellulose).

The use of ***preservatives*** is becoming more common today as formulations are containing higher levels of water to reduce the overall cost of the product. *Sodium benzoate* is not very effective at neutral and higher pH values but is used fairly widely for in-process control of microorganisms. *Potassium sorbate* and the various *parabens* also find limited usage. Novel ingredients that are finding more applications as preservatives are select flavor components such as benzyl alcohol because of their efficacy, safety profile, and relatively low impact on taste. Products today are subjected to a battery of bacteriological challenge testing to ensure microbiological robustness during manufacture and consumer use.

5. Therapeutic Ingredients

Anti-Caries Agents. Fluoride ion delivered from a compatible toothpaste base has been shown in numerous clinical trials to reduce the incidence of carious lesions in both children and adults. It acts by reducing the acid solubility of tooth enamel. Fluoride has also been shown to aid in the remineralization of white-spot carious lesions.

Sodium fluoride, *sodium monofluorophosphate*, and *stannous fluoride* are the anti-caries actives of choice. In the United States the Final Monograph[21] defines the allowable levels of soluble fluoride ion from each salt in each allowable abrasive system for anti-caries active drugs. In a broad sense the only significant change from the earlier "accepted" target of 1000 ppm of fluoride is the addition of an allowable 1500 ppm of soluble fluoride ion from sodium fluoride and sodium monofluorophosphate. Also, since toothpaste is volume-dosed, the allowable level of total fluorine has been widened to 850–1150 to account for the varying specific gravities of toothpaste products. This document goes on to describe other test criteria that must be met, including enamel caries reduction, enamel solubility reduction, and fluoride uptake.[21] Any new agent would have to be approved by FDA

through a rigorous process (New Drug Application, NDA) to determine safety and efficacy of the ingredient.

Actives for Prevention/Reduction of Plaque, Gingivitis, and Periodontitis

While most plaque control is achieved with mechanical removal such as proper and at least twice daily brushing and flossing, there is a significant portion of the population that suffers from gingivitis and periodontitis due to inadequate plaque removal, which occurs especially in hard-to-reach places. Therefore, addition of chemotherapeutic agents to oral care vehicles is necessary as an adjuvant to improve and enhance oral hygiene.

In the past two decades there have been significant advances in identifying and formulating antibacterial systems that are highly effective against plaque, gingivitis (a disease that can lead to redness and bleeding of gums), and periodontitis, which can eventually lead to tooth loss. Control of plaque especially at the gingival margin is then highly desirable in the maintenance of a healthy mouth.

Triclosan, a broad-spectrum, nonionic antibacterial agent, has been used by dentifrice manufacturers as a way to control plaque and gingivitis. A delivery system that can enhance the efficacy of triclosan is based on PVM/MA copolymer, which enhances the uptake of this antibacterial agent to hard and soft tissues in the mouth. Commercial toothpaste containing this active delivery system has been studied extensively for its safety and efficacy and has been granted the American Dental Association Seal of Acceptance for prevention against gingivitis, plaque, and cavities. It was also given approval for use in toothpastes by the U.S. Food and Drug Administration for prevention of gingivitis and cavities. Clinical studies have also shown the feasibility of prevention and control of periodontal disease using a chemotherapeutic dentifrice formulated with triclosan/copolymer system.[22]

There are other ingredients mentioned elsewhere in this chapter that have clinically demonstrated antiplaque and antigingivitis activity in either toothpaste or mouthrinses. These actives include *chlorhexidine* (mouthwash), *stannous fluoride* (toothpaste), *cetylpyridinium chloride* (mouthwash), and a mixture of phenolics and essential oils at specified levels (*thymol*, *eucalyptol*, *methyl salicylate*, and *menthol*) (mouthwash).

Other *herbal ingredients and extracts* have been reported to be active antiplaque and antigingivitis agents. Enzymes of various sorts have been used in toothpastes with claims for stain removal, whitening, antiplaque activity, and bad breath reduction. They can be denatured readily by some formulation ingredients such as anionic surfactants and temperature extremes and therefore create formulation problems.

Hypersensitivity

Dentin hypersensitivity is a common condition affecting up to 57% of adults, with highest incidence at age 30 and above. According to Brännström's hydrodynamic theory, tooth sensitivity[23] results when dentin tubule openings become exposed and fluid movement occurs as a result of tactile, chemical, evaporative, or osmotic stimuli. The fluid movement triggers mechano-receptors of pulpal nerve fibers and is interpreted by the brain as pain. Exposure of dentin commonly results from wearing away of the tooth's protective surfaces (cementum and enamel) due to gum recession, acid exposure, and/or abrasion. The management of dentin hypersensitivity has classically consisted of using dentifrices that contain an ingredient that serves to modulate nerve depolarization and disruption of neural response to pain stimuli, as the first line of action. This method does have shortcomings in that it does not address the cause of the problem (open dentin tubules) and does not provide rapid relief.

Potassium nitrate (5%) is a well-established desensitizing agent, 5% of which has now become the widely accepted standard for application in toothpaste formulations containing fluoride. Potassium nitrate depolarizes and inactivates the pulpal nerve, thus reducing hypersensitivity and pain associated with exposed dentinal tubules. This ingredient is widely used in dentifrices claiming to reduce hypersensitivity and does have a "salty" taste but otherwise is easily formulated and is compatible with fluoride. It is the only other ingredient approved for that purpose by the FDA.

Another approach, aimed at hypersensitivity relief, uses occlusion technology to plug or seal the tubules to prevent fluid movement within the dentin tubules and the subsequent pain response. Both available technologies will be explained in detail below. Occlusion technologies include oxalates, stannous and strontium precipitates, amorphous calcium phosphate (ACP), bioactive glass, arginine bicarbonate, and others. These agents have been investigated for the treatment of hypersensitive teeth, with various degrees of clinical efficiency. For example, arginine bicarbonate–based dentifrices offer instant and lasting sensitivity relief based on a significant number of published clinical studies.

Some anti-occlusion agents, such as stannous salts or bioactive glass, along with the low-water delivery systems required to maintain their efficacy, can sometimes result in compliance issues due to taste or mouth-feel issues. Recently, a newly developed and clinically proven hypersensitivity technology that is compatible with most dentifrice formulations and is based on using a bio-adhesive agent with specially designed hydrophobic silica to occlude dentin tubules has also been introduced commercially outside the U.S.[23]

6. Non-Therapeutic Ingredients for Cosmetic Benefits

Dental calculus, or tartar, afflicts the majority of adults worldwide. Dental calculus is ossified dental plaque. It is composed primarily of calcium phosphate mineral salts deposited between what remains of previously living microorganisms, and can form above and below the gum line (supra- and subgingival). These materials typically have a rough and porous surface that provides an ideal environment for stain accumulation as well as further accumulation of plaque. Brushing and flossing can remove plaque that eventually leads to tartar; however, once formed, tartar is too hard and firmly attached to be removed with a toothbrush. Tartar buildup can be removed at the dentist office with ultrasonic tools or unique hand instruments.

A comprehensive review was given by Volpe et al.[24] describes in some detail agents that have been clinically documented to prevent tartar formation as well as the most widely accepted methodology to assess supragingival calculus based on Volpe-Manhold. This review is especially intriguing since it suggests a connection between calculus and gingival recession.

The most used tartar control agents in dentifrices as well as rinses are collectively known as condensed linear phosphates, or polyphosphates. The commercially available and best-known polyphosphates are the salts of pyrophosphates, tri-polyphosphates, and hexameta (glassy) phosphates (number of phosphorus atoms equal to 2, 3, and ~21). Polyphosphates act to inhibit the crystal growth (deposition) of the minerals (mostly calcium phosphates) in the plaque on teeth by forming a soluble complex with the calcium in the plaque, and thus control the tartar formation.

Also well known to prevent tartar are zinc salts such as zinc citrate, zinc lactate, and other soluble and insoluble zinc salts. The way the zinc ion works to prevent tartar is to poison the calcium phosphate crystal growth process by replacing calcium in the crystal lattice.

B. Mouthrinses

Mouthrinses are specially formulated solutions that are meant to be vigorously swished around the entire mouth so that the rinse comes in contact with the entire mouth—teeth, gingiva, tongue, and other oral tissues. (The authors have chosen to use the term “mouthrinse” but consider the terms “mouthwash” and “mouthrinse” as having the same definition.) There are several indications for the use of mouthrinses depending on the formulations. There are mouthrinses that are intended to help to improve oral hygiene/health (kill germs, reduce plaque, and prevent gingivitis),[25] reduce oral discomfort, strengthen enamel, provide moisture to oral tissues, and fight bad breath.[26] Oral rinses may be purchased over-the-counter (OTC) or via prescription. Mouthrinses, like dentifrices or toothpastes, can be classified as

cosmetic or therapeutic, but can also be classified according to the specific end use, such as antibacterial, antigingivitis, whitening, and antiplaque. Historically, most mouthwashes have contained alcohol (6–27%) to provide the refreshing taste and help to solubilize flavor. In recent years, many major manufacturers have marketed rinses free of alcohol due the tissue irritation that occurs with alcohol and the sensitivity that some individuals have that preclude the use of alcohol.

Most of the mouthwashes have been formulated to be ready for use, while some mouthwashes (mainly in Europe) are more concentrated and are intended to be used after dilution. Mass-marketed ready-to-use mouthwashes will be the primary focus of this section.

From a usage experience point of view, a mouthwash should be a liquid of reasonable viscosity when used (not too thin or too thick), with a "pleasant" flavor. It should be safe to use on a daily basis and robust against bacterial growth during storage. Such products may be designed to foam in order to reinforce the concept of cleaning. It should be stable over the temperature range likely to be encountered during its distribution and anticipated shelf life. Although it is often a clear and single-phase solution, a few dual-phase mouthwashes, such as Dentyl pH (Fresh Breath Ltd) and Plax 2 in 1 (Colgate-Palmolive), do exist.

1. Ingredients

There is a large overlap in the ingredients used and their functions in toothpastes and mouthwashes. The approach taken in this section is to mention only those ingredients in fairly wide usage in mouthwashes.

Solvents. *Water* is the first ingredient in all ready-to-use and most concentrated mouthwashes. Purified, distilled, or deionized water is normally used to avoid interactions with other ingredients and to provide a neutral starting base for the mouthwash.

Ethyl alcohol is used in many ready-to-use mouthwashes and can be found in concentrations ranging as high as 6–27%. Alcohol helps to deliver the desired fresh-mouth feeling, and even at low levels aids in the solubilization or emulsification of the flavor, and depresses the freezing point of the formulation. It also helps to stabilize the product against microbial growth.

There was a great deal of controversy questioning whether high concentrations of alcohol were related to oral cancer. A multitude of studies have been conducted on this issue and there has been no correlation found between alcohol in mouthrinses and oral cancers.[27–29] Moreover, the American Dental Association and the U.S. Food and Drug Administration have found no indication of a causal relationship between alcohol in mouthrinses and oral cancers. Alcohol can have a drying effect on oral tissues and can be especially irritating for individuals who

have xerostomia or reduced salivary flow. Individuals with alcohol-dependency problems should not use alcohol-containing mouthrinses. For persons who need to avoid alcohol-containing mouthrinses for any reason, there are nonalcoholic cosmetic mouthrinses readily available. Many of the nonalcohol mouthrinses are as effective as the alcohol-containing cosmetic mouthrinses.[30, 31]

Flavor is the reason for choice of a mouthwash by most consumers. Predominant flavor types for cosmetic type mouthwashes in the United States are mints such as spearmint, peppermint, and to a lesser extent wintergreen. These are not usually just straight oils such as peppermint *(Mentha piperita)* oil but formulated with other flavoring compounds to provide a more complex taste profile. Herbal, woody, floral, and medicinal (or phenolic) type notes are routinely added to these compounded flavors.

Flavor preferences vary widely from country to country and are even different for demographic groups of the same country. Due to the differences in flavor preferences and the relatively high cost of some flavor oils, it is necessary to carefully formulate the flavor for a product. The best way to select a flavor for a given product is by screening the prototype flavors with a target group of consumers for the product. Beyond just the taste of the flavor, the flavor may act to mask or modify the perception of bad breath, including malodor associated with "morning mouth" or the ingestion of certain types of food, such as garlic and onion. Studies are often designed to determine the effectiveness of candidate flavors in masking or altering the perception of the target odor. A common test to determine the effectiveness of a given flavor against specific malodor involves the use of judges trained in the identification of malodor.

The *essential oils/phenolics* commonly referred to in mouthwashes include thymol, eucalyptol, methyl salicylate, and menthol. Many people consider these ingredients to add a "medicinal" or "antiseptic" character to the flavor of a mouthwash.

Synthetic coolants, often the esters of menthol, have been used in products to provide a long-lasting cooling effect without the strong aftertaste associated with menthol.

Synthetic sweeteners are usually added to mouthwashes to make them have a more desirable taste. The most widely used sweeteners are sodium saccharin, sucrolase, and potassium acesulfame.

Humectants are used in mouthwashes to aid in the solubilization of flavors, to modify the mouth-feel, to add sweetness, and to increase the osmotic pressure of the mouthwash to decrease the risk of microbial growth. Humectants that have been used in recently marketed products are essentially the same as those used in toothpastes: glycerin, sorbitol, hydrogenated starch hydrolysate, propylene glycol,

and xylitol. Higher levels of humectant are usually used in alcohol-free mouthwashes.

Surfactants are used in mouthwashes to emulsify or solubilize flavors and other non-water-soluble ingredients to obtain a clear product. These surfactants may also contribute to the cleansing effect of the mouthwash. Combination of the surfactants is often necessary to achieve product clarity. To ensure the cosmetic stability throughout the shelf life of the product, the formulation is evaluated for clarity and phase separation at both initial and simulated storage period under low- and high-temperature conditions.

Surfactants are widely used in mouthwash formulations. The more commonly used poloxamers are poloxamer 407, poloxamer 338, and poloxamer 124. Polysorbates, such as polysorbate 20, polysorbate 60, polysorbate 80, and steareth-20, are also used widely in mouthwash formulations. PEG-40 hydrogenated castor oil is another emulsifier used in some mouthwash products. Cationic emulsifiers were used in the past but have been abandoned in recent years due to the off-notes associated with these materials.

Anionics, for example, sodium lauryl sulfate and sodium lauryl sulfoacetate, have been used in mouthwashes, usually in combination with a nonionic. They are, however, not used in formulations containing cationic materials due to their incompatibility.

Buffers are used in some products to maintain the pH within a narrow range to help stabilize or improve the efficacy of certain ingredients. Common buffering systems include benzoic acid/sodium benzoate, phosphoric acid/sodium phosphate/disodium phosphate.

Antimicrobials. Besides killing "germs," the antimicrobials also help to reduce bad breath, plaque, and gingivitis. *Chlorhexidine digluconate* (CHX) is a cationic quaternary antimicrobial agent. It was first used in Europe in mouthrinses to treat gingivitis and periodontal disease and has now been used in a 0.12% concentration in the U.S. for many years (it is available in the U.S. by prescription). CHX is effective as an antimicrobial, as it 1) binds to oral hard and soft tissues (a process known as substantivity) and 2) causes the bacterial cell membrane to leak and allows the cytoplasm to leak out of the cell, causing cell death.

Cetylpyridium chloride (CPC) is also a cationic quaternary antimicrobial agent and binds to oral tissues, but not to the extent that CHX binds to oral tissues. CPC ruptures bacterial cell walls and destroys the cytoplasmic contents. The results of six-month studies of CPC have demonstrated a 24% reduction in gingivitis and a 16–28% reduction in dental plaque and biofilm.[32]

The *phenolic-related essential oils* used in mouthrinses include a combination of thymol, menthol, eucalyptol, and methyl salicylate. The action of the phenolic

causes a disruption of the bacterial cell wall and inhibits bacterial enzymes. The substantivity of phenolic-related essential oil-containing mouthrinses is poor compared to CHX. However, these essential oil mouthrinses are antimicrobial, have antigingivitis properties and have reduced bleeding upon probing in clinical trials.

The total antimicrobial effect of a formulation is a combination of various factors: the mechanical effect of rinsing debris and microorganisms from the oral cavity, possibly enhanced by a surfactant; the effect of the antibacterial agent on the oral flora; and the possible effects of the flavor components and the alcohol, if present at a high level. Synergistic effects may also occur and should be evaluated.

Other Ingredients. *Hydrogen peroxide* (1–2%) can be used as an oxidizing/bleaching agent in mouthwashes. Because of the reactivity of hydrogen peroxide, it is not compatible with certain mouthwash ingredients, such as many flavor ingredients. Formulations with hydrogen peroxide may also require special storage conditions to minimize the degradation of the peroxide.

Chlorine dioxide, or its precursor in formulations, sodium chlorite, is used as an oxidizing agent in mouthwashes. These oxidizing materials are used primarily to fight bad breath but could also provide some antibacterial effect if properly formulated.

Zinc salts, such as zinc chloride and other soluble zinc salts, have been used in mouthwashes as a tartar control ingredient. Zinc salts are known to react with volatile sulfur compounds and therefore contribute to the breath-freshening effect. Studies have shown that they also have some antiplaque activity.

Fluoride. Sodium fluoride is compatible with most common mouthwash ingredients and can be easily formulated in mouthwashes. This effective material played an active role in inhibiting demineralization and enhancing remineralization of enamel. In the United States, fluoride-containing mouthwashes are regulated by the FDA as over-the-counter drugs only for cavity protection. Limits on the level of fluoride, formula composition, and permissible claims are outlined in the FDA anti-caries monograph and should be reviewed and followed carefully during formulation development.

Preservatives are used in mouthwashes, especially alcohol free mouthwashes, to help maintain the micro-robustness during manufacturing process and throughout the product shelf life. Common preservatives used in mouthwashes include parabens (methyl and propyl-paraben), potassium sorbate, and sodium benzoate. Most of the preservatives have an optimal pH range for the best preservation effect. It is important to take this into consideration when developing mouthwash formulation.

There are many ingredients that are occasionally used in mouthwashes. These include a number of salts, polymers, surfactants, antibacterial agents, and

natural extracts. One of these ingredients is tetrasodium pyrophosphate. Tetrasodium pyrophosphate is normally used in mouthwash for its anti-tartar and stain-prevention effect. Another ingredient is xanthan gum, which is used for its film-forming properties to stabilize the foam generated by the surfactant. Sodium bicarbonate or baking soda is used in a few mouthwashes for its malodor-reducing properties.

FORMULATION EXAMPLES OF MOUTHWASHES

Alcohol-Free Mouthwash

Ingredient	**%**
Water	86.01
Benzoic acid	0.04
Sodium benzoate	0.15
Poloxamer 407	1.25
Glycerin	12.00
Sodium saccharin	0.05
FD&C Blue No. 1	0.0002
Flavor	0.25
Polysorbate 20	0.25

Alcohol-Containing Mouthwash

Ingredient	**%**
Water	76.18
Glycerin	8.00
Sodium benzoate	0.10
Benzoic acid	0.04
Sodium saccharin	0.08
Cetylpyridinium chloride	0.05
FD&C Blue No. 1	0.0002
SDA alcohol 38-B	15.00
Flavor	0.25
Polysorbate 80	0.30

2. Manufacture

Mouthwashes are generally prepared in three steps. In step one, water-soluble/miscible ingredients including actives, surfactants, some humectant, and colors are dissolved in water. In step two, flavor is dissolved in the remainder of humectant, such as propylene glycol, as a premix. Step three, premix is added to the aqueous

phase and emulsified to form a homogenous solution. The final step in production is filtration through a series of filters to ensure that no particulates are present in the final products.

3. Packaging

Both ready-to-use and concentrate mouthwashes are packed in bottles. Historically most mouthwashes were packed in glass with a re-sealable cap. Glass was considered to be nonreactive and provided "perfect" barrier properties. The trend in the beverage industry toward lighter-weight and more break-resistant plastic containers has influenced the manufacturers of mouthwash to adopt plastic bottles. Today the majority of mouthwashes are sold in plastic bottles with a re-sealable plastic cap. The resins of choice for the bottle are polyethylene terephthalate (PET) and polyvinyl chloride (PVC). Some high-density polyethylene, exotic resins and coextruded bottles also exist. In general, PET offers better product transparency than PVC.

In the United States, FDA regulation requires "tamper-evident" packaging for mouthwashes, and Consumer Product Safety Commission regulation requires child-resistant caps for certain fluoride-containing mouthwashes.

6.18.2 ORAL HYGIENE AIDS

A. Manual toothbrush

The most often-asked questions of dental hygienists and dentists are "What toothpaste should I use?" and "What toothbrush should I use?" The professional answer to both questions will depend on which of these products best fits the patient's needs. For toothbrushes, there is an added dimension—which one will be the most effective for the patient, with regard to the thorough removal of dental plaque biofilm. The most fundamental point regarding dental health and dental plaque biofilm is that it must be mechanically removed. Dentifrices and mouthrinses are adjunctive and can certainly be bactericidal. None, however are 100% effective at killing the oral bacteria and removing the strongly adherent acquired pellicle (the pellicle is the first organic film to form on the tooth surface prior to the formation of dental plaque.

1. Historical Perspective

Since ancient times people have made devices for cleaning their teeth. Chew sticks, known as miswaks or *siwaks* sticks, were made of twigs from the arak tree and were some of the first known toothbrushes, made by the Babylonians as early as 3500 BCE. The invention of the bristle toothbrush was credited to a Chinese emperor in the seventh century. Stiff boar's hair was used as bristles and attached

to bamboo or bone handles. The toothbrushes were imported to Europe by travelers to China. The Europeans found the boar bristles to be too stiff and caused their gums to bleed. The Chinese altered their toothbrush by substituting horse hair for boar's hair. Toothbrushes were not mass-produced in Europe until 1780 when they were introduced by William Addie of Clerkenwald, England.

The first U.S. patent for a toothbrush was granted in 1857 to H.N. Wadsworth, but toothbrushes were not mass-produced in the U.S. until after the Civil War, around 1885. Inventors have been actively developing new designs ever since. Wallace H. Carothere worked for DuPont Laboratories and invented nylon in 1937. By 1938 nylon-bristle toothbrushes were being marketed in the U.S. The first nylon-bristle toothbrush was marketed as Dr. West's Miracle Toothbrush. The Prophylactic Brush, made by the Florence Manufacturing Company of Massachusetts, was the first rival to the Dr. West's toothbrush. After World War II, toothbrushes were available all across the U.S.

2. Importance of Toothbrush Features

The modern manual toothbrush has gone through a tremendous evolution. Consumers can choose from a large array of toothbrush handle shapes, sizes and colors, bristle profiles, and bristle stiffness. To satisfy their individual preferences, consumers can choose from many different product features.

The effectiveness of a manual toothbrush is, to a different degree, dependent on (1) the design and materials the toothbrush is made of, and (2) how well the patient uses the toothbrush. If the patient has had toothbrushing instructions from a dental hygienist or dentist and has had their toothbrushing effectiveness evaluated during maintenance visits, the patient is more likely to be successful at thorough dental plaque biofilm removal.

The patient, and how he or she uses the toothbrush, is critical to successful brushing. That stated, there are some features of toothbrush design that have been universally accepted by dental healthcare providers. Importantly, a soft to ultra-soft bristled toothbrush is the most important feature for thorough dental plaque biofilm removal. Softer bristles will get into spaces, pits, and fissures where hard- or even medium-bristled brushes will pass over the high portions of the tooth. Additionally, soft to ultra-soft bristle toothbrushes are much less likely to cause tissue trauma or discomfort. This is especially important to the patient that has gingivitis or periodontitis or sensitive teeth or gingiva. If the bristles are stiff and cause trauma to tissue that is already sensitive, the patient will be less likely to thoroughly remove the dental plaque biofilm.

The American Dental Association has safety and acceptance guidelines for manufacturers to obtain the Seal of Acceptance. The American Dental Association has incorporated the International Standard Organization's standards for manual

and powered toothbrushes with their own standards to formulate the guidelines for the Seal of Acceptance. Some of these standards include:

- toothbrush bristles must be soft
- the bristles must be free of sharp or jagged edges
- there are specific guidelines regarding the number of bristles in each tuft and requirements for bristle retention
- manufacturers have many creative handle designs; however, the only requirement from the ADA is that the handle be durable under normal use.

With the emphasis on having patients use soft-bristled toothbrushes, these are the factors that determine toothbrush stiffness:

- the number of filaments in each tuft
- the number of tufts
- the type of material used to make the bristles
- the length of the bristles
- the diameter of the bristles.

B. Powered Toothbrush

1. Historical Perspective

Like manual toothbrushes, powered toothbrushes have also undergone enormous changes since they were first introduced. There were several "powered toothbrushes" patented as early as 1885 that were not actually "powered" by electricity.

The first actual electrically powered toothbrush was made available to consumers in the U.S. after World War II. E.R. Squibb & Sons manufactured the *Broxodent* in 1960. In 1961, General Electric produced the first rechargeable cordless toothbrush. These early electric toothbrushes had simple actions. The Broxodent had a reciprocating back-and-forth motion and the General Electric toothbrush had an accurate motion. Dental hygienists and dentists did not immediately embrace these electric toothbrushes. Most often, dental hygienists and dentists recommended these toothbrushes to patients who had a physical impairment or had orthodontic appliances or some other type of need.[33] There were fears that patients would brush too vigorously. Early clinical research revealed no difference in brushing with a manual versus and electric toothbrush. Importantly, later studies indicated that the control of gingivitis was improved with a powered toothbrush.

With the latest generations of powered toothbrushes that range from sonic to ultrasonic, there is ample evidence that when used correctly, powered toothbrushes will remove dental plaque biofilm and reduce gingivitis equivalent to or better than manual toothbrushes. It must be emphasized that no matter what type of toothbrush is being utilized, it is the user that makes the most important impact on the efficacy

and thoroughness of cleaning. Following the instructions of the manufacturer and of dental professionals is of utmost importance.

2. Types of Powered Toothbrushes

a. Range of Products

An electric toothbrush is a toothbrush where the motion of the brush head is driven rapidly by electric power. In its earliest generation, the device was plugged into regular outlets and powered by AC current. The advent of the battery-operated design removed both the physical constraint and safety concerns of the AC-powered devices in the bathroom environment.

The basic **battery-powered toothbrushes** are similar in design to regular manual toothbrushes, where the brush head delivers similar reciprocal brushing actions. The power is provided by common AA or AAA batteries.

The rechargeable type is where the device is equipped with a cradle that plugs into the wall for the battery recharge. The cradle functions as the charging base using metal tabs to connect to the rechargeable batteries. In the more advanced designs, power is being transferred through contactless induction. The brush component is usually detachable from the main body and is replaceable when the bristles are worn. Rechargeable electric toothbrushes are, in general, more advanced in the degree of cleaning actions. They use dynamic motions such as oscillating-rotating or sonic technology to provide more effective cleaning and plaque removal.

With the innovation of electronic technology, the powered toothbrush advanced beyond the basic simulation of manual motion. The latest models include the Colgate® ProClinical® Powered Toothbrushes with distinct multidirectional cleaning action. One model, the A1500, combines high-frequency mechanism, electronic sensors, and an ergonomic handle design that intelligently and automatically adjusts the speed and the motion of the brush head corresponding to its position in the mouth. Without the need of specific training, consumers gained a stronger brushing experience and better cleaning of the teeth and gums with this unique sensing and control technology.

b. Action

Most power toothbrushes are simple to use, but quite different than manual toothbrushes. Unlike manual toothbrushes, which require the patient to generate the action of the toothbrush, power toothbrushes generate the bristle action and the patient needs to merely move the toothbrush around the mouth—tooth to tooth, ensuring that each tooth is cleaned thoroughly. Power toothbrushes have a variety of ways that the bristles move in the head of the toothbrush. Examples include:

- Head is stationary; each tuft of bristles moves in counter-rotational directions
- Rotating-oscillating head; stationary bristles

- Rotating-oscillating-pulsating
- Oscillating-pulsating-pivoting
- Rotary head with interchangeable heads that include a brush with a single tuft of bristles, a hollow-cup brush, and an elongated brush tip that rotates
- Side-to-side action
- Multidirectional cleaning action

c. Sonic and Ultrasonic

Power toothbrushes can be further classified according to the speed of vibration. Sonic toothbrushes vibrate in the audible range of 30,000 brush (260 Hz) strokes per minute. Ultrasonic power toothbrushes have a high-frequency bristle motion (18,000,000 strokes per minute) at ultrasonic vibration that produces greater than 20,000 Hz.

d. Additional Features

Newer products also feature a wide variety of computer-assisted capabilities. These features include timers, a variety of settings that range from a slower speed for brushing up to higher speeds meant for stain removal and whitening, and digital screens that inform the patient that they are brushing too hard or brushing incorrectly in some fashion or not brushing long enough.

C. Interdental Cleaning Devices

The one shortcoming of the toothbrush, whether it is powered to manual, is that none of them are made so that the user can adequately clean between the teeth. The lack of any physiological interdental cleaning underscores the need and importance of interdental cleaning devices.

1. Importance of Interdental Cleaning

Despite the mantra of "brush and floss" from dental professionals, studies tracking in-home use of dental floss indicate that only 2–10% of patients regularly performed acceptable flossing and most did not floss on a daily basis.[34]

The interdental papilla, the gingival tissue that lies between the teeth, has a physiological shortcoming that makes it more vulnerable to the effects of dental plaque biofilm and bacterial endotoxins. That shortcoming is a col area that is nonkeratinized (the col area is the area of the gingiva that is anatomically in the center from the facial side [cheek side] of the gingiva to the lingual [tongue-side] of the gingiva). It does not have the tougher, keratinized tissue that covers the outside surfaces of the gingiva. This col tissue is very susceptible to being ulcerated due to endotoxins and inflammatory reactions due to the bacterial challenge. If dental

plaque biofilm is not removed at least once every 24 hours, that nonkeratinized gingiva is exposed to material that will cause it to break down and succumb to inflammation and chronic periodontal disease. Further, this interdental plaque is the source of some of the volatile sulfur-containing compounds from bacteria that cause halitosis. If patients do not believe this, all they have to do is floss their teeth and then smell the floss! They will be instant believers!

2. Dental Floss—The Shortcomings

The highest form of scientific evidence is the systematic review. There have been some excellent systematic reviews on the use of dental floss.[34–37] The information revealed in these studies is very important, but may not come as a surprise to dental professionals:

- The strength and quality of evidence in most randomized clinical trials on dental flossing is very low.[36]
- There is insufficient evidence that dental floss reduces plaque.[40]
- There are no studies that indicate that the use of dental floss reduces the risk of dental caries.[35]
- The quality of evidence to support the theory that flossing and toothbrushing reduce gingivitis is very low.[36]
- When dental professionals flossed patients' teeth, significant benefits were seen that were not seen when patients flossed their own teeth.[37]
- Many individuals do not know how to floss properly or have the dexterity to do so; for subjects researched that did use floss with toothbrushing, there was no added benefit to the flossing.[35, 36]

It makes sense that if the interdental spaces are too wide for floss to contact both sides of the interproximal surfaces of the teeth, then the floss is not going to be effective at removing the dental plaque biofilm. It is logical that any interdental cleaner that is wide enough to fill the interproximal space and reach to the interproximal surfaces will be effective in interproximal plaque removal. Dental hygienists and dentists need to be responsible for assisting patients to find the best interdental cleaning method to meet their need.[38]

3. Interdental Brushes

Interdental brushes are one of the most efficacious interdental cleaning aids available. They are available in various sizes; the very smallest interdental brushes (in diameter) will normally fit a normal, healthy interdental space that is tight. There are larger sizes and importantly, they are available in conical and cylindrical shapes. Interdental brushes are available with nylon bristles and also with elastomeric "fingers" that work similarly to bristles.

4. Additional Interdental Cleaning Aids

There are a multitude of interdental cleaning aids available, and dental hygienists and dentists must be careful in guiding patients to the aids that will be most useful for them—the recommendations should be patient-centered. It is important that dental healthcare professionals be aware of the scientific evidence behind the interdental aids and ensure that the patient has been instructed on the correct use of the interdental aid. The interdental aid that will be most successful is the one that will adequately fill the interproximal space (embrasure) without causing tissue trauma. Fortunately there are numerous types, shapes, and sizes of interdental aids available:

- End-tufted brushes
- Floss threaders
- Interdental cleaners with elastomeric "fingers"
- Interproximal (interdental brushes)
- Powered flossers
- Rubber-tip stimulators
- Soft wooden sticks
- Textured flosses
- Toothpicks
- Waterflossers (oral irrigators)

Figure 2: Floss holders

Figure 3: Powered Flosser

Figure 4: Floss Threader

Figures 2–4: illustrate various interdental cleaning aids.

SUMMARY

Oral health is taken much more seriously now that it is recognized that an unhealthy mouth with millions of disease-causing bacteria is related to systemic diseases. It is quite logical that the presence of chronic inflammation lasting for years with soft tissue and bone destruction and tissue death is going to have effects on other parts of the body and body systems. Other healthcare professionals are realizing that the mouth is connected to the rest of the body. Clearly there will be research that continues to explore the relationship between oral health and overall systemic health.

There has never been a time where so much has been known about what causes dental diseases, and this knowledge is accompanied by a wealth of products that can support successful oral preventive self-care. The use of fluoride and oral health education has worked and the payoff is tangible: senior citizens, even in nursing homes, have their teeth. The future of dental health has never been better as long as citizens of all ages have access to care.

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SENSORY CHARACTERIZATION

SENSORY SIGNALS— THE APPLIED SCIENCE OF SENSORY PERCEPTION AND ITS VALUE

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ABSTRACT

This chapter provides the reader with an objective use of the Senses and Sensory Evaluation to describe perceivable behavior in the world of personal care and cosmetic products. Sensory perception is at once fundamental and ambiguous. Each of us uses our senses to understand the world around us through vision, touch, hearing, smell, and taste. Through interpretation of those sensations by our brains, we name what we perceive, and those naming labels are influenced by our background and training. Through organized, objective understanding of product features, standardized attributes can be developed and applied for testing and decision-making.

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7.1.1 OVERVIEW

The field of Sensory Evaluation has evolved as a tool to understand and organize our perceptions. It is a scientific discipline that addresses methods to evoke, measure, analyze, and interpret human responses to properties of materials perceived through our five senses. While there is valuable basic research into how the brain interprets sensory input, the most common purpose of sensory evaluation is to conduct valid and reliable tests that provide data for decision-making. This is especially important in the development of cosmetic and personal care products whose purpose is to enhance the perception of "beauty."

Measurement of Sensory perception is an ambiguous science due, in large part, to *differences in the way we express what we perceive* as opposed to the differences in *what we perceive* (although those differences exist as well). When working with others, our individual interpretations and previous experiences may lead to our describing different sensations with the same word or to describing the same sensation with different words. This issue often causes confusion and miscommunication. The situation is all too common when describing personal care products. Researchers and marketers in the personal care and cosmetic industries have expressed frustration with this lack of uniformity since it often leads to inefficiency in development, marketing, and testing. On the positive side, sensory evaluation testing, thoughtfully done, can measure subjective consumer perceptions to products through their liking of and preferences for products. It can also provide a focus on objective measurement of important product features that are useful in further product development, product maintenance, and the generation of claims for such products.

Even with the growing use of sensory evaluation within personal care product and ingredient companies, *its applications are weighted towards product assessment based on liking* as well as preference by the target consumers, the client, or the decision-makers within a company. This chapter provides an introduction to *objective* sensory evaluation. It describes methodology for documenting the sensory properties of personal care products through use of a technical-based language called Descriptive Analysis currently in use by many in industry, academia, and consulting.

The approach to effective objective sensory evaluation is provided through use of lexicons (representative and comprehensive lists of words to describe a product or category) and protocols, methods use for assessment, as well as leveraging other sciences such as chemistry, rheology, and physiology. This system provides a harmonized framework for understanding product attributes as well as evaluation of objective techniques to characterize appearance, texture, and fragrance across a wide range of products. Examples include commercial formulations, model systems, and raw ingredients that are applied to the skin, body, and hair.

While this chapter is an introduction to this methodology, it does not replace a full descriptive analysis training; rather, it provides examples of an objective language for describing sensory perception as related to personal care products and allows users to have an improved ability to communicate with one another. It describes the process of lexicon development, provides sample lexicons for several large product categories, and demonstrates the underlying commonalities across disparate product categories. The conclusion of the chapter provides several applications of objective descriptive analysis for decision-making within companies.

7.1.2 HISTORY OF SENSORY EVALUATION

Use of our senses for learning and decision-making has existed throughout history. People categorize products as good or bad, more or less, acceptable or not acceptable, and they learn to assign intensity judgments to these categories as well as product features.

This basic use of the senses for measuring and judging was refined as trading, buying, and selling flourished. Portions of lots were viewed or sampled in order to set prices and determine quality of foods and other goods. The process of product grading continues to this day worldwide. By the early 1900s, measurement of product sensory properties extended with the addition of the professional taster and consultant (Meilgaard et al. 2007). The field grew for measurement of sensory properties in foods as well as for measurement of consumer subjective ratings for acceptance of food products, such as that conducted by the U.S. Army Quartermaster Food & Container Institute to measure food acceptance for the U.S. Armed Forces (Peryam et al. 1954).

By the late 1950s and 1960s, more scientific rigor in terms of assessor selection, testing conditions, testing procedures, and statistical analysis began to be employed at the university and industry levels (Pangborn 1964; Caul 1957; Szczesniak 1963). The Food Science Department at the University of California at Davis was one organization that advanced the field, resulting in the foundational book *Principles of Sensory Evaluation of Food* (Amerine, Pangborn, and Roessler 1965).

While sensory evaluation, or organoleptic testing (Pfenninger 1979), was initially focused within the field of consumer food products, it has increasingly been applied to personal care, home care, paper, and fabric products. Organoleptic testing was an attempt toward objective testing of products. As the field has continued to develop formalized methodology/practices for effective consumer testing relative to acceptance and preference and for objective testing for discrimination and measurement of product attributes, the term has fallen out of favor.

The first publication cited for objective sensory evaluation of personal care products was Schwartz, 1975, with Adaptation of the Sensory Texture Profile

Method to Skin Care Products, based on the Texture Profile Method developed by General Foods in the 1960s (Szczesniak 1963). Sensory Spectrum applied and expanded this methodology through the Spectrum Descriptive Analysis method in the 1980s and published two research papers on descriptive analysis techniques. One of these was for skin care products (Civille and Dus 1991) and the other for paper/fabric (Civille and Dus 1990).

A number of discrete sensory methodologies for evaluation of personal care products are in current use, with some practitioners blending methodologies. Regardless of methodology, the working sensory professional is tasked with acquisition and interpretation of valid data. Erhardt (1978) specifies seven tasks for sensory research:

- determine the project objective
- determine the test objective
- screen the samples
- design the test
- conduct the test
- analyze the data
- interpret and report results.

Sensory Evaluation methods, research, practices, and applications are documented in journals including, but not limited to those such as Chemical Senses, Journal of Sensory Studies, Journal of Texture Studies, Food Quality and Preference, Journal of Food Science, Food Technology, Cosmetic & Toiletries, and Journal of Cosmetic Science.

Conferences on the subjects include: Sensometrics (biannual), Pangborn Sensory Science Symposium (biannual), Society of Sensory Professionals (biannual), and Eurosense (biannual). These conferences are associated with professional organizations. Selected standards and guides are published by ASTM Committee E18 (www.astm.org) and the International Organization for Standardization (IS0) (www.iso.org). A growing number of independent companies and consultancies are devoted to sensory evaluation services, with some providing training courses, and several textbook stylebooks on sensory evaluation are widely available. While the majority of resources are focused on foods and beverages, sensory evaluation resources specific to "nonfoods" continue to grow rapidly.

7.1.3 DEFINING SENSORY PROPERTIES

Sensory properties are defined as the characteristics perceived through the senses; *not the liking or preference for products*. These properties exist, regardless of whether we like them or not, regardless of whether we're even aware of some

of them. They are what a product looks like, smells like, tastes like, feels like, and sounds like. They can also demonstrate what a substrate, such as the skin or hair is like, and how the interaction with a product changes that substrate during and after product use.

Meilgaard et al. (2007) provide an extensive list of references for study of the human senses and the properties measured by them.

High-level categories for sensory properties are described as follows:

- **Appearance:**
  Characteristics measured by the sense of vision to include optical and physical characteristics.
- **Aroma/Olfaction:**
  Volatiles perceived by the olfactory system through the nose or oral cavity. The biology of the nose and its sensing ability are extensively covered elsewhere in this book.
- **Flavor:**
  The complex, integrated effect of basic taste sensations (the perception of properties perceived through the taste buds), olfactory sensations, and chemical feeling factors/trigeminal nerve (the fifth cranial nerve) sensations stimulated by a substance in the mouth.
- **Texture:**
  The way something feels when touched or eaten, based on its surface and/or the way it changes when compressed or manipulated, to include geometrical, moisture, mechanical, and noise-based properties.
- **Sound:**
  Noise characteristics measured by the sense of hearing during mastication or manipulation of products.

While all senses can come into play when measuring the sensory properties of personal care products and ingredients, those properties most commonly measured are appearance, texture, and, particularly for finished goods, aroma. Products designed for use in or on the mouth, such as oral care products and lip products, are often evaluated for flavor.

7.1.4 RATIONALE FOR GENERATING TECHNICAL-BASED LANGUAGE IN ORDER TO OBTAIN OBJECTIVE PRODUCT DESCRIPTIONS

Objective description of product attributes can be expressed through technical-based language rooted in other scientific disciplines such as rheology, chemistry, and physiology. Use of a technical-based language has several advantages for developers and researchers over consumer language–based descriptions.

Consumers are very good at describing what they do and do not like. They are less adept in providing detailed reasoning for their opinions. **Typical consumer-based describing language tends to**

- Categorize perceptions into good/bad
- Compare to other familiar (to the consumer) products, without context
- Include emotions
- Be vague or inconsistent
- Include attribute terms that are integrated, having multiple technical components (e.g., creamy, slimy, gummy)

Conversely, **technical-based language is designed to**

- Consider terms as neutral descriptors, not as being positive or negative
- Identify and define physical- or chemical-based terms
- Use fundamental, single-meaning terms rather than multifaceted, integrated terms

Technical-based language can be used to "break open" perceptions that are difficult for consumers to articulate, or have multiple facets, such as "soft," "refreshing," or "creamy." Generation and understanding of an objective language for describing product attributes has the following benefits (Stapleton 2013):

- Places the focus on product properties and design features, rather than individual evaluator or decision-maker preferences for products
- Simplifies intra-company communication across business units and locations so that all parties understand attribute terms in the same way
- Streamlines development and research due to better feedback and direction
- In many cases, correlates to instrumental testing

Certainly, consumer liking and preference are of critical importance to the success of products in the marketplace. Consumer descriptions of products garnered from questionnaires, reviews, interviews, and other sources have value for marketers, developers, and sensory personnel since they provide an understanding of what consumers value, despite its often-imprecise nature. The adept researcher develops skills to translate the "fuzzy" world of consumer responses into objective parameters that can be measured and manipulated to create consumer-preferred products. Understanding products from an objective standpoint enhances the ability of the researcher to make those connections to ask consumers questions in ways that yield more useful detail.

7.1.5 INTRODUCTION TO DESCRIPTIVE ANALYSIS METHODOLOGY

Descriptive analysis is a tool that provides detailed documentation of product properties and allows more objective comparison of products for enhanced decision-making. Descriptive analysis records the applicable sensory characteristics of a product in terms of the attributes perceived and the strength of each attribute.

Descriptive analysis methods involve the detection and the description of both the qualitative (attribute terms) and quantitative (intensity of signal) features of a product by trained panels of judges prescreened for acuity in the categories of interest and absence of preexisting conditions or health issues for the panel of interest (ASTM STP758 1981).

Panelists may be recruited internally or externally through agencies, advertising, word of mouth, and more recently through social media, which to an accelerating degree in the rapidly evolving use of the Internet, is supplanting more traditional newspaper advertising. The number of judges is based on the level of training and risk level of the decisions made by the panel and varies between 5 and 25. Training involves introduction and practice in the terminology, evaluation protocols, and scaling for product evaluation. Scaling techniques involve the use of numbers, distance, or words to express the intensity of a perceived attribute across differing products (Meilgaard et al. 2007).

Choice of a scaling system is dictated by the precision needed in measuring differences, the amount of training the panel receives, the selection of a specific descriptive analysis method and its recommended scale, and the historical use of objective sensory data within a company. Scales should have the ability to discriminate small differences. Post hoc, line, and numerical intensity scales can be transformed to other scale systems. Category data such as counting how many times a motion occurs or assigning yes/no presence to a perception does not need to be transformed. For analysis, scales must be converted to numerical values.

The Spectrum™ Descriptive Analysis Method (Civille) uses technical-based language for terminology and grounds itself in the use of published and internal intensity reference scales to define intensity boundaries in sensory experiences. This method is utilized globally by a variety of finished product, cosmetic, and ingredient personal care companies. Other descriptive analysis methods are in use for evaluation of personal care products reflecting differences in training philosophy and data collection assumptions. Moreover, companies often blend methodologies to create internal, customized sensory testing programs. In this chapter, the Spectrum™ Descriptive Analysis Method serves as an exemplar of a successful modality to achieve our analytical goals.

7.1.6 THE SPECTRUM™ DESCRIPTIVE ANALYSIS METHOD: PHILOSOPHY AND PRINCIPLES

The Spectrum™ Descriptive Analysis Method for personal care products trains professionals with the goal of generating panelists that function similarly to a calibrated instrument. Panelists are screened and selected on their ability to detect and discriminate differences in appearance and tactile qualities. Fragrance evaluation can be trained within the same or a different panel; again, panelists must screen and pass screening for acuity. Panelists are trained on a universal scale that focuses on intensity or strength of the signal coupled with detailed description and definitions of sensory attributes and use of calibrated training samples. In this method, panelists are trained for visual and tactile evaluations and receive a minimum of 100 hours of training and practice prior to commissioning for sensory studies. Their training is provided across a diverse set of products within a broad category such as lotions, creams, and gels.

Attribute intensity is rated on a 100-point intensity scale, with 0 = none and 100 = very strong/very high. The intensity scale uses 1-point increments. Alternatively, a 0 to 10 scale using 0.1 increments can be employed. Panelists are trained to use the scale in a similar way across panelists and across samples. Data are collected from the individual judges with replication of sample presentation recommended. Analysis is designed for correlation with both instrumental and consumer research data. Use of a universal scale allows attribute intensity to be compared across various parameters, e.g., comparing intensity of slippery feel to intensity of sticky feel, as well as for comparison of samples within and across studies and products having shared attributes. The panel is monitored for accuracy and performance with ongoing conformance assessments. For further detail and for historical foundations of the Spectrum™ Descriptive Analysis Method, see Meilgaard et al. (2007). Project-specific orientation is provided to a working panel as needed. Following initial training, panelists are often trained in additional product categories as appropriate to the end user. Subsequent training follows the same universal scaling construct such that panelists always interpret the scale intensities in a similar way; for example, a value of 47 on a 100-point scale represents a moderate intensity for any attribute.

When training a new panel, the group may generate its own terminology based on close and repeated examination of a range of products following the guidelines for development of technical-based language, or it may rely on established terminology such as presented here. In either case, this process includes using references to clearly demonstrate each term so it is understood in the same way by all panelists. The scope of terms may be broad or focused according to the panel's purpose and the test objective. Repeated exposure and practice, along with ongoing availability of reference standards, allows attribute understanding to be

internalized and provides ongoing intensity calibration similar to that used for an instrument.

While a traditional Spectrum™ panel requires approximately 100 hours of training and practice with the terminology and scaling system before being capable of performing within expected tolerances for repeatability, reliability, and accuracy, the initial process of learning terminology, qualitative references, and protocol techniques can be introduced in a matter of ten hours or less. Companies often benefit from having their developers and marketers understand technical/descriptive language around a product category in order to focus internal product assessment on objective descriptions rather than personal liking and preferences. In such cases, these oriented persons are not intended to function as a statistically valid panel. With some additional practice beyond the initial orientation, companies sometimes use a subset of these persons to screen samples and gather initial information on competitive products.

7.1.7 FUNDAMENTALS FOR DEVELOPING LEXICONS

Lexicons are systematically created descriptor lists to objectively characterize the sensory properties of consumer products. They allow differing parties to communicate efficiently when describing products of interest (Lawless and Civille 2013).

While hundreds of lexicons exist in the literature, it is the responsibility of the user to assess the quality of any lexicon considered for use. ASTM International (www.astm.org) prescribes five steps for development of a well-designed lexicon, based on use of an established panel and valid protocol evaluation techniques (ASTM Stock #DS72 2011):

1) Select a large number of products representative of the category breadth to establish a "frame of reference"
2) Generate terms that describe the products
3) Use references that clarify the terms and definitions
4) Use examples so that the panel understands the attribute terms well
5) Develop the final list of lexicon terms

During selection of the final list of lexicon terms, attributes should be reviewed to ensure they are nonredundant and not correlated with one another (Civille and Lawless 1986). The lexicon is validated by using it to compare a small subset of products in the category as confirmation that it will describe and discriminate between the products. In addition to providing terms, definitions, and examples, lexicons should provide specific instructions for the way the attributes should be assessed (Meilgaard et al. 2007). Lexicons developed using these principles are well designed and can be successfully used by multiple panels or trained groups to describe products. They are created for use with a scaling system so that intensity

differences between similar products can be differentiated. However, lexicons can be applied to multiple scaling systems and often do not include scale intensity information apart from indicating the directionality of the scale.

All lexicons presented in this chapter were developed using the principles above. These lexicons provide a strong foundation to those developing their own lexicons and for those interested in understanding product attributes.

Figure 1: provides a synopsis for developing a product lexicon.

7.1.8 PROCESS FOR DEVELOPING PERSONAL CARE PRODUCT LEXICONS

Personal care products exist in many forms ranging from lotions to shampoo, to facial scrubs to color cosmetics and antiperspirants—just to name a few categories. It is a common misconception that each product or product category is defined by a unique set of words. In truth, many attribute descriptors, for example those related to visual and tactile texture, are common to many forms. Once one learns the terminology for one product category, many of those same attributes exist in other categories. In part, this is due to the characterization of the underlying rheological characteristics (i.e., how the product's viscosity at different shear rates and measurement times changes when dispensed, deformed, and manipulated) in the context of their use within the world of personal/skin care as well as for foods and fabrics. Table 1 demonstrates the rheological manifestations of three mechanical force related attributes across foods, personal care products, and fabrics.

Table 1: Comparison of Three Mechanical Properties across Foods, Skincare, and Fabric

Mechanical Properties: Reaction of a product to a stress		
Hardness: Force to attain a given (stated) deformation		
Foods	Skincare	Fabric
firmness (compress)	force to compress	force to compress
hardness (bite down)	force to spread	force to stretch
Cohesiveness: Amount sample deforms/bends rather than ruptures		
Foods	Skincare	Fabric
cohesive/chewy	cohesive/stringy	stiffness/roundness
crispy/crunchy		
Adhesiveness: Force to remove from a given surface		
Foods	Skincare	Fabric
sticky (teeth/palate)	tacky/sticky	fabric friction to itself
toothpack	drag	hand friction/drag

What differentiates the lexicons for various products categories are the applicable terms chosen for each. For example, shampoo, facial cleanser, and bar soap all generate foam, though each may have bubbles of different size, variation, density, and amount. None of those attributes apply to nonfoaming products such as lotions, foot creams, and facial serums. In turn, part of what differentiates lexicons for related products such as the foaming ones described above are the test substrate selected (hair tress, cheek, and arm, respectively) and the protocol instructions for assessment. A detailed description of the various substrates are described elsewhere in this book. This description entails not only the physicality of the substrate but the chemistry, biology, and molecular genetic characteristics.

From the author's personal experience, the learned understanding of the "universal" nature of attributes across product types is mind expanding. Of note was the realization that grittiness (small, abrasive particles) for sandpaper, paper towels, razor stubble, and the flesh of pears during chewing all manifested in a similar

sensory signal although the substrates and matrices differed. Noticing the commonalities among disparate product types reinforces the fundamental concept of quantitative sensory perception technology that a given attribute can exist in multiple products, albeit at varying intensities or prominence. With practice, one can learn to focus on the sensory attributes that characterize a product rather than the product's brand image, reputation, or typical nature or on the evaluator's personal degree of liking for any facet of the product.

Training Process

In learning how to describe visual and tactile properties of personal care products, an efficient introductory category is the broad lotion category that includes lotions, creams, and gels. It is efficient for training for a number of reasons:

- Wide array of products are available
  - Broad Range of Intensities Across Attributes
- Provides a substantive product laydown, allowing residues to be perceived and named
- Is a familiar product form
- Is generally easy to remove using isopropyl alcohol or mild cleansers
- Has a form similar to other product categories such as:
  - Suncare
  - Shampoo/Conditioner
  - Liquid Soap/Body Wash
  - Foundation/Primer/Serum
  - Topical Pharmaceuticals (OTC and Ethical)
  - Certain Liquid-Form Ingredients

Attributes are introduced within the framework of a *testing protocol*. The protocol provides a set of procedural directions for conducting an evaluation. A thorough protocol provides instructions for the session leader and participants regarding room conditions, test site preparation, and product presentation in addition to the techniques used to assess the attributes. A well-written protocol allows trained evaluators to evaluate product in the same way, using the same techniques and under similar conditions, yielding results that can be directly compared to one another.

Attributes are introduced and learned individually. However, product assessment is a continuous process that includes the following phases:

- Application to the test substrate
- Product manipulation on the test substrate (extent of manipulation varies)
- Feel of product on test substrate post–product manipulation (one or more time points)

Product assessment can also involve the following:

- Visual evaluation of product during or after dispensing
- Tactile manipulation of the neat product without spreading

Role of References

In training, each attribute is demonstrated using one or more references. A reference can be a product applied to the test site following the technique used for assessment, such as a measured amount of cream on a defined area of skin rubbed a defined number of times, or it can be demonstrated using the native test site itself, such as a hair tress/switch or volar forearm skin. *A reference may be qualitative*, meaning it is clear or dominant in the attribute, *or can be quantitative*, meaning it possesses a scaled intensity amount of the attribute. When quantitative references are used, it is recommended that three or more levels are demonstrated. A reference allows association of a physical (or visual or chemical, as applicable) signal with the attribute so that the attribute perception can be discussed and understood. Discussion within the group is important, so that participants can recognize where their personal vocabularies are consistent and inconsistent with the attribute explanation demonstrated, and the focus can be placed on the sensory attribute, not on personal feelings about the attribute characteristics.

Table 2 demonstrates quantitative reference scales for two attributes—wetness and product thickness.

Table 2: Example of Skinfeel Attribute Intensity Reference Scales (0 to 100 point)

Attribute	**Intensity Reference**
Wetness Intensity	
0	Talc
22	White Petrolatum
60	Vaseline Total Moisture Original Lotion
100	Water
Product Thickness Intensity	
7	Light Mineral Oil
30	Vaseline Total Moisture Original Lotion
65	White Petrolatum
86	Neutrogena Norwegian Formula Hand Cream

7.1.9 SAMPLE LEXICON AND TESTING PROTOCOL FOR LOTIONS AND CREAMS

In the following section, products are dispensed by the panel leader or technician. Panelists record requested information for each attribute on a corresponding ballot for that lexicon and protocol.

Qualitative references provided demonstrate a moderately high to high intensity of the attribute.

Appearance and Feel of Lotions and Creams

Product Appearance (Appearance of Dispensed Product)

In a polystyrene petri dish, panel leader uses a standardized orifice container to dispense the product in a spiral shape using a 2-cm circle, filling it from the edge to the center.

Evaluate for:

Integrity of Shape	Degree product holds shape [flattens ---------- retains shape]
Qualitative Reference	Petrolatum, white
Integrity of Shape (10 seconds)	Degree product holds shape [flattens ---------- retains shape]
Qualitative Reference	Petrolatum, white

Tilt petri dish to see reflective properties and evaluate for:

Gloss	Amount of reflected light from product [dull/flat ---------- shiny/glossy]
Qualitative Reference	Baby oil or light mineral oil

Additional appearance attributes can be measured, including the following:

Color Hue	The actual color name or hue, such as red, blue, etc. The description can be expressed in the form of a scale range, if the product covers more than one hue.

Color Intensity	The intensity or strength of the color from light to dark: [Light ---------- Dark]
Color Purity	The chroma (or brightness) of the color, ranging from dull, muddied to pure, bright color. Neon green is brighter than olive green. [Dull ---------- Bright]

Product Pickup (Manipulation of Dispensed Product for Rheological Properties).

Using automatic pipette or syringe, panel leader delivers 0.1 cc of product to tip of thumb or index finger. Compress product slowly between index finger and thumb one time, then separate fingers. Evaluate for:

Firmness	Force required to fully compress product between thumb and index finger [no force ---------- high force]
Qualitative Reference	Petrolatum, white
Stickiness	Force required to separate fingertips [no force/ not sticky ---------- high force/ very sticky]
Qualitative Reference	Petrolatum, white

Using the same dispensed product or wiping product from fingers and receiving another dose, compress and separate product between the index finger and thumb three times using a quick, light pressure, and evaluate for:

Cohesiveness	Amount sample strings rather than breaks when fingers are separated [no strings ---------- high strings]
Qualitative Reference	Petrolatum, white
Amount of Peaking	Degree to which product makes stiff peaks on fingertips [flat/no peaks---------- stiff peaks]
Qualitative Reference	Petrolatum, white

Rub-out (Product Application and Manipulation)

Using automatic pipette or syringe, panel leader delivers 0.05 cc of product to center of 5-cm diameter circle on volar forearm. Spread the measured amount of product within the circle using index or middle finger, using a gentle circular motion. Stroke at a rate of two strokes per second, following a metronome.

After three rubs, evaluate for:

Wetness	Amount of water perceived while rubbing [none ---------- high amount]
Qualitative Reference	Water
Spreadability	Ease of moving product over the skin [difficult/drag ---------- easy/slip]
Qualitative Reference	Baby oil or light mineral oil

After 10–15 rubs, evaluate for:

Thickness	Amount of product felt between fingertip and skin. [thin/ ---------- thick/] almost no product lots of product
Qualitative Reference	Petrolatum, white

After 15–20 rubs, evaluate for:

Oil	Amount of oil perceived in the product during rub-out [none ---------- extreme]
Qualitative Reference	Baby oil or light mineral oil
Wax	Amount of wax perceived in the product during rub-out [none ---------- extreme]
Qualitative Reference	Surface of wax taper candle; cheese wax
Grease	Amount of grease perceived in the product during rub-out [none ---------- extreme]
Qualitative Reference	Petrolatum, white

Continue rubbing and evaluate for:

Rubs to Absorbency	The number of rubs at which the product loses wet, moist feel and a resistance to continue is perceived. (Count data) [upper limit for finished formulations = 120 rubs]
Qualitative Reference	Vaseline Total Moisture Body Lotion; light mineral oil

Afterfeel—Immediate (Visual and Tactile Assessment of Product Residue)

Visually analyze the forearm test site and evaluate for:

Gloss	Amount or degree of light reflected off skin [dull/matte ---------- shiny]
Qualitative Reference	Baby oil or light mineral oil residue on skin

Tap cleansed finger lightly over application site and evaluate for:

Stickiness	Degree to which fingers adhere to residual product [not sticky ---------- very sticky]
Qualitative Reference	Petrolatum, white residue on skin

Stroke cleansed fingers (1–2 strokes) lightly across skin and evaluate for:

Slipperiness	Ease of moving fingers across skin [difficult/drag ---------- easy/slip]
Qualitative Reference	Untreated volar forearm skin; light mineral oil residue on skin
Thickness of Residue	Amount of product residue felt between fingers and skin [thin ---------- thick]
Qualitative Reference	Petrolatum, white residue on skin
Amount of Residue	Amount of product on skin [none ---------- large amount]
Qualitative Reference	Baby oil or light mineral oil residue on skin

Type of Residue	Oily, Waxy, Greasy, Silicone, Plastic/Coated, Powdery [May be rated for intensity, for proportion of signal or for presence]

Descriptions of Residue Types

Oily	Thin, slippery, continuous feel, reminiscent of baby oil
Waxy	Thin, dry, draggy, stiff, coated feel, reminiscent of candle wax
Greasy	Thick, draggy, cushioned feel reminiscent of petrolatum
Powdery	Extremely small particles having a rounded, almost continuous feel, reminiscent of fine talc or cornstarch
Silicone	Dry, slippery, silky feel that fills in crevices of skin as a continuous layer, reminiscent of silicone compounds of varying weights. May be a thin residue or thicker with a cushiony feel. Tends to coat the fingers, obscuring fingerprint feel.

Note that descriptions of residue type are based on tactile perception, not product formulation.
Afterfeel evaluations may be repeated at one or more additional time points. Twenty minutes afterfeel is standard to this core protocol.

7.1.10 SAMPLE PROCOTOL FOR SKIN PREPARATION AND MAINTENANCE DURING TESTING

The following section provides information provided to panelists on how the forearm test substrate should be prepared prior to testing as well as maintenance of the hands during testing. This supplemental information enforces testing controls to ensure panelists are treating the skin test sites similarly across panelists and from test to test.

The details of these procedures may vary somewhat based on the specific protocol and objectives of the sensory test.

Initial Skin Preparation:

1. Wash arms with a nonabrasive, low-residue cleanser not more than two hours prior to evaluation.
2. Do not apply lotions, creams, or other topical products to the cleansed arm testing sites prior to the panel session.
3. After arriving at panel, go to the sink and wash hands with the provided cleanser. Pat hands dry with paper toweling provided.

Go to assigned workstation and prepare for work. Apply the test site templates to your arms. Using the scribe and inkpad, three 5 cm × 5 cm circles are scribed on each lower forearm, avoiding the wrist and crook of arm. Circles should not overlap and should be centered on the forearm. Under standard parameters, test sites are not reused in a session.

Ongoing Skin Maintenance During Testing:

1. Clean fingers between samples or presentation of samples to remove products.
2. Afterfeel evaluation requires the use of clean fingers when touching the test site.
3. 91% Isopropyl alcohol is provided for cleansing fingers and skin and is used with cotton balls, paper wipers, and/or facial tissue. Allow alcohol to evaporate prior to evaluating.
4. Under specific circumstances, other products may be used to remove product residues, generally after completion of a sample evaluation. These include, but are not limited to, the following: edible oils, cleansers, and 5% TEALS. Instructions for use of these products are provided when used.

7.1.11 SAMPLE LEXICON AND TESTING PROTOCOL FOR EVALUATION OF HAIR TRESSES

In the following, products are dispensed by the panel leader or technician. Panelists record requested information for each attribute on a corresponding ballot for that lexicon and protocol.

Hair tresses may be of various origins and should be consistent within a study in terms of composition, weight, and size. Information presented is for a 2-g tress.

Preassessment Before Application of Leave-In Treatment Product

Measure length of hair swatch from the end of the card to the end of the hair. Record the measurement. Pull hair swatch taut and measure as above. Record measurement. Visually evaluate hair for:

Sheen (visual)	Amount of reflected light [Dull----------Shiny]

Comb through hair with rattail comb. At third stroke of combing, evaluate for:

Combability (dry) (top half of swatch)	Ease with which comb can be moved down hair shafts without resistance or hair tangling [Difficult----------Easy]
Combability (dry) (bottom half of swatch)	Ease with which comb can be moved down hair shafts without resistance or hair tangling [Difficult----------Easy]
"Fly away" hair (visual)	The tendency of the individual hairs to repel each other during combing after three strokes of combing down hair shafts [None----------Much]

Application of Leave-In Treatment Product

Using automatic pipette or syringe, panel leader delivers 0.125 cc of sample or transfers an equivalent volume of a heavy gel/solid onto edge of palm of hand. Using opposite index and middle fingers, rub onto edge of palm two–three times to distribute. Pick up hair swatch by the card. Using long, even strokes, from the top to bottom, apply to hair swatch, turning card after each stroke, rubbing ends of swatch with index and middle fingers.

Evaluate for:

Ease of distribution	Ease of rubbing product over hair [Difficult----------Easy]
Amount of residue	The amount of residue left on the surface of the hands [None----------Extreme]
Type of residue	Oily, waxy, greasy, silicone, other (list)

Evaluation of Tress Post-Application

Clean hands to remove residues before proceeding. Comb through hair swatch with a rattail comb. At the third stroke of combing evaluate for:

Combability (treated) (top half of swatch)	Ease with which comb can be moved down hair shafts without resistance or hair tangling [Difficult----------Easy]
Combability (treated) (bottom half of swatch)	Ease with which comb can be moved down hair shafts without resistance or hair tangling [Difficult----------Easy]
Stringiness (visual)	The sticking of individual hairs together in clumps [Unclumped----------Clumped]

Feel tress with fingers to evaluate for:

Wetness (tactile)	The amount of perceived moisture [Dry----------Wet]
Coldness (tactile)	Thermal sensation of lack of heat [Hot----------Cold]
Slipperiness (tactile)	Lack of drag or resistance as moving along hairs between fingers [Drags----------Slips]
Roughness (tactile)	A rough, brittle texture of hair shafts [Smooth----------Rough]
Coatedness (tactile)	The amount of residue left on the hair shaft [None, uncoated----------Very coated]
Stickiness of hair to skin (tactile)	The tendency of the hair to stick to the fingers [Not sticky----------Very sticky]

Evaluation After drying

Let hair swatch dry for 30 minutes lying on clean paper towels, checking swatch at five-minute intervals and evaluate earlier if dried. Record drying time. Measure length of hair swatch from the end of the card to the end of the hair. Record the measurement. Pull hair swatch taut and measure as above. Record measurement. Comb through hair swatch with rattail comb.

[Alternately, hair can be dried with a hair dryer, setting defined conditions.]

At the third stroke of combing evaluate for:

Combability (dry) (top half of swatch)	Ease with which comb can be moved down hair shafts without resistance or hair tangling [Difficult----------Easy]
Combability (dry) (bottom half of swatch)	Ease with which comb can be moved down hair shafts without resistance or hair tangling [Difficult----------Easy]
"Fly away" hair (visual)	The tendency of the individual hairs to repel each other during combing after three strokes of combing down hair shafts [None----------Much]
Stringiness (visual)	The sticking of individual hairs together in clumps [Unclumped---------- Clumped]
Sheen (visual)	Amount of reflected light [Dull----------Shiny]

Feel tress with fingers to evaluate for:

Slipperiness (tactile) between fingers	Lack of drag or resistance as moving along hairs between fingers [Drags----------Slips]
Roughness (tactile)	A rough, brittle texture of hair shafts [Smooth----------Rough]
Coatedness (tactile)	The amount of residue left on the hair shaft [None, uncoated----------Very coated]
Type of residue	Oily, waxy, greasy, silicone, other (list)

Alternate Evaluation—Application and Evaluation of Shampoo

This section would occur after the pre-assessment and in lieu of the leave-in treatment section.

Application of Shampoo

Water temperature is set at 35 ± 2°C. Wet tress thoroughly and squeeze tress lightly between fingers to remove excess water. Using automatic pipette or syringe, panel leader delivers 0.5 ml of hair shampoo across top of tress below card. Pick up hair swatch by the card.

Using opposite hand with thumb in front and fingers in back, squeeze thumb and fingers together through the shampoo.

Evaluate for:

Viscosity	Perceived thickness of the product [very thin_____very thick]

Use ten continuous downward massaging motions, working shampoo into hair swatch. Evaluate for:

Ease of distribution	Ease of rubbing product over and into hair [difficult _____ easy]
Lather Flash	Number of manipulations required to generate visible lather [count]

After generating lather on the tress, remove by squeezing hair between index and middle fingers. Gather all lather into a mound onto opposite fist. Evaluate for:

Lather Volume	Amount of lather produced by the product in a given period of time. [very low volume _____very high volume]
Lather Density	Measured by the visual appearance of the individual bubbles. Large, airy bubbles are characteristic of a light (not dense) lather while very tight, compact bubbles represent dense lather. [light/airy _____ heavy/dense]

Place hair swatch under running water and use a downward massaging action to rinse. Massage until hair swatch is tactilely rinsed and all visible foam is removed.

Rinse Time	Number of seconds to remove lather from hair [number of seconds]

Squeeze tress between fingers using a downward stroke to remove excess water. Place tress on toweling and cover. Pat firmly down the tress in three sections to remove additional water. Hold hair swatch at the top in one hand and with other hand start combing from the top of the hair tress, working the comb down to the end of the swatch. After the first stroke evaluate for:

Ease of Detangling	Ease to comb through hair from top to tips at the first stroke [very tangled/hard to comb _____ not tangled/easy to comb]

Continue with Evaluation of Tress Post-Application.

7.1.12 SAMPLE LEXICON AND TESTING PROTOCOL FOR EVALUATION OF LATHER AND SKINFEEL OF BAR SOAP—FOREARM TEST

In the following, each panelist is provided a preconditioned bar of soap. Panelists record requested information for each attribute on a corresponding ballot for that lexicon and protocol.

Additional information on the bar soap weight or visual look may be collected.

Preparation for Testing

Panelists refrain from using any type of moisturizing cleansers or moisturizers on evaluation days to include bar soaps, cleansers, lotions, and creams. Panelists may, however, rinse their arms with water and pat dry.

Panelists evaluate up to two samples per day (one sample per site, beginning with the left arm). For the second soap sample, repeat the washing procedure presented on the right arm evaluation site. Wash each site once only.

Baseline Evaluation of Site

Visually evaluate inner forearm skin for:

Gloss	The amount or degree of light reflected off skin [Dull----------Shiny]
Visual dryness	The degree to which the skin looks dry (ashy/flaky) [None----------Very dry]

Stroke cleansed fingers lightly across skin and evaluate for:

Slipperiness	Ease of moving fingers across the skin [Drag----------Slip]
Amount of residue	The amount of residue left on the surface of the skin [None----------Extreme]
Type of residue	Indicate the type of residue: soap film, oily, waxy, greasy, powder, silicone, other (list).
Dryness/roughness	The degree to which the skin feels rough [Smooth----------Rough]
Moistness	The degree to which the skin feels moist [Dry----------Moist]
Tautness	The degree to which the skin feels taut or tight [Loose/Pliable----------Very tight]

Using edge of fingernail, scratch a line through the test site.
Visually evaluate for:

Whiteness	The degree to which the scratch appears white [None----------Very white]

Evaluation of Lather Rheology

Water temperature is set at 35 ± 2°C. Wet soap bar thoroughly by holding under running water for a defined time. Remove from water and rotate bar in hand for a defined number of times to begin lather generation. Wet evaluation arm site thoroughly. Apply with up-down motion from inner wrist to crook of arm (one up-down lap = 1/2 second).

Amount of lather observed during application:

record for 10, 20, 30 laps	[None----------Extreme]

At 30 laps continue with

Thickness of lather	Amount of product felt between fingertips and skin [Thin----------Thick]

Bubble size variation	The variation seen within the bubble size [Homogeneous----------Heterogeneous]
Bubble size	The size of the soap bubbles in the lather (visual) [Small----------Large]

Evaluation of Wet Skin

Rinse site by placing arm directly under warm running water. Use free hand to stroke gently with up-down lap over the site. Rinse for 15 laps (1 lap = 1 second). Also rinse evaluation fingers.

Evaluate wet skin for:

Rinsability	The degree to which the sample rinses off (visual) [None----------All]

Gently stroke upward on skin site with a clean finger and evaluate for:

Slipperiness	Ease of moving fingers across the skin [Drag----------Slip]
Amount of residue	The amount of residue left on the surface of the skin [None----------Extreme]
Type of residue	Indicate the type of residue: soap film, oily, waxy, greasy, powder, silicone, other (list).

Evaluation of Skin After Drying

Dry the site by covering it with a paper towel and patting dry 3 times along the site. Also thoroughly dry evaluation finger. Evaluate for:

Gloss	Visual: amount of light reflected on the surface of the skin [Dull----------Shiny/glossy]
Visual dryness	The degree to which the skin looks dry (ashy/flaky) [None----------Very dry]

Tap dry, cleansed finger over treated skin and evaluate for:

Stickiness	The degree to which fingers stick to residual product on the skin [Not sticky----------Very sticky]

Gently stroke skin site with clean finger and evaluate for:

Slipperiness	Ease of moving fingers across the skin [Drag----------Slip]
Amount of residue	The amount of residue left on the surface of the skin [None----------Extreme]
Type of residue	Indicate the type of residue: soap film, oily, waxy, greasy, powder, silicone, other (list).
Dryness/roughness	The degree to which the skin feels dry/rough [Smooth----------Dry/rough]
Moistness	The degree to which the skin feels moist, wet [Dry----------Moist]
Tautness	The degree to which the skin feels taut or tight [Loose/pliable ----------Very taut]

Using the edge of the fingernail, scratch through test site and evaluate for:

Whiteness	The degree to which the scratch appears white. [None----------Very white]

7.1.13 SAMPLE LEXICON AND TESTING PROTOCOL FOR EVALUATION OF SKINFEEL OF ANTIPERSPIRANTS USING INNER ARM SITE

In the following, products are dispensed by the panel leader or technician. Panelists record requested information for each attribute on a corresponding ballot for that lexicon and protocol. Self-application is an alternate technique.

This protocol uses the inner arm site as an analog for the underarm. While the skin on the arm is different than that of the underarm in structure and flora, this protocol is sensitive to a range of visual and tactile signals related to product formulation. It can be modified to simulate sweating or other conditions. Use of the inner arm site minimizes the large person-to-person differences in underarm shape, hair, skin texture, and perspiration rate. Lexicon attributes shown here can be applied to an underarm site protocol, along with other relevant attributes such as pilling caused by friction.

Use of Arm Site as Analogue for Underarm

Roll-On/Solids/Gels

Preparation of Skin

Evaluation site (crook of arm) is washed with nonabrasive, low residue, nondeodorant soap no more than 1 hour before evaluation. A 6" ×2" rectangle is marked on the crook of the arm so the fold bisects the rectangle.

Baseline Evaluation of Site

Prior to application, instruct panelists to evaluate untreated sites for baseline references.

Visually evaluate skin for:

Gloss	The amount or degree of light reflected off skin [Dull---------- Shiny]
Visual dryness	The degree to which the skin looks dry (ashy/flaky) [None----------Very dry]

Stroke cleansed fingers lightly across skin and evaluate for:

Slipperiness	Ease of moving fingers across the skin [Drag----------Slip]
Amount of residue	The amount of residue left on the surface of the skin [None----------Extreme]
Type of residue	Indicate the type of residue: *soap film, oily, waxy, greasy, powder, silicone, other (list).*

Dryness/roughness	The degree to which the skin feels rough [Smooth----------Rough]
Moistness	The degree to which the skin feels moist [Dry---------- Moist]
Tautness	The degree to which the skin feels taut or tight [Loose/pliable----------Very tight]

Using edge of fingernail, scratch a line through the test site. Visually evaluate for:

Whiteness	The degree to which the scratch appears white [None----------Very white]

Application of Antiperspirant

Roll-on gels: Using automatic pipette or syringe, panel leader delivers 0.05 ml of product at two spots along the 2" bottom and top of the 2" × 6" rectangle evaluation site. Spread the product on the site using 12 rubs (6 laps) with a vinyl-covered finger. Solids/Gels: Apply the product by stroking up the arm once through the 2" ×6" rectangle (force to apply), then back down and up the arm three times (ease to spread), using a consistent pressure to get the product on the arm. A tare weight is taken of each application and recorded.

Immediately after application, evaluate for:

Coolness (somesthetic feel)	The degree to which the sample feels "cool" on the skin [Not at all cool----------Very cool]
Gloss	The amount of reflected light from the skin [Not at all shiny----------Very shiny]
Whitening	The degree to which the skin turns white [None----------Very white]
Amount of residue (visual)	The amount of product visually perceived on the skin [None----------Large amount]
Tautness	The degree to which the skin feels taut or tight [Loose/pliable----------Very tight]

Fold arm to make contact. Hold five seconds. Unfold arm and evaluate for:

Stickiness (fold)	Degree to which arm sticks to itself [Not at all----------Very sticky]

Stroke finger lightly across skin on one section of rectangle and evaluate for:

Wetness	The amount of water perceived on the skin [None----------High amount]
Slipperiness	Ease of moving fingers across the skin [Drag----------Slip]
Amount of residue	The amount of residue perceived on skin (tactile) *Evaluate by stroking finger across site.* [None----------Extreme]
Oil	The amount of oil perceived on skin [None----------Extreme]
Wax	The amount of wax perceived on skin [None----------Extreme]
Grease	The amount of grease perceived on skin [None----------Extreme]
Powder/chalk/grit	The amount of powder, chalk and/or grit perceived on skin [None----------Extreme]
Silicone	The amount of silicone perceived on skin [None----------Occluded]

Post-Application Evaluation

After 5, 10, 15, and 30 minutes, evaluate for:

Occlusion	The degree to which the sample occludes or blocks the air passage to the skin [None----------Occluded]
Whitening	The degree to which the skin turns white [None----------Large amount]

Amount of residue	The amount of product visually perceived on skin (visual) [None----------Large amount]
Tautness	The degree to which the skin feels taut or tight [Loose/pliable----------Very tight]

Fold arm to make contact. Hold five seconds. Unfold arm and evaluate for:

Stickiness	The degree to which arm sticks to itself [Not at all sticky----------Very sticky]

Stroke fingers lightly across skin on one section of rectangle and evaluate for:

Wetness	The amount of water perceived on the skin [None----------High amount]
Slipperiness	Ease of moving fingers across the skin [Drag----------Slip]
Amount of residue	The amount of residue perceived on skin (tactile) [None----------Extreme]
Oil	The amount of oil perceived on skin [None----------Extreme]
Wax	The amount of wax perceived on skin [None----------Extreme]
Grease	The amount of grease perceived on skin [None----------Extreme]
Powder/Chalk/Grit	The amount of powder, chalk, and/or grit perceived on skin [None----------Extreme]
Silicone	The amount of silicone perceived on skin. [None----------Extreme]

After 30 minutes, evaluate as follows:

Place a swatch of black cotton fabric over test site. Fold arm so fingertips touch the shoulder lightly. Pull fabric from crook.

Rub-Off Whitening	The amount of visual residue on the dark fabric [None----------Large amount]

7.1.14 SAMPLE LEXICON AND TESTING PROTCOL FOR EVALUATION OF APPEARANCE AND SKINFEEL FOR FACIAL FOUNDATION—HALF-FACE

In the following example, products are dispensed by the panel leader or technician. Panelists record requested information for each attribute on a corresponding ballot for that lexicon and protocol. This protocol does not evaluate product color attributes; those can be added.

Skin Preparation

Panelists refrain from using any type of moisturizers or cosmetics on evaluation days. Upon arriving at evaluation session, panelists wash face with low residue, nondrying, low-residue cleanser and blot dry with soft wiper. Wait ten minutes before beginning evaluation.

Panelists evaluate up to two samples per day (one sample per half-face site).

Baseline Evaluation of Site

Face is visually bisected from along the centerline of nose and chin. Look in the mirror and visually evaluate the cheek area from the cheekbone to the jaw on one side of face and evaluate for:

Gloss	The amount or degree of light reflected off skin [Dull ----------Shiny]
Visual Skin Texture	The degree to which skin lines and imperfections are visible [Not visible----------Very visible]

Stretch face by opening mouth, as if to yawn, and evaluate for:

Tautness	The degree to which the skin feels taut/tight [Loose/Pliable----------Very tight]

Stroke clean fingers (one–two strokes) lightly across skin and evaluate for:

Stickiness	The degree to which the finger sticks to skin [Not sticky ----------Very sticky]
Moistness	The degree to which the skin feels moist, wet [Dry ----------Moist]
Slipperiness	The ease of moving fingers across the skin [Drag ----------Slip]
Occlusion	The degree to which the sample occludes or blocks the feel of the skin when touched [None ---------- Occluded]
Suppleness	The degree to which the skin feels supple, pliable [Stiff ---------- Supple/Pliable]
Dryness/Roughness	The degree to which the skin feels dry/rough [Smooth ---------- Dry/Rough]

At the request of the client, more than one facial area can be assessed for applicable attributes.

Product Application

Using automatic pipette, the panel leader or technician delivers 0.075 ml of product across fingertip of your index finger. Dab to apply (apply one dot forehead, three dots to cheek, one dot to chin). Spread the measured amount of product in a circular upward and outward motion, starting at chin, cheek, then forehead until evenly distributed (chin two–four rubs, cheek four–eight rubs, forehead two–four rubs). Focusing on the cheek site, evaluate for:

Coolness	The degree to which the sample feels "cool" on the skin [Not at all cool ---------- Very cool]
Wetness	The amount of water perceived while rubbing [None ----------High amount]
Spreadability	Ease of moving product over the skin [Difficult/Drag ---------- Easy/Slip]

Thickness	The amount of product felt between fingertip and skin [Thin ---------- Thick]
Oil Intensity	The amount of oil perceived on the skin. [None ----------Extreme]
Wax Intensity	The amount of wax perceived on the skin. [None ----------Extreme]
Grease Intensity	The amount of grease perceived on the skin. [None ----------Extreme]

At the request of the client, more than one facial area can be assessed for applicable attributes.

Post-Application Evaluation Afterfeel

Immediately and 60 minutes after application, evaluate the following:

Looking at the entire test site, visually evaluate for:

Gloss/Shine	The amount or degree of light reflected off skin [Dull/Matte ----------Shiny/Glossy]
Evenness	The degree to which the sample has spread evenly over the skin [Uneven/Streaky/Blotchy ----------Even/Uniform]
Concealment	The degree to which the sample hides imperfection in the skin [No concealment ---------- Complete concealment/ Opaque]
Visual Skin Texture	The degree to which skin lines and imperfections are visible [Not visible----------Very visible]

Stretch face by opening mouth, as if to yawn, and evaluate for:

Tautness	The degree to which the skin feels taut or tight [Loose/Pliable ----------Very tight]

Tap cleansed finger lightly over cheek application site and evaluate for:

Stickiness	The degree to which finger adheres to residual product [Not sticky----------Very sticky]

Stroke cleansed fingers (one–two strokes) lightly across cheek and evaluate for:

Moistness	The degree to which the skin feels moist, wet [Dry ---------- Moist]
Slipperiness	The ease of moving fingers across the skin [Drag ---------- Slip]
Occlusion	The degree to which the sample occludes or blocks the feel of the skin when touched [None ---------- Occluded]
Suppleness	The degree to which the skin feels supple, pliable [Stiff ---------- Supple/Pliable]
Dryness/Roughness	The degree to which the skin feels dry/rough [Smooth ---------- Dry/Rough]
Amount of Residue	Amount of product on skin [none ---------- large amount]
Type of Residue	Oily, Waxy, Greasy, Silicone, Powder/Chalky (% of total residue) [none ---------- large amount]

Post-Wear Product Rub-Off (60 Minutes Afterfeel)

Place cosmetic sponge lightly against the cheek one inch from the nose, and rub (swipe) once in an outward motion along the cheekbone and evaluate for:

Rub-off	The amount of foundation on the applicator (visual) [None ---------- Large amount]

7.1.15 APPLICATIONS OF DESCRIPTIVE ANALYSIS FOR PERSONAL CARE AND COSMETICS

Descriptive Analysis is versatile and can be applied across many product categories. It provides valid and reliable product information for making sound business decisions in research and development, marketing/marketing research, and manufacturing as well as basic and applied research in governmental agencies and academia. Well-executed descriptive analysis provides clear and detailed documentation of the qualitative and quantitative features of a single product or range of products and can be used to measure a single point in time as well as multiple time points, product lots, or comparative products with a similar confidence level to that provided by instrumental data. Data tell the story of the products, allowing decisions to be made on products that the decision-makers may have not experienced.

General Applications of Descriptive Analysis include

- Document Product Attributes for Current, Target, Control and Competitive Products
- Support of Current Product Maintenance
  - Product Shelf Life
  - Quality Assurance
  - Process Changes
  - Ingredient Substitutions
  - Troubleshooting
- Support of Product Development for
  - Prototype Screening
  - Formulation Effects
  - Process Effects
  - Interpretation of and Co-Analysis with Consumer Research
- Support of Marketing and Marketing Research for
  - Questionnaire Design
  - Consumer Data Interpretation
  - Building of Predictive Models for Consumer Acceptance
  - Advertising Claims

Descriptive Analysis output may be presented numerically, graphically, or using written descriptions and may be analyzed using univariate or multivariate statistics. Several examples are provided below.

Table 3 provides a standard output table for evaluation of two lotions samples. In this example, two samples were submitted to measure any product differences between the products. Attributes with *** indicate statistical differences at alpha level of 0.05, those with * indicate statistical differences at alpha level of 0.10, and those blank are not significantly different.

Data indicate that many small but statistically differences exist between the samples. In the aggregate, Sample 784 may have a consumer perception of richer due to lower wetness, higher thickness in application and residue, and more force to spread on the skin.

Table 3: Example of Descriptive Analysis Results for Two Lotion Samples

Descriptive Attribute	Sample 489	Sample 784	P-VALUE	LSD	sig
Rub Out					
Wetness	44.6 A	39.7 B	<0.001	0.98	***
Spreadability	51.0 A	46.0 B	<0.001	0.95	***
Thickness	36.0 B	40.2 A	<0.001	1.64	***
Oil	37.7	37.2	0.589	--	
Wax	6.5 B	7.7 A	<0.001	0.37	***
Grease	42.8	43.8	0.109	--	
Rubs to Absorbency (Count)	83.6	86.6	0.361	--	
Immediate Afterfeel					
Gloss	47.8 A	39.6 B	<0.001	2.10	***
Stickiness	10.0	10.2	0.392	--	
Slipperiness	70.7	70.2	0.507	--	
Thickness of Residue	17.7 B	21.6 A	<0.001	0.69	***
Amount of Residue	26.2 B	32.1 A	<0.001	1.72	***
Oily%	23.1 A	19.2 B	0.002	2.12	***
Wax%	24.4 B	30.4 A	<0.001	1.81	***
Grease%	47.7	46.9	0.363	--	
Silicone%	4.8 A	3.5 B	0.008	0.93	
20 Minute Afterfeel					
Gloss	23.2	23.0	0.873	--	
Stickiness	4.1 A	2.7 B	0.009	1.00	***
Slipperiness	76.2	76.6	0.554	--	

Descriptive Attribute	Sample 489	Sample 784	P-VALUE	LSD	sig
20 Minute Afterfeel					
Thickness of Residue	9.1 B	12.5 4	<0.001	1.07	***
Amount of Residue	13.2 B	19.8 A	<0.001	2.06	***
Oily%	7.3 B	10.4 A	0.041	2.94	***
Wax%	52.5 A	44.6 B	0.035	7.26	***
Grease%	33.5	38.3	0.134	--	
Silicone%	6.7	6.7	1.000	--	
Pick-Up					
Firmness	32.7 a	32.1 b	0.060	0.50	*
Stickiness	25.0 A	24.0 B	<0.001	0.42	***
Cohesiveness	23.3 A	14.5 B	<0.001	2.27	***
Peaking	23.5	23.2	0.482	--	
Appearance					
Integrity of Shape	40.7 A	39.0 B	<0.001	0.63	***
Integrity of Shape (10s)	30.4 A	29.5 B	0.023	0.77	***
Gloss	83.4 b	83.6 a	0.067	0.14	*

Many audiences prefer graphical presentation of data. Figure 2 provides histograms of the same data presented in two different ways: by product attribute and by sample. Data should be presented in the way that best answers the questions posed prior to the study.

Descriptive Analysis is often used to evaluate large sets of products prior to selection of a subset, based on study criteria, for use in consumer testing. Figure 3 indicates the breadth of sensory properties for the rub-out of a range of lotions evaluated by descriptive analysis, as represented by a range graph. Overlaid on this graph are the samples selected for consumer testing and their range, based on criteria for the marketing-defined desired consumer experience for the product type.

Figure 2: Use of Histograms to Present Data by Attribute and by Sample

Figure 3: Use of Range Graphs for Perspective

Samples can also be mapped using multivariate statistical analysis techniques to generate perceptual maps. A perceptual map is a graphical representation of the relationship between samples based on correlated groups of attributes defined as "dimensions." Pairs of dimensions can be plotted against one another to illustrate product similarity and differences within the range of products tested. Product placement on the array is mathematically determined by the degree to which each sample is defined by the dimensions on the graph. For example, a sample high in Dimension 1 on the X-axis and Dimension 2 on the Y-axis will reside in the upper right-hand quadrant. A sample low in both of these dimensions will reside in the lower left-hand quadrant.

Figure 4 represents output after many weeks of testing hair treatment products using trained subjects. Due to the inherent person-to-person variability and the impact of weather conditions on the hair, several products were evaluated multiple times by the panel. While some movement of the samples is on the graphs, these sample results have sufficient stability to have confidence in the placement of the remaining samples. For this study, the test question was "Which of our prototypes provides similar Style Quality and Sleek Look to the Market Leader?" The results indicate that Prototypes 4 and 7 are most promising. This type of mapping provides a wealth of additional information about current, prototype, and competitive products that can be used for other product development and marketing decision-making.

Figure 4: Multivariate Comparison of Hair Treatment Samples Using Perceptual Mapping

Knowledge of descriptive analysis lexicons for personal care and cosmetic products can also be used for product screening. Table 4 displays the independent qualitative, objective evaluation of two hand sanitizers. The product descriptions illustrate that despite very different product forms, the post-dry afterfeel is much more similar.

Table 4: Qualitative Descriptive Analysis Screening of Two Hand Sanitizers

	Control 1-Gel with aloe and vitamins	**Control 2-Instant Foam (pump)**
Initial Feel	Product control is good – viscosity of product gives control; immediately cooling during rubbing; has a draggy (not slippery) feel while rubbing and is plastic-like; feel tautness growing; glossy	Hard to control–meager foam–wet and not well formed with a very quick breakdown to liquid – loose/"watery"; immediately cooling during rubbing; harder to spread because it dries quickly; noticeable alcohol aroma; slight taut feeling

	Control 1-Gel with aloe and vitamins	Control 2-Instant Foam (pump)
Phase Change	Happens at about 25–30 seconds; moderately high sticky-tacky feel between palms and fingers; noticeably taut	Happens at about 20 seconds; low moderate sticky-tacky feel between palms and fingers
Immediate Afterfeel	Almost completely dry by 60 seconds (between fingers a 90 seconds); dries to a thin film that is initially plastic-like but moves to silicone/ powdery feel; very slight residual tautness	Almost completely dry by 25–30 seconds (between fingers about 60 sec with low stickiness); dries to a very thin film that has a powdery and waxy feel
Additional Notes	Skin feels soft but not moisturized–not a lotiony feel; skin feels clean and not occluded (no skin smothering/masklike feel)	Skin feels almost neutral with a thin coating; not moisturized but not stripped; a clean, light feel

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DELIVERY SYSTEMS

DELIVERY SYSTEMS FOR COSMETICS AND PERSONAL CARE

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ABSTRACT

The cosmetic and personal care global markets continue to offer opportunity for the invention and discovery of unique functional ingredients. When formulated into consumer products, the final brand must offer enhanced functionality for reasonable cost. Newly discovered or invented functional ingredients are often expensive, and sensitive to degradation as a result of bodily contact and the environment. Thus, there is a strong need to enhance functional ingredient performance through ingredient protection and protection of efficacy.

Smart encapsulation of such functional ingredients is one way to achieve these goals. The development of optimal delivery systems can produce delivery of the ingredient where it needs to be; when it needs to be there; and released from the protective design at a rate that both minimizes irritation and conserves ingredient concentration. By this means, the protected ingredient can be thoughtfully targeted and delivered at the optimal efficacy. Such delivery systems are "Smart Solutions" and they provide these benefits at an affordable cost. This chapter discusses in detail the need for "encapsulation" of functional ingredients. This discussion is followed by a description of current commercial delivery systems, principles of action, and issues solved. The chapter ends with a focus on unsolved issues and an intriguing number of questions that need answering in order for the delivery-system field to continue to expand in a cogent manner.

While the number and types of delivery systems have proliferated over the last decade, we have chosen to focus on three main types of delivery systems that continue to show promise as measured by their use in commercial cosmetic, personal care, and pharmaceutical products.

These include:

1. **Powder technologies,** representing encapsulation of actives within microspheres. Examples chosen for this discussion include delivery systems with trade names that include, but not are limited to: Polypore™, Polytrap™, Macrobead™, Tagravit™ microspheres, microencapsulated fragrances, and MultiSal™
2. **Lipid-based encapsulation systems,** consisting of liposomes, solid lipid nanoparticles (SLN), SalSphere™
3. **Sugar-based cyclodextrin technology**

We discuss the need for advanced delivery systems for cosmetic and personal care active ingredients and their utility in novel formulations with enhanced performance. We also present specific cases of performance for dermatological applications and provide a comparison of the different technologies.

The chapter concludes with the technological challenges of delivery systems in the major areas of cosmetics and personal care: oral care, hair care, skin care, and perfumery applications. These challenges pave the way for future development of novel products.

Keywords: particulate delivery systems, topical application, microspheres, sub-micron spheres, liposomes, cyclodextrins, solid lipid nanoparticles, microencapsulation, functional ingredient compatibility, and skin-friendly formulations.

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8.1.1 BACKGROUND AND MOTIVATION

The world personal care industry is a multi-billion-dollar industry, with American global sales at about $38 billion dollars annually as per estimates in 2011 (*Global Cosmetic Industry Magazine*, June 2012). While the utility of single functional ingredients is well known, there has recently been an eager interest in both the cosmeceutical and nutraceutical combination of multiple ingredients for incorporation into finished products to achieve varied benefits. This trend also includes providing an aesthetic feel for formulations. Complementing dermatological with cosmetic technologies, skin care scientists and cosmetic formulators are challenged to design prototypes with the right concentration of actives and stability features. These

challenges also include: programmed-release kinetics of different actives, penetration profiles, and clinical efficacy. For this reason, there are a number of delivery systems available that can provide solutions for the major issues associated with formulation and consumer efficacy of products.

A successful cosmetic delivery system is based on the identification of a suitable methodology to formulate with active ingredients and provide enhanced stability, consumer appeal, efficacy, market impact, and performance. While numerous delivery systems have emerged over the past decade,[1] the most commonly known delivery system is that of oil-in-water emulsions. More recently, complex emulsion delivery systems have evolved. These include: microemulsions, Pickering emulsions,[2] multiple emulsions, lamellar gels, and water-oil-water emulsions.

In order to include features such as enhanced skin penetration and performance, complex delivery systems have evolved. These include, for example, vesicular liposomes and niosomes (non-ionic surfactant vesicles that are comprised of polyoxyethylene alkyl ether, polyoxyethylene alkyl ester, or saccharose diester), molecular (cyclodextrins), and particulate (microcapsules and matrix particles). More recently, nanosomes (defined as modifications of liposomes comprised of very small, single bi-layer vesicles manufactured by application of ultrasonic energy to large, multilayer liposomes) and sub-micron sphere technologies such as SalSphere™ have been developed.

Cosmeceuticals have become widely used in the cosmetic, personal care, and pharmaceutical industries. Typically, ingredients employed include alpha hydroxy acids, glucans, enzymes, and antioxidants. For these systems, it is important not only how the active is delivered to the deeper skin layers, but also, the effect of the active on the release of other ingredients in the formulation.

Difficult-to-deliver actives, such as enzymes, can be successfully delivered to skin by prevention of foreseeable and immediate skin-protein-enzyme interactions and thus, loss of enzyme activity. Sustained release of such enzymes and their interaction with the skin proteins is essential for effective delivery, and the method to achieve this is via encapsulation of the enzyme in liposomes[3] or nanoporous silica spheres.[4] Also, dextran-conjugated enzymes can be produced. Enzymes can degrade due to surfactants as well as a result of contact with other ingredients in the formulation.

The benefits of suitable encapsulation of functional ingredients/API's (i.e., Active Pharmaceutical Ingredients) for dermatological applications are:

Enhanced stability. The system can be utilized to isolate poorly soluble, air-oxidative active ingredients that may interact with the other ingredients. This provides long-term product shelf life. The system can protect functional ingredients for reducing oxidation/thermal degradation without compromising efficacy. Examples

of such difficult actives include salicylic acid, resveratrol, minoxidil, menthol, methyl salicylate, fragrances, and incorporation of acids into alkaline bases.

Reduced irritation. The encapsulation system can deliver small doses of the functional ingredient to reduce irritation and other side effects on the skin. Tools and techniques to monitor irritation include skin pH and RIPT (Repeated Insult Patch Testing). Nonorganic and alcohol-free suspensions will reduce irritation and skin dryness.

Ease of formulation. The encapsulation technology can improve formulation stability by providing stable, high-dose samples in water-based suspensions that can be readily diluted in aqueous-based formulation products.

Controlled release. The technology results in a long-lasting, sustained release of functional ingredients.

Enhanced bioavailability and efficacy. The hydrophobic/lipophilic nature of certain encapsulation agents can enhance the bioavailability of various functional ingredients. Making use of the balance of oil-loving and water-loving portions of these agents can enhance/enable penetration of actives to the intended site. This approach includes, for example: hydrophilic actives (and hence difficult to permeate through the outer hydrophobic surface of the skin), as well as actives that require a deeper penetration into percutaneous fat areas (under the dermis) by solubilizing the actives in skin-penetrating solvents (with some hydrophobic character), or by means of controlling the particle size.

Tools and techniques to assess performance and compliance typically include visual stability for assessment of uniformity of a product's physical appearance, and encapsulation efficiency by the centrifugation process. Physical characteristics are usually assessed by using microscopy, particle size analysis, rheometers, and pH meters. The level of functional ingredient can be assessed using HPLC (High Pressure Liquid Chromatography) or GC (Gas Chromatography); clinical efficacy evaluation is determined by using *in vivo* tests.

8.1.2 CLASSIFICATION OF DELIVERY SYSTEMS

Classification of such delivery systems is classically based on the following categories: powder technologies, lipid-based encapsulation systems, and sugar-based encapsulation systems.

a. Powder Technologies:

Starch-based encapsulation systems

MultiSal™ MicroSphere™ Technology

MultiSal™ is a multi-compartment encapsulation that allows the release of multiple functional ingredients at the same location. This technology incorporates

the sub-micron hydrophobic spheres discussed in the previous section into a larger, water-sensitive microsphere that averages 20–30 microns in diameter.[5] The outer microsphere can be constructed out of various ingredients. These include: modified starches, gums, and other natural or synthetic polymers, all of which lend an additional layer of stability to ingredients encapsulated in the sub-micron spheres that reside within (**Figure 1**).

Figure 1: Structure and release procedure of MultiSal™ technology

The additional outer layer also provides another "compartment" in which to incorporate ingredients that can be isolated from those ingredients contained within the sub-micron spheres. This outer layer can also have a complementary effect such as an acid that will be first in the sequence of release so as to exfoliate the skin prior to other beneficial functional ingredients being released from the sub-micron spheres. MultiSal™ can be used in most anhydrous product applications and can, depending on the type of encapsulation, be used in oil-in-water emulsion systems.

One of the main benefits of the additional encapsulation layer of MultiSal™ technology is that of stability. This system can withstand temperatures of up to 160°C before breaking down. Ingredients that would be susceptible to loss during heat processing are now protected, retained, and only released on skin when triggered by moisture in the presence of rubbing. **Figure 2** below shows the retention of menthol under heat stress when encapsulated in MultiSal™ vs. menthol in free form. The technology is able to protect and retain a significantly higher percentage of menthol, which translates to a higher aroma intensity and efficacy in a final product.

Figure 2: Enhanced stability of menthol using MultiSal™ technology

b. Improving Performance

MultiSal™ offers the benefits of two types of release: water-triggered, and sequential delivery of multiple ingredients. The outer shell of MultiSal™ is sensitive to moisture and will break down in the presence of the application of shearing action achieved by rubbing on the skin. Therefore, an ingredient such as menthol, discussed above, can be retained until triggered, thereby creating an "on demand" release kinetic. Because this technology is a multi-component system, there are two compartments in which ingredients can be stored. The ingredients in the outer shell will be released first, with the encased sub-micron spheres being deposited at the same time. Ingredients that are encapsulated in the sub-micron spheres will then release over an extended period and to deeper layers of skin since the submicron spheres are capable of penetrating deeper than the outermost layer, the stratum corneum. This approach provides an advantage in anti-aging formulations because multiple ingredients with various benefits can be delivered in an optimal sequence. For instance, BHA (beta-hydroxy acid) can be released first and exfoliate the skin to create a fresher and cleaner surface for the sub-micron spheres to deposit onto and release their agents. **Table 1** shows Salvona's MultiSal™ product names, the functional ingredients, and the applications.

Table 1: Salvona's products comprised of microencapsulated functional ingredients

#	Product Name	Functional Ingredients	Loading (%)	Functional Applications	Effect
1	MultiSal™ Retinol	Retinol	5/10	Anti-aging	Time-release technology for greater stability, longer shelf-life, and enhanced efficacy.
2	MultiSal™ Triple Action Anti-Aging	Lactic acid, Phenyl-ethyl resorcinol, Palmitoyl Tri-peptide 5	-	Anti-aging	Exfoliate, moisturize, brighten, and eliminate wrinkles from within
3	MultiSal™ Salicylic Acid	Salicylic acid	30	Anti-blemish	Daily Exfoliation for Healthy and Younger Looking Skin
4	MultiSal™ Fragrance	-	20/30	Sensory	Protection, extended release of fragrance and release-on-demand in presence of moisture;
5	MultiSal™ Menthol	Menthol	30	Cooling	Protection, extended release of menthol and cooling effect-on-demand in presence of moisture
6	MultiSal™ Dark Circle Eliminator	Caffeine	-	Skin rejuvenation	A natural solution to eliminate unsightly dark circles and puffy eyes.
7	MultiSal™ Sebum Control/ Skin Mattefyer	Acrylates/ C12-22 Alkyl Methacrylate Copolymer (and) Hydrated Silica	-	Sebum reduction	Instant and long-term matte finish on the skin to combat the appear-ance of oily skin.

#	Product Name	Functional Ingredients	Loading (%)	Functional Applications	Effect
8	MultiSal™ Skin Repair	Salicylic Acid (and) Lactic Acid (and) Hydrated Silica (and) Camphor (and) Zinc Sulfate (and) Hypericum Perforatum (and) Hamamelis Virginiana Leaf Extract (and) Glycyrrhiza Glabra (Licorice) Root Extract (and) Anthemis Nobilis Flower Extract (and) Tocopherol (and) Isomerized Safflower Acid (and) Lecithin (and) Cholesterol (and) Niacin (and) Menthol Crystals (and) Palmitic Acid (and) Centella Asiatica Extract (and) Echinacea Purpurea Extract	-	Skin rejuvenation	Smart Delivery System Helps the Skin Repair Itself

c. Gel-based encapsulation systems

CylaSphere™ Retinol from BASF, Germany (originally from Coletica, France) are acacia senegal plant-derived microspheres encapsulating 1% retinol. The product claims include usefulness in smoothing, lightening, and whitening applications. It is incorporated into emulsions at 1–10% loading. The composition is a blend of water, butylene glycol, glycine soja (soybean) oil, retinol, carbomer, tocopherol, acacia senegal gum, and propylene glycol alginate. Literature evidence shows higher epidermal residence time from the spheres vs. o/w emulsions containing

free retinol, and the determination was made using an *in vitro* Franz experimental set-up for diffusion of retinol through hairless mouse skin.[6] The product is present in Sothy's Homme Age-Defying Active Care.

d. Porous polymeric systems

One company that is well known for this type of technology is Tagra, which is part of the Elfa Chemical Industries Group. Established in 1998, it is located in Tel Chay, northern Israel. Tagra specializes in developing stabilized active ingredients for the personal care and pharma industries. The approach results in claims of successful protection of more than 40 actives in cosmetics, OTC, and pharma products. The technology is based on a solvent evaporation method of encapsulation to generate particles with average particle size of 40 microns.[7] The advantages of the technology are 1) overcoming ingredient and solvent incompatibilities, 2) preventing degradation, 3) retaining ingredient's activity, 4) prolonged shelf life, and 5) masking odor, color, taste, and feel. The release-on-demand claims are 1) solvent-removing method, 2) CTFA regulations, 3) high loading, and 4) maximum efficacy. Tagravit capsules enable encapsulation of pigments, vitamins, oils, flavonoids, and pharmaceutical ingredients. **Table 2-6** shows Tagra's product names, the functional ingredients, and the applications.

Pigments

Table 2: Tagra's products comprised of encapsulation of pigments

#	Product Name	Functional Ingredients	Functional Applications	Cosmetic Applications	Effects
1	TagraCap1	Red, Black, Yellow, White, Blue, Green	-	Makeup	Color
2	TagraCap3	Red, Black, Yellow, Brown	-	Makeup	Color
3	TagraCap4	Brown, Red, Yellow, Black	-	Makeup	Color
4	Red7Cap	Red7 Pigment	-	Makeup	Color
5	MicaCap	Pearls	-	Makeup	Color
6	CarbonCap	Carbon Black	-	Makeup	Color
7	CopperCap	Copper Oxide	Anti-acne, anti-inflammatory	Cellulite	Color

Vitamins

Table 3: Tagra's products comprised of encapsulation of vitamins

#	Product Name	Func. Ingredients	Loading (%)	Functional Applications	Cosmetic Applications
1	Tagravit A2	Retinol Palmitate	7	Wrinkle reduction, rejuvenation/ Regeneration, Anti-acne, Whitening	Anti-aging, nourishing, moisturizing, after sun care, after shave, cellulite treatments
2	Tagravit E1	Vitamin E (alpha tocopherol)	25	Wrinkle reduction, antioxidant, rejuvenation/ regeneration, whitening, anti inflammatory	Anti-aging, sensitive skin, nourishing, moisturizing, after sun care, sun protection, cellulite treatment, hand care, make up
3	Tagravit R1	Retinol	7	Wrinkle reduction, rejuvenation/ regeneration, anti acne, whitening	Anti-aging, nourishing, moisturizing, sun protection, cellulite treatment, hand care
4	Tagravit R2	Retinol	14	Wrinkle reduction, rejuvenation/ regeneration, anti-acne, whitening	Anti-aging, nourishing, moisturizing, sun protection, cellulite treatment, hand care
5	Tagravit RC	Retinol and Vitamin C	7/7	Wrinkle reduction, rejuvenation/ regeneration, anti-acne, whitening	Anti-aging, nourishing, moisturizing, sun protection, cellulite treatment, hand care

#	Product Name	Func. Ingredients	Loading (%)	Functional Applications	Cosmetic Applications
6	Tagravit C1	Vitamin C	25	Antioxidant, whitening	Anti-aging, sensitive skin, nourishing, after sun care, after-shave hand care, makeup
7	Tagravit F1	Linoleic and Linoleic Acid	14	Wrinkle reduction, rejuvenation/ regeneration, anti inflammatory	Anti-aging, nourishing, moisturizing

Oils, essential oils, volatiles

Table 4: Tagra's products comprised of encapsulation of oils

#	Product Name	Func. Ingredients	Loading (%)	Functional Applications	Cosmetic Applications
1	Tagrol TTO1	Tea Tree Oil	5	Rejuvenation/ regeneration, anti-acne, anti inflammatory	Sensitive skin, moisturizing, after-sun care, after shave, hand care
2	Tagrol Ment1	Menthol	10	calming	After-sun care, moisturizing, after sun care, after shave, hand care
3	Tagrol B1	Borage Oil	25	Rejuvenation/ regeneration, anti-acne, anti-inflammatory	Anti-aging, sensitive skin, nourishing, moisturizing, after-shave, cellulite treatment, hand care

#	Product Name	Func. Ingredients	Loading (%)	Functional Applications	Cosmetic Applications
4	Tagrol EPO1	Evening Primrose Oil	25	Wrinkle reduction, anti oxidant, rejuve-nation/regenera-tion, anti- acne, whitening, anti-inflammatory	Anti-aging, nourishing, moisturizing, after-shave, cellulite treatment, hand care
5	Tagrol H1	Hippophae Oil	25	Wrinkle reduction, antioxidant, rejuvenation/ regeneration, whitening, anti inflammatory, calming	Anti-aging, sensitive skin, nourishing, moisturizing, after-sun care, sun protection, after shave, hand care
6	Tagrol PO1	Patchouli Oil	5	Rejuvenation/ regeneration, anti-inflammatory, calming	Sensitive skin, after-sun care
7	Tagrol EC1	Lemon Eucalyptus	5	Anti-inflammatory,	Cellulite treatment
8	Tagrol GN1	Ginger	5	Antioxidant, anti-inflammatory	Cellulite treatment
9	Tagrol CM1	Chamomile	5	Antioxidant, rejuvenation/ regeneration, anti-acne, anti-inflammatory, calming	Anti-aging, after-sun care, after-shave, hand care

Flavonoids:

Table 5: Tagra's products comprised of encapsulation of flavonoids

#	Product Name	Functional Ingredients	Loading (%)	Functional Applications	Cosmetic Applications
1	Tagrol GS2	Grape Seed Extract	10	Wrinkle reduction, antioxidant, rejuvenation/ regeneration, anti-inflammatory	Anti aging, moisturizing, after-sun care, sun protection, cellulite treatment
2	Tagrol Resvera-trol	Resveratrol	20	Wrinkle reduction, antioxidant, rejuvenation/ regeneration, anti-inflammatory	
3	Tagrol Rutin	Rutin	7	Antioxidant, anti-inflammatory, capillary veins reduction	Anti-aging, nourishing, after-sun care, sun protection, cellulite treatment
4	Tagrol Licorice	Licorice	10/4	Antioxidant, whitening, anti-inflammatory, calming	Anti-aging, after-sun care, hand care

Table 6: Tagra's products comprised of encapsulation of pharmaceutical ingredients

Pharmaceutical applications

#	Product Name	Functional Ingredients	Loading (%)	Functional Applications	Claims
1	Tagravit Benzoyl Peroxide	Benzoyl Peroxide	90	Anti-acne, anti-inflammatory	1. Improves stability, 2. Improves penetration, 3. Reduces side effects, 4. No bad odor

#	Product Name	Functional Ingredients	Loading (%)	Functional Applications	Claims
2	Tagravit Peptide	Under Development	-	Anti-acne, anti-inflammatory	-

Amcol's current commercial delivery system is comprised of an allyl methacrylate crosspolymer,[8] which acts as a viscosity-increasing agent for formulations. It is an adsorbent polymer with high void volume for absorbing sebum six times its weight in certain embodiments. It has a general molecular formula of $C_{17}H_{24}O_6$ and molecular weight of 324.37 g/mol. It is also applicable as a carrier vehicle for essential oils.[8, 9]

Table 7: Amcol's products comprised of encapsulation of BPO, salicylic acid, and retinol

#	Delivery system	Composition	Functional Ingredients	Loading (%)	Applications	Claims
1	Poly-Pore 438 BP		Benzoyl Peroxide	38	Acne-gels, lotions, facial masks	Improve stability Controls sebum Sustained BPO release
2	Poly-Pore 450 SA	Allyl Methacrylates Crosspolymer and Salicylic Acid	Salicylic Acid	50	Acne-gels, lotions, makeups, facial masks	• Alcohol free • Oil free • Carbomer gel formulations • Sebum adsorption and control • No preservatives/ no surfactants
3	Poly-Pore 120RE*	Allyl Methacrylate Crosspolymer (and) Polysorbate 20 (and) Retinol (and) BHT	Retinol	20	Anti-aging, skin-renewal creams, makeups, powders, skin care formulations	• Stability of retinol • No irritation

Benefits include:

1. Simple, solvent-free, alcohol-free incorporation of salicylic acid in all types of personal care products, including gels, emulsions, creams, and loose and pressed powders.
2. Extended release of salicylic acid results in reduced irritation and long-lasting availability at the site of action.
3. Provides sebum control, reducing shine and extending wear in color cosmetics
4. Enhances emulsion stability
5. Improves stability of formulations typically destabilized by acids—such as Carbomer gels
6. Provides secondary thickening in gels and emulsions

Poly-Pore (Φ 20–40 micron) is stored at –1 to 4°C. Poly-Pore RE should be added to finished product batch during cooling (40°C). There is a delayed release of salicylic acid from Poly-Pore formulation indicated in the literature.[10]

Polytrap 6035

Table 8: Amcol's products comprised of encapsulation of blank, vitamin E, Shea Butter, cyclomethicone, dimethicone/petrolatum, tea tree oil

#	Delivery system	Composition	Functional Ingredients	Loading (%)	Applications	Claims
1	Polytrap 6603	Lauryl Methacrylate/ Glycol Dimethacrylate Crosspolymer	Blank	0	Anti-aging products, acne care, moisturizers, facial masks	• Sebum control
2	Polytrap 665		Vitamin E	65	Sunscreens, powders, and liquid makeup	• Sustained delivery • Stability of vitamin E
3	Polytrap 650	Lauryl Methacrylate/ Glycol Dimethacrylate Crosspolymer (and) Butyrospermum Parkii (Shea Butter)	Shea Butter	50	Anti-aging products, moisturizers, facial masks	• Sebum control

#	Delivery system	Composition	Functional Ingredients	Loading (%)	Applications	Claims
4	Polytrap 6035	Cyclomethicone (AND) Lauryl Methacrylate/ Glycol Dimethacrylate Crosspolymer	Cyclo-methicone	70	Lipsticks, color cosmetics, skin protectants	• Exceptional sebum adsorption capacity
5	Polytrap 6500	Dimethicone (AND) Petro-latum (AND) Lauryl Meth-acrylate/Glycol Dimethacrylate Crosspolymer	Dimethi-cone/ Petrolatum	70	Gels, powders	• Sebum control • Long-lasting performance
6	Polytrap 680	Lauryl Methac-rylates Glycol Dimethacrylate Crosspolymer (and) Melaleuca Alternifolia (Tea Tree) Leaf Oil	Tea Tree Oil	80	Skin care, gels, lotions	• Ease of formulation

Poly-Pore 120RE

Benefits

1. Significantly improves the stability of retinol, resulting in formulations that are more effective
2. Extended release results in reduced irritation and long-lasting availability at the site of action.
3. Provides sebum control
4. Enhances emulsion stability
5. Provides secondary thickening in gels and emulsions

Typical Applications

1. Anti-aging and skin rejuvenation products
2. Cellulite treatments
3. Makeups and foundations incorporating skin care benefits
4. Daily-use moisturizers and lotions
5. Facial and body masks

6. Loose and pressed powders
7. Hand and body lotions

Polytrap 6035

Benefits

1. Aids incorporation of cyclomethicone in a broad range of products including gels and powders
2. Provides sebum control in all types of formulations including lotions, gels, o/w and w/o emulsions, and both loose and pressed powders

Typical Applications

1. Facial cleansers requiring improved elegance and luxurious after-feel
2. Skin care including anti-aging products, acne care, moisturizers, and oil control treatments
3. Color cosmetics including loose and pressed powders, body powders, and liquid makeups
4. Toiletries, including deodorants, body lotions, sunscreens, baby care products
5. Other personal care sticks and gels

Polytrap 650SB

Benefits

1. Enables formulation with Shea Butter in all types of personal care products, including gels, emulsions, creams, and loose and pressed powders
2. Provides sebum control, reducing shine and extending wear in color cosmetics
3. Enhances emulsion stability
4. Provides secondary thickening in gels and emulsions
5. Evens skin tone and returns luster to skin and hair
6. Revitalizes, softens, and maintains skin moisture
7. In formulation Polytrap 650SB, penetrates deep into skin to help restore elasticity
8. Naturally rich in vitamins A, E, & F that are essential vitamins for good skin balance

Typical Applications

1. Facial treatments and skin care products including gels, emulsions, and anhydrous products
2. Makeups and foundations incorporating skin care benefits

3. Daily-use moisturizers and lotions
4. Exfoliating gels and treatments
5. Loose and pressed powders

Polytrap 680TT

Benefits

1. Aids incorporation of Tea Tree Oil in a broad range of products including gels and powders
2. Enables formulating efficacious levels in skin care formulations and powders
3. Lessens the odor of Tea Tree Oil
4. Provides sebum control in all types of formulations including lotions, gels, o/w and w/o emulsions, and both loose and pressed powders
5. Creates longer-lasting elegance through extended delivery of the oil

Typical Applications

1. Skin care, gels, lotions for the treatment of acne, athlete's foot, blisters, burns, cold sores, dandruff, insect bites, oily skin, rashes, and wounds.
2. Skin care including anti-aging products, acne care, moisturizers, and oil-control treatments
3. Color cosmetics including loose and pressed powders, body powders, liquid makeups
4. Toiletries, including deodorants, body lotions, sunscreens, baby care products

Polytrap 6500

Benefits

1. Aids incorporation of dimethicone and petrolatum into a broad range of products including gels and powders
2. Extends the skin-protectant capabilities of dimethicone and petrolatum, providing long-lasting performance not possible with the ingredients alone
3. Provides sebum control in all types of formulations including lotions, gels, o/w and w/o emulsions, and both loose and pressed powders

Typical Applications

1. Facial cleansers requiring improved elegance and luxurious after-feel
2. Skin care including anti-aging products, acne care, moisturizers, and oil control treatments

3. Color cosmetics including loose and pressed powders, body powders, and liquid makeups
4. Sun protection and after-sun products
5. Toiletries, including deodorants, body lotions, sunscreens, baby care products
6. Other personal care sticks and gels

Macrobead-Functional Exfoliation

Table 9: Amcol's products comprised of encapsulation of dimethicone and mineral oil

#	Delivery system	Composition	Functional Ingredients	Loading (%)	Applications	Claims
1	Macrobead 7100	Lauryl Methacrylate/ Glycol Dimethacrylate Crosspolymer (and) Dimethicone	Dimethicone	35	Facial, body scrubs, oil-free acne cleansers, skin polishers, liquid soaps, bar soaps	Exfoliating Macrobead with delivery of Dimethicone
2	Macrobead 6038	Lauryl Meth-acrylate/Glycol Dimethacrylate Crosspolymer (and) Mineral Oil	Mineral Oil	35	Facial, body scrubs, oil-free acne cleansers, skin polishers, liquid soaps, bar soaps	Exfoliating Macrobead with delivery of Mineral Oil

Macrobead 7100 dimethicone/Macrobead 6038 Mineral Oil

Benefits

1. Effective yet gentle exfoliation
2. Rubout that precludes overscrubbing
3. Controlled particle attrition provides sensory cue of gentleness.
4. Leaves behind a luxurious after-feel providing sensory indicator of effectiveness
5. Enables simple formulation of novel facial and body cleansers/washes featuring high levels of silicones

Typical Applications

1. Facial and body scrubs
2. Oil-free acne cleansers
3. Anti-aging cleansers
4. Skin polishers
5. Liquid soap
6. Bar soap
7. Medicated shampoos

Arch (Part of Lonza) has a delivery system comprised of microencapsulated off-white powders with claims of controlled-release encapsulation technology.

Table 10: Lonza's products comprised of encapsulation using acrylates/carbamate polymers

#	Product	INCI	Applications
1	Chronosphere G	Acrylates/carbamate polymer/glycerin	Body lotion, toner, facial moisturizer, makeup, lipstick
2	Chronosphere TRF	Acrylates/carbamate polymer/saccharomyces cerevisiae extract	Body lotion, toner, facial moisturizer, makeup, lipstick
3	Chronosphere Vitazyme ACE	Acrylates/carbamate polymer, tocopherol polypeptide, retinyl palmitate polypeptide, ascorbyl polypeptide	Body lotion, toner, facial moisturizer, makeup, lipstick

8.1.3 LIPID-BASED ENCAPSULATION SYSTEMS

a. Liposomes

Phospholipids are widely used as delivery systems due to the fact that they are comprised of a special class of phosphate lipids (phosphatidylcholine [PC], sphingomyelin [SM] and other phospholipids[11] that resemble the lipid layer surrounding keratinocytes, which facilitate penetration). The lipid component of phospholipids is hydrophobic (i.e., nonpolar) while the phosphate component is hydrophilic. When mixed with hydrophobic and hydrophilic ingredients, liposomes will arrange in a micelle structure[12] around these ingredients, with the lipid ends of the micelle surrounding the hydrophobic ingredients and the phosphate ends surrounding the hydrophilic ingredients. They are currently available in the market for cosmetic use mainly because of their ability to penetrate into the deeper layers of the skin and ease of preparation.

Liposomes have a reputation of instability, and therefore encapsulation of ingredients in liposome systems has major drawbacks. These include limited shelf-life stability, formulation issues,[12] and low loading of the functional ingredient. The shelf-life stability of liposomes in a formulation depends on the interaction of the liposomes with the other base ingredients and the ability of the liposome lipid bi- or multi-layer structure to maintain its physical integrity in the product base. If this structural integrity is not maintained, agglomeration of individual liposomes can occur, resulting in a significant increase in "particle" volume since the agglomerates consist of weakly bound multiple individual "particles," and formulation instability. In addition, liposomes have the inherent limitation in formulating where procedures as well as raw materials must be considered carefully in order to avoid adverse effects, such as shifts in pH, which could have detrimental effects on their stability.

Regardless of their reputation of instability, commercial delivery systems based on liposomes have been developed and used successfully in commercial cosmetic and personal care systems.

For example:

Moistureguard™ is a cationic liposome-encapsulating moisture-protection complex comprised of petrolatum and silicone, with claims of melting on the skin and intensely repairing dehydrated skin up to eight hours after application.

Engelhard

Table 11: Englehard BASF's products comprised of encapsulation

#	Product Name	Ingredients	Description
1	Alpha Hydroxy Acid Liposomes	acidopholus/grape ferment extract, phospholipids, xanthan gum	High potency extract of alpha hydroxy acid from fermented grapes

#	Product Name	Ingredients	Description
2	Anti-irritant liposomes	bisabolol, phospholipids, triethanolamine	Potent anti-irritant complex from kola nuts and alpha bisabolol
3	Catezomes HWP-LG	dimethicone, behenamidopropyl dimethylamine behenate, PEG-8, hydrolyzed wheat protein, hydrolyzed silk	Blend of silk amino acids, hydrolyzed keratin, wheat protein
4	Catezomes P-20 AM	panthenol cyclopentasiloxane behenamidopropyl dimethylamine behenate stearamidopropyl dimethylamine stearic acid	Used in hair and skin care formulations
5	Catezomes SI-20	dimethicone, behenamidopropyl dimethylamine behenate	Non-phospholipid vesicles, no penetration

Cytovector™ is an encapsulation technology based on liposomes comprised of quarternized soy-based molecules. *In vitro* data for Cytovector show depigmentation[13] and protection from free radicals. Micropatch Caffeine is made of acacia and alginate that claim slow-release action of caffeine all day long and render the skin hydrated and smoother in appearance.[14] A good description of these and other gums may be found in the *Rheology Modifier Handbook* by Braun and Rosen.[15]

b. Solid Lipid Nanoparticles (SLN)

Solid lipid nanoparticles are comprised of organic free, oily droplets of lipids that are solid at room temperature and stabilized by surfactants.[16] SLNs can protect the encapsulated ingredients from degradation. They can be used for the controlled delivery of cosmetic agents such as perfumes, UV blockers, retinol, and coenzyme Q10 over a prolonged period of time and have been found to improve the penetration of active compounds into the stratum corneum. SLNs have occlusive properties, making them ideal for potential use in day creams. SLNs were incorporated into Chanel's Allure perfume. The high water content of SLN dispersions can be an issue in formulations. SLN dispersions cannot incorporate high loadings of the functional ingredient and are associated with low shelf-life stability.

c. Lipid-based sub-micron technology

Structure and Mode of Action

SalSphere™ technology is comprised of sub-micron, hydrophobic (lipid matrix) spheres that encase a functional ingredient (FI) or active pharmaceutical ingredient (API) and suspend it in an aqueous medium via a hydrophilic outer shell that possesses a cationic charge (**Figure 3**). Once applied to skin, the positively charged moiety on the shell anchors the sphere for deposition while the hydrophobic matrix gradually dissolves into the lipid membranes of the skin. By this means, the contained ingredients are released in a controlled manner over time.

The above-mentioned controlled-release approach leads to various benefits for topical skin care applications.[17] First, the technology aids in stabilizing ingredients by either isolating them from other components within a formulation that they might react with. Second, the approach also shields the ingredients from potentially degrading stressors, such as oxidation, when on skin. This protective element leads to a secondary benefit of longevity . . . because not all of the active is exposed on the skin at once. The process allows the ingredient(s) to reach the skin surface upon slow dissolution of the matrix core and over an extended time period. The ingredient is therefore preserved and delivered at a more potent level than if it were to be applied in free (non-encapsulated) form. This same slow-release kinetic conversely helps to shield the skin from potentially irritating ingredients such as benzoyl peroxide or beta hydroxy acids. The release of this system can be accelerated through the application of heat or increased enzymatic activity.

Figure 3: Structure of the sub-micron spheres

Overcoming Solubility and Compatibility Issues

Certain ingredients, such as the BHA mentioned above, can cause instability in a formulation due to their low solubility. The hydrophobic sub-micron technology solves this issue and can encapsulate functional ingredients and active

pharmaceutical ingredients (FI/API) at loadings up to 30% without any phase separation or discoloration. The technology also allows maintaining assay levels, particle size, pH, and color under accelerated aging conditions of three months @ 42°C. The encapsulation causes no chemical alteration to these ingredients. Rather, the hydrophobic spheres enrobe them and suspend them in a water-based medium within shells that are composed of inert materials that carry a charge to aid in their suspension. As a result, the formulator does not need to utilize additional solvents or surfactants in order to suspend ingredients in their products. Being water-based, the sub-micron technology is easily incorporated under either rotary mixing or homogenization, depending on the viscosity of the formula. As mentioned, ingredients are encased in a hydrophobic vehicle and are therefore largely isolated from the rest of the formula. As a result, formulations using this technology can be created at pH ranges of 4.5–5.5, which is the healthiest range for human skin. This advantage alleviates the formulator's dilemma of having to limit their ingredient choices or having to use extra suspension or dissolution agents in their product.

Figure 4: Illustrations of the stability of a gel containing either BHA from SalSphere™ (**Figure 4A**) or free BHA (**Figure 4B**)

Improving Performance

In addition to the controlled-release benefits discussed above, SalSphere™ is able to better target ingredients to the skin from rinse-off applications. The hydrophobic nature of the core is more compatible with the skin and can act as a depository vehicle for both hydrophobic and hydrophilic FI. This enhanced deposition is often the first step to overall higher ingredient efficacy when combined with the slow-release kinetics discussed above.

The ability of the sub-micron technology comprised of resveratrol to provide enhanced deposition on skin was studied *in vivo* by skin extraction using an extraction apparatus (circular bulb 15.5 cm^2 in area). A measured amount of the product,

about 0.25 grams, was applied on the target area. The samples were left on the target site for one minute. The applied area was rinsed with 100 ml water and tapped dry with paper towel.

A 3-mL disposable syringe was filled with 1.5 mL of ethanol and the ethanol was placed in the skin extraction apparatus and carefully inverted over the application area, tightly holding the bulb in place and swiveled for 30 seconds. The ethanol fraction was collected into a labeled glass jar. The extraction step was repeated for a total of three times per marked area on arm.

Figure 5: Enhanced deposition of resveratrol from a rinse-off application vs. free (0.5% resveratrol loading)

Figure 5 shows the sub-micron technology resulted in 55-fold higher deposition of resveratrol onto skin vs. free. Free resveratrol has the propensity to be physically removed from the skin during the rinsing process. However, the cationically charged, hydrophobic matrix of SalSphere™ is attracted to the skin and resistant to being removed through rinse-off.

8.1.4 SUGAR-BASED TECHNOLOGIES

a. Cyclodextrins

Cyclodextrins are obtained from the enzymatic degradation of starch. These molecules are composed of polysaccharides and possess an inner radius of 0.5–0.8 nm that can host cosmetic functional ingredients.[18] On a molecular level, the outer faces of this complex are hydrophilic while the cavity is hydrophobic. The cavity is where a cosmetic active is captured and surrounded, thereby protecting these

Table 12: shows Salvona's SalSphere™ product names, the functional ingredients and the applications.

#	Product Name	Functional Ingredients	Loading (%)	Functional Applications	Effect
1	SalSphere™ Anti-Aging Lift	Polyvinyl Alcohol (and) Niacin (and) Tocopheryl Acetate (and) Glycerin (and) Resveratrol (and) Thioctic Acid (and) Dimethyl Sulfone (and) Panax Ginseng Root Extract (and) Portulaca Oleracea Extract (and) Palmitoyl Tripeptide-5	-	Anti-aging	Instant reduction of fine lines with long term anti-wrinkle therapy
2	SalSphere™ Natural Anti-Aging	Pyrus Malus (Apple) Fruit Extract (and) Resveratrol	-	Anti-aging	Blend of natural polyphenols that reverse the signs of aging
3	SalSphere™ Resveratrol	Resveratrol	1/5	Anti-aging	Natural antioxidant for younger looking skin
4	SalSphere™ Benzoyl Peroxide	Benzoyl Peroxide	25	Dermatology	An effective stable solution for acne
5	SalSphere™ Intense Hair Re-growth	Minoxidil (and) Acetyl Tetrapeptide-3 (and) Trifolium Pratense (Clover) Flower Extract (and) Pyrus Malus (Apple) Fruit Extract (and) Apigenin (and) Water (and) Butylene Glycol (and) PPG-26-Buteth 26 (and) PEG-40 Hydrogenated Castor Oil (and) Oleanolic Acid (and) Biotinoyl Tripeptide-1	-	Dermatology	Targeted hair growth with enhanced intensity and penetration
6	SalSphere™ Minoxidil	Minoxidil	2/5	Dermatology	Effective solution to hair restoration

#	Product Name	Functional Ingredients	Loading (%)	Functional Applications	Effect
7	SalSphere™ Salicylic Acid 30	Salicylic Acid	30	Dermatology	Effective acne treatment from a clear application
8	SalSphere™ Hair Rejuvenator	Olea Europaea (Olive) Fruit Oil (and) Carthamus Tinctorius (Safflower) Seed Oil (and) (and) Vitis Vinifera (Grape) Seed Oil	-	Hair	Delivery of potent botanical blend for visible hair rejuvenation
9	SalSphere™ Hair Stimulator	Caffeine	10%	Hair	Controlled Release of Caffeine for Hair Growth
10	SalSphere™ Healthy Scalp	Salicylic acid	30%	Hair	Maintain a healthy and Flake-Free Scalp
11	SalSphere™ Natural Hair Growth Promoter	Pyrus Malus (Apple) Fruit Extract	5%	Hair	Potent antioxidant for hair growth
12	SalSphere™ Style Protector	Phenyl Trimethicone	-	Hair	Prevention of frizzy, unruly and flat hair
13	SalSphere™ SalCool	Methyl Diisopropyl Propionamide (and) Ethyl Menthane Carboxamide (and) Menthyl Lactate	10%	Sensory	Long-lasting, odorless, refreshing sensation

#	Product Name	Functional Ingredients	Loading (%)	Functional Applications	Effect
14	SalSphere™ Aqua Skin	Fucus Vesiculosis Extract (and) Phenyl Trimethicone	-	Skin	Optimal moisturization for enhanced Luxurious Skin Feel
15	SalSphere™ Cellulite Eraser	Caffeine (and) Nelumbo nucifera leaf extract (and) Palmaria palmata	-	Skin	Multiple benefits from time-released caffeine
16	SalSphere™ Clear Skin	Salicylic acid (and) lactic acid	5%/1%	Skin	A skin-friendly complex of AHA/ BHA to clear blemishes
17	SalSphere™ Light	Alpha arbutin (and) Resveratrol	-	Skin	Long-lasting skin brightening
18	SalSphere™ Sunkiss	Dihydroxyacetone	-	Skin	Long-lasting sunless tanning for glowing skin

actives from thermal, chemical, and mechanical degradation; retarding loss via evaporation and retaining poorly water-soluble actives. There is a commercial formula of salicylic acid that is solubilized in cyclodextrin complexes for anti-acne applications. The claims are enhancement of exfoliation and suitable for acne treatments.

While promising, both liposome and cyclodextrin technology do not fully address the issues presented herein relating to the balance between efficacy and skin-friendly attributes. At the present time, sufficient data do not exist on the effect of these delivery systems on skin pH or their release kinetics, and how these kinetics impact performance of the functional ingredient. Basically, the formulation comprising this technology does not result in a skin-friendly pH upon topical application. The adhesion from a rinse-off is not guaranteed due to lack of suitable anchoring complexes. Slow gradual release is not validated and confirmed by published data. Thus, there is a need for an intelligently engineered delivery system that solves all of these issues.

Table 13: BASF product comprised of encapsulation using salicylic acid

#	Product Name	Ingredients	Loading	Claims
1	Beta hydroxyde ACSD	SA	50 %	Complex of acacia polysaccharides with salicylic acid using coupling technology, slows down penetration, reduces irritation, pore size reduction, non-cyotoxic, shine reduction, skin-friendly pH

Lipo Technologies manufacture a number of delivery systems that are comprised of a lipocapsule made up of Gelatin and Polyoxymethylene urea (PMU). Methoxymethyl Methylol Melamine (MMM) capsule size is of 5–3000 microns. The products employ precipitation of synthetic polymers around a hydrophobic core material. Other products by Lipo Technologies include Lipobeads, Lipopearl, Liposphere, Lipocrystal, and InstaScent (Scratch and Sniff). A cholesterol ester mixture reflects light to produce beautiful color. Lipoparticles are comprised of cyclodextrin, porous nylon, silica, and cellulose as encapsulating materials. Functional ingredients include salicylic acid, tocopherol, menthol, triclosan, optical brighteners, etc.

8.1.5 OTHER DELIVERY SYSTEMS

a. HydroSal™ Technology

Structure and Mode of Action

HydroSal™ is a sub-micron technology that utilizes a porous core as an encapsulation compartment and is utilized mainly for the controlled release of volatile sensory ingredients such as fragrances. These sub-micron spheres are suspended in water and are surrounded by an additional polymer. Once applied to surfaces ranging from skin to hair to hard surface, the aqueous carrier will dry out, leaving the secondary polymer to collapse over the sub-micron spheres and act as a secondary diffusion barrier to ingredient release. The solidification of the secondary barrier is the element that creates a controlled release of volatile ingredients such as fragrances. The fragrance notes are allowed to escape into the atmosphere, but at a greatly reduced rate that preserves their intensity but also extends that intensity over time.

(A) **(B)**

Figure 6: Structure of HydroSal™ core with a temporarily evaporated shell (A), and with the outer shell intact (B)

Improving Performance

The primary performance benefit of HydroSal™ is that it provides an "on demand" water trigger response for fragrance release. Once disturbed by moisture, the secondary barrier once again becomes amorphous and allows the entrapped ingredients to diffuse rapidly from the core. In the case of a fragrance, for instance, this rapid release creates a burst of intensity that is on par with the degree of moisture exposure. The process of fragrance burst will continue to repeat until the fragrance is depleted and will respond in kind to the amount of moisture to which the system is exposed. Besides the water-triggered delivery described above, HydroSal™ also provides malodor neutralization. When the secondary barrier is disturbed and the

fragrance vacates the core in a rapid fashion, the open sites of the core will absorb lipophilic malodor agents and, because these agents are less volatile than something like a fragrance, they will be retained in the core. HydroSal™ can readily be suspended in a water-based formulation. It is slightly anionic in character, however, and may show some incompatibility in formulas with cationic components. Aside from fragrance encapsulation, HydroSal™ has been utilized in encapsulation of cooling and hair care agents.

b. Cellesence

A subsidiary of IFF (International Flavors and Fragrances), provides smart encapsulation technologies for textiles. 8.3% of total sales are spent in innovative materials and delivery systems (taste and scent release); 170 scientists, 90 PhDs, and 320 application engineers support basic molecular discovery, new delivery systems, and new applications. The technology is comprised of tiny, polymeric microencapsulated super-moisturizers and four skin care ingredients in diabetic socks (TransDermal-DTM), long-lasting bug-killing spray (NEEM), and microencapsulated ingredients for textiles.

c. Fermenich

Has developed Durarome-encapsulated flavors that are water soluble with no surface oil exposed to oxygen; flavors such as strawberry, orange, and peppermint are encapsulated in carbohydrate matrices.[19]

d. Thermarome

Comprises of aroma encapsulation in yeast cells (spray-dried form). **Flexarome** is a technology consisting of Erythritol crystals. Symrise is the fourth-largest global supplier of fragrances and flavors; it produces fragrances using the spray-drying production method based on coacervation and interfacial polymerization. The polymeric materials used include polyurethane, polyamide, polyester, silicone resin, epoxy resin, urea-formaldehyde, and proteins such as gelatin, casein, and serum albumen. InCap is a cost-effective delivery system mainly for deodorants, antiperspirants, and powdered detergents. Body moisture triggers the release of the encapsulated fragrance or other encapsulated material. High load of 70% fragrance is obtained via a patent-protected method. Interfacial polymerization uses SymCap, which has been developed for use in laundry detergents and fabric softeners to provide long-lasting fragrance on dry fabric. The release mechanism is based on friction.

e. Givaudan

has a product called Mecha Caps comprised of thin-walled microspheres encapsulating fragrances. The product is formed by poly-condensation of urea with

formaldehyde and poly-condensation of melamine with formaldehyde. The benefits of the technology are longer shelf life, gentle friction on fabric, and controlled release of fragrance for longer time than free.[20] The major disadvantage is undesirable use of formaldehyde. In the wet and dry conditions, the microcapsules deposit higher levels of fragrance vs. the free.

f. Evoglass

Claims encapsulated flavor with high stability comprised of excellent flavor intensity, good water solubility, and a shelf life two to four years, dust free using carbohydrate matrices with particle size < 0.6 mm, 0.6 mm, or 1 mm.

Comparison of delivery systems for encapsulation of the same functional ingredient:

In this section, we discuss each important functional ingredient that has been encapsulated by various technologies and compare them in terms of factors such as structure, composition, stability, and clinical efficacy.

1. Retinol

CylaSphere™ Retinol from BASF consists of plant microspheres encapsulating 1% retinol that claim smoothing, lightening, and whitening applications. Literature evidence shows higher epidermal residence time from the spheres vs. o/w emulsions containing free retinol using an *in vitro* Franz experimental set-up for diffusion of retinol through hairless mouse skin. We have been unable to identify results on stability and clinical efficacy of the product.

Findings were that the cumulative release rate of emulsions that contained 0.2% free retinol and 1.0% Poly-Pore 120RE (equivalent to 0.2% retinol). Release kinetics of retinol from 1% Polypore RE in formulation reveal that initially very little retinol is released, but after six hours, an equivalent amount has been delivered into the receptor phase (and therefore to the skin) as compared to the free (0.2% retinol). These data prove that actives such as retinol entrapped in Polypore will have reduced irritancy because not all the retinol is released on the skin surface at one time.[21] Data on clinical efficacy and stability have not been found in the literature.

MultiSal™ retinol is composed of microspheres with sub-micron spheres (SalSpheres™) encapsulated in polymeric coating in the form of a free-flowing powder. The size of microspheres can range from 10 to 60 microns. After 50 days of stability testing at 42°C, the encapsulation technology retained 80% of the retinol compared to only 5% in the control. We have tested two formulations of MultiSal™ containing 0.05 and 0.1% retinol in night cream as compared to the same levels of free retinol. *In vivo* testing of the formulations was tested for five hours on

three volunteers to determine the release kinetics. The encapsulated retinol showed no irritation upon application to the skin of the volunteers. Results show that MultiSal™ provides a slow release, by which retinol is released for a longer time. Controlled-release product allows better skin absorption over five hours and therefore is recommended as an effective anti-aging product with reduced irritation and suitable for consumers with sensitive skin. This was confirmed with *in vivo* clinical tests that showed a dramatic reduction in fine lines and wrinkles, with no irritation using a lotion containing MultiSal™ technology vs. free for two weeks daily. *In vitro* Franz test showed significantly slower release into the receptor phase from the technology vs. free over 24 hours of testing.

2. Menthol

MultiSal™ technology is able to protect and retain a significantly higher percentage of menthol under heat stress vs. free, which translates to a higher aroma intensity and efficacy in a final product. Tagrol Menthol 10% is comprised of menthol & polymethyl methacrylate & tricaprylin. The beneficial activates of Tagrol Menthol are: a) it cools the skin, b) it provides a fresh sensation, and c) it calms and relieves irritation. Health and beauty applications include sunscreen product, cooling after-sun lotion, and after-shaves. Lipocapsules made up of gelatin encapsulate menthol (16%) for food-grade purposes and protect it from volatility and deliver the menthol.[22]

3. Resveratrol

SalSphere™ sub-micron technology resulted in 55-fold higher deposition of resveratrol onto skin vs. free from a rinse-off application. Free resveratrol has the propensity to be physically removed from the skin during the rinsing process. However, the cationically charged, hydrophobic matrix of SalSphere™ is attracted to the skin and resistant to being removed through rinse-off.

4. Benzoyl Peroxide

Tagravit Benzoyl Peroxide with 90% loading is for pharma industry. There are no available data on stability and substantiation of claims. Polypore 438 BPO Delivery System is effective at causing a significant reduction in *P. acne* counts over a 14-day period, and the study showed that 3.5% BPO entrapped in Poly-Pore was as effective as a control system that contained 10% free BPO.

SalSphere™ BPO has been proven to be effective in stabilizing BPO vs. commercial products and free BPO in finished products. It has been shown to have a controlled-release effect and thus is a useful and viable option to treat acne. The formulation-friendly product is readily available in lotions, creams, and gels.

5. Salicylic Acid

Lipo CD-SA at 5% loading has pH of 2.1; there are no data on clinical efficacy; however, there is information on formulations. It is an encapsulated form of SA at 5% in light amber liquid with unneutralized SA and no alcohol as co-solvent.[22]

Polypore 450 SA is comprised of white free-flowing powder of polymer and SA without alcohol (50% loading of SA). Extended release of salicylic acid results in reduced irritation and long-lasting availability at the site of action. Polypore 450 SA is a polymeric formulation including SA in an allyl methacrylate crosspolymer. MultiSal™ consists of solid hydrophobic nanospheres encapsulated in a water or pH-sensitive microsphere. Biogenic salicylic acid is a proprietary material that encapsulates SA, and has a roughly 25% concentration of salicylic acid. Lipo CD-SA is a proprietary material comprising 5% salicylic acid in encapsulated powder form.

Beta-hydroxyde ACSD comprises of 50% salicylic acid and has proven exfoliating activity, visibly refined pore size, general improvement of oily skin, and optimal comfort level. SalSphere™ SA 30 has been proven to reduce skin irritation, provide skin-friendly pH in clear formulations, and enhance deposition onto the skin vs. free in rinse-off applications. It is formulation friendly and has been shown to result in controlled release of SA vs. commercial products and free SA in ethanol over 24 hours of testing.

MultiSal™ SA is a microencapsulation system that provides the free acid in an alkaline base and thus converts the impossible to possible. *In vivo* tests have confirmed clinical efficacy in treatment of acne lesions using the product.

6. Fragrances

HydroSal™ technology has been proven to provide longer-lasting capability of fragrance intensity vs. free fragrance in multiple applications in perfumery and household care. Givaudan uses Mecha caps to encapsulate fragrances in thin-walled microspheres. The major disadvantage is undesirable use of formaldehyde. In the wet and dry conditions, the microcapsules deposit higher levels of fragrance vs. the free.

8.1.6 TECHNICAL CHALLENGES IN DELIVERY SYSTEMS

A variety of technical challenges remain unsolved and are important for highlighting in this chapter so that future efforts can be directed towards their solution.

1. Challenges in encapsulation of actives for efficacy and stability—how to validate encapsulation in raw samples and the finished products (i.e., is it a suspension, colloidal dispersion, or encapsulation efficiency of hydrophilic actives?)
2. Consecutive release of actives, outer-shell active release, inner-matrix active release: is it possible?

3. Co-polymeric systems for sustained release—e.g., HPMC and Eudragit for API formulations
4. Different trigger responses for cosmetics
5. Adapting pharma API tablet technologies to cosmetics and topical formulations: is it possible?
6. Surfactant-free systems; oil-water emulsion stabilization with natural/organic certified suspending agents, or even better, pickering emulsions?
7. *In vitro* and *in vivo* efficacy, how to validate it; consumer-to-consumer variability
8. Classification of medication vs. cosmetic claims; validity?
9. Cost of encapsulation
10. Concept or feasible? Can thermally unstable actives be protected? Encapsulation is not applicable to all actives. What is the alternative: a) chemical modification, or b) micelle-based encapsulation systems?
11. Leakage of hydrophilic actives into continuous phase during encapsulation-aqueous media; how to prevent it?
12. How to prevent water loss over time, enabling higher shelf-life stability?

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NUTRICOSMETICS

THE USE OF NUTRACEUTICAL INGREDIENTS IN THE COSMETIC INDUSTRY

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ABSTRACT

Skin conditions are not only related to environmental conditions such as UV exposure, temperature, humidity, and chemical and mechanical wear and tear, but they are also associated with age, gender, and the nutritional history of individuals.[1] Since introduction of the terms nutraceuticals and cosmeceuticals[2] separately in the 1980s, the term nutricosmetics was proposed in conjunction with the launch of the first oral ingredient for a beauty product called "Imedeen" in the late 1980s. This chapter provides an extensive introduction to the rapidly growing area of nutraceuticals in cosmetics and personal care. What was once a beauty industry that focused on topical treatments has now emerged in an expanded form that acknowledges and provides documentation and ingredients for the enhancement of beauty and well-being based on internal nutricosmetics as well as the synergy between both topical and internal ingredients. It is also pointed out that the beauty-from-within concept has long been known and accepted in the Asian world and that the path to individualized approaches is now being embarked upon for future product enhancements.

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9.1.1 INTRODUCTION

"You are what you eat" is a phrase that can be found in almost every culture. Traditional Chinese Medicine (TCM) has taught for thousands of years that optimal health and well-being are related to the intake of certain ingestables, which are designed by practitioners to balance the body and, in Western terms, to achieve homeostasis. TCM thinking and practice, in terms of ingestion of proper foods and herbs have, in fact, gone far ahead of the Western thinking only now emerging, that each individual is different and therefore, to achieve homeostasis and optimal health requires personalized ingestion of selected "nutraceuticals."

Achieving beauty from within is a well-accepted concept in Asia and Europe. This concept and its growing global acceptance go far deeper than the "traditional" definition of achieving the appearance of beauty by means of topical "cosmetics." Although not yet accepted from a regulatory point of view, the concept is strikingly appealing in that what is ingested will have a significant impact on health, well-being, and the appearance of the largest organ of the body: skin. The nutritional approach to improving appearance and beauty has an enormous market potential. Orally supplemented nutritional products for skin care grew into a multi-billion-dollar segment and enjoyed double-digit growth in the middle and late 2000s.[3] Looking ahead, since our intention is not only to discuss current approaches in this field, we foresee the potential for *individualized* nutritional approaches to beauty and the opportunity for incredible leaps forward in humanity's common need to be, look, and feel beautiful and young as we all move into healthy (and beautiful) aging.

There are three fundamental forces behind the use of nutraceutical ingredients in the cosmetic industry. This industry needs cutting-edge, competitive advantages for their products that can offer unique claims and beauty benefits to grow business. The nutraceutical industry is looking to expand applications and claims for their ingredients;[4] and consumers—especially baby boomers—are eager to do anything, and everything, to slow down the inevitable aging process and maintain a youthful, healthy appearance and feeling.

The extraordinary growth rate of nutricosmetics in the past ten years has been fueled by new product introductions and a strong marketing push from big-name players. Unfortunately, a lack of scientific evidence and marginal beauty benefits from many nutricosmetics has turned consumers away. Fortunately, the consumer pull has encouraged the technology push that has continued to pursue the holy grail of finding, demonstrating, and proving that the concept of "beauty from within" is an idea whose time has come.

9.1.2 NUTRACEUTICAL INGREDIENTS THAT ARE SUITIBLE FOR COSMETIC USE

One of the challenges in selecting appropriate nutritional ingredients to achieve skin care benefits is to translate existing nutritional knowledge into a science-based beauty claim and to achieve believable results in complex biological systems that vary from person to person.[5] The following general guidelines shall be considered:

- Chemical profile and active components are well characterized.
- The mechanism of action of the active components in the ingredients has direct correlations with perceived skin benefits.
- Nutritional, biological, and functional evidence available from the use of the ingredients is well established.
- Bioavailability data are available to support the oral administration and delivery of the functional components systemically—and preferably into dermis and/or epidermis.
- Optimum dosages assure efficacious levels of the active components.
- Products should be suitable for long-term use without significant safety concerns.
- Regulatory concerns for oral consumption should be addressed.
- Relevant human clinical results should substantiate the usage and skin-functional claims.
- The question needs to be dealt with as to whether one "active" ingredient or the typical multiplicity of ingredients found in nature are the causes of the observed results.

9.1.3 CHARACERIZATION OF NUTRACOSMETIC INGREDIENTS BASED ON BIOLOGICAL FUNCTION

a. Antioxidation

UV-induced skin damage such as wrinkles, skin aging, and skin cancers has been well documented. However, before such long-term damage appears, complicated structural and functional compromise has occurred at the molecular level, in skin cells, skin tissues, and even at systemic levels after exposure to solar radiation generated at both UVA and UVB wavelengths.

The changes described above include: generation of reactive oxygen species (ROS), modification of proteins and lipids, damage to DNA, and compromises of the integrity of skin-cell membranes.[6] Free radicals are produced by normal physiological processes in the body to perform critical functions of cellular defense, inflammation process, and immune responses. Damage, both genetic and epigenetic types, can also be generated from environmental sources such as ultraviolet radiation, tobacco smoke, food additives, and many other pollutants. Cells have specific mechanisms to maintain homeostasis that keeps free radical levels in check. However, if this process is out of balance, free radicals become dangerous, highly reactive, and unstable molecules that damage DNA, proteins, and components of cell membranes. Eventually this type of damage will lead to cellular damage throughout the body and play a primary role in the skin aging process. Physiological changes that occur as we age result in the loss of a homeostatic balance between the generation of reactive oxygen species (ROS) that cause oxidative damage and the production of naturally occurring antioxidants such as glutathione (GSH) or other regulatory enzymes (superoxide dismutase, catalase, and peroxidases).

Antioxidant defense mechanisms are species specific and heavily influenced by nutrition, since important antioxidants such as ascorbic acid and α-tocopherol cannot be synthesized by humans and therefore must be obtained from one's diet. Antioxidants are popular dietary supplements in the nutritional and functional food industries.[7] Nutritional ingredients promoted as antioxidants include vitamins (i.e., VC, VE, VB, β-carotene), minerals (i.e., selenium), amino acids (i.e., lysine, cysteine, n-acetyl cysteine, lipoic acid), polyphenols (i.e., anthocyanidines pycnogenol, tannins, curcumin, resveratrol,), flavonoids (i.e., catechins, EGCG, rutin, quercetine, etc.), coumarine derivatives, and many different types of botanical extracts.[8]

Even though antioxidants may reduce free radicals *in vitro* generated by radiotherapy and chemotherapy, there is limited clinical evidence suggesting that a dramatic improvement in systemic oxidative stress *in vivo* can be achieved from the oral administration of common antioxidants such as alpha-lipoic acid[9] and vitamin E even at very high dosages.[10] The failure to deliver the perceived reduction of

systemic oxidative stress from supplements of simple antioxidants may be due to the sub-optimum dosages, poor bioavailability, and lack of organ/tissue specificity from the antioxidants. Another factor that has to be taken into consideration is how to better control the macronutrients such as fat and sugar, which induce oxidative stress.[11] Only considering the food selection based on total antioxidant capacity, without standardization, was another factor cited as a possible reason that reductions of oxidative stress markers could not be achieved in a crossover two-week intervention study.[12] Polyphenols are classes of natural antioxidants that exist in fruits, vegetables, nuts, and different plant parts. These exist as free radical scavengers by neutralizing existing free radicals and/or maintaining a reducing environment around the cells and thereby preventing the formation of undesirable "aging" free radicals.[13] Natural polyphenols have a great structural diversity with antioxidation capacities higher than vitamin C and E.[14] Delivering natural polyphenols in order to meet distinctive nutritional requirements for the managing of oxidative stress phenomena has a unique advantage over the "simple" administration of classical antioxidation vitamins,[15] especially at levels approved by regulatory agencies but which may not be optimal for individuals at each stage of their aging process. One of the specific benefits for oral supplement of polyphenols is protection from UV-induced skin erythema, the increase of skin hydration,[16] and reduction of hyperpigmentation[17] with procyanidins found in apples, pine bark, and grape seeds.

Carotenoids are the most extensively studied and utilized antioxidants in nutricosmetic products. Carotenoids include different types of lipophilic natural compounds such as α, β, λ, and δ-carotenes; lycopene found in fruits such as tomato, watermelon, grapefruit, and red bell pepper;[18] lutein found in green leafy vegetables such as kale and spinach; retinoids (vitamin A, retinal, retinol, and retinoic acid); zeaxanthin from marine microalgae; and Astaxanthin. The concentrations of cutaneous carotenoids have been considered as sensitive biomarkers of oxidative stress of skin. Human skin is relatively enriched with high levels of lipophilic carotenoids such as lycopene and beta-carotene compared to dihydroxycarotenoids, lutein, and zeaxanthin.[19] Carotenoids in skin are strongly associated with the influence of stress factors such as fatigue, illness, smoking, alcohol consumption, and daily dietary intake.[20] Carotenoids have an excellent capacity to quench singlet oxygen ($1O_2$) and inhibit lipid peroxidation. However, they cannot scavenge superoxide anion radicals (O_2^-), hydroxy radicals ($OH^\cdot$), or hydrogen peroxide (H_2O_2).[21,22]

Oral supplementation of carotenoid-enriched diets can quickly increase the measured carotenoid level in skin.[23] Oral supplementation of isotretinoin for photo-aging has also been documented.[24] Many parameters of the epidermal defense against UV-induced damage were significantly improved after seven weeks of oral intake of an antioxidant complex that contained lycopene, α & β-carotene, α-tocopherol, and organic selenium.[25] The significant reductions of UV-induced

peroxides T-BARs and two markers of UV-induced genotoxicity, p53 expression, and photodyskeratotic cell number demonstrated the efficacy resulting from the combination of protection by diminishment of oxidative stress, and reduction of both UV-induced DNA damage and cell damage.[26]

Astaxanthin is a marine carotenoid that is distributed in marine bacteria, algae, crustaceans, and fish. This carotenoid is an excellent antioxidant with a unique mechanism that neutralizes oxidative free radicals in both polar and nonpolar zones of phospholipid aggregates. Out of five different carotenoids, only astaxanthin reduces peroxidation and preserves cell membrane structure. In human dermal fibroblasts under UVA exposure, astaxanthin can reduce the level of reactive oxygen species, increase antioxidant enzyme activities, reduce the expression of heme oxygenase, and reduce apoptosis.[27] Astaxanthin also shows biological functions including protection of human LDL against oxidative damage; reduction of oxidative stress marker MDA, increase in SOD and TAC, protection against oxidative DNA damage, anti-carcinogenesis, anti-inflammatory benefits, promotion of immune response, protection of nerve cells, relief of eye fatigue, anti-diabetic properties, and anti-obesity activities.[28] Recently, in two human clinical trials, orally and in combination with topically administration of astaxanthin for eight weeks at a daily dose of 6 mg significantly improved skin conditions measured by skin elasticity, trans-epidermal water loss, sebum oil level, and crow's feet wrinkle.[29]

Human clinical trials have shown clear evidence of photo-protection through oral administration of antioxidants, especially in combining multiple nutritional ingredients with antioxidation properties from different mechanisms of action. The protection of skin from UV and especially long wavelengths of light by lutein and zeaxanthin was reviewed by Roberts in 2009. Lutein and zeaxanthin are found in human skin as a result of dietary intakes. Multiple human ingestion studies showed reduced skin edema, erythema, and hyperplasia caused by UV exposure.[30] In a randomized, placebo-controlled 12-week clinical trial, 40 healthy women were divided into four groups to evaluate the effects of lutein and zeaxanthin administrated orally and/or topically. The lutein and zeaxanthin combination significantly decreased lipid peroxidation of skin and increased photoprotective activity, with the best performance from the combination of oral and topical treatment. Oral administration of lutein and zeaxanthin showed the same effects in skin hydration though topical application, which yielded a better improvement of skin elasticity than oral administration.[31]

b. Anti-inflammation

Sunlight has a significant effect on the skin, causing premature aging, skin cancer, and a host of other skin changes such as erythema and tanning. Exposure of skin to UV radiation induces biphasic reactions. Thus, upon initial exposure, an

immediate erythema reaction occurs, a weak reaction that fades within 30 minutes. A delayed erythema reaction occurs after 2–5 hours of exposure and peaks at around 10–24 hours. Enhanced prostaglandin (PGE2) and leukotriene (LTB4) is the major mechanism of action for UV-, sun-, and chemical/thermal-caused erythema.[32] Prostaglandins and leukotrienes also play important roles in the physiological and pathological processes of wounds, burns, scalds, acne, microbial infections, dermatitis, and many other diseases and conditions of the skin.[33] Many natural nutritional ingredients contain the active components that can be utilized in dermatology by inhibiting the pro-inflammatory pathways related to prostaglandin and leukotriene production.[34]

It is not surprising that dietary polyunsaturated fatty acids inhibit COX and LOX enzymes due to their structural similarities to arachidonic acid. Polyunsaturated fatty acids also can be metabolized by skin epidermal enzymes to convert into anti-inflammatory and anti-proliferative metabolites.[35] The source of the dietary polyunsaturated fatty acids are plant-based 18-carbon fatty acids with three to nine double bonds such as α-linolenic acid (n-3) from canola and flaxseed oil; linoleic acid (n-6) from soy, corn, and sunflower oils; and oleic acid (n-9) from olive oil. Fish oil contains 20 and 22 carbon n-3 fatty acids—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Dietary polyunsaturated fatty acids not only can decrease PGE–2 and LTB4 production, but also reduce the pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6.[36] Dietary polyunsaturated fatty acids have diverse dermatological functions, including maintenance of the stratum corneum permeability barrier, maturation and differentiation of the stratum corneum, formation and secretion of lamellar bodies, inhibition of pro-inflammatory eicosanoids, elevation of the sunburn threshold, inhibition of pro-inflammatory cytokines (tumor necrosis factor-alpha, interferon-gamma, and interleukin-12), inhibition of lipoxygenase, promotion of wound healing, and promotion of apoptosis in malignant cells, including melanoma.[37]

Human clinical trials showed that dietary omega-3 polyunsaturated fatty acids (omega-3 PUFA) reduce sunburn, which is an acute inflammatory response in humans, by reduction of ultraviolet-B (UV-B) induction of prostaglandin (PGE2) in healthy skin after oral supplement 4 g daily of 95% ethyl esters of eicosapentaenoic acid (EPA) for three months. Abundant clinical evidence also showed that omega-3 polyunsaturated fatty acids, especially EPA, can offer protection against photo-aging, photosensitivity, photo-immune suppression, and photo-carcinogenesis.[38] Omega-6 essential fatty acid from borage oil has been studied in 11 clinical trials for dermatitis. Though the efficacy is relatively moderate, an 8- to 12-week oral supplement of 2–4 g per day borage oil showed benefit in controlling the dermatitis and preventing flare-up in mild to moderate disease.[39] Oral supplement omega-3 and -6 fatty acid with vitamin C, vitamin E, and zinc can improve the symptoms and reduce severity scores of atopic dermatitis.[40]

Polyphenols are a widely distributed group of natural products that have been reported to have anti-inflammatory, antiallergic, antimutagenic, antibacterial, antiviral, antineoplastic, antithrombic and vasodilatory activity. Fruits and vegetables such as berries, green tea, grape seed, and soy are the primary sources of polyphenols. In the United States, the level of polyphenol intake is low due to the small amounts of fruits and vegetables consumed by Americans.[41] The anti-inflammatory effects from polyphenols are well documented at both systemic and cutaneous levels with clearly defined molecular targets, for example, by the inactivating of reactive oxygen and nitrogen species, inhibiting pro-inflammatory enzymes (COX/LOX/iNOS), and modulating inflammatory signaling pathways at gene expression levels through NF-κB and mitogen-activated protein kinase (MAPK).[42] Plant-derived polyphenols interacting with phase II detoxification enzymes plus repairing UV-induced DNA damage are also proposed as the basic mechanism of action for skin photoprotection.[43] Catechin derivatives purified from green tea and black tea, such as epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and theaflavins, showed inhibition of COX- and LOX-dependent metabolism of arachidonic acid in tissues.[44] Poloyphenols from green tea can also increase IL-12. IL-12 plays a role in the removal or repair of UVB-induced DNA damage, resulting in reduced inflammation and tumorigenesis.[45]

Curcumin, originally isolated from an Indian medicinal plant, *Curcuma longa* L. (Zingiberaceae) is the major yellow pigment in turmeric, curry, and mustard. Curcumin is one of the most extensively studied natural phenolic compounds, with anti-inflammatory, antioxidant, and chemopreventive activities that target numerous signaling molecules and pathways.[46] Curcumin is well reported with potent inhibition of NF-κB that regulates the expression of a large number of genes—such as TNF-α, IL-1β, IL-6, cyclooxygenase-2, chemokines, inducible nitric oxide synthase, and matrix metalloproteinases (MMP)—which are involved in UV-induced skin inflammation and skin aging.[47] Curcuminoids and other diarylheptanoids were also characterized as dual inhibitors of arachidonic acid metabolism by reducing PGE_2 production in the cyclooxygenase pathway[48] and down-regulating matrix metalloproteinases (MMPs).[49] Although the clinical trials on orally administrated curcuminoids for skin care are very limited, topical usage of curcumin compositions in skin diseases is well reported. The challenge for utilizing curcumin compositions for skin health is its poor bioavailability. In phase I human clinical trials and pharmacokinetic studies, oral administration of curcumin even at a dosage level above 10 grams per day, very low plasma and tissue levels of curcumin and its metabolites were reported.[50] Recent development of formulation and delivery platforms such as liposomal, phospholipid complex, capsulation, nanoparticulation, and delivery with metabolism adjuvant significantly improved the pharmacokinetic profile of curcumin.[51]

Vitamin D is associated with the regulation of cathelicidin expression, which can induce cutaneous inflammation in rosacea. Vitamin D can also regulate cancer-suppressor protein p53 with a speculation of supplemental vitamin D to prevent skin cancer and melanoma. A double-blind, placebo-control human clinical trial showed significant negative association between plasma alpha-tocopherol and symptom score of atopic dermatitis. Sixty days of oral supplements vitamin D, E, or E&D significantly improved dermatitis condition.[52]

c. Immune protection

UV-induced immune suppression is relatively more complex and less understandable in skin and systematic responses. This compromised immune function involves many signal pathways and different cell types that are directly and indirectly impacted by both UVA and UVB. The suppressive immune function by UV has been elucidated as a part of core mechanisms of photo-damage and photo-carcinogenesis. Sunscreen agents have been utilized in the protection of UV-induced skin damage, with potency measured by the sunburn protection factor (SPF). However, many sunscreens cannot block UV light, especially UVA, effectively.[53] Further, there is no good correlation between sunburn protection factor (SPF) and an immune protection factor (IPF) from sunscreens.[54] Though defining the IPF and measurement of the immune protection effect of cosmetic ingredients remains a challenging task, many international cosmetic companies and research institutes have worked on the development of concepts, measurements, and products that can address UV-suppressed skin immune function.[55] Many natural products, such as polyphenols,[56] epigallocatechin gallate (EGCG) from green tea,[57] and plant-derived oligosaccharides,[58] have been reported with the function of restoring the UV radiation-suppressed immune function. Silymarin is a plant-derived flavonoid isolated from the fruit and seed of the milk thistle. Silymarin is well known for its liver protection function in dietary supplement products. The anti-photo-carcinogenic and chemo-preventive effects observed in animal studies of silymarin are at least partially associated with its anti-inflammatory and antioxidation properties.[59] Silymarin also can decrease the production of IL-10 and reduce infiltration of MHC+ and CD11B+ cells in UV-irradiated skin that lead to preventing UV-induced immune suppression.[60] Oral supplement of immunomodulation composition containing β-glucan, melatonin, and vitamin E also provided reduction of wrinkle, age spots, and photoprotection benefits.[61]

Enzymatically activated and enriched polysaccharide from the *Aloe vera* gel were reported to restore immune function by controlling the important molecules and cytokines at gene expression level and stimulating the growth of healthy skin cells.[62] The material is an enzymatically activated polysaccharide from *Aloe vera* inner leaf gel with a molecular weight between 5 and 400 KDa exhibiting the most

potent immunomodulatory activity.[63] It has been shown to prevent UVB-induced contact hypersensitivity (CHS) suppression and, importantly, has demonstrated inhibition of UVB-induced TNF-α release from human keratinocytes. The mechanism of immune protection effects from aloe-based polysaccharides was reported due to the inhibition of immune-suppressive cytokines such as IL-10 production[64] and UVB-induced suppression of accessory cell function of Langerhans cells.[65] The immunomodulatory activity of aloe polysaccharide is also reported to have induced differentiation of immature dendritic cells.[66] Studies have shown that *Aloe vera* polysaccharides, in concert with other important aloe compounds, stimulate immune response, have anti-inflammatory properties, and promote wound healing.[67]

d. Skin hydration

Regression analysis showed an inverse association between serum vitamin A, sebum content, and skin pH. Dietary intake of fats, especially saturated and monounsaturated fatty acids, were negatively associated with skin hydration. Positive association of skin hydration and pH with beta-cryptoxanthin serum content and dietary intakes of fluids and calcium was observed only in humans.[68]

Aloe products are made from the leaves and/or inner gel of those edible aloe species (*A. vera* or *A. barbadensis*, *A. arborescens*, and *A. saponaria*) that are processed to a degree adequate for consumption, or are processed for easy intake in gel, liquid, paste, powder, granule, tablet, and capsule forms. They are known to be effective for the treatment of topical burn wounds,[69] genital herpes, dermatitis, psoriasis,[70] or internal application for irritable bowel syndrome, immune deficiency, hypercholesteremia, and type 2 diabetes.[71] Oral supplement polysaccharide standardized *Aloe vera* inner leaf gel powder formulation significantly increased water content of the stratum corneum and improved water hydration after two weeks of supplement.[72] A study was conducted by the South Korea FDA on healthy adult men and women subjects (50 years or older) at the intake dose suggested for functional health foods containing aloe products. Dermatological functionality evaluation of the aloe product on human subjects was performed on biomarkers related to skin beauty such as wrinkles, skin color, skin elasticity, and moisturization level of skin surface. The primary ingredient was *Aloe vera* inner leaf gel powder with as low a dosage as 1,200 mg ACTIValoe®/day, and as a high dosage as 3,600 mg ACTIValoe®/day. After 12 weeks of oral administration, facial wrinkles decreased significantly in the two dosage groups. Furthermore, facial elasticity increased significantly and partially in the low-dosage group after taking aloe. On the other hand, there was a marginal decrease in facial erythema in the low-dosage group, and a significant decrease in the high-dosage group.

e. Supporting healthy skin-cell renewal and rebuilding dermal structure

Skin aging is a complicated intrinsic process that involves endogenous hormonal changes, immune and antioxidation balance, external exposure to UV light, and chemical and environmental stress. One mechanism of skin aging is potentially related to the reduction of hormones such as estrogen, which occurs in aging women and leads to reduced collagen, elastin fiber, and glycosaminenoglycans (GAGs).[73] Such hormone reduction also leads to altered extracellular matrix and elasticity, decreased skin moisture and skin thickness, and increased skin wrinkles.[74] Oral supplement natural phytoestrogenic isoflavonoid for human health is well established with much less understanding of potential use in skin aging.[75] Recently, oral supplement of an isoflavone compound S-equol for 12 weeks in postmenopausal women showed significant reductions in wrinkle area and wrinkle depth, and had a beneficial effect on crow's-feet wrinkles.[76] Oral supplement soy isoflavone aglycone in food for 12 weeks significantly improved fine wrinkles and malar skin elasticity compared with control group.[77]

Hydrolyzed collagen from bovine type I collagen or chicken sternal type II collagen, collagen-derived peptides,[78] and a mixture of amino acids can stimulate dermal fibroblasts and promote chondrocytes to produce type II collagen and proteoglycans *in vitro* by acting as cell-growth factors.[79] A pilot open-label study of chicken sternal derived type II collagen plus low molecular weight hyaluronic acid and chondroitin sulfate increased the content of hemoglobin and collagen in dermis and reduced skin dryness, scaling, and global lines/wrinkles after 12 weeks of oral supplementation.[80]

f. Anti-skin aging

Bioactive natural compounds have been widely accepted by cosmetics industry to provide functional ingredients for skin protection, wrinkle reduction, skin hydration, and skin whitening, with perceived anti-aging benefits from consumers.[81] In recent decades, more and more clinical evidences have been published to support topical efficacy from natural ingredients,[82] such as polyphenols from green tea for skin immune restoration, resveratrol from grape seeds for skin protection, Co-Q10 for wrinkle reduction, etc.[83] However, the concept of orally administrated natural compounds for skin aging has not been fully substantiated. Even more challenging is to provide an explanation for the potential mechanism of action of orally administrated natural ingredients with measurable clinical changes on the physical appearance and function of human skin.

A combination of multiple nutritional ingredients in a proprietary composition is the most popular approach to deliver micronutrients for improving skin appearance. Imedeen Prime Renewal is an example, in that it contains soy isoflavonoids, marine protein and polysaccharides, polyphenols from white tea, grape

seed and tomato extract, as well as vitamin C, vitamin E, and zinc. The double-blind, placebo-controlled, randomized human study on postmenopausal females showed significant improvements of facial appearance. Skin aging parameters such as wrinkles, hyperpigmentation, sagging, under-eye dark circles, and dermal density measured by digital images and ultrasound were significantly improved after 6 months of oral supplement of the soy isoflavone, marine protein, antioxidants, vitamin, and mineral composition.[84]

Another example is DermoVite, which is a formulated oral supplement containing marine proteins, alpha-lipoic acid, vitamin C, E, B3, B5, zinc, copper, and four plant extracts from soya, red clover, tomato extract, and pine bark. No pronounced effects were observed until after four months of oral treatment. After six months of usage, the formula significantly improved skin thickness and elasticity.[85] Another composition containing marine-derived protein formulated with α-lipoic acid, isoflavonoid extracts from red clover and soy, lycopene extract from tomato, 95% polyphenol extract from pine bark, vitamin C/E/B3/B5, zinc, and copper significantly increased skin elasticity and thickness, and improved global evaluation of skin aging symptoms. An oral supplement composition containing glycosaminenoglycans, Co-Q10, β-carotene, extracts from grape seeds, pine bark, and green tea, D-α-tocopheryl acetate, selenium, and zinc also reduced the depth of skin roughness, pore size, and fine wrinkles with statistical significance against the control group.[86] An oral supplement, which contained N-acetyl D-glucosamine, glucosamine sulfate, L-proline, L-lysine, manganese, copper, zinc, quercetin, and grape seed extract, significantly reduced the number of visible wrinkles as measured by the silflo replicas, and decreased the number of fine lines after five weeks of intake.[87] A balanced micronutrient intake including lipoic acid, acetyl carnitine, vitamin K, D, B6, folate, and magnesium are speculated to prevent DNA damage, delay the mitochondrial decay, and prevent cancer and other degenerative diseases of aging.[88]

9.1.4 BIOAVAILABILITY AND CLINICAL CONSIDERATIONS

Most nutraceutical ingredients have a well-established human consumption record either from historic usage or from the past two decades of their usage in functional foods and dietary supplement products. The justification of using specific nutritional ingredients in an oral-for- beauty product is well established according to *in vitro* scientific data obtained as to their mechanism of action and usage in other systemic health conditions. However, there are very limited animal models available for evaluation potential skin-related benefits due to the significant difference between the skin type of animals and human. The most commonly reported animal models are related to skin diseases, such as: dramatically depressed immunity, UV damage, inflammation, wound healing, and carcinogenicity. It is a quantum leap

from the understanding of the *in vitro* mechanism of action of nutritional ingredients and *in vivo* animal data for safety and function, to perceived human benefits and also to the industry perspective on the safety of cosmetic products.[89]

To make the situation even more complicated, the bioavailability of nutraceutical ingredients is not well known—especially whether those active compounds could be delivered to the dermis and epidermis—even though nanotechnology is promising.[90, 91] Natural nutrients such as polyphenols generally have poor absorption, rapid metabolism, and rapid systemic elimination after oral supplement.[92] Resveratrol offers a perfect example[93] to illustrate the challenge of delivering clinical benefits even with profound understanding of resveratrol's function in reduction of oxidative stress and systemic inflammation, and regardless of the many pharmacokinetic reports.[94] Substantiation of the efficacious dosages of nutritional ingredients for skin benefits is typically extrapolated from the experience of usage for other human health indications.

Human clinical studies remain the best way to substantiate the functional claims with direct evidence for the composition, dosage, delivery vehicle, and length of time for administration. The protocols for design and selection of the subjective measurement of clinical output in the term of improvement of skin integrity, appearance, and feeling are not that easy. Well accepted clinical evidence has been collected by means of instrumental measurements obtained with several instruments supplied by Courage and Khazaka in Germany, such as Corneometer CM825 for skin hydration, Skin-pH-Meter PH900 for skin pH, Sebumeter SM810 for the sebum content, NOVA dermal phase meter for skin moisturization, ultrasound to measure skin density, and triplicate pinch and recoil measurement for skin elasticity. Photo evaluation provides less objective evidence due to the difficulties of controlling the subtle changes of external conditions in taking digital images and the potential bias from evaluators. It is essential to use a placebo or positive comparator in the clinical study design with a double blind, using randomized subjects with clear, inclusive, and exclusive criteria employed to normalize subjects in treatment and control groups and to minimize potential bias.

CONCLUSION

Continuing the momentum to accumulate a growing body of clinical evidence showing a positive association between nutricosmetics and skin health is the ultimate pathway to convince skeptical consumers and scientists. The future growth of the nutricosmetics field will be fueled by science-based innovation of nutraceutical ingredients, clinical substantiation of the marketing claims, and real beauty benefits experienced by consumers.

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MULTI-FUNCTIONAL BOTANICALS FOR NUTRICOSMETICS APPLICATIONS

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ABSTRACT:

The statement "Beauty from within" perhaps best describes the field or purpose of Nutricosmetics. Though considered a niche market in the West, the realization that skin health is affected by inner health is not new. The skin is the largest organ of the body and it not only provides cover and protection to inner organs, it also acts as a secondary organ of elimination. Whatever we eat or enters through the gastrointestinal tract is in some way expressed on the skin, hence the phrase "We are what we eat" makes sense. Even ordinary skin problems such as acne, rashes, dryness, or oily skin can be traced back to the food we eat. Today Nutricosmetics is viewed as a hybrid field of Cosmeceutical and Nutraceuticals. In this chapter we will be discussing the nutricosmetic potential of selected botanicals, their activities, and the formulation of concepts using these botanicals.

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9.2.1 INTRODUCTION

Though the concept of nutricosmetics is quite old, the idea of capitalizing on the concept of beauty foods is relatively new. One of the first nutricosmetic products introduced in the market was Imedeen, by Swedish biochemist Ake Dahlgren in the 1980s. The product contained certain marine extracts for restoring the structure and function of skin (F. Heule et al. 1992). Since then the nutricosmetic market has come a long way, with new innovative products getting launched into the global market every year.

9.2.2 GLOBAL MARKET

Emphasis on skin care and healthy aging has been a prime driving force for the sales of nutricosmetics. Greater appreciation of role of nutrition in the skin's health is one of the causes of a booming market in Japan. The beauty market of the cosmetics and nutricosmetic industry has been showing a steady growth, with beauty food accounting for almost 19% of dietary supplement sales there. Total sales for 2010 were estimated to be 1.3 billion U.S. dollars.

Japan dominates the nutricosmetic market, with sales of over a billion dollars in 2010. Being an aging society, Japanese consumers are more concerned about skin beauty and aging.

Not far behind is China, with sales of 813 million U.S. dollars as reported by Eurometer (*Nutraceutical World* 2011).

The driving force for the growth of nutricosmetics is customized solutions provided to consumers, as in cosmetics. There has been a growing trend of segmenting the products based on requirement for skin health. Hence one can find products that are age, skin-condition, and even gender specific.

9.2.3 AYURVEDA AND NUTRICOSMETICS

The concept of beauty in Ayurveda, or "science of life," is not limited to external beauty but also to inner beauty. The use of herbs both externally and internally has been advised in Ayurveda for good skin health. Ayurveda also believes in customized skin care, based on the fact that there is more than one kind of skin, depending on the constitution of the person. "One-size-fits-all" solutions don't exist.

According to Ayurveda, skin health and youthfulness are dependent on a number of factors such as balance in the three *doshas*—Kapha, Vata, and Pitta. Doshas are primal metabolic forces as conceptualized for understanding the action of five basic elements (earth, water, fire, air, and ether) and for therapeutic application in diagnosis and treatment. The term *doshas* itself means "fault," and represents the ways that a particular energy is out of balance. Skin can also be classified on the basis of doshas into three types—Vata, Pitta, and Kapha skin (K.P.S. Khalsa and M. Tierra 2008).

Thus for a youthful skin there should be a harmony in these three doshas—proper moisture balance (Kapha balance), proper functioning of metabolic mechanisms that coordinate all the chemical and hormonal reactions of the skin (Pitta balance), and efficient circulation of blood and nutrients to the different layers of skin (Vata balance).

9.2.4 MULTIFUNCTIONAL NUTRICOSMETICS

In the following section we will be discussing a few of the potential nutricosmetic ingredients obtained from nature or nature-derived resources. Apart from their nutricosmetic benefits, they also affect the health when administered orally as food or dietary supplements. For example, Indian gooseberries are well known for their digestive, detoxifying, and immune-boosting health benefits.

a. Amla

Amla or *Emblica officinalis* is one of the most revered fruits in Ayurveda. It is regarded as one of the best rejuvenating herbs in the Ayurvedic tradition and has been used as an adaptogen in several Ayurvedic formulations. Amla can also be used as a nutricosmetic for its antioxidant and anti-aging potential.

Emblica officinalis has been used for a variety of health conditions in Indian folk medicine such as metabolic disorder, liver diseases, stomach ulcers, and also skin disorders. *Emblica officinalis* has also been studied for its collagen-promoting action as well as its inhibitory action on matrix metalloproteinase enzyme in the human skin.

Amla has shown to increase pro-collagen type I C-peptide and tissue inhibitor of metalloproteinase-1 (TIMP-1) production and to decrease MMP-1 production, concomitant with elevated mitochondrial activity in the fibroblast in a concentration-dependent manner (Fuji et al. 2008). This activity has potential in both cosmeceutical and nutraceutical applications.

In a 2010 study, its efficacy to inhibit UVB-induced photo-aging was studied in human skin fibroblast cells. *Emblica officinalis* not only stimulated fibroblast proliferation, but also protected the pro-collagen-1 against UVB, and inhibited inflammatory enzymes such as hyaluronidase. The study results showed that *Emblica officinalis* can effectively inhibit photo-aging in human skin fibroblasts (M.D. Adil et al. 2010).

Anticarcinogenetic activity of *Emblica officinalis* on DMBA (7,12 – dimethyabenz(a) anthracene)-induced skin cancer was studied in Swiss albino rats. Results showed that antioxidant activity of *Embica officinalis* can help to restore the imbalance between oxidative stress and antioxidant defense and limit oxidative tissue damage (G. Sancheti et al. 2005).

Saberry®, a biostandardized extract of Amla, has been developed with high ORAC activity, which is also a measure of antioxidant activity. The results from the ORAC assay on Saberry® are given in Table 1.

Table 1: ORAC activity assay on Saberry®

ORAC Hydro (µmol TE/g)	ORAC Lipo (µmol TE/g)	ORAC Total (µmol TE/g)	HORAC (µmol CAE/g)	NORAC (µmol TE/g)	SORAC (Kunits SODeq/g)	SOAC (µmol VItE/g)
2678	4	2682	345	904	102	1351

Saberry®, a light-colored extract standardized for beta glucogallin, is obtained from a proprietary extraction process enabling the preservation of natural actives in the amla.

Saberry® is a completely water-soluble and light-beige-colored extract, making it a suitable ingredient for **Beauty Beverages**. When dissolved in water it imparts sweetness to the beverage. Saberry® is also a good constituent to be added in collagen beauty supplements because of its MMP-1 inhibitor activity, which assists the pro-collagen formation.

Saberry®, with self-affirmed GRAS status, can also be added into the beauty food or beverages. It is stable in beverages as well as solid-dosage formulations. Saberry® is completely soluble in water and gives a sweet aftertaste to beverages. The recommended dosage range is between 100 and 500 mg daily.

b. Green Tea

Fig 1.: Epigallo catechin gallate

Green tea, consumed as a beverage around the world, has many health benefits associated with it. Green tea is produced by steaming fresh leaves at high temperature, thereby inactivating the oxidizing enzymes and leaving the polyphenolic content intact (F. Liudong et al. 2011). Polyphenols such as epigallo catechin gallate (Fig. 1) present in green tea are regarded as active constituents and are used for standardization of green tea extracts. Green tea is also known to possess anti-inflammatory, antioxidant, and anticarcinogenic qualities (S.K. Katiyar 2003). Green tea has potential application as an anti-aging as well as a photo-protective ingredient in nutricosmetics.

It was observed that oral consumption of green tea polyphenols inhibits chemical carcinogens or UV radiation-induced skin carcinogenesis in animal models (S.K. Mantena et al. 2005); green tea is thus advocated for its UV protective action. Based on the results of the study, it was suggested that daily oral consumption of green tea may provide protection against the harmful effects of UV radiation on skin.

In a 2005 study on 40 women subjects with moderate photo-aging, both topical and oral supplementation with green tea were studied for their effect on photo-aging skin. In the study, subjects were randomized to either a combination of 10% green tea cream and a 300-mg green tea supplement twice a day. The results showed that subjects who were treated with a green tea regimen of both topical and oral supplementation had improved elastic tissue content in the skin. The results suggest that green tea polyphenols (catechins) could help to mitigate UV radiation damage in skin (Chiu et al. 2005).

In a very recent study done on humans, it was found that metabolites of green tea catechins are actually incorporated into human skin upon consumption of green tea extract, and can protect the skin against UV radiation-induced cutaneous inflammation. A study performed at the photobiology unit of Manchester University, UK, found that green tea catechins were able to reduce the UV radiation-induced 12-HETE (hydroxyeicosatetraenoic acid). However, the PGE2 levels were unaltered.

This study was carried out on 16 healthy human subjects who were given 540 mg of green tea catechins and vitamin C (50 mg) supplement daily for 12 weeks. The buttock skin was exposed to UV radiation both pre- and post-supplementation of green tea, and resultant erythema and inflammatory markers were quantified.

The study results show that oral consumption of green tea catechins may contribute to protection against sunburn, erythema, and perhaps even long-term UV radiation-induced damage to skin (L.E. Rhodes et al. 2013).

The above studies show us that green tea catechin has a potential role as a nutricosmetic for protecting skin against harmful UV damage. Green tea supplements along with green tea-based topical products can provide essential antioxidant benefits and protect skin from UV damage. Green tea extracts are available in several strengths and are standardized to polyphenols or specific catechins. The HPLC analysis is more dependable, as it provides information on the specific catechin content and is not interfered with by the presence of other green tea components. Green tea extracts are also available as decaffeinated products to avoid the side effects of excess caffeine intake. As water-soluble extracts, they can be formulated in a variety of ways and supplements such as RTD tea, or in dry formulations such as tablets, caplets, or stick packs.

c. Ellagic acid (Pomegranate)

Pomegranate is considered a superfruit in India. It is also cultivated in Iran, the Mediterranean belt, the Middle East, tropical Africa, North Africa, and the Indian subcontinent. It is also extensively grown in China and Southeast Asian countries. Though not native to Japan, pomegranate is used for bonsai because of its unusual twisted stem. It is mentioned in several ancient texts such as Ayurveda, the Book of Exodus, and the Quran. In the Ayurvedic system of medicine, pomegranate fruit has been the source of traditional remedies since ancient times against diarrhea, for toning skin, treating hemorrhoids, and as a tonic for yjr heart (Ch. Murli Manohar 2002). Even the flowers of pomegranate are used as antidiabetic remedies in Ayurveda (J. Wang et al. 2012).

Pomegranate is often standardized for ellagic acid, which is a polyphenolic compound present in fruits.

Fig. 2: Ellagic acid structure

Ellagic acid is commonly found in red raspberries, walnuts, strawberries, and pomegranate. Ellagic acid is the principal polyphenolic compound in fruits of pomegranate (Bell and Hawthorne 2008). Ellagic acid is a polyphenolic acid that is formed as lactonization of hexahydroxydiphenic acid of ellagi tannins (H. Akiyama et al. 2001).

Ellagic acid is well known for its antioxidant activity (Bell and Hawthorne 2008), antibacterial, and antiviral properties (H. Akiyama et al. 2001). It has shown anti-proliferative or anti-cancer activity in preclinical models (Y. Hagiwara et al. 2010). A small clinical test has shown cholesterol-reducing activity in patients with metabolic syndrome (Basu et al. 2009).

Ellagic acid has also shown potential for providing benefits to the skin, when taken orally.

Based on previous studies that demonstrated the topical benefits of ellagic acid on chemically induced skin tumorogenesis (P. Lesca 1983; R. Chang et al. 1985), H. Mukhtar et al. (1986) designed a study to investigate whether the parental administration of ellagic acid in drinking water can provide protection against 3-methylcholanthrene-induced skin tumor in BALB/c mice. Oral administration of ellagic acid showed protective effect against tumor induction, perhaps due to inhibition of metabolic activation of polycyclic aromatic hydrocarbons (PAH) by ellagic acid. Results suggested that dietary supplementation of ellagic acid was able to reduce the risk of skin carcinogenesis in the test animals.

Ellagic acid was found to have a photoprotective effect on collagen breakdown and inflammation in the skin induced by UVB irradiation. In this study, the human dermal fibroblast cells were used to study collagen degradation in skin cells. Ellagic acid was able to block the matrix metalloproteinase production in the UVB-exposed cells (J.Y. Bae et al. 2010).

In 2006, in a human clinical trial performed in Japan with ellagic acid (supplied by Sabinsa Corporation), researchers found that ellagic acid extract can have an inhibitory effect on UV-induced pigmentation on skin.

In this four-week study, 13 volunteers were randomized to three groups—control, low dosage of ellagic acid (100 mg/day), and high dosage of ellagic acid (200 mg/day). Each volunteer was exposed to 1.5 MED (minimum erythema dose) of UV irradiation.

The whitening or protective effect of ellagic acid against UV-induced pigmentation was studied using a spectro-colorimeter for luminance in skin and a mexameter for measuring melanin and erythema in the skin.

Results showed that oral consumption of ellagic acid-rich pomegranate extract has an inhibitory effect on slight pigmentation in human skin caused by UV irradiation.

Ellagic acid is well known for skin-lightening activity when applied topically. It is used as a quasi-drug in cosmetics in Japan for treating hyperpigmentation on the skin. 0.5% ellagic acid-containing cream was shown to be effective against the UVB-induced hyperpigmentation (R. Kamide et al. 1995).

d. Curcumin C3 Reduct® (Turmeric)

Turmeric has been traditionally used since ancient times for its health benefits. The powdered rhizomes of turmeric are commonly used in preparation of curries. It has also been used in Ayurveda and other Asian medicine for generations for management of disorders such as inflammation, skin wounds, hepatic and biliary disorders, and certain tumors (Lazaro 2008). Turmeric's role as anti-aging agent and antioxidant is worth exploring for its nutricosmetic benefits. The benefits of turmeric come from its active molecules—curcuminoids. The Curcuminoids are natural phenols in structure (Fig. 3) and contain two α, β unsaturated ketonic structures. Curcuminoids are present as a mixture of three related structures. Curcumin is the main curcuminoid compound and the other two derivatives are demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). Following are the structures of curcuminoids.

Fig. 3: Structure of Curcuminoids

Curcuminoids and curcumin in particular are very strong antioxidants. Osawa et al. (1994 and 2004) isolated a hydrogenated derivative of curcumin—tetrahydrocurcumin. The studies have also revealed that the tetrahydrocurcumin derivative of curcumin possessed the strongest antioxidant activity among all the curcuminoids (Fig. 4). The tetrahydrocurcuminoids are also known as white curcuminoids as they do not have a characteristic yellow color and lack the dyeing nature of curcuminoids.

Fig. 4: Antioxidant activity of curcuminoids

Curcumin and its derivatives, including tetrahydrocurcuminoids, can be supplemented to prevent aging, as they may work in the form of dietary intervention with externally applied cosmeceuticals to slow the skin's aging process. As natural antioxidants, they quench free radicals in the system and may help to potentially inhibit visible signs of aging and photo-aging.

Studies on curcumin have shown its protective effect against skin melanoma. The study done on mice with skin tumors induced by 7,12 dimethylben[a]anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol-13-acetate showed that dietary curcumin significantly reduced the number and volume of tumors in the mice (Limtrakul et al. 1997).

In a recent animal study, the protective effects of oral and topical forms of curcumin were compared against skin squamous cell carcinoma. The skin squamous carcinoma is the most common type of cancer in the USA, which has also

been associated with the use of tanning booths causing photo-damaged skin. In this study, Sabinsa's Curcumin C_3 Complex® was used to prepare both topical and oral formulation. Curcumin at 15 mg dosage was able to suppress tumor growth in the mouse skin cancer model, emphasizing the use of oral or topical curcumin in preventive care for skin-related conditions due to increased skin exposure. This protective effect of curcumin was related to its anti-inflammatory activity (K. Sonavane et al. 2012).

In a similar study performed on SKH-1 mice, curcumin showed inhibition of skin cancer formation when administered orally or topically. SKH-1 mice were pretreated with curcumin either in topical or oral form for 14 days. The UVB radiation was then given to them to induce skin cancer. It was noted that the time for tumor onset was longer in mice taking oral or topical curcumin as compared to control group animals. Fewer tumors were formed in the topical and oral curcumin group as compared to their controls. The study result thus showed that curcumin not only inhibits skin cancer formation but also prolongs the time for tumor onset when administered topical or orally (J. Phillips et al. 2013).

The Sabinsa Corporation provides curcuminoids as Curcumin C_3 Complex®, and tetrahydrocurcuminoids are available as Curcumin C_3 Reduct®.

e. Cococin™ Coconut Water

Coconut water or the liquid endosperm of green coconuts (*Cocos nucifera*) is a refreshing and natural rehydration medium for the body. Coconut water offers higher amounts of electrolytes such as potassium and magnesium, and can be considered as a natural isotonic drink.

Coconut water in tender green coconuts is rich in proteins, amino acids, sugars, vitamins, minerals, and growth factors that play a pivotal role in supporting tissue growth (M. Majeed et al. 2009).

Coconut water can help to regain the water lost from the body and also rehydrate the skin. This rehydrating property of coconut water can be explored for its nutricosmetic function. The intrinsic rehydration with coconut water can also help to augment the function of topical moisturizers. Coconut water is an excellent rehydration solution, as it provides electrolytes such as potassium and magnesium in an isotonic form.

Sabinsa provides Cococin®, a stable preparation of coconut water obtained from a proprietary lyophilization process that conserves the inherent biological activity of coconut water. The lyophilization process ensures that protein and other environment-sensitive actives are protected.

The coconut water solids in Cococin® can support cell growth, and hence can be used for supporting growth of tissues such as hair follicles and fibroblast cells; it also supports healthy aging (M. Majeed et al. 2009).

In 2008, Cococin® received a self-affirmed GRAS status as determined by an independent panel of scientists and toxicologists. The GRAS status allows the use of Cococin® in foods and beverages. Cococin® is available in both oral grade as well as beverage grade, and hence can be used in preparing yogurts, tea, and other beverages with beauty functions.

CONCLUSION:

Nutricosmetics is still a new concept in the market, which holds a lot of potential for growth. Newer ingredients are coming into the market, which is a welcome sign for the industry. This is a segment that needs self-regulation in terms of claims one can make on the product. It is important that new ingredients coming into the market should be able to substantiate their claims with proper studies.

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NANOCOSMETICS

NANOCOSMETICS

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ABSTRACT

In recent years, the need for novel cosmetic and personal care products has escalated significantly. In the search for new avenues of technology to produce ingredients that can address consumer needs, the field of nanomaterials has become the focus of new directions. Not only has this field produced a host of new phenomena but, along with them, concerns about maintaining desired, and required, standards of safety around the world. This chapter provides a thorough description of these issues for the relatively new field known as Nanocosmetics. Readers looking for innovative ideas, features, and benefits will find this field enormously thought-provoking and a fertile ground for new directions.

Nanocosmetics are products that capitalize on nanotechnology as their principal advantage for maintaining or enhancing the appearance of the skin, hair, and nails. A broader definition appropriately includes products that contain any nanomaterials on their ingredient list, rather than the active or key ingredient.

In the $200 billion global cosmetic industry, the current global market for nanotechnology is estimated to be $62 million, and, barring curtailment through regulation, is expected to grow annually at a 16% rate and attain well over $150 million by 2015. Sunscreens are expected to account for the bulk of the initial growth. However, other cosmetics and personal care products are likely to follow, if, as expected, they prove superior to current competitors. The growth of anti-aging products within the cosmetics arena is also a significant growth area for nanocosmetics and is expected to grow significantly in view of the aging population and in spite of the current recessionary period. To be competitive, the cost of nanocosmetics is not expected to be a barrier to their growth in view of their competitive pricing when compared to procedures. In this chapter, the breadth of nanomaterials that have been used for enhancing cosmetics will be reviewed and specific uses highlighted.

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NANOTECHNOLOGY AND ANTI-AGING

10.1.1 INTRODUCTION

In its broadest sense, Nanotechnology is the purposeful design and manipulation of nanoscale materials for applications in a variety of disciplines.[1] Nanotechnology-based materials, by definition, have one dimension that is equal to or less than 100 nanometers, and, at this scale, demonstrate novel intrinsic properties not found in bulk samples of the same material.[2-4] To date, such materials are utilized in several disciplines, including medicine, scientific research, engineering, environmental technology, and manufacturing. As the field is young but vigorous, a continuing exponential expansion is expected [2].

Nanoparticles are increasingly used in sunscreens, cosmetics, electronics, sporting goods, tires, paints, and foods.[5] In the medical field they are being utilized for diagnostic modalities, imaging studies, and drug delivery.[3,5] The ultra-small size of nanoparticles confers specific physical, chemical, mechanical, and electrical properties that are advantageous for tailoring specific products to meet consumer needs.[2,6] In personal care products and cosmetics, the formulation vehicle significantly determines the degree to which the applied product penetrates the skin. The depth of penetration is a property that is of great importance both technically and from a regulatory point of view, as nanoparticles are increasingly used in the cosmetic industry and have impacted sun protection, hair care, makeup, emollients, and anti-aging treatments.

10.2.2 NANOMATERIALS FOR SKIN DELIVERY: NANOEMULSIONS, LIPOSOMES, AND NANOPARTICLES

A variety of different nanomaterials exist, and each material has different properties that confer special features and benefits for their utility in cosmetics. Nanoemulsions, liposomes, and nanoparticles are detailed below. Figure 1 As the field of Delivery Systems has expanded in order to provide selected active ingredients to target layers within the skin and then deliver their payload when it will do the most good *as well as delivering* at a rate that typically slows down the transfer, nano-based materials have emerged as a viable modality.

Figure 1: Nanomaterials. Adapted from Zhang *et al.*[108]

Lipid-based nanoparticle delivery systems, including liposomes, nanoemulsions, and solid lipid nanoparticles, are becoming more widespread in study and use because they are made of well-defined and biocompatible molecules, are simple to characterize, and can be synthesized for different methods of delivery. These modalities are particularly useful for the delivery of oily, hydrophobic actives, making them advantageous for use in cosmetics and pharmaceuticals.[7] Oily compounds are often included in topical formulations for the purpose of protecting the skin and improving its inherent barrier function. The delivery vehicle must be a well-tolerated system, and given the biocompatible and nontoxic nature of lipids, they can be easily utilized in nano-approaches.[7]

a. Nanoemulsions

Nanoemulsions are similar to traditional emulsions in that both are composed of water and oil droplets that have the tendency to break into their constituent ingredients upon topical application to the hair or skin.[3] Of course, high-shear forces typical of those imparted between hands and skin, with almost no gap between them, are significant to their impact on the stability of the interfacial barriers between the hydrophilic and hydrophobic components. Nanoemulsions are typically comprised of a combination of traditional cosmetic ingredients including water, oils,

and surfactants in a two-phase or multiple-phase emulsion system. These systems contain small droplets with diameters of 50 to 100 nm dispersed in an external phase.[3] The presence of ultra-small droplets confer special properties to nanoemulsions, including transparency and a pleasant texture and sensory experience when applied topically.[5] Nanoemulsions are beneficial in cosmetic applications due to their rapid penetration past existing barriers and for having suitable textures as well as biophysical properties such as hydration capability.[8] A wide variety of cosmetic products have been synthesized utilizing nanoemulsions: lotions, milks, and clear gels, all with slightly varying, and controllable, physical behavior.[8]

b. Liposomes

Liposomes and niosomes are globular vesicles whose diameters range from 25 to 5,000 nm. These nanomaterials are composed of amphiphilic molecules that associate into a double layer or multiple double layers, forming unilamellar and multilamellar vesicles, respectively.[3] While liposomes are primarily composed of phospholipids, niosomes are mostly generated from nonionic surfactants, such as alkyl ethers or esters.[9] Both can incorporate hydrophilic or lipophilic substances within them. The structure of liposomes is analogous to that of mammalian milk, which contains milk fat globular membrane surrounding nano-sized fat droplets.[10,11] When applied topically, liposomes and niosomes tend to break into their individual components, similar to nanoemulsions.[3,5] These vesicles play an important role in cosmetic applications because they improve the stability and tolerance of the encapsulated active ingredients. They are also advantageous in that they are well tolerated and can be easily produced on a large scale.[12,13]

c. Nanoparticles

Nanoparticles are defined as single particles with at least one dimension less than 100 nm, although their agglomerates may be larger in size.[3] There are several subtypes of nanoparticles, which are described below.

Polymeric Nanoparticles

Depending on their composition and molecular structure, polymeric nanoparticles can be classified as nanocapsules or nanospheres. Nanocapsules are vesicular structures that function as carriers for lipophilic materials that can then effectively penetrate the skin via slow absorption into the *stratum corneum*.[14] In contrast, nanospheres are matricial structures, in which the active ingredient is encapsulated during the synthetic polymerization process. These nanoparticle systems can enhance active component penetration of the skin barrier, which would otherwise demonstrate inadequate penetration in their unencapsulated form.[15] Nanoencapsulation can also increase the bioavailability[16] and the stability of the topically

applied compounds.[17,18] Moreover, nanoencapsulation can protect the substances from degradation in storage, as well as photodegradation.[14]

Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLN) were developed in the 1990s as the first generation of lipid nanoparticles [19] and served as an alternative carrier system to other nanomaterials such as emulsions, liposomes, and polymeric nanoparticles already being utilized.[12] SLN are synthesized by replacing the liquid oil in an oil-in-water emulsion with a lipid that is solid at both room and body temperature.[12] SLN have several advantages as carriers for active ingredients as compared to emulsions and liposomes. Firstly, encapsulating the active ingredient within the matrix of the SLN shields the ingredient from degradation in solution. In addition to this protective aspect, the solid matrix of the SLN can be modified to control the rate of release of the active compound.[20] Active compounds can be incorporated homogenously throughout the SLN matrix, concentrated into a compound-enriched outer shell, or integrated into the core of the SLN.[20] Formulation parameters and compound concentrations can be varied to yield different release profiles from SLN.[20] Cosmetic ingredients and active pharmaceutical ingredients (APIs) can subsequently be incorporated into these carriers, providing the potential for a wide variety of topical applications.[19]

SLN exhibit properties that are advantageous for dermal application: they are colloidal carriers that permit controlled release of many active substances, as well as enabling enhanced tissue distribution and targeting.[21] In addition, they have low toxicity profiles and are well tolerated. Because SLN possess occlusive properties, they also increase skin hydration when applied cutaneously. These lipid nanoparticle carriers also increase the stability of active compounds that are sensitive to light, oxidation, or hydrolysis. Additionally, SLN stability in body fluids can be amplified by modifying their surface with hydrophilic molecules of varying length such as polyethylene glycol (PEG).[21] This hydrophilic coating decreases phagocytic uptake of the nanoparticles while minimizing unintended interactions with other proteins.

After SLN synthesis, component lipids partially crystallize in high-energy modifications. During storage, however, these modifications can transform to the low-energy, more highly ordered crystal lattice form.[12,22] The high degree of order in these crystals decreases the number of imperfections in the crystal lattice and results in an increased expulsion of the active ingredient. The potential for SLN formation of a perfect crystal lattice has been compared to the structure of a brick wall comprised of unbroken bricks of one size. Such a structure has no imperfections and little space to accommodate added ingredients. We contrast this tight, uniform structure to the image of a wall made of stones of different type, size, and shape. Such a wall, or crystal, is less ordered and has more space within itself

to accommodate active ingredients. In other words, a less-ordered crystalline lattice contains more imperfections and therefore a higher drug or active ingredient loading capacity.[12] Thus, despite the advantages of SLN, their tendency to form a highly structured lattice precludes higher maximal incorporation of the active ingredient.

Nanostructured Lipid Carriers

In light of the limitations of SLN, a second generation of nanoparticle carriers, nanostructured lipid carriers (NLC), was developed in an effort to better encapsulate and increase the load capability of active ingredients as well as to prevent release during storage.[21] These carriers differ from SLN because rather than using solely a solid lipid component, they contain blends of both solid and liquid lipids at varying ratios.[22] By using a combination of solid and liquid lipid components in NLC, the resultant lipid matrix is less highly ordered, has more imperfections, and thus enables greater loading of active nanoparticles.

Three types of NLC have been developed: imperfect type, amorphous type, and multiple type.[13] Imperfect type NLC have an imperfectly ordered solid matrix achieved by blending structurally different liquid and solid lipids. This synthetic method leaves large distances within the lipid matrix that can accommodate encapsulation of active ingredients in random clusters.[21] Amorphous type NLC are synthesized by combining solid lipids with special lipids (e.g., hydroxyoctacosanylhydroxystearate, isopropyl myristate, or medium-chain triglycerides). This combination yields a noncrystalline lipid matrix.[23] Multiple type NLC are synthesized with nano-solids and oil components in a water carrier. These NLC can be utilized when the solubility of an active ingredient is not adequate for achieving the desired concentration.[21] Adding a higher fraction of liquid lipid will modify the solid matrix to allow for greater solubility of active ingredient in the lipid regions.[24] These liquid regions, known as oily nanocompartments, prevent expulsion of the active ingredient, while maintaining the solid matrix structure of the NLC.[24]

Both SLN and NLC are appropriate for use on inflamed or damaged skin because they are composed of non-irritant and nontoxic lipids.[25,26] SLN and NLC have been investigated using a variety of encapsulated compounds, including Vitamin E,[20] tocopherol acetate,[27] retinol,[15] and ascorbyl palmitate.[28] Yet, evidence suggests that NLC are advantageous over SLN in that they have a higher loading capacity for active ingredients and lower expulsion rate of the active ingredient during storage,[29] providing increased utility for topical administration. NLC are also superior to polymeric nanoparticles because of their low toxicity, biodegradability, controlled release, and avoidance of organic solvent use during production.[19] SLN and NLC can achieve successful encapsulation of active compounds within their structure and offer an economical and simple administration route for patients.

10.1.3 NANOTECHNOLOGY AND PHOTOPROTECTION

Excessive skin exposure to ultraviolet (UV) radiation causes a variety of adverse effects including sunburn, dry skin, photo-aging, wrinkles, photo-immunosuppression, and photo-carcinogenesis.[6,30] Protective clothing such as long-sleeved clothing and hats to shield the face are one way to decrease sun exposure. However, sunscreen remains the most widely used form of photoprotection.[6] The degree of photoprotection offered by a particular sunscreen depends on its sun protection factor (SPF), which corresponds to the duration of time for which the sunscreen will prevent reddening of the skin.[30] Despite their benefits, molecular sunscreens can penetrate the skin and can cause photo-allergies, skin irritation, and phototoxic reactions.[31]

Titanium dioxide (TiO_2) and zinc oxide (ZnO) are two of the most common inorganic UV filters utilized in sunscreens.[2,32] These inorganic sunscreens are effective filters of UVA and UVB light[2] and have long been considered safe and effective methods of photoprotection because of their photostability and low photo-allergic potential.[32,33] Yet historically, their use was limited because of poor aesthetics: the large size of the TiO_2 and ZnO particles caused a white film to remain on the skin after application.[2,6] Additionally, these inorganic particles did not disperse well in their carriers and left a grainy feeling on the skin when applied.[6] This resulted in decreased consumer desire to utilize these products.

Sunscreen safety can be improved by means of employing carriers that possess sunblocking characteristics themselves, such as SLN.[31] These decrease the concentration of molecular UV blockers required in the sunscreen formulation, provided that these carriers have a beneficial safety profile. SLN do in fact demonstrate sunblocking activity by scattering UV light. This intrinsic sunblocking activity is thought to correlate with the degree of crystallinity of the lipid matrix. Therefore, sunscreens can be formulated with a decreased quantity of molecular sunblocking agents combined with these crystalline lipid matrices.[31] An *in vitro* study evaluated the efficacy of a highly crystallized nanoparticle carrier for benzophenone-3, a liquid lipophilic molecular sunscreen. When compared to a standard sunscreen emulsion, the crystalline lipid nanoparticles more strongly scattered incoming UV rays; when benzophenone-3 was incorporated into these nanoparticles, there was a synergistic effect on photoprotection.[31] More importantly, when incorporated into nanoparticles, the concentration of benzophenone-3 could be reduced by 50% and still maintain the same degree of photoprotection as the reference formulation. This study demonstrated that highly crystallized lipid nanoparticles are effective carriers for molecular sunblocking agents (as opposed to particulate agents) and that they are capable of conferring increased photoprotection.[31]

TiO_2 and ZnO insoluble nanoparticles were incorporated into sunscreens in an effort to reduce the disadvantages traditionally associated with these inorganic

compounds and thus increase effectiveness and consumer use. Nano-sized TiO_2 and ZnO enabled sunscreens to be transparent upon skin application, resulting in increased consumer acceptance and use.[5] Nano-sized TiO_2 and ZnO were first patented in the 1980s; the United States Food and Drug Administration (FDA) approved nanoparticle use in sunscreens in 1999.[2] Today, one of the largest applications of nanoparticles is in sunscreen products.[3,5] The proposed benefits include not only improved aesthetics, but also superior UV protection[6,30] when compared to micron-sized particles.[34] Their enhanced UV blockage properties are thought to be due to their ability to scatter light efficiently.[30]

TiO_2 and ZnO nanoparticles exist in three states: the primary nanoparticles, aggregates of these nanoparticles, and agglomerates. The first stage in manufacturing requires the production of the TiO_2 and ZnO primary nanoparticles, which range in size from 5 to 20 nm. These primary particles cluster to form aggregates ranging from 30 to 150 nm in size. The driving force for the clustering is similar to that seen in macroscopic aggregation of larger particulates. The aggregates are the smallest units that are present in sunscreens. However, as a result of the drying and heating process during sunscreen formulation, aggregates clump into loosely bound agglomerates with a size greater than 1 micron.

The degree of UV absorption by inorganic nanoparticles such as TiO_2 and ZnO depends on the size of the aggregates[3,6]; SPF will vary significantly if different sunscreen formulations are made with varying size of inorganic nanoparticles, even if the concentration of those particles is the same.[6] Large agglomerates of TiO_2 and ZnO nanoparticles are not effective UV attenuators and therefore must be broken back down into nanoparticle aggregates and maintained in this form in the final sunscreen formulation. To do this, inert coating materials such as aluminum oxide or organosilicone oils (polydimethyl siloxane) and other variations of silicone polymers and copolymers are added to the sunscreen to maintain small aggregate size, prevent agglomerate reformation, and improve nanoparticle dispersion.[3,5,6] This process ensures that the targeted SPF will be maintained in the final sunscreen. In contrast, organic UV filters absorb UV light as a function of their concentration; therefore, different concentrations of such filters will confer different SPFs.[6] Regulations around the world vary as to the acceptability of organic versus inorganic UV filters.

Despite the cosmetic benefits of nano-sized TiO_2 and ZnO in sunscreens, which include dispersal in a less viscous vehicle, increased transparency, and skin blending,[2] safety concerns have emerged. While particle size is fundamental to their utilization, size is also the cause for concern regarding their potential toxicity,[4] including the potential for reactive oxygen species (ROS) generation upon UV exposure,[2,6] percutaneous penetration of the particles,[6] their ability to evade the host's immune defense mechanisms and complex with host proteins, and the potential respiratory tract consequences resulting from inhalation.[2]

Overall, both TiO_2 and ZnO have an excellent safety record and have been utilized for decades in a variety of consumer products, including skim milk, cottage cheese, lotion, toothpaste, baby powders, and shampoo. However, because TiO_2 and ZnO are known photocatalysts and emit electrons upon UV exposure, there has been concern about the generation of free radicals and oxidative damage to living cells.[2] Several *in vitro* studies have suggested that TiO_2 and ZnO generate ROS that cause subsequent DNA damage as a result of UV exposure. However, there are limitations to these studies. For example, one study did not confirm particle size in the sunscreen; therefore it is unclear whether they truly were nanoparticles in the formulation.[35]

Another study failed to describe its methodology and therefore the results cannot be appropriately analyzed or reproduced.[36] A commonly cited study by nanotechnology proponents refutes the potential dangers of nano-sized TiO_2 and ZnO. Investigators applied nano-sized ZnO in the dark, either under simultaneous irradiation with UV light, or to cells pre-irradiated with UV light. Pre-irradiated cells later treated with ZnO and cells given concurrent ZnO and irradiation both demonstrated the same type and incidence of chromosomal aberrations. Researchers therefore concluded that ZnO is not phototoxigenic, and that the DNA damage resulted from UV-mediated enhanced susceptibility to ZnO.[37] Regardless, in response to the many *in vitro* studies demonstrating ROS generation, manufacturers began to coat the surface of these nanoparticles to decrease free radical generation upon UV exposure.

Oxidative damage due to TiO_2 and ZnO exposure to UV radiation should only be considered a valid concern if the nanoparticles are proven to penetrate the epidermis.[2] In healthy human skin, nanoparticulate TiO_2 or ZnO do not penetrate further than the upper layers of the *stratum corneum*.[2,38-44] The *stratum corneum*, the outermost layer of the epidermis, consists of nonviable keratinocytes and represents the rate-limiting barrier against absorption and percutaneous penetration of materials applied topically.[5] Figure 2 The barrier protection provided by the *stratum corneum* is thought to prevent deeper penetration of nanoparticles and inhibit their migration to living epidermal and dermal cells. (See Chapter 1 for a detailed description of the skin structure.)

Figure 2: Structure of the skin.

Although nanoparticles do deposit within the *stratum corneum*, the continuous shedding of this epidermal layer prevents deeper nanoparticle penetration into the epidermis. However, nanoparticles have been shown to penetrate the skin via the follicular ostia. TiO_2 nanoparticles have been detected within hair follicles after topical application of sunscreen; however, this does not represent nanoparticle penetration into living skin layers.[39] Furthermore, sebum flow from the pilosebaceous glands and follicular epithelium turnover effectively eliminate insoluble particles collected in follicular orifices.[3] Thus, despite the safety concerns regarding the toxic effects demonstrated by TiO_2 and ZnO nanoparticles on living cells, available evidence demonstrates that when applied to healthy, intact skin, TiO_2 and ZnO nanoparticles do not have the capacity to penetrate living skin cells; therefore the risk of ROS generation and subsequent DNA damage is minimal.[2] However, more research is warranted to examine the effect of nanoparticle application to damaged skin, as no conclusive evidence exists in the literature.[2,6]

While it may be intuitive to assume that compromised skin has increased susceptibility to penetration by small particles, this may not be the case. In fact, skin inflammation often produces epidermal thickening, which may in turn enhance the barrier function of the skin, rather than reduce it.[5,45] For example, psoriasis results in hyperkeratosis, which can decrease penetration of topically applied substances. In contrast, eczema and similar cutaneous pathology involves breaks in the stratum corneum, effectively reducing the barrier function of the epidermis and thereby permitting increased penetration of topical treatments.[3] Similarly, sunburned skin slightly enhances TiO_2 and ZnO nanoparticle penetration in sunscreen formulations in *in vitro* and *in vivo* models, but transdermal absorption was not detected.[46]

Nanoparticle carriers have also been employed for encapsulating sunscreen agents to improve efficacy and minimize side effects. Examples of organic UV filters include avobenzone and oxybenzone.[6] Oxybenzone is an aromatic ketone that provides broad-spectrum UV coverage and was first approved by the FDA in the early 1980s.[33] Despite its effectiveness as a sunscreen agent, it is the most common cause of photoallergic contact dermatitis[19] and systemic absorption of oxybenzone following its topical application has been reported.[47] Sanad et al. developed NLCs of oxybenzone to enhance its photoprotective efficacy as well as safety.[48] The synergistic effect of NLCs' and oxybenzone's UV blocking power is sixfold more efficient in protecting against UV radiation, while simultaneously minimizing skin irritancy traditionally seen in oxybenzone.[48] Today, it is one of the most widely used organic UV filters used in sunscreens produced in the United States.[33]

10.1.4 NANOTECHNOLOGY AND HAIR CARE

Hair covers much of the skin's surface area, with the exception of glabrous zones such as the palms, soles, and vermilion border of the lips. Appendageal hairs consist of pilosebaceous apparati coupled to either terminal or vellus hairs. The abundance of human hair lends it to being a focus of many cosmetic enhancements and treatments.

Hair care consumer products are designed for maintenance of hair health, cosmetic enhancement, or alleviation of hair damage or disease. Hair health maintenance entails preservation of hair color, texture, shape, or shine. Cosmetic enhancements include hair coloring, styling, and volumizing, as well as growth of missing hair or removal of unwanted hair. Mitigation of hair damage may be accomplished via strengthening, lengthening, or volumizing hair. Hair and scalp disease may be alleviated with topical agents. This subject has been discussed in detail elsewhere in this book.

Current hair care products have some limitations including chemical content, potential irritancy, or allergenicity[49-51] and potential side effects from systemic absorption.[51] Hair dyes consist of a range of chemicals whose composition and duration of effect varies among dyes.[49] Contact sensitization to hair dyes is a well-known safety issue and is most common from professional exposure.[51] There has also been increasing awareness regarding *p*-phenylenediamine (PDA), a potent contact allergen found in permanent hair dyes.[49,50] Delayed-type (and rarely, immediate) hypersensitivity reactions have been reported following exposure to PDA and its related compounds.[49,50] When tested in murine models, PDA-containing hair dyes induced local inflammation, Th1 cell proliferation[50] and increased levels of inflammatory cytokines such as interferon-gamma and interleukin (IL)-17.[52] The field of nanotechnology offers unique solutions to some of these hair care challenges, as significant strides have been made in the development of new and useful hair care tools. For example, PDA-incorporated nanoparticles were less cytotoxic to human skin keratinocytes compared to PDA alone.[49]

Hair-coloring products generate $12 billion per year with an estimated 50% of adults using hair dyes at some point in their lifetime.[49] Incorporation of nanotechnology into hair-care products has improved such products. Nanostructured liposomal emollients penetrate the hair shaft better, protect chemically unstable ingredients against degradation, and permit controlled and sustained release of the dye's active ingredient.[53] An *in vitro* study on porcine skin compared the degree of penetration of dye-containing nanoparticles to the penetration of the same quantity of dye in the nonparticle form. The dye-containing nanoparticles demonstrated much deeper penetration into hair follicles, especially if massage was applied. However, without mechanical manipulation of the skin surface,

there were no differences in penetration between the two groups.[54] It is thought that hair shaft movement induced by massage acts as a pumping mechanism to facilitate particle translocation into the hair follicle. Figure 3. Under *in vivo* conditions, this presumed pump mechanism is thought to occur without massage via continuous body movement.[54,55] Additionally, this study found that the nanoparticles remained stored in the hair follicle for a much longer period of time compared to the non-nanoparticle dye.[54] This study demonstrated that dye nanoparticles possess improved penetration into and storage capacity within hair follicles compared to nonparticle dyes; thus demonstrating the positive impact that nanoparticles can have on hair dyes.

Figure 3: Structure of a hair follicle.

Other studies have demonstrated that topically applied nanoparticles preferentially accumulate in hair follicles[52,55,56] in a time-dependent and size-dependent manner,[55,56] with smaller nanoparticles accumulating in higher concentrations within follicular openings.[56] This follicular targeting upon topical administration has potential implications for delivery of cosmetics and pharmaceuticals.

In addition to their utility in hair dyes, several studies have demonstrated the potential for nanoparticles in the promotion of hair growth. Subcutaneous implantation of gelatin nanofibers that contain silver nanoparticles stimulated development of secondary hair follicles,[57] and hinokitiol-containing nanocapsules, when included in shampoo and hair tonic, stimulated hair growth.[58] Additionally, fullerene nanomaterials have been shown to potentiate hair growth in mice and human skin sections.[59] Several new nanotechnology-based anti-dandruff products are currently being developed; however, no studies supporting the efficacy of these products have been published to date.

Today's shampoo formulations are not used solely for cleansing purposes, as additional benefits are expected by purchasing consumers. Such benefits include conditioning and hair surface smoothing. This has proven difficult as silicone oil, a major constituent of hair conditioners, is removed from hair during shampooing.[50] Furthermore, during hair washing, hair shafts are rendered hydrophilic, thus repelling the silicone oil prone to accumulate on the scalp and preventing the conditioning effect the oil has when it deposits on the hair shaft. In response, a nanoemulsion was created that utilizes a nonionic surfactant, which promotes the delivery of this silIcone oil from the scalp to the hair shaft and thus facilitates hair conditioning.[50] This nanoemulsion demonstrated thermodynamic and physical stability during storage, suggesting it is suitable for use as a conditioner on human hair.[50] Additionally, the nanoemulsion-containing shampoos demonstrated improved silicone oil deposition on hair surfaces compared with traditional control shampoos. This is likely due to the fact that traditional shampoos experience phase separation during extended storage time and increased storage temperature.[50] Nanogels also have the potential to be utilized in shampoos and conditioners. With their small size, the nanogels disperse well in the formulation, conferring a thickening property to the product while delivering sustained release of a fragrance, for example, over time.[49]

10.1.5 NANOTECHNOLOGY AND MAKEUP/COVERUP

Nanotechnology is of great use and potential in the makeup industry. For example, nanoparticle utilization in cosmetics generates products with bolder colors, improved texture, longer duration of effect, and increased skin penetration of specific ingredients such as antioxidants and vitamins.[2,60] In addition, nanoparticle formulations in cosmetic products improve the stability of the ingredients encapsulated within the nanomaterial as well as make the cosmetic product more aesthetically pleasing.[61]

Because of their purported benefits, nanomaterials are frequently found in modern cosmetic products in the form of nanoemulsions, nanocrystals, nanocapsules, nanosomes, liposomes, niosomes, micelles, polymeric nanocapsules, SLN

and NLC, carbon nanotubes and fullerenes, and dendrimers.[5,61] Nanoemulsions are used in conditioners and lotions applied to the skin and hair. As described above, combined traditional cosmetic ingredients including water, oils, and surfactants and are manufactured as two-phase systems with ultra-small droplets dispersed in an external phase. This composition confers benefits to the resulting nanoemulsions including transparency and pleasant texture.[3]

A variety of modern cosmetics contain nanomaterials, including makeup, moisturizers, and hair care products. For example, L'Oreal has utilized niosomes in their anti-aging topical treatments since 1986. Similarly, Christian Dior employs liposomes for transdermal delivery with the expectation that increased concentrations of active agents will be delivered to the epidermis without resultant toxicity.[61] The first two cosmetic products containing lipid nanoparticles were introduced to the cosmetics market in 2005; currently there are about 30 such products on the market.[22]

The efficiency of a cosmetic product not only depends on its active ingredient, but also on the vehicle that carries the active ingredient.[30] These carriers are also important determinants of the penetrating capability of the applied product. Due to increased skin penetration and efficacy, SLN and NLC are used as carriers for various cosmetic and dermatologic purposes.[31,61] Lipid nanoparticle carriers provide improved stability of active ingredients, controlled release of the active ingredient, and lack irritant properties and toxicity.[30,61] and reduction in cost since many active ingredients are quite expensive and by slowing down the rate of delivery, their efficacy is maintained, while at a more reasonable cost. Many cosmetic ingredients, including coenzyme Q10, ascorbyl palmitate, tocopherols (vitamin E), and retinol (vitamin A) have demonstrated controlled release properties over a prolonged period of time when attached to solid lipid nanoparticle carriers.[22] They also possess occlusive properties that help to form a film on the skin, and have the potential to block UV radiation.[30]

Prolonged release of active ingredients is important in both cosmetics and pharmaceuticals that are applied topically; this is especially true for traditional fragrances and those incorporated into cosmetic products.[22] Wissing et al. compared the release profiles of SLN-loaded Allure perfume compared to a nanoemulsion of identical lipid and surfactant content and composition. Results demonstrated that although initial release was similar, SLN-loaded Allure resulted in delayed and prolonged release of the perfume from the solid lipid matrix of SLN. After six hours, 75% of the perfume was released from the SLN, but 100% was released from a nanoemulsion.[22] Another study demonstrated that when incorporated into

NLC, Kenzo perfume demonstrated a longer release profile compared to emulsion and conventional shampoo.[22]

10.1.6 NANOTECHNOLOGY AND EMOLLIENT THERAPY

Multiple external insults can lead to disruption of the skin barrier and a subsequent increase in trans-epidermal water loss. Figure 4 A variety of nanomaterials have been developed in an effort to repair the barrier and prevent water loss at the skin surface. Nanoemulsions are commonly used in conditioners and lotions[5] because the small droplet size in this nanomaterial confers transparency and a satisfactory sensory consistency. Liposomes are also frequently utilized in cosmetic products because they improve the stability and skin tolerance of cosmetic ingredients.[5] Nanoparticles play a role in emollient therapy because, as previously mentioned, the occlusive properties of lipid nanoparticles reduce transepidermal water loss, thereby providing increased skin hydration when such particles are topically applied.[22] An advantage of SLN is that they can be mixed with an already commercially available topical treatment and via the increased occlusivity mechanism, can improve its efficacy.[13] A 2003 *in vivo* study examined the skin hydration effect after four weeks of application of an oil/water cream containing SLN, and a traditional, non-SLN-containing cream. The cream containing SLN increased skin hydration significantly more than the conventional cream.[22] While topical therapies with occlusive properties exist, many have an undesirable appearance when applied.[22,62] The SLN-containing creams not only provide enhanced hydration, but also demonstrate more acceptable aesthetics.[22] In 2005, Cutanova Cream Nano Repair Q10 cream was the first lipid nanoparticle-based cosmetic product introduced to the cosmetics market.[22] Investigations have found that this Q10-containing NLC cream had superior skin hydration properties compared to a conventional oil/water cream.[22] This NLC dispersion provided a fivefold higher occlusive factor compared to an oil/water emulsion.[22] Furthermore, it achieved a similar degree of occlusion as highly occlusive liquid paraffin while avoiding a glossy appearance of the skin.[63] Finally, NLCs used in Cutanova Cream Nano Repair Q10 were analyzed for the degree of coenzyme Q10 penetration through the stratum corneum. Study results showed that coenzyme Q10-loaded NLCs demonstrated improved skin penetration compared with a reference emulsion.[63]

Figure 4: Damage to skin barrier leading to transepidermal water loss.

Though traditional emollients and creams have been used as topical therapies for cutaneous disorders, they are often ineffective vehicles for delivering particular active ingredients to a targeted active site.[63] To bypass this difficulty, oil/water nanoemulsions have been used as carrier systems for a wide variety of applications. For example, in the case of diminished *stratum corneum* lipids, of which ceramides are the major species,[63] nanoemulsions containing ceramides can be utilized to restore the lipid barrier.[64] In part due their inherent function as part of the water-impermeable skin barrier, ceramides are exceptionally insoluble compounds.[65,66] To allow for effective topical application of a lipid-based active ingredient, the carrier vehicle must be able to penetrate the *stratum corneum*. Because of their

lipophilic core, nanoemulsions have the potential to carry water-immiscible active ingredients through aqueous environments and function in drug delivery.[16,67]

Several studies have investigated supplementing native epidermal lipids for the purposes of treating dry and aging skin.[68,69] The highly ordered lipid arrangement of the *stratum corneum* lends itself as a fortified barrier preventing water loss; it also impedes the entrance of drugs and foreign toxins through the outermost layer of the skin.[69] Abnormal lipid structure has been associated with compromised skin barrier function and diminished skin water content; both are qualities seen in dry and aging skin.[69]

The structure of synthetically prepared liposomes can emulate that of the *stratum corneum* and has been developed as a means of delivering supplemental lipid compounds to compromised skin. A study by De Pera et al. evaluated the impact of topically applied internal wool lipids in the form of liposomes on disrupted skin. Prior work demonstrated that using lipids analagous to that of the stratum corneum, such as internal wool lipids (IWL), improved the retention of the liposomes in the *stratum corneum* barrier, more so than conventional phospholipids.[70-72] Conventional lipids have a tendency to instead penetrate transdermally, and, as such, have difficulty being maintained in the *stratum corneum* barrier. It was found that with daily application of these IWL-containing liposomes, the skin barrier was bolstered and trans-epidermal water loss (TEWL) was diminished.

Another study, by Coderch et al., investigated the topical application of two different liposomes with *stratum corneum* lipid composition in the treatment of dry and aging skin.[68] Two liposome formulations, synthetic *stratum corneum* lipid mixtures (SSCL) and IWL, when topically applied to the skin limited TEWL and improved skin capacitance.[68] These outcomes demonstrate the beneficial effects of skin lipid supplementation with SC-resembling lipids and the emollient capabilities of topical liposomes in the treatment of the disrupted barrier in dry skin. Furthermore, as augmented barrier integrity has been seen in intact skin as well, this further supports the possibility of incorporating liposomes in new cosmetic and pharmaceutical products.

The occlusive properties of lipid nanodispersions as topical products have been further investigated in several studies. As discussed previously, the skin has a low permeability due in part to its *stratum corneum* lipid-containing barrier. In order for a topically applied formulation to be effective, it must be able to penetrate the skin and accumulate in the deeper layers. Using smaller particles, such as nanoparticles, ensures better penetration into the *stratum corneum*.[7] When applied to the skin, nanoparticles form a monolayered lipid film and thus achieve better occlusion and hydration.[7] Figure 5.

Figure 5: Emollient properties of nanoparticles. Adapted from Müller *et al.*[12]

Lipid nanoparticles can be formulated into a dispersion that when topically applied will retard the loss of skin moisture and function as an effective emollient.[73] Several other studies further confirm that the emollient properties of nanoparticles correlate not only with their size, but also with lipid composition. As discussed previously, the authors found that the smaller size allowed for better skin penetration and improved emollient efficacy.[7,27] Furthermore, lipid nanoparticle dispersions when topically applied also improve skin hydration by fusing to form a dense film. The evaporation of water enhances the occlusive barrier because as water evaporates, the nanoparticles appear to fuse due to capillary forces during water withdrawal; this further enhances lipid nanoparticle film formation on the skin.[27]

10.1.7 NANOTECHNOLOGY AND ANTI-AGING

Aging skin exhibits wrinkles, increased dryness, and pigmentation changes.[74] Environmental factors, such as UV radiation from the sun, exposure to pollutants, and cigarette smoke all contribute to the generation of an inflammatory process in the skin.[75] These insults initiate a cascade of events that begins with the depletion of cellular antioxidants, DNA damage leading to thymidine dimer formation, and the generation of pro-inflammatory mediators in a variety of skin cells.[74] Free radicals and ROS generation are thought to be major initiators of an intracellular cascade in keratinocytes and fibroblasts. This is followed by up-regulation of matrix metalloproteinases (MMPs), which are responsible for the degradation of dermal fiber components. The process described ultimately leads to wrinkle formation.[75]

In addition to the protease damage, the inflammatory cascade and ROS injure cellular lipids, carbohydrates, and proteins; the degradation products accumulate in the dermis and epidermis, augmenting the pathophysiology of aging.[74] Various strategies exist for inhibiting this damaging cascade at different levels: decrease UV penetration into skin via sunscreen, reduce inflammation via anti-inflammatory compounds, suppress ROS damage via antioxidants, and inhibit MMP and elastase activity.

Coenzyme Q10 (2,3-dimethoxy-5-methyl-6-decaprenil-benzoquinone), also known as ubiquinone because of its ubiquitous presence in nature, plays a variety of roles within cells. It acts as an electron carrier in the mitochondrial respiratory chain for ATP production;[76] it also functions as a powerful antioxidant that protects cells from ROS.[77] Cutaneous signs of aging are directly related to both oxidative stress and diminishing levels of innate coenzyme Q10. Neutralizing free radicals and thus lessening oxidative stress is a key target in impeding the progression of damage in aging skin.[78,79] Because of these characteristics, coenzyme Q10 has been an appealing focus for cosmetic development.[80] Coenzyme Q10, however, is susceptible to photodegradation, and this chemical instability limits its utility in topical cosmetic formulations.[76] Inconsistent skin penetration is another coenzyme Q10 limitation. Hoppe et al. noted 20% penetration of coenzyme Q10 into the epidermis and only 2% penetration into the dermis.[76] However, nanomaterial technologies pose a solution to these therapeutic barriers. For example, nanoemulsions containing coenzyme Q10 demonstrated improved incorporation into suspension;[81] while a topically applied SLN had a more consistent biphasic release profile.[82] A surfactant-free nanoparticle showed steady delivery of coenzyme Q10 for a two-month period.[83]

With regard to stability, Kwon et al. synthesized polymeric nanoparticles containing coenzyme Q10 and demonstrated that after exposure to high temperatures and UV radiation, coenzyme Q10 degradation was much reduced.[84] Thus, nanoencapsulation of coenzyme Q10 for use in cosmetic formulations is feasible, and concerns about its stability and release can be alleviated by nanomaterial fabrication techniques.

Topical administration of coenzyme Q10-containing nanoproducts has been demonstrated to diminish photo-aging and wrinkle depth.[14] Coenzyme Q10 was demonstrated to hamper cytokine production and MMP generation in *in vitro* studies with cultured human dermal fibroblasts.[74] The same authors evaluated a coenzyme Q10-containing cream in a clinical trial; after five months, wrinkle score grade was reduced, as evaluated by a dermatologist.[74] Together, these *in vitro* and *in vivo* studies demonstrate that coenzyme Q10 likely inhibits downstream MMP upregulation in dermal fibroblasts, and as such, diminish the incidence of wrinkles and revitalize aging skin.

Lipid-based nanoparticles have been investigated as vehicles for delivery of molecules such as ascorbyl palmitate[85] and coenzyme Q10.[82] Both of these active ingredients are often used in anti-aging products because of their ability to reduce photo-oxidation and wrinkle depth.[7] However, a major hindrance to the utility of these components in anti-aging treatments is their instability in aqueous formulations. Several studies have found that by incorporating these active ingredients into the matrix of SLN, the lipophilicity of the compounds is no longer a problem for solubilization.[82,85] Nanoparticle encapsulation confers improved chemical stability and allows for the incorporation of these lipid-based ingredients into a variety of anti-aging cosmetic products.[7]

An NLC product containing both omega-3 and omega-6 unsaturated fatty acids (i.e., oil-based components) as well as coenzyme Q10 was developed and investigated for efficacy. The NLC product had comparable penetration into the skin as a similar SLN product,[86] as well as good chemical stability.[87] Omega-3 unsaturated fatty acids have been shown to inhibit the expression of MMP-type 1 after UV exposure.[86] Since matrix metalloproteases type 1 participate in the degradation of collagen and elastin, vital structural skin proteins,[88] the activity of the unsaturated fatty acids against this enzymatic degradation was shown to maintain skin vitality by protecting collagen.[86] This NLC vehicle successfully combined the anti-aging effects of omega unsaturated fatty acids and coenzyme Q10 to prevent the natural degradation of skin structure seen in aging.

- Coenzyme Q10
- Ascorbyl palmitate
- Omega-3, Omega-6 unsaturated fatty acids
- Alpha-tocopherol (vitamin E)
- Retinoids (vitamin A)
- Alpha-lipoic acid

Figure 6: Anti-aging compounds

Other products have been encapsulated and studied as topical anti-aging treatments. Figure 6. It is well known that the skin protects itself via antioxidants such as vitamin E, also known as α-tocopherol.[89] Vitamin E predominantly acts to protect lipid structural components against oxidation in the skin.[89] The critical role of α-tocopherol in protecting the *stratum corneum* makes it an ideal therapeutic agent for protecting the skin against this oxidative damage and subsequent aging.[90] For example, Dingler et al. studied an anti-aging formulation consisting of vitamin E-loaded solid lipid nanoparticles.[20,91] The SLN developed were physically stable in hydrophilic solution as well as when incorporated into a topically applied cream. When topically applied in a human subject, the particles produced an occlusive

skin barrier, which further facilitated penetration of vitamin E into the skin. Larger studies are needed to further confirm the efficacy of vitamin E-loaded SLN, but the initial results demonstrating occlusion and adequate penetration of the active ingredient are promising. Given the known antioxidant and anti-aging properties of topical vitamin E, this successful SLN formulation demonstrates potential for further advancing the efficacy of topical anti-aging products.

Retinoids are a class of vitamin A (all-*trans*-retinol) derivatives.[92,93] The oxidation of all-*trans* retinol yields all-*trans* retinoic acid, or tretinoin, thought to be the most potent vitamin A metabolite, which has been the gold standard for some time since it has shown the most promise for the treatment of intrinsic aging and photo-aging.[92,94] Various studies have investigated natural and synthetic retinoids, demonstrating histologic and clinical success in skin treated with topical tretinoin since the 1980s.[95-97] Since that time many cosmeceuticals have been developed utilizing tretinoin for anti-aging treatments.[98] However, though retinoids have shown success in addressing skin aging, adverse skin effects such as erythema, burning, irritation (the so called "retinoid dermatitis") and increased photosensitivity have limited their wide-scale use and patient tolerance of treatment.[93,99,100] This skin irritation is particularly prominent with the use of tretinoin products, as compared to other retinoids.[93] In addition, tretinoin demonstrates low water solubility and great instability on exposure to light, air, and heat, possibly limiting its efficacy in topical application.[98] In an effort to decrease these adverse effects, a variety of new drug delivery vehicles for tretinoin have been created.

Nanotechnology has been especially successful for enhancing drug delivery. In particular, nanoparticles have demonstrated good results in the encapsulation of active ingredients for anti-aging. For example, Shah et al. developed and characterized an SLN encapsulating tretinoin.[98] The SLN significantly improved the photostability of tretinoin and prevented isomerization of the compound. Furthermore, *in vitro* studies indicate that tretinoin-encapsulating nanoparticles maintain equivalent permeation through skin as compared to control tretinoin cream. In addition, the results of skin irritation studies in an *in vivo* animal model demonstrate that SLN tretinoin yield significantly fewer side effects than topical treatment with a non-nanomaterial-based tretinoin cream.

Several other studies describe the development and characterization of similar SLN delivery vehicles for the encapsulation of retinoids, and demonstrate successful encapsulation as well as chemical stability of retinoids.[101-103] Further *in vivo* studies are necessary to confirm the clinical efficacy of these products, but the success of these nanomaterial-based carriers in encapsulating and delivering retinoids, well-known anti-aging compounds, demonstrates promise for further advancement of nanocosmetics for anti-aging purposes.

Alpha-lipoic acid is a natural molecule that is found in foods and also produced by the human body.[104] It is known to be a powerful scavenger of free radicals[105] and as such possesses anti-inflammatory capacity. It is increasingly being studied and utilized in anti-aging skin care products because it is less injurious than other active ingredients such as tretinoin or hydroxy acids; it may be practical for topical application to delicate skin, e.g. peri-orbital areas.[104] In a controlled study of an alpha-lipoic acid-containing cream for treatment of photoaging skin, response to the cream in woman was assessed via clinical, photography, and laser profilometry methods.[106] By all four metrics of assessment, there was a statistically significant improvement in the cream-treated half of the face.[106] The subjects demonstrated improved clinical characteristics related to photoaging of the skin, suggesting the therapeutic efficacy of lipoic acid-containing creams in anti-aging cosmetics. Another study developed and characterized an SLN system containing alpha-lipoic acid.[104]

Because lipoic acid is chemically labile with odorous degradation products, the authors encapsulated the active ingredient to improve its stability and appeal as a cosmetic formulation. The SLN synthesis yielded 90% encapsulation efficiency for the formulations, and the particles were stable up to three months at storage temperatures of 4 and 20 degrees Celsius. After successful encapsulation, these alpha-lipoic acid SLN can be incorporated into a cream, lotion, or gel formulation for different cosmetic needs,[107] and the well-known anti-aging effect of lipoic acid can be further harnessed for a more pleasing topical formulation.

CONCLUSION

Various types of nanoscale materials have proven to be efficacious carriers for dermal delivery of cosmetic products. Nanomaterials demonstrate unusual physical characteristics compared to the same material in bulk form[4] and therefore possess interesting new properties that can be utilized for the development of new and exciting cosmetic and personal care applications. Nanoemulsions, liposomes, and nanoparticles (polymeric, SLN, and NLC) have been developed in various forms to be used to contain cosmeceuticals. Inorganic and organic sunblocking agents, when incorporated into nanoparticles, have provided improved cosmetic aesthetics and consumer satisfaction. Additionally, nanoparticles themselves can be utilized as photoprotective agents in that the nanocarrier may possess inherent photoblocking activity.

Nanomaterials have also proven useful in makeup products because their composition confers benefits such as transparency and pleasant texture. In addition to their ability to serve as vehicles for active ingredient, nanoscale carriers demonstrate increased skin penetration and efficacy, as well as minimal irritation and

toxic side effects. In the arena of topical emollients, lipid nanoparticles and oil/water emulsions reduce trans-epidermal water loss, due to their occlusive properties, providing increased skin hydration. Ceramides and native lipids such as internal wool lipids can also be incorporated into these nanoscale emollients, further augmenting their efficacy. Anti-aging cosmeceuticals have also benefited from the incorporation of active ingredients such as coenzyme Q10, unsaturated fatty acids, α-tocopherol, and retinoids into nanomaterial products.

Besides novel skin applications, nanotechnology also offers several unique advantages for the consumer hair marketplace. For example, it has permitted the synthesis of dyes that accumulate in, and penetrate the follicle more efficiently. Additionally, nanotechnology can promote hair growth. This provides a potential route for transcutaneous drug delivery that allows for improved drug targeting and cosmetic application. Nanostructured liposomal emollients demonstrate improve hair shaft penetration and protect active ingredients from degradation, while permitting controlled and sustained release of the active substance.

Though nanomaterials have been used for many years in cosmetics, more recent advances in research and development have yielded a wider variety of approaches to the development of novel products with claims and results far beyond those achieved at the early stage of nano-developments. The field continues to progress rapidly, and more complex and efficient methodologies are being utilized to develop new formulations. Concerns about toxicity have been largely alleviated, and as such, the small size and unique physical properties of nanomaterials can be harnessed to develop specifically tailored products to meet consumer needs.

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PART 11

TESTING, CHARACTERIZATION AND PERFORMANCE CLAIMS

PART 11.1

METHODS TO ASSESS SKIN BARRIER INTEGRITY: EFFECTS OF CLEANSING PRODUCTS

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Ashland Specialty Ingredients

ABSTRACT

Protecting skin barrier integrity is vital to maintaining the health of human skin. While providing skin hygiene, surfactant-containing cleansing formulations can damage the skin and reduce its natural defensive barrier function. Such formulations can also cause dry, flaky, or itchy skin as a result of irritation due to surfactant absorption and penetration. Several mechanisms have been proposed to describe the interaction of surfactants with the *stratum corneum* (SC). These include protein denaturation, natural moisturizing factor (NMF) removal, selective SC lipid removal, and disruption of SC lipid organization leading to increased permeability through the SC.

In this chapter, we discuss the effects of cleansing stresses (e.g., surfactants, pH, and cleansing product temperature) on skin barrier integrity. We also describe some practical methods that can be used for evaluating the extent of skin barrier damage induced by cleansing formulations, as well as the advantages and disadvantages of each measurement technique.

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11.1.1 INTRODUCTION

a. Skin structure and functions

In addition to being the largest organ of the human body, the skin is the interface between the organism and the external environment and protects the body from external insults. In the design of skin care products or formulations, the ultimate consumer experience, as well as skin health and appearance, are determined by formulation ingredients and their material properties. The physical and chemical activity of these ingredients and their interactions with each other influence both skin/*stratum corneum* (SC) structure and their biochemistry. In order for the cosmetic scientist to improve skin condition or reduce the skin damage caused by cleansing products, an understanding of skin structure and function is essential. This is especially true concerning the basic physiology of the outer epidermal layer, SC.

There have been many articles that reviewed skin structure and functions in detail, which is however not the primary focus of this chapter[1–3]. Briefly speaking, skin is a complex multilayered organ consisting of three components. Looking from

the outside towards the inside, these components are the viable epidermis, the dermis, and hypodermis. Major skin functions include: i. the protective and defensive barrier (its vital function) to prevent uncontrolled water loss and to protect the body from a variety of environmental insults (e.g., abrasion, microorganism invasion, UV or other radiation damage, exogenous chemical agents such as surfactants and solvents, and other daily insults); ii. thermo-regulation function through blood flow and sweating to maintain normal body temperature; iii. immune system function; iv. sensory function via the nervous system; and v. psychological function for skin visual appearance and social acceptance.

In practice, it is generally accepted that topical skin care is primarily concerned with protecting the skin barrier[4]. A widely employed analogy for the SC organization is a brick-mortar (corneocyte-lipid) model[5]. Overwhelming evidence indicates that SC lipid composition and organization governs skin permeability and plays a critical physiological role in regulating water loss/uptake through the skin. In healthy skin, lipids are typically in a lamellar bi-layer crystalline state. The organization of the SC lipid membranes is very complex and is comprised of three major components: free fatty acids (FFA), ceramides (CER), and cholesterol (CHO). Together they form the highly ordered lipid phase of the SC.

The SC lipid matrix, and also the mixed SC lipid model systems that have been widely used to study the SC, do not exist as homogenous or uniformly mixed single phases, but rather form separate domains (and bi-layer phases) in the SC lipid membranes[6–8]. Although the exact ratio of the phases has not been determined experimentally, fluid (liquid crystalline) and solid phases (both hexagonal and orthorhombic) of lipids do coexist, which is a crucial requirement for the barrier properties and flexibility of the SC lipid layers.

b. Cleansing and commonly used surfactant systems in cleansing formulations

As part of their daily routine, consumers frequently use surfactant-containing cleansing products such as soap bars, detergents, as well as liquid hand soaps, body washes, cleansing lotions, liquid face washes, (hand) dish wash, etc. These surfactant-based preparations are efficacious cleansing systems that help remove unwanted materials such as dirt, oil, sweat, debris, pathogenic microorganisms, and other excess body and cutaneous serums from different substrates (skin, hair, fabrics, dishes, etc.). Cleansing efficacy is very important for hygiene and is achieved by means of surfactant properties such as solubilization/emulsification of grease in liquids, and dispersion or suspension of solids in liquids. These properties arise from the ability of surfactants to concentrate at the interface, leading to a reduction in interfacial tension. Because of their characteristics, surfactants are among the most important cosmetic ingredients.

Surfactants are amphiphilic (two-part) molecules that are composed of a hydrophobic portion (tail group, almost always a hydrocarbon structure) and a hydrophilic portion (head group). When the solution concentration of a surfactant reaches its critical micelle concentration (CMC), surfactant molecules tend to form association structures of dynamic aggregates such as micelles. These micelles are typically spherical in nature with the hydrophobic portion facing inwards. As a result of this structure, they can dissolve oils easily by incorporating oil within the inner portion of the micelles and by associating their hydrocarbon tail with the oil surface, thereby causing the solubilized oil to lift up from the surface of the substrate. Depending on the composition or charge of the surfactant molecule (head group), surfactants can be classified as anionic, cationic, nonionic, and amphoteric or zwitterionic (the charge of the head groups changes with pH) surfactants.

For most skin-cleansing formulations, anionic surfactants with hydrocarbon chains of various length especially those with C12 chain length are typically used as primary surfactants (e.g., sodium lauryl sulfate [SLS] or sodium laureth sulfate [SLES]) due to their desirable detergency, foaming, and lather features. Amphoteric surfactants, such as betaines, are normally used as co-surfactants in liquid cleansers that are milder with respect to the skin barrier. Nonionic and cationic surfactants are less frequently used in skin-cleansing systems. With regard to the extent of harshness, the following order is well known: anionic surfactants (highest) > amphoteric surfactants > nonionic surfactants. Among the anionic surfactants, besides soap surfactants that are originated from animal or plant sources (e.g., SDS [or SLS], ALS, or SLES), synthetic detergents (syndets) are also frequently used as they are milder surfactants. For a surfactant with given chain length, the larger the head group size, the lower its tendency to cause skin damage due to steric hindrance. Other factors that affect harshness of a surfactant include its critical micelle concentration (CMC) and micelle size.

c. Skin damages by cleansing products

Due to personal cleansing activities, human skin is constantly exposed to surfactants, which are well known to compromise the essential barrier function of healthy skin. Skin barrier damage can be accompanied by after-wash skin tightness, dryness, scaly skin, or even erythema, irritation, and itch due to surfactant absorption and penetration into the skin. These surfactant-induced, undesirable visible manifestations of skin and uncomfortable skin feeling are greater when the ambient temperature and humidity levels are relatively low in the winter environment. These undesirable side effects are also interrelated.

In addition to adverse effects, the alteration of skin barrier function by surfactants can be managed to enhance the penetration of topically applied materials, such as transdermally delivered drugs. In the case of surfactant use, a careful

balance has to be maintained between maximum drug penetration enhancement and minimum skin irritant response, since any surfactant that has the capability to reduce the cutaneous barrier function for certain drugs could also enhance the permeation of other ingredients, including any excipients used as well as the surfactants themselves.

To understand the interactions of surfactants with the skin and their effect on skin barrier integrity—especially with the outer SC—it is essential to develop qualitative and quantitative measurement methods (both *in vitro* and *in vivo*) to predict, evaluate, and demonstrate the effect of different surfactant chemistries, formulation ingredients, and cleansing conditions.

In this chapter, we discuss surfactant-skin interaction mechanisms and the effects of cleansing stresses (e.g., surfactants, pH, and cleansing product temperature) on skin-barrier integrity. We also describe some practical methods for evaluating the extent of skin-barrier damage induced by cleansing formulations as well as the advantages and disadvantages of each measurement technique. We conclude with a discussion of employing these methods to assess the benefits provided by harshness-reducing technologies applicable in skin-cleansing formulations.

11.1.2 PHYSICOCHEMICAL INTERACTIONS BETWEEN SURFACTANTS AND SKIN

As a system having a heterogeneous surface as well as a bulk material phase, the skin is very complex and surfactants applied to its surface can interact with many different targets in the skin structure in multiple ways. Only a few skin components will be discussed in this chapter: the SC (including intercellular SC lipids and dead cells of protein-containing corneocytes), keratin, and living cells of the epidermis.

It is generally accepted that surfactant-SC interactions and disruption of skin-barrier properties occur when surfactants have penetrated into the skin barrier. Several mechanisms have been proposed regarding the subsequent interaction of surfactants with the SC and skin components. These include, but are not limited to: enzyme disruption, skin permeation and penetration, protein denaturation and dissolution, SC swelling that causes enhanced water penetration, and removal of natural moisturizing factor (NMF) from corneocytes[9–10]. Such insults lead to reduced water-holding capacity and dry skin, selective SC lipid extraction or removal, and disruption of SC lipid organization/structure. They can also lead to a change of lipid composition, which generates an increased permeability through the SC lipid bi-layers that are responsible for the SC barrier function[11–12]. The above phenomena may also trigger some biological responses from the skin, such as the scenario described in the “dry skin cycle” model by Rawlings[13]. If the cleansing stresses (e.g., surfactant concentration, application period, pH, temperature, etc.) are strong enough, or the skin is not rinsed sufficiently, surfactant residue left on the skin

will continue to penetrate into the viable epidermis, and perhaps even into the dermis. This process will cause further disruption of living skin cell homeostasis, ultimately leading to an inflammatory response. It is worth noting that the above-mentioned mechanisms are not independent from each other but rather related in a cycle.

Most of the mechanisms described above also involve water loss, either directly or indirectly. In other words, surfactant-based cleansing products cause water loss and reduce the water-holding capability of the SC by disturbing the skin's normal mechanisms for regulating water content[14]. In fact, maintaining of optimal water content is a key factor (if not the most important one) for sustaining the practical function of the SC[15–16].

a. SC protein binding, denaturation, dissolution, and SC swelling

Surfactant interaction with skin proteins can adversely affect skin hydration and viscoelasicity. It is widely reported that surfactants can bind to skin keratin with high affinity[17–22]. This binding reduces the ability of skin protein to bind and retain water, thereby causing dry and tight skin. This binding could also lead to the development of repulsive forces, causing some protein denaturation (separation of the protein matrix, uncoiling of the filaments, and exposing more water-binding sites), SC conformation change, and transient swelling/hyperhydration. These events are often followed by water evaporation causing skin drying[17–18]. This effect is thought to saturate at about the CMC since protein binding and denaturation are associated with surfactant monomers, although this hypothesis remains controversial[11, 19–22].

In general, surfactants that bind more strongly to SC proteins have a higher potential to cause significant protein denaturation and severe skin-barrier damage. For anionic surfactants, the skin swells as water content increases with increase in charge—protein unfolding and dissolution may occur. An increase in water uptake only hydrates the skin temporarily, while eventually it will reduce the skin's water-holding capability due to surfactant adsorption onto protein hydration sites. After rinsing, as the water evaporates, the hydration level of the skin is lower than the preexposure level. As the result, the skin will be even drier than before cleansing due to additional water evaporation and SC swelling. For other surfactant systems, there is little change with increase/decrease in surfactant charge and therefore minimal SC swelling is observed. Indeed, SC swelling can be reduced by the addition of secondary surfactants (such as betaine) even though the total surfactant concentration is increased by mixing[23]. This phenomenon can be explained by the reduced binding of the harsher surfactant due to the competition between them for binding sites, and the lowering of the CMC by the addition of a surfactant with lower CMC. Another way to reduce SC swelling is to increase the size of the head

or polar group of surfactant, which will provide an additional barrier in the form of steric hindrance.

Surfactant and even water adsorption on the SC may dissolve some components of the water-soluble natural moisturizing factor, leading to a reduction in their levels in skin[24]. NMF is a heterogeneous mixture of hygroscopic amino acids in the corneocytes and it is one of the major components responsible for water holding (other two major water-holding SC factors are intercellular lamellar lipid organization and corneodesmosome function)[25]. NMF adsorbs water from the atmosphere to provide sufficient hydration to help keep skin flexible and smooth, as well as to support a variety of enzymatic reactions in the dermis. It is very water soluble and can be very easily leached by water if the protecting lipid bi-layers surrounding the corneocytes get damaged, as for example, by surfactant exposure. Consequences of reduced NMF include reduced hydration of the SC, reduced activities of SC enzymes leading to reduced desmosome breakdown, increased stiffness of the SC leading to the possibility of cracking around joints, and erythema due to inflammation associated with an undesirable biochemical response.

b. SC lipid extraction and selective removal

SC lipids are the major barrier to permeability of the SC and play a critical role in skin functions ensuring cellular cohesion, skin-barrier properties, water-holding capability, and normal healthy-looking skin. Indeed, skin or SC with disordered lipid structure or abnormal composition would be more water permeable and more vulnerable to external attacks. Mainly due to the effect on the lipid layers, surfactant exposure would increase the ability of exogenous compounds to penetrate the skin and to increase water loss through the skin.

It is reported that lipid extraction is possible above the CMC, and cholesterol and free fatty acids (not ceramides) are generally removed[24, 26–29]. In one study, Loden concluded that lipid extraction/solubilization was caused by surfactant micelles and therefore is concentration dependent[30]. When the SDS concentration is relatively high, more surfactant molecules are available for skin binding and penetration and thereby “consume” the lipid matrix. This concept is used to explain the mechanism of damage to SC by surfactants at concentrations above their CMC[21, 31].

The solubilization and removal of SC lipid by a large amount of absorbed surfactant molecules has been reported in lipid films[32]. Lipid content in the skin SC is normally observed to decrease because of delipidization after cleansing with soap products[33]. In a study of recovery in SDS-treated skin by daily topical application of SC lipid fractions, Imokawa and colleagues strongly supported deficiency of intercellular lipids as an essential mechanism in

surfactant-induced skin damage[27]. The concept of disruption of the lipid matrix (degreasing) is the dominant hypothesis for explaining the damage induced in SC lipid organization by surfactants. A few groups have challenged this idea of lipid extraction by conducting experiments suggesting that only very small amounts of lipids are removed from the SC by surfactants, even at high concentrations (above CMC), if the surfactant treatment time is not excessive (less than a few minutes)[28–29].

c. Disruption of SC lipid organization/structure and change of lipid composition

While extraction of SC lipids by surfactants remains controversial and may not be the critical factor affecting lipid barrier function, alterations in lipid phase behavior and organizational structure, and disruption of the integrity of the lipid bi-layer barrier have attracted more attention. Surfactant exposure can cause expansion of intercellular spaces of the lipid lamellar structure and surfactant molecules will intercalate into the lipid network[34]. It is also reported that anionic surfactants induce intercellular lipid structure disruption, disordering, and, possibly lipid fluidization[32, 35]. Changes in lipid organization will impact skin irritation and alter the penetration of irritant molecules in the SC. This process also includes affecting desmosomal cleavage between corneocytes[9].

Prolonged water and surfactant contact, although very different in their chemical nature, are both deleterious to the skin barrier since they disrupt SC lipid structure[24, 36]. In many situations human skin is exposed to both stresses, as for example during cleansing, as well as in many workplace environments. Lipid disruption not only leads to increased water loss through the skin, but also enhances penetration of other chemical substances including toxic and irritating compounds through the skin.

Long-term exposure to surfactants will also modify the lipid composition and cause phase transitions and changes in the overall phase behavior of the lipid bi-layer systems[37]. Lipid composition is of paramount importance for the SC organization and its barrier properties[38–40]. In fact, it is known that lipid composition in the SC is different between individuals, between anatomical skin sites, and a function of skin damage and diseases[41]. The alteration of the ratio of the three major lipid components may also cause phase separation of lipids at the surface of the *stratum corneum* and result in crystallization of the lipid matrix[42]. These phase changes in turn will affect the water-holding capability and barrier properties of the SC, causing dry skin and/or skin irritation[37, 43–44]. Evidence indicates that diminishing amount of ceramide and increasing levels of fatty acid (nearly doubled) are related to soap-induced winter xerosis[42].

Based on literature reports, SC lipid-surfactant interactions likely progress as follows: (1) initial water loss due to osmosis or surfactant-induced water swelling or hydration to open up the lipid structure; (2) surfactant penetration/insertion into the lipids that further alters lipid phase, structure, and even composition, leading to extra water uptake by the lipid layers; (3) after extended exposure to surfactant solutions for a long period, part of the lipid matrix is removed by dissolution and the lipid barrier function is further damaged, causing water loss and dry skin due to reduced water-holding capability; and (4) SC disruption by surfactants stimulates SC formation and this new formation typically proceeds too quickly, leading to abnormal architecture and inadequate barrier function thereby causing low hydration and abnormal desquamation (scaly appearance).

d. pH effect

Another factor that can aggravate the deleterious effects of surfatcant-skin interactions is the pH of the cleansing formulation. It is well known that pH plays a very significant role in the alteration of skin intergrity by surfactants. The effect of pH on SC barrier function and skin permeability to drug molecules has been the subject of many studies[9, 45–51]. The results of these studies demonstrate that the enviromental pH to which skin is exposed has a significant impact on SC lipid composition and organization. Further it impacts SC cohesion, protein swelling, and skin irritation. Indeed, a predictable relationship between the solution pH and skin irratancy has been demonstrated, although it has been reported that the influence of pH on the chemistry of surfactant-based washing compositions is more important than that of the direct contribution of pH on the SC[52].

Since the pH of a healthy SC is acidic (generally around pH 5), the attaction and penetration of basic surfactant solutions is more favorable. Under basic pH conditions anionic surfactants bind primarily by hydrophobic functional groups to hydrophobic sites on the skin to minimize the repulsion of negative charges, thus leading to swelling of the SC in the presence of more alkaline surfactants[35]. The effect of surfactant pH on SC was further demonstrated to negatively influence the barrier repair mechanism by the daily use of soap-based cleansers with greater pH[53].

Soap-based cleansers are alkaline in nature (pH 10, e.g., for regular soap bars) while milder synthetic detergents (syndets, such as Dove bar) are mostly neutral (pH ~7 for Dove soap bar). The fact that detergents with higher pH are less mild is not surprising since there is more binding to keratin by anionic surfactants[52]. It has been reported that alkaline pH alone, in the absence of surfactants, induces an increase of SC swelling and irreversible lipid phase changes in SC[9, 45, 54].

11.1.3 APPROACHES AND METHODS TO ASSESS THE EFFECTS OF CLEANSING STRESSES ON SKIN-BARRIER INTEGRITY

Requirements from consumers have stimulated the development of milder cleansing products, which makes it difficult to differentiate minor variations between products. Exaggerated and more progressive application tests (i.e., "accelerated" testing), where test conditions are modified to be harsher (e.g., more concentrated, elevated temperature, longer exposure period, etc.), are usually conducted to enhance the skin reactions.

For clinical tests utilizing human subjects, the procedures are regulated by ethical recommendations and test guidelines to ensure safety and standardization. More details may be found in a published series of testing procedures and guidelines by the European Group for Efficacy Measurements on Cosmetics and Other Topical Products (EEMCO) for tests related to different skin properties, such as dry skin and xerosis[55–56], skin hydration by electrical methods[57], TEWL in cosmetic sciences[58], skin surface pH[59], tensile functional properties[60], skin color[61], skin microcirculation[62], and skin topography[63].

While it is the most straightforward method to assess the harshness of skin-cleansing products, consumer perception and expert grading is not objective and, therefore, not always reproducible or reliable[35, 64]. As a result, a large number of subjects are required and different consumer habits and practices have to be considered. It is also difficult to distinguish the small differences between relatively mild cleansing products using such methods. These problems make it difficult, expensive, and time consuming to directly compare skin-cleansing products solely by consumer perception tests. Consequently, various exaggerated methods and approaches have been developed for formulation screening and product development, leaving consumer testing as a tool mostly used for claim substantiation of final products.

Noninvasive biophysical and bioengineering methods offer insights into the different aspects of skin structure and functions. These methods are primarily based on sophisticated scientific instrumentations and have been used to measure skin characteristics following environmental exposure (e.g., UV exposure) and cosmetic treatment (e.g., surfactants, skin care products, etc.) as well as monitoring skin aging and diseases. They are sensitive, easy to use, fast, mostly portable, and not costly. They are practical approaches that can be used to monitor and assess topical treatments for *in vivo* human skin in real time and to obtain objective, quantitative, and reproducible information on skin conditions after surfactant challenge.

In the last several decades, various experimental approaches have been utilized to better serve different application needs. There are *in vivo* methods where

human skin is subjected to various imaging and instrumental methods. Often tape stripping is applied to provide more in-depth information. Frequently *in vitro* approaches are employed. In these cases, full thickness human or porcine skin, isolated *stratum corneum*, or extracted lipids may be used. Porcine skin is commonly employed due to its physiological similarity to human skin. Other than real skin samples, some model materials have been recently developed to study surfactant interactions with skin, SC, lipid, or proteins. These include lipid models, protein models, synthetic membrane substrates (vitro skin, vitro corneum, episkin®, silicone membrane, etc.).

To model the interactions of surfactants with the skin and their effect on skin-barrier integrity, especially with a focus on the outer SC, it is essential to develop qualitative and quantitative measurement methods (both *in vitro* and *in vivo* approaches) to predict, evaluate, and demonstrate the effect of different surfactant chemistries, formulation ingredients, and cleansing conditions.

Because surfactants can interact with skin in a variety of ways, there are many properties of skin that can be characterized to assess cleanser mildness. Each single property cannot represent the comprehensive skin condition by itself. In the discussion below, the effects of surfactant-based cleansing products on the skin-barrier integrity will be discussed according to the following three categories: surfactant-skin/SC interactions, surfactant-lipid interactions, and surfactant-protein interactions.

a. Surfactant-skin/SC interactions

1. Sensory testing and substantiating instrumental methods

Sensory methods such as visual or tactile methods through consumer perception and expert grading after actual use are often used to evaluate various skin parameters and assess skin/SC damage by cleansing products, evaluating parameters such as after-wash dryness, roughness (scaling), tightness, erythema (redness), and irritation/itching or even pain. This approach is generally rapid and inexpensive but it is subjective and not quantitative. Skin damage has to be significant and visible, therefore it may not indicate subclinical skin damage.

With the assistance of modern image analysis techniques, skin surface texture, dryness, and roughness can be visualized by skin replicas (commonly silicone replicas). This replica methodology can be used to provide an imprint of the skin surface and the scanned images of the replicas can be quantitatively analyzed with image analysis.

Skin color change and erythema can be evaluated by expert grading. This can also be conducted by color measuring instruments based on the quantification of skin's optical and thermal properties. These portable instruments (such as

Erythema Meter from Dia-Stron, Minolta Chroma Meter, and DermaSpectrometer from Cortex) monitor skin reflection with different light or utilize the three-dimensional color coordinate system (L, a, and b) to measure skin redness[65–66]. Another way to characterize skin redness is by measuring blood flow and vascular status of skin (e.g., utilizing laser Doppler technology).

Based on measurements of redness or erythema, the harshness and irritancy of surfactant systems or cleansing formulations can be assessed by exaggerated exposure tests such as the soap chamber test or patch test, where the volar forearms of human volunteers are treated with surfactants and subsequently the condition of the skin barrier is determined. For example, the soap chamber test has been used to compare the beneficial effects of syndet bars over regular soap bars[67–68].

2. Microscope, video microscopy for skin surface topography

Optical or light microscopy is most commonly used to visualize the skin surface and study the effects of environmental and physiological (e.g., aging) factors on the appearance of the skin surface. With recent technological advancements, high-quality images with high resolution and large depth of field can be obtained and quantified.

Digitalized optical microscopes (e.g., Scopeman Video Microscope from Moritex and Visioscope from Courage & Khazaka) can collect images of the skin surface, allowing real-time visualization of skin condition and texture after image analysis[69]. The introduction of noninvasive confocal microscopy imaging (e.g., VivaScope from Lucid and Vectra 3D imaging systems from Canfield) and advanced image analysis software now provide specialized photographic 3-D skin surface images with high optical resolution and contrast to facilitate skin studies and enhanced understanding of skin at the cellular level.

Profilometry is another commonly used method to quantify skin surface topography such as skin scaling and roughness[70]. Skin surface changes induced by cleansing products and the benefits from cosmetic treatments can be visualized in three-dimensional images.

Compared to optical microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscope (AFM) can provide microscopic images of the SC ultrastructure with greater resolution[24, 36, 42, 71–74].

3. Skin penetration/permeability by Franz cell and impedance measurements

Skin penetration and permeation by surfactants can be measured using Franz diffusion cells. In a typical experimental protocol, a skin sample is placed as a diffusion barrier between two components of a diffusion cell. The amount of surfactant

that permeates through the SC membrane after a certain period of exposure can be measured by several techniques (e.g., HPLC)[75].

Skin's electrical impedance can be used to evaluate skin integrity and skin-barrier damage and recovery[12]. Skin-barrier perturbation due to exposure to different chemicals can be quantitatively expressed by measuring changes in skin impedance. When the skin barrier is perturbed, skin electrical impedance is expected to decrease since the transport rate of ions flowing across the skin is higher. It has been shown by different research groups, and for different applications, that skin impedance correlates well with skin permeability[12, 21, 76]. Thus, for example, Ghosh and Blankschtein[21] have reported a positive relationship between *in vitro* manitol transdermal permeability and *in vitro* skin electric current when exposing pig skin to sodium dodecyl sulfate solutions. In other studies of drug permeation across human skin, Karande et al.[12] demonstrated a correlation between skin permeability and skin impedance for different solutes in various formulations. They also showed the feasibility of using impedance as an alternative indicator for increased skin permeability induced by chemical penetration enhancers.

With relatively straightforward *in vitro* skin impedance studies and high-throughput or combinatorial technologies, it is possible to quantitatively examine *in situ* skin-barrier perturbation induced by surfactants, and other physical or chemical stresses including temperature, time of exposure, and pH. Previous reports have demonstrated that results from *in vitro* impedance measurements are consistent with *in vivo* findings in terms of ranking the extent of surfactant-induced skin-barrier damage[77]. Using this approach, one can simultaneously examine, screen, and rank many simple formulations and dramatically reduce the time and efforts needed for skin-related formulation improvements including product development and marketing.

In a recent study, *in vitro* skin electrical current (impedance) measurements were conducted in a Franz diffusion cell (Figure 1) to quantitatively monitor changes in skin-barrier integrity as a function of surfactant chemistry and concentration[54]. In addition, time of exposure, temperature, pH, and the addition of chemical constituents (such as glycerin) were also evaluated. One example is shown in Figure 2, where skin damage by SDS and SLES at different pH values is compared. Overall, the data are in very good agreement with results obtained from a range of other *in vitro* and *in vivo* studies.

As a straightforward and relatively rapid approach to screen formulations for their impact on skin-barrier integrity, the skin impedance method has also been used to evaluate the harshness of cleansing formulations such as soap bars and syndets, shower gels with or without harshness-reducing skin-conditioning ingredients, shaving preparation formulations, hand dishwash formulations, baby wash products, etc. Again, the results correlate and are consistent with other *in*

vitro biophysical and *in vivo* clinical studies[78]. In summary, these results demonstrate that skin impedance measurements can be used as a direct, quantitative method for characterizing skin-barrier integrity for a wide range of skin-cleansing formulations.

Figure 1. Experimental setup of the Franz diffusion cell to measure pig skin electrical impedance *in vitro*[54]. Pig skin is secured between the donor and the receptor compartments with the SC side facing upwards. An AC sinusoidal signal is applied across the skin. The electric current is measured and the skin impedance can be calculated by Ohm's law.

Figure 2. (A) Average relative normalized impedance (the higher, the less damage to the skin barrier and the milder the surfactant system is) versus the SDS solution pH when the porcine skin samples are exposed to 14.3% SDS surfactant solution with different pH values for five hours at room temperature; (B) Average relative normalized impedance versus SLES solution pH when the skin samples are exposed to 14.2% SLES surfactant solution with different pH values for five hours at room temperature[54]. Note the scale in the two plots is different.

4. Mechanical behavior of skin

The skin is a material that demonstrates both elastic and plastic characteristic behavior (viscoelasticity). The mechanical and material properties of skin (e.g., SC stress, after-wash tightness, elasticity, and friction) are affected by exposure to surfactants or other stimuli. Surfactant damage causes dry skin and loss of elasticity, which often leads to cracking of the skin[1]. However, very little is currently understood about this subject since multiple skin layers, including *stratum corneum*, viable epidermis, and dermis are involved.

Most instruments designed to assess the mechanical behavior of skin (*in vivo*) apply a known stress to the skin and measurement of the resulting strain, which can be monitored in different ways. By applying a sinusoidal stress to the skin surface, the viscoelastic characteristics and complex modulus of skin can be studied both *in vivo* and *in vitro* with a gas-bearing electrodynamometer[79–81]. Torsion devices (such as Dermal Torque Meter from Dia-Stron) were developed to study the elasticity parallel to the skin surface by measuring volumetric deformation after applying a twisting force[82–83]. Suction systems, on the other hand, were designed to

measure the elasticity perpendicular to the skin surface[84]. These systems measure vertical deformation or stretching and the recovering of skin based on the electrical capacitance of skin (examples include DermaFlex from Cortex Technologies and BTC 2000 from DermaLab) or by using optical systems (e.g., Cutometer from Courage & Khazaka) to measure skin's elevation into a probe due to suction pressure[85]. A device based on shockwave propagation (Reviscometer from Courage & Khazaka) was developed to study skin viscoelesticity (specifically in the SC) by measuring the resonance running time of an acoustical shockwave[86]. This approach is very sensitive and able to differentiate between cosmetic treatments, and has been correlated to consumer perception[87].

As a thin film material, the SC can be investigated by using *in vitro* methods based on biomechanics, dynamic mechanical analysis (DMA), and wafer curvature techniques[88–89]. The drying stress under various conditions such as temperature, relative humidity, and topical cosmetic treatments can be compared and differentiated.

Changes in skin smoothness and friction by surfactants and other cosmetic products can be characterized by a friction meter[90–91]. Currently the Newcastle Friction meter is the only commercially available instrument for this evaluation.

5. Bioengineering methods to measure water flux and water content of skin

Water plays a very important role in physically and biologically maintaining healthy skin. At the same time, a key function of the SC is to act as a semi-impermeable membrane barrier to retain and keep water from leaving the body. Water flux and water content of the outer skin, therefore, are excellent indicators of skin-barrier integrity.

Over the last several decades many noninvasive biophysical and bioengineering instruments have been developed to objectively and quantitatively characterize various skin conditions (skin moisturization, skin diseases, skin aging, sun-mediated stress, etc.) as well as to evaluate skin protection and repair by cosmetic products.

There exists a concentration gradient in the SC, which decreases from the boundary with the viable epidermis to the outermost layers of the surface. This water diffusion flux depends on skin permeability, as well as on the water activity gradient between the human body and the external environment. More hydration at the SC surface will lead to a smaller concentration gradient and a lower transepidermal water loss (TEWL) value[92]. TEWL measurements can be taken to determine water flux by passive diffusion through the SC layer and can be considered as a measure of skin's intrinsic barrier properties. Higher TEWL values normally indicate a damaged or ineffective SC barrier due to chemical (such as surfactant) exposure or diseased skin conditions.

There are two TEWL measurement techniques: open-chamber and closed-chamber. The most known commercially available open-chamber TEWL meters

are the Tewameter, ServoMed Evaporimeter, and DermaLab TEWL systems. Due to the vulnerability to disturbance from environmental air movements for the open-chamber systems, the measurements have to be conducted in a well-controlled laboratory environment and subjected to strict testing guidelines. It should be noted, however, that these instruments are quite reliable when used properly[93–95]. Currently closed-chamber TEWL meters that are free from environmental impact are becoming more popular since they are feasible for common workplace and dermatological laboratories. There are two different closed-chamber TEWL instruments available on the market. The VapoMeter from Delfin is a portable and battery-powered unit based on the unventilated concept while AquaFlux from Biox is a bench-top, AC-powered unit based on the condenser-chamber concept[96–97]. TEWL measurements have been used to study and predict skin irritancy following exaggerated surfactant exposure procedures, and the results show a positive correlation between TEWL measurements and visual assessment of skin condition[66, 72, 98–100].

Several instrumental methods have been developed to measure skin hydration and the SC water content (skin surface moisture change) by measuring skin permeability to an alternating electric field. Depending on instrument designs, different signals and parameters can be obtained. NOVA Dermal Phase Meter (based on impedance measurements, which are related to resistance to the flow of alternating current), Skicon200 skin surface hygrometer from IBS and Skin Sensor from DermaLab (based on high-frequency conductance measurements), and Corneometer (based on capacitance measurements) from Courage & Khazaka are among the most popular instruments that are portable and easy to use[37, 41, 66, 69, 98]. Measurements of skin hydration using these devices to study surfactant-skin interactions have been reported and, again, a good correlation between these results and visual dryness/irritation grading has been shown[29, 101–103].

It has to be noted that although TEWL is related to the water content on the skin surface, measurements of TEWL and skin hydration do not always directly correlate. It has been reported that visible skin dryness correlates well with low levels of skin hydration but is not always accompanied by an increase in TEWL readings[24]. In fact, TEWL indicates whether the skin-barrier integrity is physiologically or biologically broken down, which is not a prerequisite for skin dryness, while skin hydration provides information about the physical condition of the skin surface layers. Nevertheless, if the skin is too dry, the surface may physically break down and crack, causing barrier damage and ultimately leading to higher TEWL.

Washing procedures also affect skin/SC pH, which is correlated with skin/SC hydration. The pH of normal skin is between 4 and 6. This acidic environment of the skin surface is very important for maintaining epidermal barrier function and the restoration of the damaged skin barrier. Higher pH has been observed for skin under pathological conditions or after exposure to detergents[33, 104]. The skin/SC pH

can be measured by a commercially available instrument (glass electrode) skin pH meter PH 900. However, the concept of SC pH is somewhat problematic, as normal SC does not have free water.

To obtain useful and reliable information regarding the interaction between surfactant-based products and skin, and to better understand the effect of surfactants on skin-barrier function to support strong claim substantiation, it is highly recommended to combine measurements that include several electrical probe methods and TEWL measurements[105].

b. Surfactant-lipid interactions

Skin-barrier function resides primarily in the heterogeneous organization of ordered lipid lamellae of the *stratum corneum*. Surfactant-lipid interactions play a critical role in damaging skin-barrier function and in repairing the barrier after cleansing stress.

Although it is possible to study lipid behavior in intact skin or isolated SC, the complicated structure of the SC and the inherent variability of biological samples make the detailed analysis of lipid phase behavior in real SC lipid organization very challenging[106]. A widely employed approach for investigating skin-barrier biophysics is to use model lipid systems that consist of a mixture of free fatty acid (FFA), ceramides (CER), and cholesterol (CHO) to mimic the SC lipid structure/phase and anticipate its barrier function. Such lipid models of the SC barrier have been widely used and validated by many studies using diverse biophysical experimental methods[107–111]. While the model lipid system is certainly not the same as the lipid composition and organization in the SC, this approach is a simple and useful starting point in which the major SC lipid classes are represented.

1. Differential scanning calorimetry (DSC)

DSC investigates the changes in the liquid-crystal organization of the lipids (from SC or lipid model) by monitoring the shift of molecular structural transition temperature (the melting temperature T_m or the glass transition temperature T_g). Fluidity and disordered arrangement of lipid structure and selective lipid extraction induced by surfactant application can be studied by DSC[112–114].

2.Vibrational spectroscopy (FT-IR)

Fourier Transformed Infrared (FT-IR) spectroscopy measures the vibrational energies (wavelength and intensity) of molecules by light absorption in the infrared range. The IR spectra are affected by neighboring molecular environments and can be used to provide powerful and simultaneous information on both intra- and intermolecular interactions in lipid model systems as well as isolated SC or even skin *in vivo*[115–117]. This technique is very sensitive to molecular composition,

structure, and interactions between neighboring molecular components. Lipid chain dynamics, chain packing/organization, conformational order and structure (gel, crystalline, liquid crystalline), fluidity (molecular interactions in lipid bi-layers), domain formation, and phase behavior (orthorhombic, hexagonal, disordered or liquid) in model lipid systems with various compositions can be quantitatively examined since certain IR spectra regions of the SC or SC lipids are very sensitive to these parameters. Figure 3 contains typical FT-IR spectra of a lipid model system at different temperature ranges. Typically one would examine the symmetric CH_2 stretching/rocking (νsCH) around wavenumber 2850 cm^{-1}, and/or CD_2 symmetric stretching/rocking (νsCD) parameters for fatty acid around wavenumber 2090 cm^{-1}. For illustration, Figure 4 contains a plot of the position of the CH_2 stretching band as a function of temperature, allowing us to elucidate the phase behavior of a selected lipid system. The νsCH mode is very suitable for probing the behavior of ceramide chains since it is an intense and relatively narrow band with very limited cholesterol interference[43]. The νsCD mode on the other hand comes only from the fatty acid component and can be used to probe the conformational order of fatty acid through the behavior of the deuterated acyl chains[118].

Figure 3. Typical FT-IR spectra of a lipid structure at different temperature ranges 119.

Figure 4. SC lipid conformation order (i.e., bi-layer fluidity) can be determined from the peak position of the symmetric CH_2 stretching mode of the lipid chains from the IR spectra of the SC[9, 119].

FTIR spectroscopy has been applied extensively to study the interactions between surfactants and *stratum corneum* lipids[9, 24, 69, 120]. *In vivo* and *in vitro* attenuated total reflectance Fourier-transform infrared (ATR-FTIR) and transmission-mode IR spectroscopy of skin, isolated SC, or lipid model systems can be used to examine the characteristics of the SC and/or SC lipids (fluidity and integrity of lipid domains) following surfactant exposure and sequential tape striping[9, 24, 120–122]. Integrity of the intercellular lipid domains and damage to SC lipid barrier bi-layer by surfactant exposure as well as recovery can thus be investigated. The effect of pH of the surfactant systems on the SC barrier integrity can also be determined. ATR-FTIR can be used to investigate delivery and monitor deposition (mapping or imaging) of skin-beneficial ingredients (*in vivo*) in skin-cleansing formulations, which correlates well with electrical measurements[41, 69]. With the help of tape stripping, one may determine the distribution profile of molecular components deposited or topically applied across the SC. For isolated *stratum corneum*, transmission-mode FT-IR can be used to study damage and restoration of the skin barrier *in vitro*[109,123].

3. Quartz crystal microbalance (QCM)

The quartz crystal microbalance (QCM) detects very small changes in mass via a piezoelectric mechanism and has been broadly utilized as a physical, chemical, electrochemical, and biochemical sensing tool in various applications[124]. As a simple mass measuring technique with nanogram resolution, it can accurately monitor *in situ* subtle surface coverage changes including penetration and diffusion of molecules, and monolayer sorption onto thin films. This oscillator technology is straightforward and convenient to use, especially for complicated solid-liquid interfacial phenomena.

For skin penetration studies, particularly with a focus on SC lipids, the SC mass change is normally very subtle while the skin system is very heterogeneous and complicated. Hence, accurately detecting mass changes due to penetration of exogenous molecules through the SC layer, or studying the effect of chemical penetration on SC lipid phase behavior and structure is very challenging. When experimentally feasible, QCM provides a more sensitive and quantitative approach to such problems than traditional gravimetric measurements[19–20, 32].

Recently, water vapor uptake and surfactant sorption onto SC lipid model layers cast on a quartz crystal were studied using the QCM technique[32, 125–126]. In a very recent article, *in vitro* water vapor uptake and surfactant absorption onto skin lipid model films were quantitatively studied using the QCM[111]. Figure 5 outlines this technique for measuring surfactant adsorption to a model lipid system. The results show that barrier properties of the lipid model system may be damaged by surfactant absorption, as well as by long-term water exposure, due to alterations to the lipid film structure. Surfactant absorption is found to be concentration dependent even beyond its critical micelle concentration (CMC). QCM results for different surfactant systems are consistent with reported clinical data in showing that a clinically milder surfactant, such as sodium lauryl ether sulfate (SLES), does not perturb the film as much as a clinically harsh surfactant, such as sodium dodecyl sulfate (SDS), as shown in Figure 6. These results indicate that this experimental approach is valid and valuable in understanding the effect of surfactants on skin-barrier lipid organization, and hence skin-barrier function.

Figure 5. Experimental setup for surfactant absorption measurement of a lipid model coated QCM 111.

Figure 6. Comparison of QCM absorption profiles of model lipid systems in sodium lauryl ether sulfate (SLES) and sodium lauryl sulfate (SDS) solutions as a function of surfactant concentration[111]. The results show that surfactant absorption into the lipid matrix, and the associated water uptake, are significantly diminished in the case of SLES. Also almost double the concentration of SLES is needed to achieve the maximum mass increase.

c. Skin-protein interactions

Since proteins and surfactants are both amphiphilic materials, surfactant molecules would bind to proteins through interactions between both hydrophilic and hydrophobic groups in a stereochemically constant structure. This binding is dependent on the surfactant chemistry, concentration, temperature, pH, etc. The protein structure will become altered by surfactant binding and protein denaturation can be used as an indication of skin-barrier damage or irritation caused by surfactants. In fact, a correlation between surfactant-induced skin damage or irritation and the interactions of the surfactant molecules with *stratum corneum* proteins[127]. Various protein models (albumin such as BSA, NMF model, zein, etc.) have been used to study surfactant-protein interactions.

1. BSA denaturation test

As a globular protein, bovine serum albumin (BSA) contains 76% sequence identity with human albumin. The denaturation of BSA has been used as a model protein to human skin protein for harshness evaluation. Correlation between

surfactant-induced BSA denaturation and skin roughness, after exposing to detergent solutions, was demonstrated[128–129].

Different parameters can be measured to study protein (BSA) denaturation by surfactants. Conductivity and zeta potential of the surfactant-BSA solution can be measured to measure the charge change of the mixing system[78, 130–131]. FTIR spectroscopy, and Raman spectroscopy, and DSC can be used to determine changes in protein secondary structure and investigate the thermal unfolding of BSA caused by surfactants in solution[78, 132–133]. Fluorescence spectroscopy studies have been applied to obtain information on the nature of protein-surfactant aggregates and to monitor changes in the tertiary structure induced by interactions with ionic surfactants, which can result in the exposure of tryptophan residues[134–135]. Since protein denaturation is normally accompanied by a rise in pH and changes in viscosity and surface tension, monitoring these parameters of the BSA solution with the addition of surfactant solution would provide some information about the configuration of BSA and the surfactant-protein interactions[130, 132, 136]. *In vitro* assays based on these parameters (e.g., pH rise) have been developed to predict potential of surfactant-induced skin irritation[137].

2. Zein solubility assay

Dissolution of zein, a protein from corn, can be measured in surfactant solutions to correlate surfactant-induced protein damage with soap harshness or skin irritation. Zein is a water-insoluble protein that becomes water soluble to some extent if it is denatured by surfactants. Theoretically, there is a positive correlation between zein solubility and skin harshness; the more zein that is solubilized, the higher is the tendency to irritate human skin. The study of surfactant interactions with insoluble proteins such as zein can yield valuable information about the effects of surfactants on skin and hair. Model experiments made with zein reveal that the ability of the surfactant to denature and solubilize this protein correlates very well with the dermal irritancy potential of the surfactant[138]. This material has been extensively used in the skin care industry as a model system to understand the effect of surfactants on skin, and as a measure of surfactant-protein interaction, and to predict and screen skin irritation tendency and harshness of soap or surfactant ingredients in skin-cleansing formulations[137, 139–140]

Various surfactant systems including mixing surfactant systems, as well as cleansing formulations (such as soap bars, clear shower gels, shaving preparation formulations, and hand dishwash formulations) with different ingredients have been studied with this zein solubility assay. The results correlate well with other *in vitro* methods and *in vivo* clinical test results.[78, 141] One example is shown in Figure 7, where dissolved zein concentration is plotted as a function of soap concentration[78]. As expected, dissolved zein concentration for milder synthetic Dove soap bar is significantly lower than that for other regular soap bars.

Figure 7. Zein solubility of various soap bars as a function of soap concentration.

3. NMF level measurements

Cleansing changes in NMF levels in SC can be measured *in vivo* by ATR-FTIR spectroscopy[142] and IR imaging with tape stripping[143].

By IR spectroscopic imaging and confocal Raman microspectroscopy[25, 144], information on the molecular composition and distribution of NMF components can also be obtained.

CONCLUSIONS

The objective of this chapter has been to provide an understanding of the various phenomena impacting the nature and degree of surfactant effects on skin during cleansing practices. Once these fundamentals have been understood, we then provide practical guidance to measurement methods that can be used to study surfactant-skin interactions and to thereby assess the effects of surfactants on skin-barrier integrity. By doing so, some aspects of surfactant damage to skin caused by cleansing products have also been discussed.

Skin structure is complicated, and all the mechanism of surfactant-skin interactions is not yet fully understood. Because of this, developing methods to assess the multiple facets of surfactant-skin interactions, and to comprehensively examine damage induced by surfactant-based cleansing products, remains challenging. Besides *in vivo* and clinical methods for human skin evaluation, the development of many noninvasive *in vitro* and instrumental methods in recent

years has considerably increased the level of objective and quantitative performance discrimination for cleansing product screening. Yet each method only deals with one or a few of the many aspects of surfactant-skin interactions. Each specific method cannot be used as the sole method to accurately and comprehensively evaluate the harshness of cleansing formulations. Since no single methodology or approach has gained universal acceptance, a combination of many methods is recommended to provide a reliable assessment of the harshness of cleansing formulations and their impact on the integrity of the skin barrier[82]. Ideally, at this stage of our knowledge, choice of the measurement approaches, their combinations, and relative weighing, are of critical importance to providing guidance to formulators and experts in generating useful surfactant structure-efficacy information as well as useful claim substantiation.

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IMAGING TECHNIQUES AND ANALYSIS FOR QUANTIFICATION OF SKIN APPEARANCE

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ABSTRACT

Imaging technologies have become increasingly important in the evaluation of cosmetic ingredients and their effects on skin. This need has been greatly fueled by requirements for the technical evaluation of the effects of new ingredients and formulations intended for use in anti-aging, skin whitening, pore size reduction, and anti-acne products. This chapter provides an in-depth focus of imaging technologies that are utilized to evaluate the appearance of skin from the outside as well as to look inside the skin to assess its overall health state. In addition to obtaining a vast description of instrumentation and techniques used for such applications, the reader will also gain insight into the fundamental physical principles underlying these technologies.

In the last two decades, a number of imaging modalities have been developed to provide quantitative assessment of the skin's topographical structure. Commercial instrumentation based on fringe projection techniques is now available and provides us with a close-up three-dimensional view of the skin's surface, which is especially useful for quantifying furrows and pores. Other skin imaging technologies have also advanced by leaps and bounds, especially in the area of polarized light and fluorescence photography, which are commonly used in conjunction with image analysis techniques. In fact, it is now quite common for laboratories to carry out multi-spectral multi-modal imaging, which combines polarized light, fluorescence, and other modes of photography under one umbrella.

Novel thermal imaging technologies allow us to gather temperature distribution maps of skin, providing us with anatomical differentiation as well as the ability to focus on specific areas of interest that have undergone treatment with active cosmetic ingredients. Below the surface of skin, great strides have also been made in the evaluation of the skin's internal structure. Examples of techniques useful in this regard include, but are not limited to, reflectance confocal microscopy and ultrasonography. There have been many advances in these imaging techniques over the past 10–15 years that have been coupled with a flurry of *in vivo* studies. In fact, many of these technologies are used to carry out important evaluation of skin lesions, both benign and malignant, as well as other important phenomena that occur in skin.

In this chapter we also discuss high-resolution microscopy methods, which in many respects have started to reach their maturity and offer many insights into phenomena that occur in skin. Equally important, image-processing and analysis techniques that are associated with all of these imaging technologies provide us with quantitative descriptions of skin-related measurements. This is especially true for histological and immunofluorescent staining techniques, which are exceptionally useful to monitor the effects of skin care active ingredients. In most cases, treatment of skin with active ingredients leads to the upregulation or downregulation of specific proteins and other biomolecules as measured in cell culture systems *in vitro* and in the various morphological components of skin as monitored *in vivo* or *ex vivo*. Traditionally, histological and immunofluorescent images were qualitatively assessed by a researcher or pathologist. Thanks to advances in image analysis software, such evaluations can be achieved quantitatively. The overall goal of this chapter is to touch upon many important facets of this technology and provide the reader with a general overview and the current state-of-the-art.

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11.2.1 SKIN SURFACE IMAGING AND ANALYSIS

Skin topography is an extremely important component of the consumer's sensorial experience, mostly perceived by visual observation and touch. Since the stratum corneum is the outermost layer of skin, it is the first part of the integument seen by the observer. The stratum corneum is comprised of corneocytes, which exfoliate from the skin. The exfoliation pattern depends on the overall health state of the skin. For example, in dry, scaly skin, corneocytes tend to exfoliate in large clusters. This is known as scaling and is frequent in dry skin as well as many common dermatological diseases, such as psoriasis. On the other hand, in smooth, moisturized skin corneocytes will shed off in much smaller clusters, ideally as individual cells. Skin ashing is a term frequently used to describe skin characterized as dry with decreased luster properties.[1] The skin's microrelief pattern also contributes significantly to its topography. Fine grooves (furrows) intersect with each other, forming many types of shapes including squares, rectangles, triangles, rhomboids, and trapezoids that contribute to the skin's glyphic patterns. Glyphic patterns provide the skin with the necessary flexibility to accommodate various ranges of motion, especially in the joint regions. Glyphic patterns should be distinguished from wrinkles, which are genetically determined and develop during adulthood as a result of dermal matrix deterioriation.[2] The formation of wrinkles can be accelerated by various environmental factors including exposure to solar radiation. In addition to the stratum corneum and its components, there are a number of appendages (e.g., hair and pores) in skin that also contribute to its three-dimensional landscape.

In this section of the chapter, we describe many of the methods used to measure skin topography. These include polarized light photography, skin replica methods, and fringe projection techniques. In addition, we examine various factors associated with quantification of pore appearance, which contribute greatly to skin topography, especially in the facial region. Lip health is another area of great importance to the consumer and therefore of great interest to cosmetic scientists attempting to improve its appearance. For this reason, we discuss several techniques to measure not only lip properties, but also the influence of both cosmetic and active ingredient treatments. A detailed discussion of lip structure can be found elsewhere in this book in the major section on substrates.

Other imaging techniques have also been used to a great extent to characterize various features of skin. For example, ultraviolet reflectance photography is used to better visualize areas of hyper- and hypopigmentation in skin. Fluorescence reflectance microscopy is also capable of generating pertinent information about internal structures of skin, such as collagen, horn, porphyrins (due to acne vulgaris), etc. The emergence of these techniques over the last two decades has led to the development of commercially available instruments to conduct multi-spectral multi-modal facial imaging.

a. Polarized light photography

Surface and subsurface features of the skin can be accentuated with polarized light photography.[3,4] Important surface features measured by polarization photography consist of wrinkles, furrows, and pores. Subsurface features, on the other hand, include erythema, vascular lesions (e.g., portwine stains, rosacea, etc.), and pigmented lesions.[5] Polarized light photography has been employed in clinical dermatology and for the evaluation of personal care products for more than two decades.

Before delving into the particularities of polarization photography, there are a few principles of optics that should be considered. There are two types of light reflection: specular and diffuse reflection. **Specular reflection** is associated with glare and has a shiny appearance, which in the case of skin results from direct reflection from the outermost surface of the stratum corneum. The angle of incidence equals the angle of reflection in specular reflection, similar to the phenomenon that occurs when light reflects from a smooth surface. A familiar example of this in nature is when one observes a lake with a mountain in the background with the mountain's reflection projected on the smooth lake's surface. In skin, only about 4–7% of the incident light on the surface of skin undergoes specular reflection.[6]

Diffuse reflection, on the other hand, is light scattered at various angles due to imperfections at the surface where the angle of reflection is not equivalent to the angle of incidence. Light also penetrates into the skin where it may be absorbed by melanin and other chromophores, or it interacts with other structures such as collagen fibers causing the light to scatter (changing its orientation of polarization) prior to reflecting back outside of the skin. This latter process is known as backscattering and accounts for approximately 93–96% of incident light that interacts with skin.[6]

During polarization photography, the diffuse and specular components can be separated using linear polarization filters. Normally, one filter is placed in front of the light source and the other is mounted on the lens. In our laboratory, we typically use polarization paper that can be cut to size in order to cover the light source. When the polarizers are aligned parallel to each other we observe specular reflection, similar to the image of volar forearm skin shown in Figure 1a. Notice the accentuation of the surface features and crosshatched nature of furrows by maintaining the same degree of polarization of light that illuminates the subject and is read by the camera sensor.

In contrast, orienting the polarizing filters perpendicular to one another allows us to view diffuse scattering (see Figure 1b). Images obtained of skin in this manner will contain information resulting from the backscattering phenomenon, especially that caused by vascularization. These images were obtained from a relatively young individual (35 years old) with Fiztpatrick Type II skin, little history of photo-damage, and from a region of the body (volar forearm) that is generally protected from UV rays.

(a)

(b)

Figure 1: Photographic images of the volar forearm of a subject with Fitzpatrick Type II skin; age 35. Polarizers were placed in front of the illumination light source and the lens. (a) Image obtained with polarizers oriented parallel to each other. (b) Image obtained with polarizers oriented perpendicular to each other (cross polarizers). The small dark spot in the middle of the field of view is a marking reference point.

In addition to qualitative visual observation of the images, typically one would quantify various features in the image. This is especially true in the case of the image obtained with parallel polarizers. Normally, one is interested in the density

and directionality of the furrows in a given image. Usually, computer-generated algorithms or image-processing and analysis software are utilized to isolate the furrows from the original image. In Figure 2, several typical steps are shown using commercially available software (Image Pro Plus and Adobe Photoshop) in order to isolate the furrows so that further measurements can be made.

Figure 2: (a) Image from Figure 1a subjected to a series of image-processing steps to isolate furrows including: (b) flattening and conversion to grayscale, (c) sharpening and contrast adjustment, and (d) inversion and skeletonization.

The mounting of illuminating lights is a key factor in obtaining properly lit images. The very first systems used for this purpose consisted of a flash unit fixed in the hot shoe mount of the camera. Later, more sophisticated flash housing units were developed, placing two light sources on each side of a macro lens. The camera and lighting system shown in Figure 3 is a typical macrophotography flash system manufactured by Nikon. Similar systems are also available through other camera manufacturers, such as Canon. In these systems, polarization paper can be cut-to-size and inserted underneath the front cover of the speed light. A linear polarization filter can also be mounted directly on the lens. In more recent years, a multipurpose system known as the Visia Complexion Analysis (VISIA-CR, Canfield Scientific, Fairfield, NJ) was developed allowing researchers to conduct visible light, polarized light, UV reflectance, and fluorescence reflectance photography (www.canfieldsci.com). These other modes of photographic investigation and the multi-spectral multi-modal facial imaging system are described in the sections below.

Figure 3: Photograph of a Nikon DLSR (Model D300), which contains the Nikon R1 Wireless Close-Up Macro Speedlight Flash System.

b. Imaging Techniques with Skin Replicas

Skin replica techniques are among the oldest of the trade.[7] They are relatively quick procedures that are easy to carry out, and they provide very useful information about the surface structure of the skin. To a large extent, innovative instruments capable of noninvasive (noncontact) three-dimensional imaging have captured the spotlight and in many cases have become the preferred method of topographical analysis of skin. However, these instruments are often very costly and not available to most laboratories. Thus, researchers still find utility for replicas in the analysis of skin topography.[8,9] Skin replicas can capture topographic features such as deep wrinkles, furrows, pores, and, even, droplets of sweat beads.[10,11] Utilizing digital imaging techniques, replicas may be imaged and, subsequently, analyzed with image analysis software.

The most common skin replica experiments are carried out using a dental impression material made of vinyl polysiloxane (Silflo, Flexico Developments Ltd., Potters Bar, UK) in combination with a solvent thinner and catalyst. These materials are available through CuDerm Corporation (Dallas, TX; www.cuderm.com) and are used in conjunction with a replica locator. Silflo is mixed with catalyst, usually 1 mL of the impression material with 1 drop of catalyst, and then poured into a replica locator, which is placed at the site of interest on the skin. The ensuing polymerization reaction results in a paste that hardens with time.

Historically, replicas were analyzed by taking a photograph with a film-based SLR camera or by conducting profilometry. It should be noted that the obtained replica is a negative impression of the skin surface. However, in many cases researchers take an additional step and create a positive impression of the mold. In the case of contact or laser profilometry, one can generate a three-dimensional image of the surface. Contact profilometry generates a topographic map of the surface by rastering a stylus on the positive impression.[12,13] Laser profilometry, on the other hand, operates on the principle of laser triangulation, does not require contact with the sample, and generates a three-dimensional image of the replica by performing vertical (z-axis) measurements of the height while scanning in the lateral directions (x- and y-axes).[14] Depending on the desired level of magnification, scanning electron microscopy could also be used to capture surface details of replicas.[15,16] Nowadays, most groups perform the analysis on the negative impression rather than going through the additional step of generating a positive impression. In part, this is due to advances in image-processing techniques, which allow for the inversion of the negative impression image.

With the most common analysis, a digital image of the replica may be obtained by mounting a digital SLR camera (containing a macro lens) above the sample. A typical setup would include a macro copy stand that includes a camera mount and,

in some cases, illumination lights (see Figure 4). If illumination lights are not provided with the system a remote flash system can be used with the camera (Nikon and Canon provide macro flash units that serve this purpose fine). Fiber optic illuminators are also often employed. Regardless of the light source, the replica must be illuminated with oblique light at an optimal grazing angle of 25°. Another alternative for imaging the replica is to use a flatbed scanner. This technology allows high resolutions to be obtained and provides greater depth of field imaging than that available with a standard macro lens used in combination with a DSLR camera.

Figure 4: Illustration of a macro copy stand that includes a camera, macro lens, and illumination source to provide oblique lighting of a skin replica.

Skin replicas are usually analyzed to determine the degree of roughness and density of wrinkling of the skin. It has been most successfully used to measure

photo-damaged skin and premature aging of skin subjected to tobacco smoke.[9,10] A typical replica analysis would be carried out on the perioribital region adjacent to the eye. From the generated image one may perform optical profilometry in which the light intensity of the furrows is measured. Important parameters include the average depth of the furrow, variance between the altitude of the highest peak and lowest peak of the furrows, and the density of furrows.[9]

Skin replica analysis can also be extended to other phenomena in skin, such as monitoring sudoriferous behavior. As an example, Figure 5 contains a skin replica image (obtained with a flatbed scanner) of the volar forearm of an individual exposed to a high-temperature environment (45˚C) for 30 min to induce sweating. Small protruding beads of sweat on the surface of the skin result in dark areas in the image, which can be isolated and quantified using several image-processing and analysis techniques. In this way, the number of active sweat glands per given area can be quantified.

Figure 5: (a) Photograph of the volar forearms of a subject containing replica rings arranged in a four-quadrant layout. (b) Digital image of a cast from the volar forearm region along with (c) its image-processed counterpart containing isolated sweat profusions on a white background (counted). Reprinted from McMullen et al. with permission from the Society of Cosmetic Chemists, © 2013.[17]

c. Fringe Projection Methods to Measure Skin Topography

In addition to replica methods and photography, **skin topography** is also frequently measured using **fringe projection** methods. As shown in Figure 6, a digital projector is employed to project a phase-shifting fringe pattern on an object of interest.

Meanwhile, an image of the fringe pattern on the three-dimensional object is obtained with a camera. Due to surface topography, the fringe pattern is deformed and can be analyzed by a computer algorithm allowing for the generation of a three-dimensional image. For evaluating the surface features of skin the two most popular commercially available instruments are the PRIMOS® (GFMesstechnik GmbH, Teltow, Germany) and DermaTOP® (EOTECH SA, Marcoussis, France) systems.[19] The principal difference between the two instruments is the manner in which the fringe patterns are produced. In the PRIMOS® system, fringe patterns are generated with micro-mirrors while the DermaTOP® system utilizes a template to generate the shadows.[18,20] Optimally, the DermaTOP® FOV HE-25 system performs a measurement on a 20 × 15 mm^2 field of view with 2 μm vertical resolution, 15 μm lateral resolution, and 1 s acquisition time. On the other hand, the PRIMOS® premium system gathers data from a 24 × 14 mm^2 field of view with 2.4 μm vertical resolution, 24 μm lateral resolution, and 70 ms acquisition time.[21] The reader should note that these specifications are only for two specific models. It should be mentioned that each company has a series of instruments with various specifications.

Figure 6: Schematic of a fringe projection (interferometry) profilometer. Reproduced from Lagarde et al. with permission by John Wiley & Sons, © 2002.[18]

Fringe projection techniques are used to measure skin topography directly *in vivo* and also *in vitro* using skin replicas. Skin topography, also referred to as skin relief or texture, is an important feature of the skin that reveals its state of health. Most studies focus on measuring skin roughness and other topographical features,

such as wrinkles and furrows.[22] In addition, these techniques may be also utilized to monitor the skin's health state as associated with lesion malignancy, laser remodeling, radiation treatment, treatment with cosmetic products, and hydration state of the skin.[21] For illustration, Figure 7 demonstrates the use of the DermaTOP® system to monitor the difference between the temple skin (periorbital region obtained with a replica technique) of two subjects, one 73 years old and the other 23 years old. The deep furrow in the middle of the three-dimensional plot can clearly be discerned in the elderly subject (septuagenarian). On the other hand, in the vicenarian (23-year-old) pores are the dominant feature as there are no discernible deep wrinkles or furrows.

Figure 7: Three-dimensional image of the temple (periorbital region) of (a) a 23-year-old and (b) 73-year-old subject. Reprinted from Lagarde et al. with permission from John Wiley & Sons, © 2005.[22]

For further illustration, Figure 8 contains three-dimensional representations (in two-dimensions) of the furrow distribution in the volar forearm region of both subjects (also of a skin replica), along with image analysis markings to quantify the anisotropy of the furrows (i.e., the quantity of furrows oriented in different directions). In the vicenarian subject, it is clear that the furrows form a crosshatched structure where the level of isotropy is high while in the septuagenarian subject the orientation of the furrows is more anisotropic. Findings from these studies can be summarized as follows. Roughness, peak-trough amplitude, and anisotropy of the furrows decrease with age. The density of the furrows, on the other hand, decreases with age.[22] Other important skin surface parameters that can be measured with fringe projection devices include the maximum roughness (R_m), the mean roughness (R_a), mean depth of roughness (R_z), and waviness (W_t).[23] Overall, these techniques are being employed by many different laboratories around the world as newer, more portable devices are becoming available.

Figure 8: Two-dimension representation of a three-dimensional image of the volar forearm of (a) a 23-year-old and (b) 73-year-old subject. Furrow markings generated by an image analysis package are shown. Reprinted from Lagarde et al. with permission from John Wiley & Sons, © 2005.[22]

d. Pore Measurements

Over the last decade, increasing attention has been given to the topic of skin pores—also often referred to as facial pores. In this case, the term "pore" evolved from the lay population to describe an opening in the skin associated with a pilosebaceous unit, which consists of the sebaceous gland and hair follicle. These "pores" are clearly evident to the human eye and are especially visible in certain anatomical regions of the body, such as the face, where they often present themselves in areas adjacent to the nasal region. Such pores are generally associated with excessive sebum production.[24] In most cultures, the visible appearance of these pores is undesirable for many individuals, and for this reason, there have been considerable efforts in recent years by the personal care industry to develop products that reduce pore size.

Not to be confused with pilosebaceous pores, sudoriferous pores also open up at the surface of the skin. Eccrine glands secrete a clear isotonic solution of sweat on the skin's surface to provide an evaporative cooling apparatus for the body. The gland is connected to the surface of the skin by means of the eccrine duct—a long tubular structure. These pores are located on the top of furrow ridges in skin and are not visible by the naked eye. On the other hand, facial pores associated with pilosebaceous units are generally much larger. They can be classified into three different types: visible skin pores (0.1–0.6 mm^2), enlarged skin pores (0.3–0.6 mm^2), and blackhead embedded pores (tonality is lower than the rest of the skin surface).[25] It should be noted, however, that there is not a universal agreement on the classification of pores. There are also such classifications as invisible pores (< 0.04 mm^2), visible pores (0.04–0.07 mm^2), and enlarged pores (> 0.07 mm^2).[26]

Surprisingly, there has not been a great deal of research investigating the nature of facial pores until recent years. The origin and formation of pores is dependent upon a variety of factors including hereditary factors, seborrhea, acne, comedogenic xenobiotics, aging, and chronic UV light exposure.[27]

Recent studies suggest that pore size is greatly influenced by gender and sebaceous activity—males tend to have greater quantities of pores, possibly due to greater androgen activity.[25] In females there is no correlation linking age with pore size or quantity. On the other hand, while acne-prone skin tends to contain greater quantities of pores, there appears to be no correlation between severities of acne and pores.[25] From a different perspective, treatments intended to reduce the size of facial pores are primarily concentrated in the dermatological arena and include topical retinoid therapy, chemical peeling, and laser therapy.

Quantitative Assessment of Pore Size and Quantity

Assessment of pore size and quantity may be achieved utilizing several different imaging techniques. The most basic of these include visual grading,

macrophotography, and evaluation of silicone replicas. Visual grading may be completed in the presence of a panelist or utilizing a photograph. In most cases, it is imperative to correlate visual grading to one of our quantitative techniques. Macrophotography is a rapid, noninvasive strategy for quantifying pores; however, it is very difficult to obtain reproducible results due to challenges with illumination conditions. Nevertheless, utilizing a carefully controlled facial positioning and lighting system, one may accurately count the number of pores and their size. These can be estimated from the area measurements of two-dimensional photographs. Figure 9 contains a photograph obtained with an optical image analyzer called Facial Stage (Facial Stage DM3, Moritex Saitama, Japan). The original photograph and its image-processed counterpart are shown in the figure. The yellow circles in Figure 9c, the enlarged processed image, are representative of facial pores.

Figure 9: Image of visible skin pores obtained with the Facial Stage optical image analyzer illustrating (a) the origal image, (b) processed image, and (c) enlarged region of the selection in (b). Reprinted from Kim et al. with permission of John Wiley & Sons, © 2014.[26]

As already mentioned, silicone replicas are also a useful and inexpensive modality for quantifying pores, although special care must be taken to ensure extremely precise quantities of the silicone resin are combined with the hardening catalyst. Since this is a kinetic reaction, timing is of the utmost importance. Differences in the thickness or flow properties of the silicone-catalyst mixture during application to the skin will affect the polymer's ability to penetrate deep into the pore. To reiterate, silicone replicas can be evaluated by generating macrophotographic images in the presence of oblique light. Alternatively, one may utilize a flatbed scanner to generate the digital images. Our laboratory's experience is that both techniques work equally well.

Fringe projection techniques that generate three-dimensional images of the skin surface topography are also used for quantifying pores and provide an estimate of pore volume. An example of skin pore measurement with this technique is provided in Figure 10 utilizing the PRIMOS® premium system. Studies of pore volume with this instrument demonstrated very good correlation with visual grading by trained panelists, illustrating the contribution of pore volume to the overall appearance of facial pores.[26] Stereoimaging with an optical topometer is also a viable route for quantifying pores.[28]

Figure 10: Analysis of pore volume with the PRIMOS premium system. Reprinted from Kim et al. with permission from John Wiley & Sons, © 2014.[26]

Confocal laser scanning microscopy may also be used to investigate the dimensions of pores.[29] While this technique is extremely valuable for investigating the anatomy of the pore, it is not the instrument of choice for quantifying attributes of large numbers of pores due to its limited imaging area. Nevertheless, pertinent information relating the fine morphological structure of skin with pores is invaluable and may provide avenues for future research focused on the reduction of pore size.

e. Skin Thermography

The ability to measure the temperature distribution and changes in skin is extremely useful not only from a practical point of view, but also for a more profound

understanding of processes occurring within the skin. Skin thermography, or digital infrared imaging, affords the researcher such an avenue to explore various phenomena involving the state of health of the skin. Infrared cameras are designed to measure infrared radiation in the short- to mid-wave (3–5 μm) and long-wave (7–14 μm) regions of the electromagnetic spectrum. These devices essentially measure the emitted energy of the sample. Kirchoff's law provides us with a general relationship between emitted (E), transmitted (T), and reflected (R) energy:

$$E+T+R=1 \qquad (1)$$

In many circumstances, the transmitted energy is close to zero and E+R=1. In general, objects that are good emitters are poor reflectors and good reflectors are poor emitters. Not surprisingly, E=1 in the case of a black body—something that absorbs all incident electromagnetic radiation—while most objects in nature result in E<1. In the case of skin, it is both a good absorber of energy and also a good emitter, where E=0.98. In contrast, aluminum foil is a good reflector (R=0.98), but a poor emitter (E=0.02). Nevertheless, skin is a very good object for conducting thermography measurements.

The use of infrared thermography in the medical field has expanded significantly in recent years due to the increasing availability of infrared cameras. Such studies provide data related to inflammatory conditions (injuries, infections, etc.) as well as vascular activity (angiogenesis, varices, etc.). Typically, infrared images, or thermograms, of skin represent thermal energy emission stemming from the outermost portion of the epidermis down to the dermis, roughly 5 mm deep.

In the personal care arena, there are also various applications of infrared thermography. For example, it can be utilized to monitor skin surface temperature when conducting antiperspirant efficacy studies. In a study described in the literature, subjects were first acclimatized in an environmentally controlled room at 28°C and 40% RH for 30 min, and then placed in a sauna at 45°C and 30% RH for an additional 60 min.[17] During the test, axillae sweating and eccrine sweat pore activity was monitored by gravimetric analysis and a skin replica technique; however, infrared thermography was utilized to monitor the overall anatomical temperature distribution of each subject. Figure 11 contains a series of images obtained for one of the subjects upon entry into the environmental room, then after 30 min in the room, followed by a period of time spent in the sauna chamber. In Figure 11a, the overall surface temperature of the skin is lower than in Figures 11B–11D. This phenomenon occurred because the subject was acclimatized in an office for several hours beforehand at a much lower temperature than in the environmental room. After 30 min in the environmental room (Figure 11B), the surface temperature of the subject begins to increase, especially in the rostral (facial) region and the superior torso. After 10 min in the sauna (Figure 11C), the peak temperature is

reached at the rostral region, superior torso, and lower limbs. The subject begins to sweat profusely after 10 min in the sauna, resulting in an overall cooling effect over all surfaces of the integument as shown in Figure 11D. Subsequent images were obtained at 30, 40, and 50 min, which demonstrated similar behavior as that observed at 20 min. In summary, these data demonstrate that the 30-min acclimatization in the environmental room followed by the first 10 min in the sauna chamber is sufficient to bring the subject to a state of profuse sweating, which can be monitored from that point onward by other tests, such as gravimetric analysis and skin replica techniques.

Figure 11: Infrared thermograms of a subject undergoing antiperspirant studies. Images were obtained to monitor the global temperature distribution of the subject during the test. Images were collected upon (a) entry into the environmental room, (b) after 30 min acclimatization in the environmental room, and after (c) 10 min and (d) 20 min in the sauna. The scale for all of the images is provided on the right and ranges from 28.1°C to 37.6°C. Precise measurements of temperature can be made at every pixel in the image. Reprinted from McMullen et al. with permission from the Society of Cosmetic Chemists, © 2013.[17]

Infrared thermography may also be used to monitor the skin's response to environmental stress, such as exposure to hot and cold temperatures. For example, one may wish to investigate the use of an active ingredient to mitigate the skin's response to stress. To carry out such an experiment, one would treat skin with an active ingredient-containing vehicle in the form of a cream at a dose of 2 mg/cm^2 to the inner forearm of subjects twice per day. After 3 weeks, baseline readings should be

obtained and subjects could be subjected to stress first with a heating pad for 10 min followed by cold pad stress for 10 min (see Figure 12). After application of stressors, thermograms may be obtained every 2–3 minutes for 1 hour. Figure 13 contains an example of a baseline reading and one obtained after exposure to both hot and cold stresses. Since there may be some temperature gradient due to vascularity, care must be taken to obtain temperature readings from selected regions of the images.

Figure 12: Application of (a) hot and (b) cold pads used to induced stress in skin.

Figure 13: Infrared thermograms of the volar forearm comparing (a) a baseline reading versus (b) the arm after it had undergone a hot and cold stress cycle. Images kindly provided by Mihaela Gorcea, Ashland, Inc.

f. Ultraviolet (UV) Reflectance Photography

Skin pigmentary disorders, such as melasma, vitiligo, and hypopigmentation, as well as disorders resulting from photo-damage, are commonly detected with **UV light photography**.[30] This mode of photography is based on the

photo-physical properties of melanin, which absorbs much greater in the UV region of the electromagnetic spectrum than the visible region. Therefore, pigmented and depigmented lesions are more discernible than they would be using visible light photography.[31] In fact, the use of UV light photography in dermatology has been in practice for many years. Using Wood's lamp, which provides broad UVA spectral output with a maximum at 365 nm, dermatologists have been diagnosing pigmentary disorders since the early part of the twentieth century.[32,33] This technique is especially useful for monitoring photo-damage or melasma in subjects with Fitzpatrick Skin Types I–III in the case of hyperpigmentation. On the other hand, it readily views hypopigmentation disorders, such as vitiligo, in subjects with Fitzpatrick Skin Types V and VI.

In general, UV photography is conducted with the same type of cameras employed for light photography with some modifications. In order to carry out such experiments, one should employ a camera that is sensitive to UV light. In film photography, high-speed (e.g., ISO 1600) black-and-white film is used due its enhanced sensitivity to the otherwise weak UV signal, and because color film contains a UV-absorbing protective coating. At present, most digital SLRs can be modified so that the CCD or CMOS sensor is sensitive to UV light. Digital SLRs contain a UV and IR filter pack that only allows visible light to reach the sensor. This can be removed by a specialty camera shop and replaced by a UV transmitting filter in front of the sensor (providing a dedicated UV camera) or over the lens.[34] Lenses that are able to transmit UV light are readily available, and most macro lenses (made of fluorite and quartz glass) that are used for dermatological photography are suitable for this purpose. Finally, xenon flashes are a good source of UV light as long as the flash diffusers are removed from the flashes (these attenuate UV light) and replaced with UV pass filters.[31] The strategic positioning of several flashes around the subject's face is key to obtaining proper illumination conditions.

By definition, UV photography infers that one is working in the spectral range of 300–400 nm. However, in dermatology the combination of UV and fluorescence photography are often carried out in conjunction with each other. In this case, UV (300–400 nm) and visible (due to fluorescence) light is captured in the final image. In any event, UV images of skin provide a sensitive technique for the localization of melanin.

g. Fluorescence Reflectance Photography

Based on similar principles as UV reflectance photography, fluorescence reflectance photography refers to photographic images that are generated by illuminating the skin with UV light (either broad band 300–400 nm or a narrower band in

this range) and then capturing an image in the visible region of the electromagnetic spectrum. In this modality, incoming UV light is absorbed by chromophores, which then fluoresce at longer wavelengths. This type of photography was pioneered at Massachusetts General Hospital by Nik Kollias and colleagues in the mid-1990s to evaluate acne as well as hyperpigmentation in photo-damaged skin.[31,35] In their experiments, chromophores were excited in skin by placing a UV band pass and an IR block filter over the illuminating flashes, allowing only a broad band of UVA light (340–380 nm) to reach the skin's surface. As shown in the diagram in Figure 14, the reflected light from skin passes through a UV block filter before arriving to the sensor (or film) in the camera. In this way, we only view visible light resulting from skin fluorescence.

Figure 14: Diagram of a typical apparatus used for conducting fluorescence reflectance photography.

The illuminating UVA light, centered at about 365 nm, results in absorption of light by melanin (dark in the image) as well as by collagen, which results in fluorescence at 420 nm due to collagen cross-links in skin. In the acquired image, fluorescence due to collagen appears bright, while dark is representative of the presence of melanin. In the evaluation of acne vulgaris, fluorescence occurs due to protoporphyrin IX produced by *Propionibacterium acnes* in open comedones, follicles, and inflammatory lesions.[35]

h. Multi-Spectral Multi-Modal Facial Imaging

Within the last two decades, sophisticated imaging systems have been developed to collect **clinical** (standard white light), **cross-polarized** (diffuse reflectance), **porphyrin fluorescence**, **horn fluorescence**, **collagen fluorescence**, and **excitation light images**.[36] One of the most commonly used imaging systems in dermatological and cosmetic research to capture such images is the VISIA-CR system manufactured by Canfield Scientific (Fairfield, NJ). Utilizing a professional DSLR camera in combination with xenon strobe lights and various filters, images are obtained and then processed with image-processing and analysis software.

Figure 15 contains various images collected with the VISIA-CR system. The standard white light image can be used to analyze furrows, wrinkles, and other topographical and surface textural features of the skin. The diffuse reflectance image, obtained with crossed-polarizers, provides information about backscattered light. This is light that enters the skin, becomes scattered and depolarized, and then exits the skin. Before exiting the skin, the light interacts with skin's internal structures, such as melanin, collagen, hemoglobin, and carotenoids. Diffuse reflectance images provide us with information related to skin pigmentation, erythema, infiltrates, and blood vessels.[4] In fact, the use of cross-polarization photography has been applied to study erythematic inflammatory lesions in acne vulgaris.[3] It should be noted, however, that erythema is observed with much more facility in fair-skinned individuals (Fitzpatrick Types I and II) than those with a darker complexion.

A key feature of the VISIA-CR and related technologies is the capability to resolve fluorescent images resulting from fluorescence of important biochemical components in skin in an automated fashion. Fluorescent photography has been used extensively in the evaluation of acne vulgaris. Table 1 identifies the various filters that are used in the VISIA-CR system to obtain fluorescence images corresponding to specific biochemical features in the image.

Table 1. Excitation and band pass filters in the VISIA-CR system allowing for the visualization of various features in skin.

	Excitation filter	Captured emission image
Porphyrin	405 nm ± 10 nm	Long pass filter (≥ 620 nm)
Horn	405 nm ± 10 nm	Band pass filter (540 nm ± 40 nm)
Collagen	405 nm ± 10 nm	Band pass filter (450 nm ± 40 nm)

As stated in the previous section, porphyrin fluorescence corresponds to protoporphyrin IX produced by the bacteria *Propionibacterium acnes*, which is present in acne vulgaris–afflicted skin. The porphyrin image is red (see Figure 15d) and the brighter orange-red fluorescence, especially noticeable around the nasal region, is attributed to protoporphyrin IX. Cutaneous horn is an outward growth on the skin

surface that is characterized by its keratin content and its similarity to hard keratin structures, such as nails. It is typically observed in patients with acne vulgaris and also in other individuals in areas of the skin typically exposed to the sun, such as the back of the hands and the face. Due to the presence of keratin in horn, excitation at 405 nm results in green fluorescence emission that is captured at a band centered at 540 nm. The green fluorescence image is shown in Figure 15e, with the darkest shades of green in the image corresponding to horn. In the current case, these are observed in the cheek region of an acne vulgaris–afflicted patient in the form of small acne lesions. Collagen fluorescence images obtained with the VISIA-CR have a blue tone due to emission centered at 450 nm. Collagen cross-links are believed to be the source of the fluorescence, although there is some debate in the literature that additional photo-physical studies of collagen are required to better understand its fluorescence.[37] Nevertheless, in dermatological fluorescence photography this phenomenon can be used as an indicator of the skin health state. For example, in acne vulgaris patients, the development of acne lesions can lead to areas where collagen growth is disrupted. In the image in Figure 15f, darker shades of blue in the image correspond to damaged collagen. Likewise, it is well known that photo-damage results in lower fluorescence intensity of collagen cross-links in skin.[38]

Figure 15: Facial images of an acne vulgaris patient generated with the VISIA-CR multi-spectral multi-modal facial imaging system: (a) standard white light image (clinical photograph), (b) standard white flat-lit image (analysis image), (c) cross-polarized image (diffuse reflectance image), (d) porphyrin fluorescence image, (e) horn fluorescence image, (f) collagen fluorescence image, (g) excitation image, and (h) VISIA-CR imaging system. Images kindly provided by Canfield Scientific (Fairfield, NJ).

i. Photographic Analysis of Lips

Imaging techniques are frequently used to evaluate lips; often examining the efficacy of active ingredients or the cosmetic properties of lip products. Often, the evaluation of lipsticks is the first product assessment to come to mind. Typically, product developers are interested in obtaining a quantitative measure of the **color**, **gloss**, **covering power**, **long-lasting effects**, and **streakiness**.[39,40] Traditional color measurements of lips are carried out by reflectance spectrophotometry in the visible region of the electromagnetic spectrum or by reflectance stimulus colorimetry. While these methods are accurate assessments of the lips' color, they are hindered by the small sampling area—generally on the order of mm^2 to cm^2—and because they require contact with the substrate.

Photographic image analysis, on the other hand, allows noninvasive evaluation of the products on the lips and the ability to analyze larger areas. A major challenge with utilizing imaging techniques for the lips is the ability to carefully control lighting conditions and the positioning of the subject. For lipstick and makeup applications, one should utilize a lighting control system that corresponds to daylight (e.g., CIE Illuminant D65) and a suitable table mount for the subject to position the face, such as an opthalmic table (similar to the assembly in Figure 14).[39,41] In any event, reproducibly positioning the subject's facial region is especially challenging as contours, anatomical features, and even changes in facial expressions can affect the way light interacts with the skin surface. Researchers must also decide other factors associated with light sources, such as the use of polarized light to enhance specular reflection or soft light to visualize diffuse reflection. During the course of a day, the consumer can experience of variety of lighting conditions. On a sunny day, direct sunlight causes strong highlights and shadows, corresponding to adjacent specular and diffuse regions on the lips. In contrast, an overcast sky produces more even illumination and a more even tone. Indoors, lighting may also present a variety of situations depending on the light source and if diffusers (commonly found in office environments) are placed in front of or below the light source.

The actual determination of color by image analysis is another unique challenge in which careful calibration procedures must be employed. One study conducted with lipsticks demonstrated the utility of photographically generated images to yield color values in line with that obtained with well-established spectrophotometric and colorimetric techniques.[39] However, this requires a color extraction procedure by applying algorithms to the R, G, and B values obtained from the digital photographs.

j. Hyperpigmentation Measurements of Skin

The development of age spots, hyperpigmentation, and melasma are all forms of melanogenesis. A great deal of research has been completed in this area as many institutions strive to design ingredients that lighten skin. This is especially true

for the Asian market where much of the population suffers from the development of age spots. Age spots develop in the facial region and can be quantified by digital photography in combination with image analysis. Such measurements require reproducible lighting and positioning systems so that serial measurements can be conducted in order to follow the effects of a given treatment regimen. The ultraviolet reflectance photography and multi-spectral multi-modal facial imaging techniques described in the sections above can be employed to quantify hyperpigmentation. Quantifying age spots can be quite challenging and for this reason there is considerable interest in developing reproducible methods to measure spots on the face.[42]

k. Imaging of Cellulite

Cellulite has a high rate of occurrence and is usually found in the buttocks and thigh regions of females. It has a number of medical descriptors—including adiposis edematosa and orange peel syndrome—and is caused by protrusions of fat into the dermis. The formation of cellulite results in a very uneven disposition of the skin surface, which can be captured by generating topographic maps of the surface utilizing fringe projection techniques.[43] Photographic imaging is also a reasonable alternative, although special care must be taken to illuminate the buttocks or thigh region reproducibly in all areas. This may be achieved by utilizing a circular illumination system that surrounds the leg and is coupled to a photographic recording device.[44] Insights into the internal structure of cellulite-afflicted skin have been achieved with ultrasonography, magnetic resonance imaging, optical coherence tomography, and reflectance confocal microscopy.[43] Applications of these methods to study various phenomena in skin are discussed in other sections of this chapter.

11.2.2 *IN VIVO* IMAGING OF INTERNAL FEATURES OF THE SKIN

So far, most of our discussion has centered on imaging surface features of skin. There are also many important phenomena that occur below the surface of skin that we may wish to visualize. In recent years, there has been a plethora of published research demonstrating the use of sophisticated instrumentation to gather information about processes occurring in the skin. The most commonly used techniques to carry out such analyses are laser Doppler perfusion, optical coherence tomography, magnetic resonance imaging (MRI), reflectance confocal microscopy, and ultrasonography. All of these techniques are employed to carry out *in vivo* skin measurements to: monitor blood flow in the case of erythema (laser Doppler diffusion); evaluate skin lesions, inflammatory diseases, and parasitic infestations (optical coherence tomography); measure skin thickness and identify skin lesions (ultrasonography); and for the evaluation of the constituents of the stratum corneum, epidermis, dermis (MRI and reflectance confocal microscopy).[45]

Unfortunately, this entire subject is too vast to review in the current text; however, we shall provide some insights into the use of reflectance confocal microscopy and ultrasonography—the most popular techniques—in the evaluation of the health state of the skin.

a. Reflectance Confocal Microscopy

Confocal laser scanning microscopy, also known as reflectance confocal microscopy, allows for the collection of high-resolution optical images. These images are collected at selected depths within the sample by a process known as optical sectioning. The images are gathered *en face* (horizontal rather vertical) as if one were looking down onto the skin from an aerial view. In the case of opaque samples (not allowing the penetration of light), one can generate a three-dimensional surface image of the specimen. For samples that are not opaque in which light is able to pass through them, such as the skin, interior structures may be imaged. The advantage of reflectance confocal microscopy, in contrast to normal light microscopy, is that the depth of focus is controlled and very limited for each lateral optical section. It is a noninvasive technique allowing for *in vivo* measurements, thereby providing information that would normally require histology. The use of reflectance confocal microscopy to study the skin has rapidly expanded in the last two decades, which can be primarily attributed to technological advances in instrument design. It is mostly used for the diagnosis of benign and malignant lesions associated with nonmelanoma and melanoma carcinomas as well as pigmentary disorders.[15,46-51] The methodology offers the researcher an inside view of the skin at the histological level, allowing one to perform examinations at the cellular level. Nevertheless, reflectance confocal microscopy offers much promise for applications in cosmetology. It has found use in measuring phenomena associated with photo-damage, chronological aging, pigmentary disorders, cellulite, hair biology, wounds, allergic dermatitis, and contact dermatitis.

The principle of reflectance confocal microscopy is illustrated in the diagram in Figure 16. The specimen is illuminated with infrared laser light, which by way of a beam splitter and condenser lens is focused on the sample. Light from the focal plane and out-of-focus planes is reflected towards the detector. However, the use of a pinhole aperture in front of the detector only allows light from the focal plane to reach the detector. In this way all extraneous light—not from the focal plane—will not take part in the formation of the optical section image. The laser light is focused on a small area of the sample. To generate an optical section the laser scans in the entire x- and y-plane. Multiple optical sections are obtained by adjusting the z- location of the focal plane. In this manner, one may generate thin optical section images of the skin, scanning from the stratum corneum interface down to the reticular dermis. For more details on this technique, specifically with reference to the skin, the reader is referred to Reference[51].

Figure 16: Diagram of a typical confocal scanning laser microscope. Reprinted with permission from Carl Zeiss.[52]

For illustration, a photograph of a confocal scanning laser microscope used quite frequently for the noninvasive *in vivo* evaluation of skin is provided in Figure 17. This particular system is a Vivascope 1500 manufactured by Lucid, Inc. (Rochester, NY). The optical resolution in the horizontal direction (*x*- and *y*-axis) is < 1.25 μm while the vertical resolution (*z*-axis) is < 5.0 μm. The field of view is 500 μm × 500 μm. The laser utilized in this system is 830 nm. As indicated in the figure, the probe on the optical head of the instrument is placed in contact with the skin. However, one must first prepare the site of examination by cleaning it with isopropyl alcohol, then applying an oil droplet to the instrument probe and ultrasound gel to the skin site. This approach increases image quality by avoiding large mismatches in refractive index that would otherwise be experienced by imaging directly in air.

Figure 17: Photograph of a common reflectance confocal microscope employed for *in vivo* skin measurements: (a) Vivascope 1500 manufactured by Lucid, Inc.; (b) close-up view of the instrument probe placed in contact with a subject. Photographs kindly provided by Lucid, Inc. (Rochester, NY).

When imaging the skin, the most striking features appear in images obtained from varying depths within the epidermis. Figure 18 contains images obtained at various depths in the skin starting with (a) the stratum corneum, which contains very bright features in the image. The very black areas in the image correspond to wrinkles and fissures in the skin that form part of its normal topography. The extraordinary brightness in this first image is due to the difference in the refractive index between the stratum corneum and the immersion medium (gel), which results in a great deal of backscattered light.[51] It should be noted that in confocal reflectance microscopy, contrast in the images occurs due to variations in refractive indices of organelles and ultrastructural components of the tissue, which in turn leads to variations in the degree of backscattered light. The polygonal-shaped corneocytes shown in the figure are 10–30 μm.

Figure 18: Reflectance confocal images of healthy skin. (a) Images of the stratum corneum are highly refractive (due to location at the air-skin interface). Dark in the image corresponds to folds. (b) A honeycomb structure of the keratinocytes is clearly apparent in the stratum granulosum. Cell nuclei (dark) are surrounded by cytoplasm (grainy). (c) Likewise, the individual cell structure of the stratum spinosum can be clearly discerned. (d) In the case of the stratum basal layer, cells are also clustered. The brightness in the image is due to the presence of both melanin and keratin. (e) Papillary rings (bright – arrow) due to keratinocytes surrounding dermal papillae (dark – asterisk) are found at the dermal-epidermal junction. (f) In the papillary dermis, one may discern reticular fibers associated with capillary vessels (asterisk) surrounded by bright rings (due to basal layer keratinocytes). Reprinted from Tavoloni Bragaa et al. with permission from OMICS Publishing Group, © 2012.[53]

Immediately below the stratum corneum, one may view (b) the stratum granulosum, which features a honeycomb pattern that consists of polygonal-shaped keratinocytes (25–35 µm) that are outlined by their faint cytoplasms that contain dark nuclei in the center of each cell. (c) The stratum spinosum also contains a honeycomb pattern and appears very similar in structure to the stratum granulosum,

although the keratinocytes are smaller in this case. In (d) the basal layer, the basal cells (7–12 μm) are closely assembled together and appear much brighter due to the presence of both keratin and melanin in these cells. An image obtained from (e) the dermal-epidermal junction clearly identifies the presence of dermal papillae that can be identified as the dark regions (asterisks) surrounded by bright rings (arrows) due to basal keratinocytes. In the last image, one may view some features present in (f) the papillary dermis. Bright circles (arrow), representative of reticular fibers, surround capillary vessels (asterisk).[53]

To reemphasize, the power of confocal reflectance microscopy lies in its ability to noninvasively obtain *in vivo* images of the various strata of the epidermis extending down until the upper layers of the dermis. It provides us with pertinent information concerning a variety of disease states in skin. There has been an extensive amount of research over the last two decades investigating the skin with confocal reflectance microscopy, and continuing research in this area should help address many problems facing clinicians and researchers, both in the medical field as well as the personal care arena.

b. Ultrasonography

Ultrasound is an important diagnostic tool in general clinical medicine that allows clinicians to examine the health state of internal organs by the use of a noninvasive procedure. Over the last several decades, advances in ultrasound instrumentation have been developed allowing for the diagnosis of various skin conditions including benign and malignant skin tumors resulting from melanoma and nonmelanoma skin cancer as well as inflammatory diseases (e.g., psoriasis, morphea).[54] More recently, it is has also found use in lipoablation techniques (e.g., to eliminate cellulite) as well as collagen rejuvenation therapy in patients that have undergone surgical procedures.[55,56] Within the realm of the cosmetic industry, it is commonly used to measure skin thickness as well as to differentiate between chronologically aged and photo-damaged skin.[57] Overall, there has been a flurry of research utilizing ultrasound in the dermatologic community.[58] In addition to diagnosing skin ailments, the methodology has found extensive use as a research tool to investigate various phenomena confronting researchers of the skin.[59-63]

Ultrasonography techniques operate on the principle that high frequency sound waves generated by an ultrasound instrument propagate into the tissue and upon interaction with different physiological components return back to the instrument

as echoes. The propagated wave is generated by a piezoelectric device, which converts electricity into sound waves, and the echo (reflecting from the tissue) is also detected by piezoelectric devices, which can also convert sound waves into electric signals. In practice, a ring is placed on the surface of the skin onto which a gel solution is placed. This limits the dissipation of sound waves into the air, which has much lower acoustic impedance than skin or water. In general medicine, frequency ranges of 2–15 MHz are used in ultrasound imaging. This frequency range is useful for imaging structures deep below the skin; however, for skin imaging higher frequencies must be employed. Typically, frequencies of 13.5–100 MHz are employed for imaging the skin, with most high-resolution imaging conducted at 20 MHz.[54] The higher the frequency, the higher is the resolution of tissue closer to the instrument transducers. However, this comes at a cost of imaging range, which decreases with increasing frequency. Extremely high frequencies (e.g., 500 MHz) have been employed to image *ex vivo* skin sections, although the high energy associated with these waves damages tissue and, therefore, is unsuitable for *in vivo* investigations.[64]

The basis of ultrasound measurement relies on the differences of how tissue reflects sound waves, which is greatly influenced by tissue composition and structure (e.g., vascularity and density). In skin, reflective differences can be ascertained for morphological structures based on their keratin, collagen, and water content. It is especially sensitive at the interface of such structures. In a typical B-mode scanning procedure—the preferred mode for skin studies—reflected sounds waves are transformed into two-dimensional luminosity images where low echo regions (hypoechogenic) are dark and high echo regions (echogenic) are light.[54]

For illustration, Figure 19 contains an ultrasound image of melanoma on the ventral forearm. The tumor (black oval structure—middle left) is clearly distinct as hypoechogenic from the surrounding tissue. The epidermis and dermis are clearly distinct in the echogram. In the dermis, the papillary (echo rich) can be differentiated from the reticular dermis. The subcutaneous region is echo poor. This is further illustrated in Figure 20 where we compare (a) undamaged skin with (b) skin that has experienced major photo-aging. The most apparent difference between these two echograms lies in the reticular dermis region, where many echo-poor regions can be found in photo-damaged skin corresponding to zones where connective tissue (e.g., collagen, elastin) has been degraded.

Figure 19: Echogram of a full-thickness section of skin containing a melanoma lesion. Image provided courtesy of Cortex Technology (Denmark).

Figure 20: Echograms of (a) undamaged and (b) photodamaged skin. Images provided courtesy of Cortex Technology (Denmark).

11.2.3 HIGH-RESOLUTION MICROSCOPIC TECHNIQUES FOR IMAGING SKIN

Often, in order to understand real-world phenomena one must delve into the realm of things not readily seen by the naked eye. Like most other tissues and organs, this is also true for skin. In addition to its morphological complexity, there are a number of anatomical structures that occur at the micron and submicron levels. Starting in order of resolving power for the imaging techniques presented in this section, our first discussion involves **reflected light microscopy**. Corneocytes are easily imaged from tape-stripping and cyanoacrylate surface biopsies with this technique. In addition to epi-luminiscence with visible light, we also discuss the application of **fluorescence microscopy** utilizing immunofluorescent staining techniques to the study of protein expression in skin. This is introduced in this section, but much of the associated detail is described in the final section of this chapter.

While light microscopy is less costly and requires less sample preparation, there are times when other modalities, such as scanning or transmission electron microscopy, are preferred. There are certainly advantages and drawbacks to each technique. For example, light microscopy is limited by depth of field, which can sometimes make it challenging when imaging topographical features of skin. In the case of most **scanning electron microscopy** studies, we are hampered by the requirement to coat the sample with a thin metal coating and to operate the instrument under high-vacuum conditions. However, recent advances in scanning electron microscopy technology allow for analysis at low-vacuum and normal climatic conditions (e.g., relative humidity). As discussed below, this offers us a lot of opportunities in the evaluation of the skin. **Transmission electron microscopy** is also an important means to investigate phenomena that occur in the skin. Below, we discuss several examples of its use to investigate the structure and function of skin. Finally, we touch upon some applications of **atomic force microscopy** to image and conduct surface roughness measurements of both individual corneocytes and thin sheets of stratum corneum.

a. Reflected Light Microscopy (Epi-illumination)

Reflected light microscopy, also known as **epi-illumination**, is the technique of choice when performing fluorescence microscopy or investigating samples in which we wish to study topography. In fluorescence microscopy, the sample is first treated with fluorescent dyes. Once in the microscope, the sample is illuminated with a well-defined band of light that corresponds to the wavelength where the dye absorbs light. Its fluorescence can then be monitored utilizing a filter that only allows light corresponding to the emission wavelength of the probe to pass through to the camera (Figure 21). Examples of studies completed with fluorescence microscopy (both *in vitro* with skin cell cultures as well as with thin sections of skin) can be found in the last section of this chapter on histology, immunofluorescence, and image analysis. In fluorescence microscopy the thin skin section or cell culture

system is treated with a fluorescent dye; or a primary antibody along with a secondary antibody that contains a fluorescent probe covalently bound to its structure (the antibody technique is known as immunofluorescence). Whichever probe is utilized, the dye or the dye-antibody combination, these molecules will interact with some molecules in the skin section or cell culture system. Usually, immunofluorescence studies are carried out in order to monitor the upregulation or downregulation of a particular protein. Direct fluorescent dye staining (without the use of antibodies) is used to directly monitor the presence of certain molecules in skin cell cultures and skin sections. A typical example would be the use of Nile red, which is commonly employed to detect lipids (see the example in the last section of this chapter).

Figure 21: Schematic of a fluorescence reflectance microscope.

Typically, reflected light microscopy and fluorescence microscopy are distinguished from each other. In reflected light microscopy (nonfluorescence mode), the sample is illuminated from above with a wide band of visible light and an image is produced from the reflected light, both specular and diffuse. A key advantage to utilizing reflected light microscopy is the ability to resolve surface structural features. Unlike scanning electron microscopy, limited depth of field is a large hurdle with reflectance light microscopy. To circumvent this issue, many microscope manufacturers have designed mechanically controlled optical stages that allow the user to gather "stacks" of images. Multiple images are gathered along the *z*-axis, thus forming a "stack" of images. Essentially, each image (optical section) contains some element of the sample in focus. When all of the images are combined with a computer algorithm, only those elements in focus from each image are salvaged in the final composite image. Thus, by this means we greatly reduce limitations that are normally imposed by limited depth of field in reflected light microscopy. In "close-up" studies of the skin, this is particularly important due to its rich topographic structure.

A traditional reflected light microscope, such as that described in the preceding paragraph, is most useful for studying full-thickness *ex vivo* skin, thin histological sections of skin, or corneocytes. Full-thickness skin can sometimes be challenging, as it is not very flat and depth of field can be an issue. Histological sections of skin are normally treated with a dye that binds to specific entities, such as melanin, in skin. One such example is provided in the final section of this chapter where the Fontana Masson stain is used to detect melanin. *In vivo*, corneocytes can be removed from skin by tape stripping. The size of the corneocytes as well as various other dimensional parameters can be determined by image analysis. Examples of corneocyte images are provided in Figures 22–23. In this case, corneocytes were obtained by tape-stripping the volar forearm. In fact, the images actually show the bottom side of the corneocytes. As illustrated in Figure 22, corneocytes can be removed from the skin in clusters or as individual cells. This, of course, depends on the health state of the skin, its degree of hydration, and other factors. In this particular sample, most of the cells were removed in clusters. Image-processing and analysis procedures may be employed to determine the size and shape of the clusters. Figure 23 contains higher-magnification images of corneocytes obtained by the same method in the (a) dry and (b) wet state. Outlines of the corneocyte cells can be seen in Figure 23a due to the topographical nature of the specimen, which is presumably rougher in the dry state. In Figure 23b, the folds of corneocytes are more evident due to swelling in the wet state.

Figure 22: Optical image of corneocytes obtained by tape-stripping the volar forearm. Images were obtained with an Olympus BX-50 microscope with a 20x objective resulting in an overall magnification of 200x.

(a) (b)

Figure 23: Optical images of corneocytes obtained by tape-stripping the volar forearm in the (a) dry and (b) wet state. Images were obtained with an Olympus BX-50 microscope using a 100x objective resulting in an overall magnification of 1000x.

In a traditional reflected light microscope, it is not feasible to conduct *in vivo* measurements. There simply is not enough space between the translation stage and the objectives of the microscope. For this reason, many portable microscopes with the ability to capture *in vivo* readings have been developed in the last several decades (e.g., Scopeman). It is more challenging and costly to find such portable devices that can reach the resolution levels of traditional microscopes, which with a combination of objectives can attain up to 1500–2000x magnification. Nevertheless, such devices are important clinical and research tools that are used frequently due to their portability and ease of use.

b. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is a commonly used technique for examining the surface of samples. It is especially useful in cases where a large depth of field is required, such as that found in the skin. SEM has it downfalls as well. For example, most SEM instruments operate under high vacuum and require coating nonconductive samples with a thin layer (10 nm) of metal (e.g., gold, platinum, etc.) prior to analysis. The metal coating makes the surface conductive and prevents charging (white hot spots) on the surface. The principle of SEM is based on the use of an electron beam, either an electron gun or a tungsten filament source, which is focused through a series of electromagnetic lenses onto the sample. Electrons are either backscattered from the sample and collected with a backscattered electron detector or collected with a secondary electron detector. Images captured with the backscattered detector show differences in composition of the sample, while those captured with the secondary electron detector reveal morphology and topology. Most SEM images that we are accustomed to seeing are imaged using the secondary electron detector. In recent years, advances have been made in SEM instrument technology—ultimately leading to the development of the environmental SEM (ESEM)—allowing researchers to study biological samples at low pressure, in the wet state, and without metal plating.

Most of the work in the area of skin care related to SEM has focused on the evaluation of the outermost stratum corneum structure.[65,66] In most cases, SEM images are generated from positive casts constructed from skin replicas and examined for general topology and exfoliaton patterns of stratum corneum cells.[67] Corneocyte boundaries and points of tear or disengagement of stratum corneum cells from each other can easily be identified. In the more recent literature, the use of cryo-SEM shed light on the activity of moisturizing agents, specifically revealing that all stratum corneum cells are not hydrated to the same extent. In fact, centrally located corneocytes are more susceptible to hydration than those in the upper and lower regions of the stratum corneum.[68] Figure 24 contains representative cross-sectional images obtained for skin treated with various moisturizers. As illustrated in the figure, the stratum corneum can be broken down into three different zones of water uptake capacity. The upper and lower stratum

corneum are nonswelling regions while the middle region has a greater capacity to swell. Overall, the cryo-SEM technique demonstrates the ability of SEM-based techniques to provide information not available with traditional SEM. Looking forward, both cryo-SEM and ESEM offer much promise in the evaluation of skin in terms of its health and to study the impact of novel ingredients and delivery technologies.

Figure 24: (a) Cryo-SEM image of a dermatomed skin cross-section equilibrated to 80% RH after cryo-fixation and cryo-planing. The stratum corneum (SC), viable epidermis (EPI), and dermis (DER) can clearly be distinguished. Arrows in the epidermis indicate the presence of keratinocyte nuclei. (b) The stratum corneum can be divided into three distinct regions based on its hydration properties: upper non-swelling region (UNSR), swelling region (SR), and lower non-swelling region (LNSR). (c) Cross sections of skin treated with various moisturizers: isostearyl isostearate (ISIS), isopropyl isostearate (IPIS), glyercol monoisostearate (GMIS), Vaseline (VAS – petrolatum), glyercol, glucose, hydroxyethyl urea (HEU), and saccharose. Reprinted from Caussin et al. with permission from John Wiley & Sons, © 2007.[68]

c. Transmission Electron Microscopy (TEM)

Many of the intricate details we know about skin structure have come about from **transmission electron microscopy** (TEM) studies. One need only to peruse some of the classic literature about skin structure and function to fully appreciate its contributions to the field.[69-71] Transmission electron microscopy is a remarkable high-resolution technique in which electrons are focused by electromagnetic lenses onto an extremely thin specimen and then arrive on the other side to create an image on the detector. The image projected on the detector depends on how the electrons interact with the thin specimen. The sample preparation in TEM is fairly involved and in most cases requires that the specimen be stained with vaporized heavy metals, such as osmium tetroxide or ruthenium tetroxide. For the most part, TEM has been used to elucidate the ultrafine structure of the epidermis, dermis, and skin appendages. Our current understanding of the process of cellular differentiation in the epidermis was made possible due to pioneering work with TEM. It is capable of imaging cellular organelles, macromolecules, and lipids with extremely high resolution. In fact, many of the details we know about stratum corneum lipid structure have come from TEM studies.[72] The current view of the organization of the structural proteins (elastin and collagen) of the dermis and dermal-epidermal junction are in part due to early studies conducted with TEM.[71] Regrettably, there are not as many research groups that specialize in TEM analysis of skin as there used to be; probably due to cumbersome sample preparation procedures and budgetary constraints. This is unfortunate, as many of the ingredients used in modern-day skin care formulations claim to modify the ultrafine structure of skin.

For illustration, Figure 25 contains a TEM image of (a) the viable epidermis and (b) stratum corneum stained with osmium tetroxide. Essentially, a portion of the stratum spinosum, stratum granulosum, and stratum corneum are shown in Figure 25a. The lowest stratum of skin (in this case stratum spinosum) is shown in the bottom left corner of the image, while the stratum corneum may be observed in the upper right corner. The stratum granulosum is sandwiched between them. It is difficult to discern the boundary between the stratum spinosum and stratum granulosum; however, one may notice the cell nuclei of one of the stratum spinosum layer keratinocytes in the bottom left of the image. The stratum granulosum is the area in the middle of the image that runs diagonally from the upper left corner of the image to the lower right corner. Keratohyalin granules, which contain profilaggrin, can be seen as electron-dense islands within this layer. In Figure 25b a higher magnification view of the stratum corneum is shown—in this case staining was carried out with ruthenium tetroxide. As evident in the image, ruthenium tetroxide is a better stain for picking up lipids. Figure 25c provides a closer look of the region indicated by the arrow in Figure 25b in which

the arrangement of lipid bilayer lamellae in the stratum corneum can be clearly visualized. We should also note that the bright white regions in Figure 25b are an artifact of the staining procedure where the ruthenium tetroxide has digested portions of the corneocyte.

Figure 25: TEM images of various regions of the epidermis obtained from thin sections of human skin. In the first image (a) portions of the stratum spinosum, stratum granulosum, and stratum corneum are shown. In the bottom left corner of the image (arrow), one can discern the nucleus of a stratum spinosum keratinocyte. The region extending diagonally from the upper left corner to the lower right corner in the image corresponds to the stratum granulosum. Notable features in this layer are the keratohyalin granules (darkly stained regions). The stratum corneum is mostly confined to the upper right corner of the image. (b) A magnified view of the stratum corneum in which three corneocytes are separated by a thin light band corresponding to lipid lamellae. Dark regions along the lamellae correspond to desmosomes. (c) Higher magnification of the stratum corneum highlighting the lipid bilayer lamellae between corneocytes. Image (a) provided courtesy of Dr. Gopi Menon, Ashland, Inc. Images in (b) and (c) reprinted from Menon and Elias with permission from Springer, © 2001.[73]

Figure 26: TEM image of a thin section of epidermis that underwent a tracer study. Colloidal lanthanum is an electron-dense tracer that leaves a dark trail in the image. It was injected in the dermis and can be seen migrating from the bottom to top of the epidermis through extracellular space in the viable epidermis. It does not reach the stratum corneum. The insets in the bottom right corner illustrate the ultrafine structure of lamellar bodies. Reprinted from Menon and Ghadially with permission from Wiley-Liss, © 1997.[74]

Another example of the use of TEM in the investigation of the epidermis is shown in Figure 26. In this case, a tracer study was carried out utilizing colloidal lanthanum. It is an electron-dense substance that upon injection into the dermis follows the nutritional supply line from the dermis up until the upper layers of the viable epidermis. The electron-dense tracer is present in the dark regions (indicated by the arrows) in extracellular space surrounding the keratinocyte. It stops its migration at the region of the stratum granulosum where the lamellar bodies are secreted. To provide further perspective, insets of the lamellar bodies (much higher magnification) are shown in the bottom right corner of Figure 26.

As an illustration of the use of TEM to investigate the properties of cosmetic ingredients, the image in Figure 27 contains a lipid barrier function enhancer sold under the trade name ProLipid 141 (Ashland, Inc.) which contains various ingredients: glyceryl stearate, behenyl alcohol palmitic acid, stearic acid, lecithin, lauryl alcohol, myristyl alcohol, and cetyl alcohol. The product was deposited onto a nylon membrane and allowed to dry. Close inspection of Figure 27 reveals lipid bilayer lamellae, which present themselves as light bands within the larger dark bands of the image. In this case, the sample was stained with ruthenium tetroxide, allowing for clear visualization of the lipid layers.

Figure 27: TEM image of a specialty ingredient product used in the cosmetic industry to fortify the stratum corneum lipid barrier. It is comprised of the following ingredients: glyceryl stearate, behenyl alcohol palmitic acid, stearic acid, lecithin, lauryl alcohol, myristyl alcohol, and cetyl alcohol. The product was dried on a nylon membrane. The lipid bilayer lamellae are evident as white bands within the black bands in the image. Image courtesy of Gopi Menon and David J. Moore, Ashland, Inc.

d. Atomic Force Microscopy (AFM)

Scanning probe microscopy (SPM) is a plethora of techniques used to study the surfaces of substrates. Most of the SPM modes are quantitative and operate by measuring the interaction of a stylus probe with the investigated specimen. **Atomic force microscopy** (AFM) is one of the techniques under this umbrella and consists of measuring the deflection of the cantilever portion of the stylus as the tip comes into contact with the sample.

As illustrated in Figure 28, a laser is reflected on the back of the cantilever, and as it moves in the *z*-direction due to topography of the sample, the position of the reflected laser on the photodetector is monitored. Therefore, by scanning in the *x*-*y* plane we can generate a three-dimensional image of the skin surface. One should be forewarned that imaging intact *ex vivo* skin is extremely challenging. Most AFM studies of skin have been carried out on individual corneocytes or isolated sections of skin.[75-77] After tape stripping, corneocytes can be isolated from the tape by dissolution in hexane and then mounted on an AFM stud. Various features of the corneocytes can be quantified including topology, rigidity, and friction.[76]

Figure 28: Schematic of a typical AFM instrument.

Figure 29 contains the images from (a) corneocytes obtained from the upper stratum corneum and (b) from the lower stratum corneum. Surface height measurements of the corneocytes (straight horizontal line in Figure 29) illustrate distinct topology for corneocytes obtained from the most superficial layers of stratum corneum, which have much rougher surfaces than corneocytes obtained

from deeper layers. Differences in corneocyte topography have also been discerned for corneocytes isolated from distinct anatomical regions as well as samples obtained from subjects representing various age groups and individuals with pathologic skin conditions.[75] Other AFM studies have focused on characterizing structural proteins of skin, such as collagen, in terms of adhesion and elasticity.[78] A number of studies have also been conducted to better understand stratum corneum lipid organization, although these studies were carried out on lipid models of skin lipids.[79,80]

Figure 29: AFM images of corneocytes obtained from (a) superficial and (b) deeper layers of the stratum corneum. In each image a horizontal line is drawn to demonstrate the utility of height measurements, which are shown in the corresponding graphs below. Images kindly provided courtesy of Dr. Guojin Zhang, Ashland, Inc.

As already indicated, imaging intact skin is extremely difficult due to its extremely high roughness profile owed to furrows and fissures on the surface. In many circumstances, an AFM probe will twist on its side due to the height differences between peaks and valleys in the skin's topography. With care and precision, stratum corneum sheets can be isolated from *ex vivo* skin utilizing a trypsin digestion method.[81] Figure 30 contains an image of isolated porcine

skin stratum corneum that was (a) treated with water or (b) treated with sodium dodecyl sulfate. A clear distinction can be made between the two samples. In the sodium dodecyl sulfate treated sample, the boundaries of individual corneocytes can clearly be discerned due to solubilization of skin surface structural lipids. In many instances, AFM is used as an imaging device. However, it should be emphasized that its true power is in quantitative measurements of micron- and nanoscale phenomena. For example, it would be extremely instructive to perform nano-indentation measurements with AFM, yielding modulus information and other pertinent information to better characterize skin—both to investigate contributions from various morphological features as well as to evaluate the influence of treatments. In any event, there are a number of scanning probe microscopy tools (or modes) that have not yet been explored in the investigation of skin. Hopefully, these technologies will help those researchers embarking on future imaging adventures with the skin.

Figure 30: AFM images of sheets of porcine stratum corneum treated with (a) H_2O and (b) 50 mM sodium dodecyl sulfate for 20 minutes. Images kindly provided courtesy of Dr. Guojin Zhang, Ashland, Inc.

11.2.4 IMAGE ANALYSIS TO QUANTIFY HISTOLOGICAL AND IMMUNOFLUORESCENT STAINING OF *EX VIVO* SKIN AND *IN VITRO* SKIN CELL CULTURES

Numerous *in vitro*, *ex vivo*, and *in vivo* tests exist for the determination of expression of biological molecules as related to skin immunological status, pigmentary changes, and skin aging. Often, these tests are carried out utilizing *in vitro* cell

cultures, such as keratinocytes, fibroblasts and adipocytes, or with *ex vivo* skin. After a treatment protocol is administered, the substrate to be investigated may be treated with a histological stain, or an immunofluorescent dye, allowing certain features in the sample to be observed with an optical microscope. Typically, these features are the result of the interaction of the probe with biological molecules, which play a vital role in cellular function. Traditionally, qualitative comparison of images obtained from microscopes was the widely accepted analytical approach. However, the advent of image-processing and analysis methods offers great utility for conversion of microscope analysis from a qualitative to quantitative technique. In this section, we review how image analysis may be employed to quantitatively characterize data obtained from microscopic images of *in vitro* cultures of skin cells and thin sections of *ex vivo* skin. Through the use of various algorithms and image analysis techniques, specific features of interest in the image may be segmented and properly measured.[82-84]

In general, there are three typical steps involved in image analysis, which include image capture, segmentation, and measurement. Image capture involves the acquisition of an image, which, in the examples shown below, was obtained utilizing a light or fluorescence microscope equipped with a charge-coupled device (CCD). Segmentation consists of isolating objects of interest in an image that we wish to quantify. Some of the segmentation procedures discussed in this section consist of image threshold (conversion to binary image), color segmentation, or the use of image filters (e.g., Gaussian, Unsharpen mask, etc.). In terms of measurements, we are most interested in measuring the stained area in a given field of view, measuring the length of an object, and quantification of image histograms.

a. Measuring Pigmentation of Histological Skin Sections

The Fontana Masson staining technique is commonly employed to measure pigmentation effects such as skin whitening, tanning, and hyperpigmentation disorders. The staining procedure consists of treating a thin histological section of skin with silver nitrate ($AgNO_3$). As a result of treatment, $AgNO_3$ reacts with melanin to produce metallic silver (Ag) resulting in a black stain that can be visualized with a light microscope. Figure 31 contains photomicrographs of *ex vivo* skin sections that were treated with the Fontana Masson stain. The skin in Figure 31a was treated with a placebo, whereas that in Figure 31b was treated with a skin-whitening active ingredient. Upon visual inspection, one may deduce that less staining occurs in the active ingredient treated sample.

Figure 31: Photomicrographs of thin sections of skin stained with the Fontana Masson dye. Dark features in the images correspond to the amount of melanin present. Photomicrograph images for (a) placebo and (b) active ingredient treated samples are shown to demonstrate the skin whitening effect of an active ingredient. The placebo (c) and active ingredient (d) samples are then subjected to image-processing steps consisting of segmentation and grayscale conversion. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.[84]

A first step in the image analysis of the micrographs described above is to perform image segmentation. In this case, the image is segmented by isolating the epidermis on a white background by carefully selecting everything above the stratum basal layer up to and including the intact stratum corneum. The dermis and the layers of the stratum corneum that become dismembered (an artifact of the skin-sectioning technique) are removed so that their contributions are not considered in the analysis. The histogram of a grayscale image provides a distribution of the pixels in an image as a function of luminosity, in this case on a scale of 0–255. Histograms for both images (from Figures 31c and 31d) are shown in Figure 32. In

each case, a large peak at high luminosity values is apparent in the histogram and corresponds to the white background in the images. In agreement with the histogram, the Area Under the Curve value obtained for the placebo (40 ± 7) is greater than that for the active ingredient (29 ± 7) treated sample. As we were interested in quantifying the dark features of the image that result from Fontana Masson staining, the Area Under the Curve was calculated within the limits of 0–175. The value of 175 was chosen after performing image thresholds to determine at which point in the histogram the dark features corresponding to the stain started to appear. Care must be taken when choosing this value as it can be influenced by experimental factors, such as light source intensity and quantity of stain employed. Therefore, it is advisable to calibrate the image analysis measurements, CCD, and microscope by taking an image of a photographic Macbeth card prior to each imaging session.

Figure 32: Image histograms corresponding to the processed images in Figures 31c and 31d. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.[84]

b. Immunofluorescence Staining Quantification in Fibroblast Cell Cultures

A common technique for the analysis of protein expression in cell culture systems is to utilize a primary antibody in conjunction with a fluorescent-labeled secondary antibody for fluorescence microscopy visualization. In the example provided, it is demonstrated how cytochrome c, in this case the antigen, can be detected by first using a primary antibody to bind to cytochrome c and a secondary antibody (containing a covalently attached fluorescent probe, Alexa Fluor 488) to bind to the primary antibody. Figure 33 contains fluorescent photomicrograph images of (a) control and (b) active ingredient treated samples of skin fibroblasts. The interest in monitoring cytochrome c expression is because of its function as an electron carrier in the electron transport chain present in the inner membrane of mitochondria. It has been shown that increasing the level of cytochrome c expression in the mitochondrial inner membrane results in a corresponding increase in the production of adenosine triphosphate (ATP), a critical source of energy for many cellular events. Comparison of the two images in Figure 33 reveals greater expression of cytochrome c in the active ingredient (Figure 33b) treated fibroblast cell culture system than the placebo (Figure 33a). As an alternative to the approach presented with the Fontana Masson staining experiment, the fibroblast images can be converted to grayscale images and then to binary images that contain only black and white, in which case white corresponds to the green fluorescent stain (see Figure 34). One may then determine the area occupied by white pixels and normalize this value to the number of cells present in the field of view.

Figure 33: Fluorescent photomicrographs of fibroblasts treated with an antibody labelled with Alexa Fluor 488 to monitor mitochrondrial cytochrome c expression in (a) placebo and (b) active ingredient treated samples. The total number of cells present in the field of view was quantified. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.[84]

Figure 34: Conversion of images shown in Figure 33 to binary images corresponding to (a) placebo and (b) active ingredient treated samples. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.[84]

c. Image Analysis of Triple-Stained Normal Human Keratinocytes (NHKs)

Often, one may wish to monitor the presence or expression of several different molecules in cell culture. One component that is almost always monitored is the cell nucleus, which is stained with DAPI, a molecular probe characterized by λ_{ex} = 358 nm and λ_{em} = 461 nm. DAPI binds to the inner groove of DNA present in cell nuclei and results in a blue emission that can be seen in the fluorescent microscope. In this particular example, there are two other stains: Nile red and AlexaFluor 488, which result in red and green fluorescence, respectively, in the cell culture. These stains are normally viewed by inserting filters in the microscope allowing for each component stain to be visualized individually. The Nile red stain penetrates lipid droplets and is used as an indication of lipid bodies present in the cell culture. As discussed in the section above, Alexa Fluor 488 is covalently attached to a secondary antibody which binds to a primary antibody. *The primary antibody interacts with the antigen*, in this case, HMG-CoA reductase. It is a regulatory enzyme in the mevalonate pathway, which eventually leads to the biosynthesis of cholesterol. Thus, HMG-CoA reductase expression correlates to normal human keratinocyte (NHK) cholesterol levels.

An example of a triple-stained NHK is provided in Figure 35. Staining of DAPI in cell cultures not only allows us to visualize the cell nuclei, but also permits facile quantification of the number of cells in a given field of view. This allows us to normalize the data obtained from the Nile red and Alexa Fluor 488 stains. A representative example of the image-processing steps and subsequent counting of cell nuclei stained with DAPI is shown in Figure 36. First, the original image is subjected to a color segmentation procedure in which a range of blue colors are selected in the image and are shown in red in Figure 36a. Then, the cell nuclei are isolated by converting the color-segmented image to a binary image. In the next step—conversion of Figures 36b and 36c—the binary image undergoes an image inversion. This step is performed for aesthetic reasons rather than necessity. In the last stage (Figure 36d), all of the nuclei in the field of view are counted.

Figure 35: Triple-stained normal human keratinocytes using (a) DAPI, (b) antibody-labelled with Alexa Fluor 488 and (c) Nile red. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.[84]

Figure 36: Series of segmentation steps for the analysis of DAPI staining: (a) color segmentation, (b) isolation of nuclei, (c) image inversion and (d) cell counting. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.[84]

The Nile red and AlexaFluor 488 fluorescence are quantified in a manner similar to that already-discussed Sections A and B. The micrographs are transformed to grayscale images followed by generation of image histograms. This step allows for the quantification of the area under the curve, which is normalized by counting the number of nuclei (DAPI fluorescence) in a given field of view. Although the data are not shown, this type of analysis is extremely useful for testing NHKs treated with active ingredients that upregulate HMG-CoA reductase (green fluorescent image) and lipids (red fluorescent image).

d. Measurement of Collagen IV Expression at the Dermal-Epidermal Junction

The dermal-epidermal junction is the boundary between the dermis and epidermis, and serves a structural function of paramount importance in binding the stratum basal layer cells with the underlying papillary dermis. Principally, it is composed of structural proteins such as laminins and collagens. From the collagen family, collagen IV is one of the most predominant members present and provides much of the structural architecture for the dermal-epidermal junction. Therefore, it is of interest to measure the amount of collagen IV present in *ex vivo* skin sections, as it is indicative of the health state of this segment of the skin. Figures 37a and 37b contain images of skin sections, stained with DAPI and a primary-secondary antibody system tagged with Alexa Fluor 488, for a placebo and active ingredient treated sample.

To prepare the images for image analysis, they were both segmented by removing all portions of the image below the dermal-epidermal junction and above the stratum corneum. The remaining viable epidermis and dermal-epidermal junction were isolated on a black background. The segmented images are shown in Figures 37c and 37d. The images were also calibrated so that distance measurements could be obtained along the length of the dermal-epidermal junction. The next image-processing step was to color segment the images by selecting the range of green colors that appear in the image because of Alexa Fluor 488 fluorescence, corresponding to the presence of collagen IV. The selected color-segmented portion of the image was then transferred to a black image background, as shown in Figure 38, resulting in a binary image. The next step would be to generate histograms of the processed images to quantify the luminosity.

Figure 37: Fluorescent photomicrographs of thin skin sections stained with DAPI and antibody-labelled Alexa Fluor 488 to monitor collagen IV expression at the dermal–epidermal junction. Images are shown for (a) placebo and (b) active ingredient treated samples. The (c) placebo and (d) active ingredient treated sample are also shown after image segmentation and calibration. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.[84]

Figure 38: Color segmentation of the fluorescence that appears at the dermal–epidermal junction (as a result of collagen IV expression) in Figure 37. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.[84]

CONCLUSION

The world around us has changed so much over the last two decades. Advances in technology have changed the way that we communicate and socialize on a grand scale. Imaging science has benefited greatly due to these strides in technology. Thinking back to the late 1990s, it was still not possible to acquire a DSLR camera with resolution on parity with its film SLR counterpart. Nowadays, it is common for most laboratories and clinical centers to have a multi-spectral multi-modal image analysis system offering advanced imaging solutions in the evaluation of skin aging, melanin production, erythema, and acne vulgaris.

The development of fringe projection methods revolutionized the way we look at skin topography. Other complex instruments, such as the reflectance confocal microscope, experienced a boom in development leading to many new ways to look inside the skin. Now we can perform *in vivo* studies that provide histological-type information to clinicians and researchers. Likewise, image analysis techniques have also come a long way thanks to advances in computer software and hardware technology.

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BIOPHYSICAL MEASUREMENT AND EVALUATION OF SKIN ELASTICITY AND TOPOGRAPHY

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ABSTRACT

The skin is the only organ of the human body that allows an assessment by sensory perception: seeing and feeling. However, the quality of this subjective assessment strongly depends upon the clinical experience of the investigator and while "one picture is worth a thousand words," it is, unfortunately, prone to bias and inter-observer variation. Further, some subclinical morphological changes and physiological processes of the skin are not visible to the naked eye or cannot be assessed by tactile senses. **Biophysical measurement systems** fill this gap, as they allow a highly objective measurement of functional and morphological skin conditions. Since cosmetic science has become a field of high-tech research and development, biophysical measurement systems are an essential part of the evaluation process to determine the efficacy of cosmeceuticals.

Nowadays, a broad range of highly specialized skin care products are available on the market for any imaginable indication and consumer need. *However, anti-aging products that target the signs of aging are still the most important category when it comes to cosmeceuticals.*

Aging of the largest organ in the body, the skin, is a complex process that comes along with the natural aging process of the human body and results in several functional and aesthetic changes of the skin.

Two main mechanisms, atrophy and changes in the architectural organization of the dermal connective tissue, are involved in lifetime alterations of the human skin. They lead to a progressive loss of elasticity and the development of facial wrinkles. These signs are considered to be the most visible changes due to aging, and are therefore strongly related to the development of numerous anti-aging treatments and cosmetic procedures. In this context, biophysical measuring methods to objectively evaluate skin topography and elasticity are of particular interest for the assessment of anti-aging skin care.

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11.3.1 SKIN TOPOGRAPHY

The skin surface is a polygonal relief consisting of furrows, wrinkles and wavelike structure representing the three-dimensional organization of epidermis, dermis, and subcutis. It is influenced by a wide range of morphologic characteristics such as the thickness of the *stratum corneum*, the amount of collagen in the dermis, or the size and structure of subcutaneous fat cells [1; 2].

The skin surface relief is characterized by typical patterns of intersecting lines and regularly dispersed follicular and eccrine duct openings. Primary and secondary lines form a criss-cross pattern consisting of triangles and parallelograms that are particularly well marked on the back of the hand and forearms. The tertiary lines correspond to the edges of epidermal corneocytes and are,

with a depth of <0.5 µm, not visible to the human eye. The quaternary lines are also exceedingly thin, forming a discrete trabecular network on the corneocyte membrane itself [3; 4].

Due to the aging process the skin relief changes strongly (Figure 1); whereas some lines become more marked which results in the development of wrinkles, other lines flatten and disappear with aging. Although, a generally accepted definition of the term "wrinkles" does not exist, they loosely can be classified in crinkles and linear rhytides [4; 5]. Whereas crinkles disappear when the skin is slightly stretched, linear rhytides will still be visible [6].

Figure 1: Skin topography, imaged by Visioscan®**, at the forearm of young (left) and older (right).**

a. Quantitative assessment of skin topography

Over the years, many quantification methods have been developed to objectify skin topography, including two-dimensional (2D) morphological analysis of skin replicas as well as fringe projection method that can be used directly without replicas [1; 7]. Recently, three-dimensional (3D) photogrammetry, also known as stereophotogrammetry, has been added to the armamentarium to noninvasively assess skin topography.

b. Replica-based methods

Profilometry [8] and shadowing method [9] represent techniques using two-dimensional analysis of replicas to evaluate skin surface topography. The most widely used material in the preparation of skin replicas is a monomeric silicone polymer (Silica®, J. & S. Davis, Hertfordshire, UK) that is mixed with a thinner and a catalyzer. Fluidly applied, it spreads onto the skin surface by virtue of its low interfacial energy. At body temperature, it quickly polymerizes to a rubbery mass without noticeable heat of reaction. As the silicone is not hard enough to allow the mechanical measurement, a hard counter-replica has to be prepared [4].

A **mechanical profilometer** is a stylus instrument that is used to measure skin surface roughness [8; 10]. The profilometry technique scans the microrelief of the skin replica with a diamond stylus moving at a constant speed horizontally across the samples. Therefore, deflections and movements of the stylus's tip are directly transformed into an electrical signal as the stylus is moved across the surface. Usually the diamond stylus has a conical shape with a diameter of 5 to 20 µm [1]. The precision of the measurement directly depends on the size of the diameter; the larger the diameter, the more imprecise the measurement, so the narrowest lines or steep increases cannot be precisely scanned [4; 11].

Figure 2: Schematic representation of mechanical profilometry

An advancement over mechanical profilometry is **optical laser profilometry** [12]. This noncontact technique is based on the principle of dynamic focusing: a lens focuses a spot of reflected laser light onto a photodetector, which generates a signal that is proportional to the spot's position on the detector. As the target's surface height changes, the image spot shifts due to the parallax [1].

Figure 3: Schematic representation of laser profilometry

The profiles of roughness and waviness assessed by mechanical or optical profilometry are calculated by a computer software such as MapVUE® (KLA Tencor, Milpitas, USA) or Nanovea 3D (Irvine, USA). This technique generates a variety of useful surface parameters that correspond to the international standardization ISO 4284/4287. They include parameters such as Ra (mean value of roughness), Rmax (maximum single depth of roughness), Rz (mean of five measurements of depth of roughness) [1].

Profilometry techniques have the advantage of assessing skin topography with a high degree of accuracy and precision (<0.5 µm), but the scanning of skin replica is very slow [4] and large skin structures such as cellulite cannot be evaluated. Due to the mechanical load on the skin replica, material properties can be changed so that further measurements might be defective [11]. In addition, the preparation of replicas is often defective, and there is a high risk of losing accurate morphological information during the replication process [1; 9].

A more rapid method employed to assess skin topography is the **shadowing method** (also: shadowing casting method), which was developed in the early 80s. This imaging technique is contact free and is also based on the evaluation of empirical parameters generated from skin replicas [9].

The replica of the skin surface is irradiated by a parallel-beam light source with a defined incident angle. Bot, the light intensity and its incident angle, significantly influence the measurement and have to be chosen according to the depth

and density of the relief [4]. The generated pattern of light and shadows is optically recorded by a camera. In order to assess the structure of the whole skin replica, the object is slowly rotated and imaged after every movement [13]. The underlying technique is based on the principle of triangulation.

The principal advantage of the shadowing method is that the imaging survey is about 15 times faster by comparison to the profilometry technique—whereas at the same time, a larger area can be assessed. It is further contact-free, so that the skin replicas are not altered during the measurement. A disadvantage of the method is that small structures can probably not be assessed precisely because large structures obliterate smaller structures [9; 14]. Also, the individual adjustment of the angle of the incident light as well as the intensity of lighting in general might contain errors [4].

Figure 1: Working principle of the shadowing method

c. 3D Photogrammetry

Recently, digital 3D photogrammetry, also known as **stereophotogrammetry,** has become available for the noninvasive capturing of skin topography. This method is based on a group of cameras that capture two or more images of the same subject from different angles simultaneously. The digital processing of the captured

images allows the three-dimensional reconstruction of the surface, which can be quantified in terms of angles, surface areas, and volume [15; 16]. For the assessment of anti-wrinkle products, the volume parameter has become one of the most important parameters as it is most suitable to show a change in overall wrinkle severity before and after a treatment. While the technique is only minimally sensitive to changes in the skin reflection, hair and other small objects on the skin surface are a common cause of artifacts and should therefore be removed carefully before images are taken.

Several devices with this technique are commercially available. The most commonly used system in cosmetic science is the VECTRA® M3 Imaging System (Canfield Scientific, USA). This system consists of three pods with two cameras and one flash each and a connected computer system. Synchronized 2D images of the subjects with 36 megapixels are captured with the six cameras within 3.5 milliseconds. The subsequent 3D reconstruction process is displayed on the monitors and takes up to several minutes. After completion, the 3D model will be mapped with 2D textures and the captured structure can be rotated in all three dimensions. The acquired 3D coordinates can be used to calculate distances between points allowing the measurement of volumetric changes [17; 18]. Several 3D models can be matched by manually selecting landmarks to provide the system with reference points [15; 16].

3D photogrammetry is capable of accurately reproducing the surface geometry of the human face, and map realistic color and texture data to the geometric shape [19; 20]. However, published studies evaluating reproducibility and accuracy of the 3D analysis show a measuring accuracy of less than 1 mm [15; 18; 20]. As age-related changes in skin topography are characterized by typical patterns in the micrometer range, stereophotogrammetry is not suitable as a measuring device for wrinkles or small volume changes yet [21]. Nevertheless, photogrammetry systems already have a great impact in the field as they are particular suitable for the clinical rating of skin statuses before and after a treatment [16; 18; 22].

d. Fringe projection method

The latest generation of 3D measuring instruments makes it possible evaluate morphological structures of human skin rapidly, precisely, and directly *in vivo*. This so-called **fringe projection method** is based on the digital projection of a striped pattern in a defined angle onto the skin *in vivo*. By analysis of the distortions of the striped pattern it is possible to reconstruct the three-dimensional structure of the skin [23–25] (Figure 4).

Figure 2: Schematic representation of the fringe projection method

The most commonly used device in the field of cosmetic science is the PRIMOS®-System *(Phaseshift Rapid In Vivo Measurement of the Skin)* produced by GFMesstechnik GmbH (Teltow, Germany). An alternative device based on the same principle is the 3D Breuckmann Scanner (Breuckmann GmbH, Germany).

The PRIMOS®-System consists of a digital micromirror device (DMDTM) as a projection unit, and a charge-coupled device (CCD) camera used as a recording

unit (1000 × 620 pixel). The DMD consists of an array of 1024 × 768 square 16 × 16µm² mirrors. Each mirror covers one cell of a complementary metal oxide semiconductor (CMOS) static random access memory (RAM). Depending on the data in the underlying memory cell, each mirror can be titled by means of electrostatic forces either +10° (ON) or –10° (OFF). In the ON position, light is reflected through a projection lens on the surface, whereas mirrors set to the OFF position reflect the light to an absorber. This technique allows projecting a high-resolution pattern of parallel stripes on the skin surface, which is recorded by CCD video technology [26].

The PRIMOS® offers a field of view of 24 × 14 × 13 mm² [23] and measures wrinkle properties from a wrinkle depth of 40 to 50 µm [7]. The measurement of smaller folds *in vivo* is not possible due to pulsating blood flow and slightest body movements. *Skin replicas can be used for the measurement of wrinkles smaller than 40–50 µm*.

To simplify repositioning in follow-up visits the PRIMOS® system provides an overlay function. Therefore, a ghost picture representing the reference localization overlays the live picture—both pictures can be aligned with each other until the images are perfectly congruent. For data evaluation PRIMOS®-System offers also a matching function: 3D-information of both first and follow-up measurement are compared and matched to each other, so that overlapped image areas can be analyzed.

Figure 4: Example of the clinical manifestation of forehead lines of male subjects including images of 3D fringe projection method.

The biggest advantage of the fringe projection method is that the measurement is very fast and can directly be performed *in vivo* so that skin replicas become superfluous [25]. Overlay and matching function facilitate the exact repositioning to guarantee high reliability and reproducibility [21]. A disadvantage might be that the fringe projection method is not able to assess wrinkle properties less that 40–50 µm *in vivo* [7; 27].

The dedicated computer software allows the calculation of ISO 4284/4287 surface parameters. Besides, the PRIMOS software offers also a special wrinkle algorithm to assess skin wrinkles. Therefore, several parameters are particularly useful such as overall average wrinkle depth (W_d), average maximum ($maxW_d$), and maximum largest wrinkle depth (lW_d). Total wrinkle volume (W_v) and wrinkle area (aW_a, pW_a) are the most suitable parameters to assess age-related changes, especially wrinkles [27].

Table 1: Overview of PRIMOS® parameters.

Parameter	Unit
Overall average wrinkle depth (W_d)	µm
Average maximum wrinkle depth ($maxW_d$)	µm
Maximum largest wrinkle depth (lW_d)	µm
Number of wrinkles (W_n)	no
Total wrinkle volume (W_v)	mm^3
Absolute wrinkle area (aW_a)	mm^3
Percentage wrinkle area (pW_a)	%
Wrinkle shape (W_s)	arbitrary units
Wrinkle length (W_l)	µm
Arithmetic average roughness (Ra)	µm
Maximum profile height (Ry)	µm
Arithmetic average of maximum roughness (Rz)	µm

11.3.2 SKIN ELASTICITY

One important function of the human skin is to provide protection against mechanical influences. To enable such protection, the skin exhibits both flexibility and relative resistance to deformation, thus permitting movement and allowing temporary compression and distention of a part at the same time [28]. The tensile functional properties of the skin are due to structural and qualitative components of the epidermis, dermis, and subcutis [29; 30]. However, in terms of mechanical properties, the human skin can be understood as a five-layer structure.

- The **very top layer** is formed by the compact membrane of the *stratum corneum*.

- The **proximal part of the epidermis,** with its underlying desmosomes and the dermo-epidermal junction, represents the second layer.
- Papillary dermis, which is built of loose connective tissue, is the third layer.
- The strong connective tissue of the reticular dermis represents the fourth layer.
- The subcutaneous adipose tissue is the deepest layer [31].

These layers together, consisting of fibers, colloidal substance, motile liquids and a stiff epidermal layer, and their interaction at the various interfaces determine the mechanical properties of the skin [32]. Because of this complex composition of elastic solids and viscous liquids the mechanical properties of the skin are neither elastic nor viscous, but rather viscoelastic [33].

Although biophysical measuring methods have been used to assess skin elasticity for more than 30 years, it is not yet possible to assign elasticity parameters to one single structural element of the skin. Evidence is given that the elastic fibers permit the skin to return spontaneously to its initial shape after deformation [34; 35]. Thereby, not only the absolute amount of elastic fibers is relevant, but also their length and orientation. Usually elastic fibers are vertically oriented as a network and link the dermo-epidermal junction with the horizontally orientated collagen fibers. If this architectural structure is impaired due to an excessive and uncontrolled elastin proliferation, the elastic recovery capability of the skin is partly or totally lost [36]. Collagen fibers do not have any elastic properties, but rather are essential for mechanical stability and its resistance to deformation [28]. In unstretched skin collagen, collagen fibers are organized as disordered bundles. As the skin is stretched, the bundles unfold until the collagen fibers reach their maximum length. Therefore, a lack of collagen fibers, or a degenerated collagen architecture due to intrinsic and/or extrinsic skin aging or skin disease, results in a high distensibility, slackness, and a higher sensitivity regarding mechanical tension [31; 37].

Besides the role of elastin and collagen fibers, the hydration level of the epidermis and dermis also influence skin elasticity. It is known that a moistening of the epidermis with water results in an increase of distensibility and skin fatigue—but at the same time skin elasticity decreases. Also, a lack of hydration correlates in clinical evaluation with a loss of elasticity [31].

a. Quantitative assessment of skin elasticity

The noninvasive assessment of the mechanical properties of the human skin *in vivo* can be performed by distinct methods mainly characterized by the evaluation of the nature and orientation of skin deformation after an imposed force [28]. Thereby, the available techniques can be grouped into several different classes. These include: tensile with electric dynamometers [38], the torsion method [39], and

impact testing (balistometrique technique) [40]. The elevation testing approach by means of the suction chamber method is actually the most frequently used tool to assess skin mechanics [28; 31].

b. Tensile Testing

Tensile testing is based on recording skin extension following the imposition of a parallel force to skin's surface [28]. Therefore, tensile testing with electric dynamometers is the oldest commercialized device for investigating mechanical properties of the skin [38]. In this procedure, based on uniaxial tension, a small disk sticks on the skin and is set into forward and backward motion by the electric dynamometers. The stretching of the skin is recorded on the *x*-axis as superimposed ellipses (Figure 7). The steepness of the slope of the ellipse gives information about the mechanical properties of the skin such as stiffness, elasticity, and viscoelasticity [41; 42].

Figure 3: Typical curve of tensile testing with electric dynamometers

c. Torsion technique

The measuring probe of the torsion method consists of a central disk and a peripheral ring that is glued firmly onto the skin with double-sided adhesive tape. Due to the application of a constant torque applied by the disk to the skin, it is deformed and the degree of deformation is dependent on the mechanical properties of the skin (Figure 8) [39; 41]. Analysis of the resulting rotation angle (in degrees) of the disk versus time (seconds) provides information about the standard deformation parameters including skin elasticity, firmness, or the mechanical fatigue of the skin [43]. The assessment can be adapted by variation of number, duration, intensity, and rhythm of the twisting as well as by the dimension of the rings. To examine the mechanical properties of the epidermis a ring with a diameter of 1 mm is used, whereas rings with a higher diameter are used to include underlying skin layers [41].

Figure 4: Schematic overview of skin deformation due to torsion method

d. Impact technique

The impact testing of the human skin, also called ballistometrique technique, was designed to approach specific tensile functions of the skin involving the deeper skin layers [28]. The balistometrique technique records the successive rebounds of a small hammer being dropped on the skin's surface with defined energy [40; 44]. The more kinetic energy is transformed into rebound, the more elastic is the surface [41]. Because of the viscoelastic properties of the human skin, parts of the rebounding energy are lost with every restitution phase, so that height and duration of the hammer decrease with every rebound. The induced rebounds are transduced into electrical signals that can be quantified and evaluated in terms of their amplitude and frequency [28]. The main parameter of the balistometrique technique is

the so-called rebound-coefficient, which is calculated from the ratio between the heights of the first and second rebound. The rebound-coefficient depends mainly on skin elasticity and viscosity [28; 41].

e. Elevation Technique

Elevation technique using suction chamber devices are commonly used to determine noninvasively the mechanical properties of the skin [45–47]. The underlying principle of the suction chamber method is to assess skin extension caused by a negative pressure [31; 48–50]. Based on the measurement of the extension in comparison to the height and the duration of the vacuum, several skin elasticity parameters can be calculated. These include: skin distensibility, elasticity, or firmness [31].

Besides the Dermaflex® (Cortex Technology, Denmark), the Cutometer® (Courage & Khazaka, Germany) is the most used measuring device to assess skin elasticity in vivo. It measures the vertical deformation of the skin when it is pulled by a controlled vacuum into the probe (Figure 5). The depth of the penetration is assessed by a contact-less, optical measuring method containing of an infrared light source, a receiver and two facing prisms that project the light from sender to receiver. The intensity of the projected light depends on the penetration depth of the skin into the probe [51].

Figure 5: Schematic representation of the suction chamber method.

The Cutometer® has a measuring probe which is connected over a hose to the vacuum pump and the measuring system. The opening of the suction probe has a standard diameter of 2mm, alternatively probes with a larger diameter up to 8mm are available. While the 2mm probe is primarily suitable to assess the elasticity of the epidermis; 4 to 6 mm can be used to evaluate upper skin layers up to the papillary dermis, and the 8mm opening can be used to assess all skin layers [51]. The negative pressure can be adjusted between 20 to 500 mbar and the pressure buildup can be generated abrupt or gradual. Suction and relaxing time can vary between 0.1 and 60 seconds with a maximum repetition up to 99 times.

The suction chamber method represents the deformation of the skin in terms of the time and offers different measurement modes due to pressure variations. In scientific practice a pre-set constant negative pressure of 200 to 500 mbar is commonly used. Therefore, frequent practices are measurements with 3 to 10 cycles and suction and relaxing time of 2 to 5 seconds [33]. Elongation and retraction of the skin is presented on an external monitor already during the measurement.

A typical measuring curve consists of four phases (Figure 8). The first phase is characterized by a sharp increase, which explains the elastic extension of the skin. The second phase, representing the viscoelastic proportion of extension, is shown by flattening of the curve. Upon pressure discontinuation the curve sharply declines. This third phase represents the elastic proportion of recovery, which is followed by a flattening of the curve, which represents the viscoelastic proportion of recovery.

Figure 6: Skin deformation assessed by suction chamber method.

The evaluation of the stress-strain and strain-time curves obtained with the Cutometer® allows the calculation of several tensile variables representing skin's elasticity, distensibility, or recovery after deformation [28; 52] (Table 1). Parameters that are particularly suitable to assess age-related changes of the skin including the ratio of elastic recovery to distensibility (Ur /Uf), as well as the gross (Ua/Uf) and net elasticity (Ur/Ue) for evaluation of aging effects on the mechanical properties of skin [53].

Table 2: Most suitable parameters to evaluate skin aging.

Ratio of elastic recovery to distensibility	Ur/Uf
Gross elasticity	Ua/Uf
Net elasticity	Ur/Ue
Maximum recovery	Ua
Immediate recovery	Ur
Skin distensibility of the last curve	Uf5
Skin distensibility	Uf
Immediate distensibility	Ue

CONCLUSION

The use of reliable and accurate noninvasive biophysical measuring methods is an integral part of dermatology and cosmetic science nowadays. Only the objective examination of skin *in vivo* makes it possible to assess both subclinical morphological and functional changes of the skin needed for the neutral monitoring of treatment success.

However, even though the specificity and sensitivity of most biophysical measuring methods is very high, those instruments may suffer from a series of biases that can falsify the results. For instance, environmental factors such as inadequate laboratory conditions, unfavorable selection and preconditions of subjects, but also poor study design and application errors can notoriously affect most of the measurements. *Also, the interpretation of the data needs adequate expertise to understand both the technical aspects and the physiological parameters under consideration.*

In conclusion, in the era of evidence-based skin care, biophysical measuring methods have become an essential part of cosmetic science and provide new avenues for evaluation of the effectiveness of ingredients and formula-

tions designed to develop anti-aging products. However, it should be kept in mind that even the best technology is only as precise (and accurate) as the set-up and the people using it allow it to be. Or as already the great Albert Kligman, MD, inventor of the term "cosmeceutical," used to say, "A fool with a tool is still a fool."

The authors encourage the reader to become familiar with the techniques described and proficient in their use as they represent exceedingly useful and promising techniques for development of anti-aging products and ingredients.

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GLOSSARY

Polygonal: Any closed curve consisting of a set of line segments connected such that no two segments cross. The simplest polygons are triangles (three sides), quadrilaterals (four sides), and pentagons (five sides).

Subcutis: Subcutaneous tissue (from Latin subcutaneous, meaning "beneath the skin"), also called the hypodermis, hypoderm (from Greek, meaning "beneath the skin"), or superficial fascia. The subcutis is beneath dermis which is beneath epidermis. It is used mainly for fat storage.

Eccrine glands: (/ˈɛkrən/, /ˈɛˌkraɪn/, or /ˈɛˌkrin/; from ekkrinein "secrete"; are the major sweat glands of the human body, found in virtually all skin. They produce a clear, odorless substance, consisting primarily of water and NaCl. They are active in thermoregulation and emotional sweating (induced by anxiety, fear, stress, and pain).

Trabecular: A small, often microscopic, tissue element in the form of a small beam, strut or rod, generally having a mechanical function.

Rhytide: Also known as a wrinkle, is a fold, ridge or crease in the skin. Rhytides typically appear as a result of aging processes.

Counter-replica: A positive replica of the skin created by covering the first (negative) replica with a second silicone material. Counter-replicas are often harder and more resistant to deformation.

Microrelief: Skin is characterized by a structure called micro-relief. This relief reveals a network of triangles and diamond shapes dotted with hair follicle and sebaceous gland openings.

A SURVEY OF TEST METHODOLOGY USED IN EVALUATING THE DAMAGE, PROTECTION AND REPAIR OF HAIR

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ABSTRACT

Human hair is continually exposed to various damaging factors that result in a negative impact on its cosmetic properties. Common sources of hair damage include daily use of shampoo detergents and normal grooming processes. In addition, there are more severe sources of damage. These consist of weathering from the UV-Visible components of sunlight, the action of chemically active hair products (such as oxidative hair dyes, hair relaxers, permanent waves), and the use of thermal styling appliances to create extreme hairstyles.

In order to understand the mechanism of the damaging process, as well as the actions of treatment compositions that protect or repair damaged hair, appropriate test methods need to be employed. These methods allow the study of hair from the macroscopic level (where whole hair attributes are assessed) to the determination of changes at the morphological and molecular levels. This chapter describes frequently used test methods for evaluating fiber damage and demonstrating the efficacy of protective or repair treatment systems.

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11.4.1 INTRODUCTION

"I am under a lot of stress" is a frequently heard complaint. Despite the advanced technology that tries to make our life easier, such as microwave ovens, dishwashers, voice-controlled devices, and the like, we still suffer from too much to do and trying to accomplish it all under a limited time frame. But, semantically speaking, are we actually under stress? Rather, it can be argued that we are being strained. Strain is an outside influence such as an ominous deadline that must be met and stress is our reaction to the applied strain. Some people are frazzled by life's complexities, whereas others can inherently adapt to more strain. Consequently, one's tolerance for internally felt stress is unique to factors such as the current psychological, biochemical, and social states of the individual. We can all relate to this on a human level; but in order to fully relate to the impact of stressors on hair it is imperative to gain an understanding of the physical relationship between stress and strain.

The relationship between stress and strain depends on whether force or distance is used to probe a material. If strain, or deformation of a material, is the mode of action, then stress in the system is the reactionary response. If stress, or force per unit area, is the mode of action, then the degree of strain is the measurable response. A simple model can demonstrate this easily. Take a rubber band and stretch it. The distance, or strain, applied to stretch the rubber changes the way the atoms in the solid are assembled. In reaction, the new physical configuration of the rubber band affects the resistance (i.e., stress response) to the applied deformation. Ultimately, at a critical strain, small irreversible fissures in the rubber begin to form, and applying further strain to the band induces stresses that ultimately cause a catastrophic break in the rubber.

During the course of explaining some of the test methods to determine the mechanical properties of hair, there will be stress-controlling devices that monitor changes in physical dimensions, and strain-controlling devices that measure the stress response of the deformed fiber; however, stress and strain do not have to be mechanical in nature. It is conceivable to expand the definition to include factors such as chemical, thermal, and environmental insult. In analogy, there are different limits to individuals tolerating a polluted city, or to resisting the sweltering heat, and ultraviolet radiation of the summer sun.

The subject of this chapter deals with human hair and the many factors that subject hair to stress. These include the various chemical and thermal treatments applied to hair in order to improve its cosmetic appearance, as well as environmental factors, especially UV radiation from the sun. The integrity and physicochemical state of the hair impacts the resilience of the fiber and its coinciding resistance to stress. Obviously, if the hair is compromised in some way, either chemically or structurally, it will not hold up to subsequent treatments.

An example of stress put upon hair by the strain of environmental conditions is the work done by Ratnapandian et al. where they studied the mechanical properties of hair after exposure to a controlled dose of solar radiation [1]. As reported by the authors (see **Table I**) the hair strength is compromised, as indicated by a positive percent loss in mechanical properties. The increase in the magnitudes of the turnover point (defined in **Table I**) and strain to break, as indicated by a negative percent loss values, actually represents hair weakening since they are due to the breakage of disulfide cross-links that consequently increase the mobility of the molecular chains. A full description of the mechanical properties of a hair fiber from the stress-strain curve and the molecular changes that occur at each stage can be found in Feughelman's treatise on the subject [2].

Table I: Percent loss of mechanical properties of hair with simulated solar radiation (300 hours; 70% RH). Data from Ratnapandian et al. [1].

Mechanical property from stress-strain curve	**% Loss**
Work to 20% extension	46.51
Stress at 20% strain	43.18
Initial modulus	46.69
Post yield modulus	60.63
Work to break	23.14
Stress to break	49.06
Turnover point*	- 36.54
Strain to break	- 43.14

* The intersection of the yield and post-yield part of the elongation curve; see **Figure 42b**.

In this experiment, the treatment of hair with a controlled time and intensity of solar radiation exposure is an example of straining the fiber, with weathering as the active agent. Also, the measuring device for evaluating the mechanical properties, which in this case is the tensile strength tester, is also a strain-controlling device since the rate of deformation imparted to the hair fiber is held constant.

In order to study the nature of hair damage from the multitude of strains and the consequent stressful factors that it is subjected to, as well as determining the efficacy of ingredients and ultimately products for protection and repair, appropriate test methods need to be employed. This is not a simple task. Hair has a very complex chemical makeup and morphology, and the imparted stresses and strains vary as a result of the type of cosmetic treatment being employed.

This chapter provides an explanation of the nature and utility of methods that are used in the assessment of hair damage, its protection, and repair. Methods are described in enough detail for the cosmetic chemist to utilize them appropriately and to make sound conclusions from the generated data. Sometimes an understanding of the damage mechanisms can be achieved with one method; however, it is prudent to use a combination of methodologies to build a case for proving product efficacy, especially with regard to claim substantiation. Throughout the course of explaining each method, an underlying theme is to keep in mind whether the stress-strain relationship is physical or chemical in nature. With regard to human hair, it will provide a reference for understanding the factors in the degradation of hair, its interaction and response to cosmetic ingredients, and the measurement of these effects with appropriate test methods.

11.4.2 THE NATURE OF HAIR DAMAGE

The major stresses that hair experiences are:

1. Shampoo fatigue—The distal ends of shoulder-length hair could be up to two years old and have gone through literally hundreds of shampoo cycles. Even if a mild shampoo had been used during the course of the lifetime of the hair, the surfactant slowly extracts the lipid of the hair as well as smaller-molecular-weight fractions of protein that are the result of other damaging treatments. This slow erosion of hair compromises its structural integrity over time.
2. Mechanical Damage—Combing or brushing of hair imparts shear stresses within the fiber. As a comb is pulled through a tress in the direction from root to tip, the bending of the hair fibers is also forced to propagate from root to tip. That is, it is a dynamic bend and not a static one. These shear stresses produce fine fractures within the interior of the cortex, which upon further combing, result in either a split end, or worse still, breakage. Compression, tensile, and torsional forces are also present from continual bending and add

to the stresses at work during combing and resultant fracture. The situation of fracture becomes magnified when hair is in the wet state. Hair that is wet is more extendable due to changes in hydrogen bonding between adjoining structural protein elements of the hair. Mechanical stress, such as towel drying or combing out a snag in the wet state, will also cause breakage.

3. Weathering—Sunlight affects hair in several ways. Light of shorter wavelengths and higher energy, designated as UVB, degrades disulfide bonds as well as structural components of the hair such as the lipids of the CMC. Once these are degraded, important hair attributes are compromised, resulting in a decrease in cosmetic effects, including shine and elasticity. The natural pigments of the hair, eumelanin and pheomelanin, defend against this by absorbing UV light and protecting the chemical components of the hair. Hair that is light in color, such as natural blonde or bleached fibers, does not have this natural defense and is more prone to damage.
4. Chemical processing—The chemistry of bleaching, oxidative hair dyeing, permanent waving, and relaxing have been well described in the literature; a recommended reference is by Robbins in *The Chemical and Physical Behavior of Human Hair* [3]. All of these chemical treatments have multiple damaging effects on the structural components of hair. When chemical bonds are broken, functional groups change their character and influence the cosmetic behavior of hair. One example is the removal of the lipid layer from the surface of hair, which is essential for important hair attributes. Another is the formation of cysteic acid groups, which makes hair more hydrophilic, and decreases hair characteristics such as detangling and hair manageability. Many other examples can be cited based on the varied chemical components of the hair.
5. Thermal Styling—Styling appliances configure hair through both mechanical manipulation and heat. Examples of these include curling irons and flat irons. It will be shown in this chapter that the excessive heat induced by these appliances tends to denature structural proteins in the cortex and cuticle of the hair. Although this treatment initially imparts attractive cosmetic effects, with continued use the hair becomes less manageable and suffers from a decrease in its cosmetic appeal. The result is that the consumer has to use intensive conditioning treatments to conceal the damage.
6. Thermal/mechanical (wet to dry styling)—After adding a styling gel or cream to damp hair, it is usually styled with a round brush and blow dryer. The subsequent insult is not only from the heat of the blow dryer, but also from the brushing process. Combing and brushing are damaging to dry hair, but when hair is wet, it is in a weakened state and is more prone to breakage during combing. If the styling product goes through a tack phase, brushing becomes very difficult and results in a large extent of breakage.

Damage imparted to hair can range from the molecular level, to the subassemblies of the fiber. The deterioration alters the complex structure of proteins in the microfibrils of the cortex and extends to macroscopic structures, including the cuticle and cortical cells. There are test methods appropriate for studying hair damage at each level. The ultimate level is observing changes in damage impacting whole hair attributes, where many fibers interact as an assembly. Cosmetically acceptable attributes from fiber interaction include, for example, curl definition, body, and shine; whereas the damage state exhibits a host of negative attributes including frizziness, poor comb through, and dullness.

Unfortunately, the state of hair is a bleak one since it cannot renew itself. As the dead fiber suffers more and more stress, there is a vicious downward trend until the fiber fractures and breaks. Since hair grows from the scalp, the hair's age increases along the fiber from root to tip so that the tips usually have the highest degree of damage. As hair is combed, the shear stresses imparted to the hair will be most destructive to the ends, with the result that the ends will fracture or produce split ends. Therefore, a fiber's ability to respond to multiple stresses depends on its current level of damage. Another factor is the morphology of hair. Since hair is a biological outgrowth of the expression of a person's genes, each individual then has a different hair type. In general, hair can be categorized by racial descent so that Asian hair is characterized by a larger diameter, and Caucasian hair is generally finer. Of course there is overlap of hair types between the races, but it can be concluded that morphological differences allow hair to be more or less hardy in its ability to handle the stress of damaging treatments. For example, during the use of a curling appliance, the thermostat has to be put on high to "curl-up" the larger diameter of Asian hair.

If hair was a living structure, then certain treatments may rejuvenate the broken structures so that hair can revert to its undamaged natural state, such as when it first emerges from the follicle. Unfortunately, this strategy cannot be employed and consumers resort to treatments that condition, protect, and what is more challenging, repair damaged hair so that they can continue with their usual styling behaviors. Some of these treatments are cosmetic and temporary in that the results from the treatment are usually washed away in a subsequent shampooing. Protective treatments help hair to take the insult of aggressive styling processes, such as hot flat ironing, so that damage is alleviated and the breakage of hair is postponed to a later date. But again, based on the dead nature of hair, its fate is sealed; ultimately there will come a day when the hair fiber will break.

Luckily, from a biological standpoint, there is hope. Each fiber is constantly renewing itself by generating new hair inside the follicle. Those follicles that are most active, those that are in the anagen phase, are producing keratin cells that mature in the follicular canal and then emerge from the scalp in its natural undamaged state, only to be cast into the world to suffer the stresses and strains of wear and tear.

11.4.3 SIMULATING DAMAGING HAIR TREATMENTS FOR STUDYING THE ALLEVIATING EFFECTS OF PROTECTIVE AND REPAIR INGREDIENTS

The manner in which hair is artificially damaged, and with respect to what has been described above, imparting a controlled strain on hair, depends on the particular objective of the experiment. Once the goal of the experiment has been established, then the scientist designs the experiment to maintain control of important variables. For example, some variables are held constant and one or more variables are purposely manipulated with the primary intent of determining effects.

a. Treatment Schedules

For each of the stresses that hair suffers from, a treatment can be designed to simulate the stress. These are usually performed on hair tresses of a particular configuration and hair type, such as fine or bleached fibers, for example. These treatments can be termed treatment regimen because the tress is purposely exposed to successive treatments that simulate what a consumer would do in real life. A simple example would be to wash and dry a hair tress with a shampoo to determine the damaging effects of the surfactant after multiple washes. Damage can be assessed periodically during the course of washing using, for example, a Scanning Electron Microscope to determine the state of the cuticle. Also, contact angle measurements with a tensiometer will indicate to what extent the protective lipid layer of the hair was extracted by the detergent of the shampoo. More complicated treatment regimens have been designed to study the effects of other strains on hair. Several examples from the literature will follow that have been utilized for assessing the effects of UV degradation by sunlight, thermal effects from appliances, color wash-fastness of permanently dyed hair from multiple washing cycles, and mechanical fatigue. These will serve as examples to demonstrate how to design a treatment regimen to study the effects of the key variables under study. These should not be considered as standard procedures, but as part of the experimental design to control and manipulate variables to determine effects.

1. Thermal Exposure

Hot flat irons that are used to produce a straight style can reach temperatures of up to 450°F. The styling procedure consists of taking about an inch-wide section of hair and applying the hot iron close to the root section and moving the iron from root to tip without hesitating. This procedure is continued until all of the hair on the head is treated. The thermal insult to hair can be done on hair tresses in order to run controlled experiments. A treatment regimen can consist of first washing and then treating the hair with a pretreatment to study its effect on alleviating the damage incurred to the hair from the hot ironing process. After the hair is dry, the

hot flat iron is heated to a specified temperature. Then, in a controlled fashion, the iron is applied to the root section of the hair and moved down to the tip in a specified length of time. This is repeated a number of times to achieve a total time of heat exposure. Intermittent shampooing and re-treating with the experimental composition can be done as well. Measurements are taken initially, during the course of the treatment regimen, and then at it end. Alleviating damage of the hair due to the experimental composition can be determined by comparing results versus an appropriate control. A treatment regiment is illustrated in **Figure 1** that was utilized by Zhou et al. to study the thermal-protective effects of polymeric pretreatments and their alleviation of hair damage resulting from the hot ironing process. Methods utilized, which will be described in more detail in a subsequent section of this chapter, were Differential Scanning Calormetry (DSC), a specialized anti-breakage method, microscopic techniques, FTIR spectroscopic imaging, Dynamic Vapor Sorption (DVS), and thermal image analysis [4]. Results from this work indicated that several synthetically derived polymers were effective in thermal protection, especially those that were hydrophobically modified.

Figure 1: Treatment regimen used in the thermal insult of hair tress to study thermal damage and effects of protective pretreatments.

A similar treatment regimen was designed by McMullen and Jachowicz [5] in their efforts to study the effects on alleviating the damage to hair from curling irons. Again, the methodology consisted of exposing the hair to the heat from the curling irons for periods of time with intermittent shampooing and re-treating with the protective composition. However, in this case the mode of exposure of the curling iron was different than that of a flat iron in that the curling iron was clamped onto one section of the hair for a specific period of time. The window of exposure was then assessed to show the damaging effects of the heat as well as the alleviating effect of the pretreatment composition. This was accomplished by detecting a reduction in combing forces as tested through a mechanical combing device, and a reduction in the level of Tryptophan, an indicator of hair damage, by spectrofluorimetry.

There are many manually operated parts of the hot ironing process that are a source of variability in thermal treatment regimens. Maintaining a constant rate of descent of the hot iron from the root to the distal part of the tress is very difficult,

especially if the polymer treatment on the hair creates a drag for the iron. The tension that the operator applies when pressing the plates of the iron against the bundle of fibers is a compound variable. As the operator uses more force to press the jaws of the iron against the fiber bundle, more force is applied to pull the iron down the tress to keep the rate of descent constant. This results in the fibers being stretched longitudinally. To control and monitor these variables, an automated apparatus was designed by Dussaud [6]. It consists of a tress mounted on the arm of a texture analyzer so that the rate of movement of the tress in relation to the hot flat iron is controlled. The two plates of the iron are brought into contact with the bundle of fibers and the tension is monitored by a force sensor. The stretching force is kept constant, as measured by the texture analyzer, by applying a certain contact force of the iron jaws against the hair. Hair temperature is monitored by the use of an infrared thermometer. Measurements by Dussaud after controlled thermal treatments with this automated apparatus included DSC, wet elasticity, and birefringence [6]. From this study, interesting conclusions on the permanent straightening of curly hair are described.

2. UV Exposure

References in the literature indicate that solar exposure degrades various components of the hair. These changes affect lipid components [7] and proteins [8]. Other aspects of the photodegradation process include understanding the role of melanin [9] in photoprotection, as well as other variables including moisture [1] content, and the degree of damage through processes such as bleaching, perming, and dyeing with an oxidative hair color [10].

A key piece of equipment requisite to performing a UV exposure study is a solar simulator. One could subject hair tresses to natural sunlight; however, variations in cloud cover, sun angle due to the time of day, as well as change of season, puts many obstacles into performing a controlled study. The solar simulator allows the experimenter to control important variables such as light intensity, wavelength, temperature, and humidity. The simulator consists of a light source such as a Xenon lamp, a filter that will subject the hair to a particular part of the electromagnetic spectrum, and a chamber that can control temperature and preferably humidity as well. The filter will allow simulation of normal daylight, or in more refined studies allow just certain bands of UV light to impinge on the hair such as UVA, UVB, or a combination of both.

As with thermal degradation, photodegradation studies also follow a treatment schedule. The experimenter will shampoo and pretreat the hair with a protective composition and then expose the hair for a certain length of time while keeping necessary variables, such as temperature, humidity, wavelength, and radiation intensity, held constant. The treatment and exposure cycles are usually repeated to

represent the most practical consumer use conditions. In this way Locke studied the effect of a substantive UV photofilter by treating tresses with a protective composition and then putting the tresses into a sample holder that had an exposure window. Tresses were treated and exposed for eight hours. This was repeated for a total of 32 hours of exposure. Exposure consisted of placing tresses in a weatherometer that had the irradiation adjusted to simulate the same intensity of sunlight found during the day in Florida in April. Measurements were taken in the exposed and unexposed regions of the tress to determine and compare the degree of photodegradation and resultant protection by the UV photofilter. Measurements in reduction in combing and tryptophan degradation were conducted [11].

There are many references to help the experimenter with designing a protocol for study. One is by Signori where the author reviews the current understanding of the effects of ultraviolet and visible radiation on hair structure and options for photoprotection. Among some of her advice, she mentions that correlating the length and intensity of the simulated light to real-world conditions is an important factor to consider [12]. Also, many questions can be answered by referring to one of the manufacturers of the solar simulator [13].

3. Color Wash-fastness Techniques

Peroxide containing oxidative hair color is designed to "lift" the natural color of the hair and at the same time react dye intermediates inside of the hair. The intentions of this process are primarily for covering gray and/or changing one's natural color from brown, for example, to red. Although these products are termed permanent, a certain amount of fading takes place due to continuous cycles of washing and conditioning. Primarily, this is due to the surfactants used in these products that tend to slowly leach the water-soluble artificial pigment or dye out of the hair. This results in a change in both the intensity and hue of the hair, which is unacceptable for a consumer. Many products on the market consequently claim retention of the vibrancy of color with the use of protective ingredients.

To design a treatment regimen to test for color wash-fastness, hair tresses are typically first dyed and then cycled with a regimen of products. The regimen consists essentially of a shampoo, and may be followed by a conditioner and leave-in styling/conditioning products. Color measurements are then taken throughout the treatment cycle and measurements are compared to the initial state of the hair. Percent protection of the product in question is determined by comparing to appropriate controls. Such a treatment schedule was utilized to determine the color wash-fastness effect of a hydrophobically modified polymer in a treatment regimen of shampoo, conditioner, and leave-in treatment. In this case, bleached hair was dyed red, a color that is most prone to fading from shampooing, and cycled with a regimen of formulas both with and without the hydrophobic polymer. Results

were substantiated with colorimetry measurements and image analysis. More sophisticated methods were used, such as water contact angle and FTIR spectroscopic imaging, to help propose a theoretical mechanism of action of the active ingredient [14].

4. Mechanical and Thermal/Mechanical Damage

Combing imparts mechanical strain to hair, i.e., hair gets stressed by the strain of combing. There are different components to this stress. **Figure 2** illustrates a hair tress being pulled through a comb that is being held stationary under a stereomicroscope. The various strain components are labeled and consist of shear stresses in the internal parts of the hair through dynamic bending, i.e. bending that is propagated from root to tip, longitudinal tension though extension, abrasion, impact loading, compression/expansion through bending, and torsional strain. As **Figure 2** illustrates, the combing process will include all of these strains. Specialized instruments have been designed and employed to isolate and test each component or combination of components of the multiple mechanical stresses that hair is subjected to during grooming to determine their effects on hair damage. The strength of hair, and in the case of cosmetic treatments, the alleviation of weakening of hair with its consequential breakage or reduction in cosmetic attributes, is quantified after a set regimen of specific strains. These strains can be designed to be in a longitudinal (tensile), radial (torsional), or tangential (bending) direction. Abrasion and impacting are other components that have been studied. One complicating factor is that other stresses, such as chemical, UV, and thermal exposure, tends to weaken the hair so that there is a greater propensity to break under mechanical strain. Many treatment schedules found in the literature, therefore, have as their regimen a combination of these stresses imparted to the hair. The application of these stresses as described in the literature will be described in the next section.

Figure 2: Mechanism of Hair Breakage-Various Stresses During Combing

11.4.4 INTRUMENTATION AND EXPERIMENTAL METHODS IN STUDYING THE DAMAGE AND PROTECTION OF HAIR

a. Physical-Chemical

1. Differential Scanning Calorimetry

Excessive thermal, chemical, weathering, and mechanical strains induce damage to the hair surface as well as the core of the fiber. Whereas surface analysis techniques, such as SEM, ToF-SIMS, and contact angle analysis, are all valuable in corroborating measurable stresses to the quality of the integument surface, thermoanalytical techniques, including Differential Scanning Calorimetry (DSC), may be used to probe the state of the subsurface fiber chemistry, such as the health of the cortical proteins.

The anatomy of a modern computer-controlled DSC instrument includes separate sample and reference holders, as well as vendor-specific cell closures, thermocouples, and gas purge inlets. The two principal types of DSCs are *heat-flow* and *heat-flux* instruments. Heat-flow instruments are based on the double-furnace design developed by Perkin-Elmer in the 1960s [15]. As a material passes through a thermal transition, the instrument uses local temperature readings and a feedback mechanism to ensure that the sample and reference pans are maintained at equivalent temperatures (i.e., $T_S=T_R$); hence, the amounts of power to the sample and reference furnaces are independent and are used to directly measure heat flow in or out of the sample. In heat-flux instruments, a single-furnace simultaneously heats both the reference and sample. Calibrations and temperature flow differences are then used to calculate heat flow during a thermal event (i.e., $T_S \neq T_R$).

In a DSC experiment, the impact of thermal energy on phase transitions, such as melting (T_m) or glass transition (T_g) events, is logged as a function of applied temperature. Heating a sample causes changes in the ability of the material to hold onto thermal energy—where the increase in kinetic energy is distributed to optimize the vibrational, rotational, and translational states of the molecules that define motion within the material. The ability of a material to hold heat, which is called its *heat capacity* (C_p), is subsequently used to monitor excess, or *differential*, heat flow. Depending on the category of the thermal event, excess heat flows to the sample, or from the sample, relative to the empty sample pan. Put another way, to maintain the sample and reference cells at equivalent temperatures in a heat-flow instrument, excess energy is either *added* to (endothermic) or *subtracted* from (exothermic) the sample pan. For example, heating a thermoplastic polymer from subambient to the decomposition temperature will display the T_g and T_m events as endothermic; whereas crystallization and some curing processes appear as exothermic peaks (see **Figure 3**).

Figure 3: Peaks represent positive or negative heat flow (single-furnace). Characteristic DSC thermal transitions scanned from low to high temperature (exotherm points up): (a) glass transition temperature (T_g); (b) crystallization temperature (T_c); (c) melting temperature (T_m); (d) curing event; (e) thermal decomposition/oxidation (adapted from TA Instruments Thermal Analysis brochure, US).

In contrast to the aforementioned thermogram of a model synthetic thermoplastic, the human hair fiber is a very intricate composite and its intrinsic complexity typically adds intrigue to DSC data interpretation. Complexity aside, because applications of excessive heat, damaging chemicals, mechanical elongation, weathering strains, and combinations of each may alter the matrix cross-link density and/or irreversibly denature the secondary structure of the α-keratin to random coils and β-domains, DSC analyses of the endothermic denaturation temperature and enthalpy transitions are invaluable in quantifying the status of the stressed fiber cortex. That being said, consideration for the morphological complexity of a hair fiber is central in choosing whether to use "dry" or "wet" DSC methodology. In dry methodology, hair snippets are charged into a traditional, nonsealed aluminum DSC pan and water is evolved from the pan as samples are heated to decomposition. As a result, bookkeeping complexities arise

as the denaturation (T_D) and decomposition peaks inconveniently overlap into a poorly resolved doublet (between 230 and 250°C). However, the doublet may be happily resolved by testing in excess water and taking advantage of the water-plasticization of the matrix, which conveniently shifts the hair denaturation peak (120–150°) clear of interference from the 250°C pyrolysis peak [16]. This result begs the question: Why does water plasticization—which is a property typically affiliated with amorphous materials—influence the magnitude of T_D for the crystalline α-helix?

The short answer is that water plasticity, in combination with prior chemical alterations, shifts the T_D of the α-helix due to viscoelastic changes in the morphological components of the *fiber composite*. The cortex of human hair is principally compartmentalized into macrofibrils, microfibrils, and the cell membrane complex. Various researchers have further postulated kinetic and physical models to both simplify and relate the fiber chemistry and morphology to water plasticity and/or thermally induced phase changes [16–27]. For example, in Feughelman's two-phase model (**Figure 4**) the intermediate filaments (IFs), or microfibrils, are composed of partially crystalline α-helical keratin bundles that are embedded in a highly cross-linked amorphous matrix [2, 17]. Typically, in most semi-crystalline polymeric materials, water interacts less with crystalline domains and more intrusively with amorphous, hydrophilic moieties; *however*, Wortmann suggests that, in human hair, water *absorbs* into the matrix and *adsorbs* to the periphery of the IFs equally well, meaning that water distributes within the cortex "quasi-homogeneously [18]." Therefore, as *isolated* IFs in hard keratins are soft and pliable when extended in water, it is clear that the surrounding matrix, which is also covalently bound to the IFs, plays a large part in the rigidity of hard keratins [16, 19]. Consequently, the two-phase model conveys that the magnitude of T_D (i.e., thermal stability) for α-keratin is kinetically controlled by the cystine-based crosslink density and the viscosity of the nonhelical *amorphous matrix*, whereas the energy to denature (ΔH_D) correlates with the structural rigidity of the *α-helices* in the IFs [20, 21]. In an augmented interpretation of the two-phase model, Istrate uses a variety of technical arguments to weigh the limitations of the Feughelman model. Instead, a third physical phase is introduced that links chemical changes (i.e., rate limiting S-S scission) in the nonhelical terminal domains of keratin filaments to the thermal stability of the IFs [22]. In a final example, Greenberg et al. studied hard keratins from various sources and concluded that an important function of the matrix is to keep the IFs in a semi-dehydrated state—suggesting that an intact matrix is key to maintaining the mechanical strength and stability of the IFs [19].

Figure 4: Diagram of a transverse section of hair fiber showing the traditional Feughelman (two-phase) morphological model where the composite fibrous intermediate filament(s) phase is immersed within a highly cross-linked amorphous phase.

As an illustration for the utility of DSC in evaluating cortical damage, **Figure 5** shows the impact of hot flat ironing on virgin and polymer-treated fiber assemblies. The overlay was produced by following the methodology of Wortmann and Deutz [21]. Polymer-treated hair samples were prepared by applying model formulations to hair tresses. The tresses, including a freshly prepared virgin tress, were first blow-dried and next thermally treated with a timed regimen utilizing a high-temperature commercial flat-iron [4]. The virgin and thermally treated hair tresses were sectioned into small snippets and equilibrated at 60% RH overnight to normalize hydration history. Five to ten milligrams of the equilibrated snippets were subsequently charged into stainless-steel, pressure-resistant, large-volume DSC capsules, and 50 μL of water was added to each capsule. After sealing each pan, the hair snippets were hydrated overnight prior to charging into the DSC autosampler. In terms of the robustness/precision of the method, fastidious attention to the preparation protocol is critical to developing data worthy of inter-study (i.e., historical) comparisons. Specifically, art and tedium are intrinsic to the preparation of snippets, and variables such as water quality, heating rate, and application of all pretreatments must be precisely controlled.

Now that the hard part is done, it's time to let the machine earn its keep. Depending on the thermal history, *hydrated hair fibers* characteristically denature between 110 and 150°C; hence, DSC investigations are accomplished by heating samples at 2–5°C/min over the 60–185°C temperature range. For example, thermograms *a* and *e* (**Figure 5**) depict the resultant outcomes of high-pressure DSC scans for wet, untreated virgin, and wet, thermally treated virgin hair snippets, respectively; and curves *b-d* and the trends in **Table 2** show the effects of various

commercially available polymeric pretreatments on the preservation of the secondary α-keratin structure. The diminishing magnitudes of the T_D and ΔH_D, which is the area of the endothermic peak after exposure of the hair tresses to the rigors hot flat-ironing, reflect the impact of thermally imposed strains on the integrity of the amorphous matrix and embedded α-helices. Chemically speaking, it has been suggested that the imparted stresses and subsequent shift in T_D are related to a decrease in the matrix crosslink density, where the culprit is an increase in the number of thermally-induced disulfide bond scissions [22, 6]. Additionally, relative to flat-ironed virgin hair, the addition of selected polymeric treatments (e.g., *b* and *c*) to virgin fibers appears to mitigate the damaging effects of excessive thermal insult. Thus, for example, although the precise efficacy mechanism of "thermoprotective additives" is complex and currently debatable, the trends in **Figure 5** and **Table 2** suggest that treatment *b* is more successful than treatment *d* in hindering the irreversible progression of thermally induced α-keratin denaturation.

Figure 5: Effect of thermal styling treatment (232°C) on the α-helix melting temperature and associated enthalpy change (offset on *y*-axis for clarity). (a) Profile of untreated virgin hair; (b-c) flat-ironed hair/cationic polymer composites; (d) flat-ironed hair/non-ionic polymer composite; (e) flat-ironed virgin hair. Shifts in the peak minima from higher to lower temperature and decreased peak areas indicate presence of thermomechanically induced cortex damage (courtesy of D. Koelmel, Ashland Specialty Ingredients, US).

Table 2: Effects of thermal treatment on the denaturation temperature and denaturation enthalpy of European Dark Brown Hair

Sample Description	**Shift in TD (°C)**	**ΔHD Loss (%)**
Virgin	—	—
Flat-ironed + B-treated	8	22
Flat-ironed + C-treated	10	12
Flat-ironed + D-treated	17	53
Flat-ironed Virgin	23	81

Although thermal damage has been exemplified in portraying the merit of DSC methodology in measuring stresses in the fiber cortex, the influence of chemical [6, 17, 20, 22–26] and weathering strains [27], which also inflict damage to native cortical protein conformations, can also be discerned by DSC analyses. In extension, because the tensile mechanical properties of hair fibers are tied to the structural integrity of the cortex, thermal analysis enables a measurable connection between protein structure, bulk fiber performance, and the inception of novel keratin-preserving prototypes.

2. Surface Tension, Wetting, and Contact Angle Analysis

Interactions of a liquid and hair at the interface may be used to probe the physical and chemical states of the hair surface. The physicochemical state of the hair typically varies with hair length as weathering, cosmetic treatments, aging, and genetic strains induce measurable stresses on the fiber's perimeter [28]. Depending on the extent, type, and location of damage, chemically unique segments of the F-layer, epicuticle, A-layer, exocuticle, endocuticle, cell membrane complex (CMC), and exposed cortex may all contribute to the measurable fiber-fluid interface. For instance, water is a very polar liquid and its wetting interaction with the hair surface depends on the summation of the accessible chemistry of the interface—explicitly, undamaged virgin hair fibers are lipophilic, whereas damaged fibers are on average more hydrophilic. Consequently, depending on the state of the hair, certain fluids tend to spread across the hair with film-like behavior, whereas others will bead on the surface, or form patchy domains on the hair's heterogeneous landscape. Hence, the efficacy and performance of hair-damage repair treatments are affected by the current condition of the fiber, as well as the surface activity of the applied treatment formulation. Specifically, any treatment that lowers the tendency of the applied fluid to form cohesive and spherical droplets will increase the likelihood

of that fluid wetting the hair surface. When done carefully, comparative measurements of the contact angle between the applied fluid and different substrates facilitate a description of comparative wetting behavior.

Insights into the likelihood of a fluid spreading across a surface may be gleaned by the parameters of the Gibbs equation (Eq. 1). Ignoring the intimidating squiggles of calculus, one only needs to focus on a couple of variables to see that *surface tension* (γ) is key to wetting a surface—and, via a mental extrapolation, that *wetting* is described by the magnitude of the *contact angle* of a fluid at the solid-liquid interface. The Gibbs equation relates the extent of adsorption of one phase (e.g., water) onto another (e.g., hair fiber) and suggests that a system that reduces the surface tension (γ), such as a surfactant, will have the propensity to be adsorbed onto a surface,

$$\mathbf{\Gamma}_i = -\ 1/RT\ (\partial\boldsymbol{\gamma}/\partial \ln \mathbf{a_i})_{T,P} \tag{1}$$

where Γ_i is the surface concentration of the spreading fluid, a_i is the concentration of the adsorbing phase, T is the absolute temperature, and R is the gas constant.

Hence, prior to discussing the importance of contact angle in quantifying wetting processes on hair, it is useful to introduce surface tension, which, by dimensional analysis arguments, is equivalent to the *surface free energy*. As depicted in **Figure 6**, the atoms at the surface of a liquid are in a different environment than those in the bulk. Bulk molecules interact with neighboring molecules equally; however, the molecules at the surface of a liquid may only interact with neighboring surface molecules and with adjacent subsurface molecules. The net result of the asymmetry in attractive forces is that interfacial molecules are pulled inward until molecular collisions with bulk molecules arrest their contraction [29]. The surface is now analogous to a tight skin covering the bulk fluid, and the tight skin of the contracted interface requires energy to penetrate it or to extend its area. Thus, surface tension (or surface free energy, G), which has units of N/m (or J/m^2), is defined as the force (or work) applied parallel to the surface of the fluid divided by the length (or area) over which it acts,

$$\gamma = F/l, \text{ or } \gamma = (\partial G/\partial A)_{T,P,n} \tag{2}$$

where, in either form, Equation 2 infers that it requires energy (ΔG) to extend the surface (ΔA) and to bring molecules from the lower energy bulk to the stretched, higher-energy interface. Another way to view the surface ensemble is to envision that parameters affecting hydrogen-bond packing influence the cohesiveness and consequently tension of the interface. Conventionally speaking, the term "surface tension" is typically applied to forces at the liquid-vapor interface; because elastic forces confound and dominate the energy needed to expand the surface of a solid, measurements at the liquid-*solid* and vapor-*solid* interfaces are typically described by *surface free energy*.

Figure 6: Model of an air-condensed phase interface. ○ =interfacial molecules. ● =bulk molecules. The interfacial molecules are not solvated equally and are in a higher energy state than those in the bulk fluid.

Surface tension measurements are typically accomplished with a *surface tensiometer* and Wilhelmy Plate or du Noüy ring methods [30]. In surface tensiometry, the high-energy and pristine surface of the plate or ring is inserted into the solution to form a meniscus. As the probe is slowly removed, a microbalance follows the maximum force needed to overcome the wetting force, which is proportional to γ (or σ in **Figure 7**).

Figure 7: Wilhelmy plate method for determining surface tension. L=wetted perimeter; F=force exerted on microbalance; σ=γ=surface tension; θ=contact angle. θ is typically zero and, hence, cos 0 = 1 (courtesy of Krüss GmbH).

Wetting is an intuitive term and refers to the process of a liquid displacing a gas to make intimate contact with the surface of a solid. When interacting with a solid, poorly wetting liquids tend to bead, while exceptionally wetting liquids spread evenly across the exposed surface. The process of wetting a surface is related to the molecular interactions between the liquid and the solid. For example, if the liquid-liquid attractions within the droplet are stronger than the molecular attractions at the liquid-solid interface, the liquid will exhibit *cohesive* properties and will tend to bead at the surface of the solid. Conversely, if the molecules of the liquid and solid interact strongly, the liquid will counter *adhesively* by stretching its volume uniformly across the interface (**Table 3**).

Table 3: Description of cohesive and adhesive properties and relation to contact angle [courtesy of ramé-hart Instrument Co.]

Contact Angle	**Wetting Description**	**Solid-Liquid Interaction**	**Liquid-Liquid Interaction**
0°	Complete	Strong	Weak
60°	High	Strong	Weak
90°	Moderate	Weak	Weak
120°	Low	Weak	Strong
180°	None	Weak	Strong

The *static contact angle* of a fluid on a solid is typically measured with a modern Drop Shape Analyzer (DSA). In a DSA experiment, a *sessile drop* (i.e., liquid drop on solid) is carefully deposited on the substrate using a syringe. A mounted video camera is then used to capture an image of the drop on the surface, and accompanying image analysis software is used to measure the angle (i.e., contact angle) between the solid-liquid interface line and the tangent line at the liquid-gas interface. **Figure 8** shows a water drop at equilibrium on a solid surface—a hair fiber. The equilibrium contact angle is the result of the sum of the interplay of three surface tensions, or *surface energies*: γ_{sl} is the surface tension of the solid-liquid interface, γ_{sv} is the surface tension of the solid-vapor interface, and γ_{lv} is the surface tension of the liquid-vapor interface. By definition, at equilibrium the vector sum of the three tensions, which sums along the parallel of the fiber-droplet interface, must be zero, and rearranging produces the *Young equation* (Eq. 3).

$$\cos\theta = (\gamma_{sv} - \gamma_{sl}) / \gamma_{lv} \tag{3}$$

As indicated in **Table 3**, at $\theta > 90°$ the solid-liquid interface is at higher energy than the solid-air interface. In this instance, the vapor preferentially wets the surface and the liquid beads (i.e., minimal wetting). At $\theta = 0°$ (i.e., $\cos\theta = 1$) there is perfect wettability and, due to deviation from equilibrium, the Young equation no longer holds true [31, 32].

Figure 8: Top: 100 pL static water drop on surface of hair fiber (courtesy of Krüss GmbH). Bottom: Model of the interaction of liquid-vapor (γ_{lv}), solid-liquid (γ_{sl}), and solid-vapor (γ_{sv}) interfacial tensions.

The advantage of the sessile drop method is that multiple measurements on the sample surface may be used to gauge surface energy heterogeneity. That is, if measurements are done carefully, the variation between contact angle measurements will be indicative of the wetting variability as well as the gradation in surface topology as a function of x-y space. However, to make things interesting, by definition the static drop and associated Young equation apply to smooth and homogeneous surfaces; because a hair fiber is naturally porous, has variations in sebum and integral fatty acids, and has a jagged cuticular structure with natural and damage-dependent chemical and morphological

inhomogeneity, good sense must be implemented when characterizing the meaning of the data and/or applying them to surface-free energy models. Further, DSA experiments are sensitive to droplet rearrangements (i.e., lateral motion), humidity, static electricity, volatility and/or sorption of the liquid—especially if applying low-volume droplet sizes to hair fibers. Hence, in the literature the use of the liquid drop method for determining contact angles on hair fibers is rather sparse.

Prior to recent advances (e.g., **Figure 7**) in video technology and drop volume [33] control, the application of DSA to the rough and chemically heterogeneous surface of hair rendered contact angle measurements difficult and unreliable [34]. Hence, historically speaking, the prevalent method in the literature for measuring the contact angle of a fluid at the interface with a single fiber is based on *force tensiometry* and the single-fiber Wilhelmy balance method. The Wilhelmy technique is considered a *dynamic contact angle* method because, unlike the sessile drop approach, the phase boundaries and shape of the meniscus are in perceivable motion as the fiber is advanced or receded from the fluid. In the application of the Wilhelmy method to hair, a small section of the hair fiber is affixed to a thin wire hook and suspended from the microbalance. The mass of the fiber is tared, and a moveable stage containing the fluid, such as high-purity water, is subsequently raised or (lowered) until a prescribed length of the fiber is submerged (e.g., 2–5 mm). As the fiber moves into the liquid, the wetting of the fiber produces a maximum force that is related to the *advancing contact angle* by Equation 4,

$$F = \gamma P \cdot \cos\theta_{a,r} + W - \rho g y A, \tag{4}$$

where F is the maximum measured force, P is the wetted perimeter of the fiber, θ_a is the advancing contact angle, ρ is the density of the fluid, g is the gravitational constant, y is the immersion depth, W is the weight of the fiber, and A is the cross-sectional area of the hair fiber [34]. The second and third terms of Eq. 4 are ignored because the fiber mass is initially tared, and because the buoyancy of a human hair is negligible. Subsequent retraction of the fiber from the fluid stretches the meniscus along the wetted fiber surface, and the maximum force produced is related to the *receding contact angle* (θ_r) by Eq. 4. **Figure 9** depicts the Wilhelmy balance model and **Figure 10** shows a force versus distance profile for a typical experiment. As with DSA, force tensiometry is sensitive to extraneous vibration, temperature fluctuations, and surface contamination. Also, for the Wilhelmy methodology, the wetting perimeter (**Figure 11**), the direction of scales (**Figure 10** inset), and the rate of introduction or removal of the fiber from the interface affect equilibration processes and must be carefully controlled [35].

Figure 9: Diagram of dynamic contact analysis on hair using Wilhelmy methodology. Contact angle analysis is reduced from Eq. 7 to cos θ= F/γP, where *P* is the wetted perimeter of the solid, *θ* is the advancing or receding contact angle, and *F* is the wetting force corrected for buoyancy and fiber mass. *P* is approximately 2πr for a hair fiber.

Kamath et al. [36] employed the Wilhelmy balance technique to study the effects of weathering, chemical, and mechanical insults on the water wettability of the fiber surface. The extent of daily mechanical strains and natural weathering processes (i.e., UV damage) was inferred by sectioning 10-inch-long fibers and next measuring the wetting forces as a function of root-to-tip distance. **Table 4** suggests that daily UV and grooming processes introduce cuticular stresses that augment the hydrophilicity of a hair fiber as a function of time, or hair length. Hair that emerges from the scalp (zone 6) shows nonwettability properties, while zones 3–6, which are closer to the tip, show wettability traits similar to those seen in bleached fibers [34].

Table 4: Effect of root-to-tip distance on the magnitude of the advancing contact angle [38]

Tip	Fiber Partition	Advancing contact angle (θa)
1	1	72
2	2	73
3	3	67
4	4	99
5	5	101
6	6	103
Root		

Using Wilhelmy techniques, Molina et al. [37] studied the impact of chemical strains on the physicochemical properties of the integument. They evaluated the application of alkaline solutions with and without 0.6% H_2O_2 and compared to untreated hair. Because hydrogen peroxide is known to oxidize cystine to cysteic acid residues, the assumption is that this influences the hydrophilicity as well as the number of ionizable moieties on the cuticular surface. Attempts were made to augment wetting trends by characterizing the *physical* (dispersive) and *chemical* (acid-base) contributions to the work of adhesion across the interface,

$$W_{SL} = W_{SL}^{LW} + W_{SL}^{AB}, \tag{5}$$

where W_{SL} is the equilibrium work of adhesion, W_{SL}^{LW} is the Lifshitz-van der Waals (i.e., dispersive or hydrophobic) component, and W_{SL}^{AB} is the contribution of acid-base interactions between the liquid and solid. W_{SL}^{AB} may subsequently be solved for by applying Equation 6 to the empirical data [37],

$$W_{SL}^{AB} = \lambda_L(1 + \cos\theta) - 2(\lambda_L^{LW} \cdot \lambda_S^{LW})^{1/2}, \tag{6}$$

where λ_L is the surface tension of the dry solid, λL_{LW} is the dispersive component of the surface tension for the liquid, and λS_{LW} is the dispersive component of the surface tension for the dry solid. Not surprisingly, the results confirmed that untreated hair is less wettable than alkaline-treated and/or peroxide treated fibers. Further, by controlling the aqueous pH during tensiometry measurements, it was shown that alkaline-treated fibers have an increase of ~2 times increase in W_{SL}^{AB}, and oxidized fibers have an increase of 3–4 times increase in W_{SL}^{AB} relative to untreated fibers—confirming that alkaline and/or oxidative treatment increases the number of hydrophilic and ionizable functionalities on the hair surface.

Figure 10: Wetting force as a function of immersion depth for a virgin hair fiber. The fibers are typically mounted in the against-scale direction. (a) Fiber penetrating the liquid interface; (b) fiber advancing into liquid surface; (c) fiber receding from interface; (d) meniscus breaks from fiber. The insets depict one reason for contact angle hysteresis (θ_a-θ_r). During advancing of fiber into liquid, the meniscus interacts with cuticle surface; in the receding step, the meniscus strongly adheres to scale edges [34, 36, 37, 39].

Using Zisman's approach [38] to find the *critical surface tension* (λ_C), where plotting cos(θ) versus λ_{lv} with test liquids and extrapolating to θ=0 to find λ_C, Kamath [37] detailed the λ_C wetting argument into dispersive and polar terms,

$$\lambda_C = \lambda^d_S + \lambda^p_S, \tag{7}$$

where λ_C is the critical surface tension, and λ_{dS}=24 mN/m and λ_{pS}=4 mN/m are the polar and dispersive components for untreated hair, respectively. The low-magnitude dispersive component reflects the dominating *average* hydrophobic nature of virgin hair, and also intimates that very high surface tension fluids will bead on the pristine hair surface. However, intuitively, the ensuing chemical effects of oxidation significantly increase λ_C and λ^p_S– and, therefore, increase the likelihood of additional homogenous chemisorption interactions between higher surface tension conditioners, such as water-soluble cationic polymers, and the modified higher-energy surface.

Figure 11: Consequence of the magnitude of the wetting perimeter on the profile of the force curve (see Eq. 4). Nonwetting solids are less dependent on fiber diameter. Virgin hair has a negative wetting force, and θ_A is approximately 103° [34, 36, 37].

Kamath et al. [35], and also Lodge and Bushan [34], studied the wetting properties of virgin, chemically treated, and subsequently conditioned, hair tresses. Lodge observed a statistically significant (p=0.05) reduction in the dynamic contact angle (Wilhelmy balance) with permanent waving (70±7°) and with the application of conditioner (88±9°) to virgin hair (103±4°); however, application of commercial conditioner to permed hair (79±1°) caused an increase in the contact angle, relative to the permed hair. To explain the trends, both Kamath and Lodge speculated that the state of the fiber may dictate how the conditioning surfactant associates with the hair. For example, on damaged hair, the cationic groups will electrostatically bind more strongly to the copious cysteic acid moieties and the lower-energy alkyl chains of the surfactant will be directed towards the surface (i.e., higher contact angle, decreased wetting). Yet, on application of cationic conditioner to virgin hair, with a presumably intact F-layer, the hydrophobic alkyl chains of the surfactant

may physisorb with the bound lipids and the higher-energy cationic moieties will be exposed to the surface (i.e., lower contact angle, increased wetting).

Lodge [34] also rationalized that contact angle hysteresis ($H = \theta_a - \theta_r$) could be used to study the location of the deposition of conditioners on a hair fiber. Relative to the permed-fiber curve, very little contact angle hysteresis was seen for the bleached-and-treated fiber. This indicated that the conditioner deposited significantly on the cuticle edges, producing a larger θ_r than expected because the intrinsic hydrophilicity of the scales was "hidden." This result was corroborated by Dupres et al. [40, 41], who used noncontact AFM to examine the *microscopic* heterogeneity of wetting behavior for a range of solvents on hair fibers—including silicone oil (21 mN/m), decane (23 mN/m), and squalane (31 mN/m). Results indicated that only silicone oil wets the hair surface completely; however, the cuticle edges, which are proteinaceous and more hydrophilic than the cuticular surface, were wet homogenously by the oils (**Figure 12**). Using critical surface tension arguments, this clearly suggests that the cuticle edges have a higher λ_C than the cuticle surface, and that the hydrophobic oils have λ less than the λ_C of the cuticle edge.

Trends in surface tension, wetting, and contact angle trends are valuable tools for following the deleterious effects of chemical, mechanical, thermal, and weathering strains on the integrity of the cuticle. Due to chemical and surface heterogeneity, hair fibers are a challenging substrate for both static and dynamic contact angle measurements. Advances in DSA technology have rendered small-volume, sessile-drop DSA measurements on single fibers feasible, although the surface heterogeneity of hair renders challenges to evaluating instrument precision. Due to an abundance of examples in the literature, the Wilhelmy balance method is still the established method for measuring the wettability of single fibers. By employing surface energy models to contact angle results, it is possible to gain insight into the chemical modifications of the surface and to accordingly develop treatments to ameliorate the compromised wetting properties of damaged hair.

Figure 12: Preferential wetting of virgin hair by squalane. Squalane spreads uniformly across the relatively polar cuticle edge, whereas sparse wetting is visible on the hydrophobic cuticle surface.

3. Dynamic Vapor Sorption (DVS)

Prior to the advent of commercialized Dynamic Vapor Sorption (DVS) technology, the interaction of relative humidity (RH) with a sample was principally studied by exposing pre-dried samples to isothermal air over a series of saturated salt solutions (42). Samples were then periodically removed from the desiccators and weighed to study sorption kinetics and to obtain the equilibrium sorption isotherm. Understandably, the methodology was both tedious and time consuming due to the lack of automation. Furthermore, it was more difficult to accurately monitor the rate of sorption and to strictly control the environment of the samples within the cumbersome desiccators—including temperature, dry gas type, sorbent vapor type, vacuum, and the logistics of transferring highly hygroscopic materials between the desiccator and balance.

In general, modern DVS analysis is a fully automated, gravimetric technique used to monitor the interaction of a gaseous probe molecule (e.g., water, cyclohexane) with a substrate of interest (e.g., polymer film, hair). The relative vapor pressure (i.e., RH for water vapor) is accurately controlled by streaming a mixture of dry (e.g., nitrogen, air) and vapor-saturated gases in correct proportions using mass flow controllers. The mass changes of the sample are monitored with an ultra-sensitive microbalance, and changes in the chemistry of the sample can be simultaneously followed with NIR spectroscopy, Raman spectroscopy, and/or optical microscopy. Depending on the instrument model, one or several very small samples (< 30 mg) can be dried at 0% RH, pre-heated to high temperatures (e.g., 100–300°C), pre-humidified, cooled or heated, exposed to stepped or oscillating humidity, or influenced by reduced pressures—all automated and all *in situ*. Because all aforementioned processes can be programmed into the DVS control software using sequences, and without altering the position or controlled environment of the sample, both equilibrium and kinetics trials may be used to fully differentiate comparative sorption processes. During water vapor DVS analysis, which is the method typically used in the literature to study interactions with hair fibers, the relative humidity in the sample compartment is varied stepwise with time and changes in mass are continuously monitored. A subsequent plot of the *equilibrium* sorbed vapor mass (i.e., mass regain) against the relative humidity at a controlled temperature is termed a *sorption isotherm*. The resulting *hysteresis* plots, which are generated by plotting the mass difference between the sorption and desorption isotherms at each isohume, are insightful in contrasting characteristic substrate traits such as equilibrium moisture content, bound versus unbound water, surface hydrophobicity, and porosity. Although fully equilibrated experiments are inherently time consuming for porous or swellable biomaterials, proper equilibration between the *adsorbent* (substrate) and *adsorbate* (vapor) facilitates the determination of accurate equilibrium moisture contents and diffusion coefficients. In

addition, application of comparative isotherm data to theoretical sorption models may be used, albeit controversially (43), to visualize the allocation of *adsorbed* and *absorbed* vapor at the surface and within, respectively, a porous substrate—such as a hair fiber.

Figure 13: Modern DVS Instrumentation (courtesy of Surface Measurement Systems, UK).

Hair fibers are hygroscopic, yet an undamaged hair fiber with endogenous sebum and intact integral fatty acids has a low surface tension and effectively repels water (44). Although contradictory, the net result is that hair has a penchant for slowly absorbing and slowly desorbing water vapor, as indicated by its characteristic sigmoidal DVS isotherm and noted hysteresis between the sorption and desorption steps. The hysteresis lag and the exothermic heats of sorption suggest that once water negotiates a path through the cuticle and into the cortex (i.e., *absorbed* water) it is tightly held by the hydrophilic peptide residues within the fiber (45). As absorbed water induces radial swelling of the fiber and affects the free volume and mobility of α-keratin, any treatment that affects hair's water transport processes will induce transient changes in properties such as dye mobility and mechanical strength (46). Hence, DVS analysis is a tool to forecast the impact of weathering and the efficacy of protective or repair remedies on the quality and health of a keratinous fiber.

Bleaching and perming are processes that impart chemical strains to the hair. Bleaching is a harsh oxidizing process, whereas perming involves the reduction of disulfide bonds followed by an oxidative process to reform the bonds in a newly styled position. Barba et al. (47) contrasted the water transport processes of untreated, bleached, and permed natural red hair using DVS. Subsequent fitting of the sorption data to the Guggenheim, Andersen, and De Boer (GAB) sorption model was used to calculate the GAB monolayer capacity (W_m) and GAB energy constant (C_g) (48, 49). Loosely speaking, W_m relates the number of vapor-binding sites to the mass of hair; C_g is related to the enthalpy of sorption, and is a measure of how strongly water vapor adheres to the binding sites in the hair fiber (50). The GAB sorption model is an enhanced BET sorption model and has been shown to give a physics-based connection between the water absorbed and the number of hydrophilic sites on the substrate (51).

Table 5: DVS Results from Sorption Studies on Untreated, Bleached, and Permed Natural Red Hair by Barba et al. (46)

Hair Treatment	**Max. Regain, 95% RH (%)**	***Wm* (%)**	***Cg***	**t_T (min)**	**D_A (min-1)**
Untreated	25.87	7.487	6.706	3204.22	0.0163
Bleached	24.60	6.630	6.803	2895.12	0.0203
Permed	29.97	7.518	6.597	3297.16	0.0175

The authors rationalized the results in **Table 5** by noting that oxidative degradation mechanisms corrupt the surface and the way hair naturally intermingles with water; therefore, although perming significantly increased the maximum regain, bleaching is considered to have the most detrimental effects on hair by rendering the fibers more permeable to water. Bleaching lessens *Wm*, increases the apparent diffusion rate (D_A) of sorption/desorption, and decreases the amount of time it takes for the sample to reach equilibrium mass (t_T); however, neither treatment significantly affects *Cg*. In a 2009 report by the same research group, bleach-damaged hair was subsequently treated with isolated wool intermediate-filament peptides and proteins. Relative to bleached fibers, the DVS results, including improvements in D_A, t_T, and *Wm* data, all suggest that the keratin treatments reestablish the original water-management properties of the untreated virgin hair (52).

High-temperature flat ironing introduces deleterious structure and chemical effects that alter the water management properties of the hair—including cuticle cell lifting; thermal softening; and the formation of deep holes, cracks, and fractures of the cuticle structure caused by protein denaturation and the rapid expansion of water vapor within the fiber structure (53, 54). Moreover, protein denaturation

and changes in lipid structures may significantly affect the accessibility of water-binding residues. Hence, as conduits to water-vapor sorption may be closely tied to physicochemical damage of the hair fiber, DVS analysis is a logical asset for studying the efficacy of remedies for diminishing thermal damage.

Following the work of Zhou et al. (4), tresses of virgin European dark brown hair were washed with 10% SLES, then blow-dried to remove dampness, and subsequently hot flat-ironed (232°C) for 12 seconds. The process was repeated three times over a 12-minute period. If a protective treatment was applied, the treatment was added after washing, and prior to the blow-drying step. To minimize the experimental cycle time, the chosen method revolved around time constraints and comparative vapor penetration kinetics (i.e., samples were nearly equilibrated). Rather than introducing edge effects (**Figure 14**) to the sorption processes, the sample preparation consisted of carefully wrapping 20–30 strands of hair fibers into a loose coil (**Figure 14**) and loading them into the DVS apparatus (55). All DVS experiments were performed at 25°C and used the following procedure:

1. Preconditioning: Prior to drying of the loops, samples were exposed to 95% RH for 1 hour to minimize the variations in "humidity history" (56).
2. Initial drying: The loops were dried at 25°C and 0% RH for 12 hours.
3. Absorption curve: The humidity was ramped from 0% to 90% in 10% RH steps. Each sorption step was 4 hours in duration.
4. Desorption step: The humidity was ramped from 90% to 0% in 10% RH steps. Each sorption step was 4 hours in duration.

Figure 14: Formation of DVS hair loop and the potential implication of edge effects. Coiled fiber bundles were used instead of chopped hair segments to model "real-life" cuticle-permeation kinetics.

Figure 15 shows the DVS isotherm and D_A overlays for the unheated, heated and untreated, and Polyquaternium-55 (PQ-55) treated and heated hair tresses (PQ-55, **Figure 16**). Note that the maximum water vapor regain trend is unheated > PQ-55 treated and heated > heated and untreated (Fig. 3a). This suggests that the PQ-55 treated and heated composite sustained less thermomechanically imposed

damage to the microstructures involved in water binding than did the heated and untreated tress. **Figure 15b** echoes the same trends seen in the isotherm overlay and implies that, in the desorption process (upper portion of diffusion curve), water diffuses most quickly from the heated and untreated hair at moderate humidity levels (40–80% RH). With the aid of supplementary empirical evidence, the authors speculated that changes in the water-management properties of the damaged heated and untreated fibers were due to combined surface and cortex damage. First, the hydrophobic boundary mediating the transport of water vapor to the cortex was compromised through the introduction of cracks, holes, and the formation of micropores in the cuticula. Further, on a molecular scale, degradation of protein moieties and conformational changes in the keratin structure, such as α-keratins denaturing to β- or to other unfolded forms, may have reduced the number of water-binding sites within the cortex.

Figure 15: DVS results for unheated, heated and untreated, and PQ-55 treated and heated hair fibers; (a) isotherm overlay depicting maximum % regain values; (b) overlay of D_A showing effect of thermal treatment on kinetics of water management.

Whereas untreated and heated samples were irreversibly damaged by thermal insult, the PQ-55 heated and treated fibers exhibited less protein degradation and denaturation and, for this reason, retained structural elements critical for effective hydrogen-bond stabilization of the α-keratin helices. The resilience in physicochemical structure resulted in the preservation of natural water sorption processes—which were deemed important for the preservation of moisture retention, moisture restoration, and subsequent thermal protection properties (4).

Figure 16: Repeating unit structure for Polyquaternium-55

Keis et al. (57) used DVS to study the retention of moisture after the application of oil films to surfactant-washed hair. The results indicate that the moisture regains for the coconut, sunflower, and mineral oils were 23.7%, 22.5%, and 22.1%, where the maximum regain (R_{max}) is a function of the dried hair mass (M_D) and the equilibrium mass of the fibers (M_v) at 95% RH:

$$R_{max} = Mv / M_D * 100 \quad (8)$$

At low humidity levels, calculated hysteresis plots demonstrate that oil increases the retention of water, which is suggested to be an effect similar to moisturization of the hair. The authors proposed a diffusion barrier mechanism wherein the pathway for permeation of water vapor into the hair is primarily through the endocuticle and the Cell Membrane Complex (CMC). The oils are thought to accumulate at the cuticle edges before subsequently penetrating into the endocuticle, CMC, and possibly the cortical envelope: thereby slowing the rate of sorption and lowering R_{max} (relative to virgin hair, where R_{max} = 27.4%).

By means of empirical pathways, many researchers have speculated on the mechanisms of applied oxidative and/or thermal damage and the resulting stresses subsequently acquired by the maltreatment of hair's chemistry and microstructure (4, 5, 22, 53, 54, 59–65, 66). Others have made efforts to critically speculate on how and why effective barrier oils and polymeric additives function in thermal

protection applications (5, 58, 67). Although the complete damage and protection mechanisms are not fully understood, it is clear that a glaring symptom of applied weathering strains is a corruption in the natural water management processes of hair.

4. Streaming Potential

Streaming Potential, or *Dynamic Electrokinetic and Permeability Analysis* (DEPA), incorporates the measurement of electrochemical properties while a flowing test solution interacts with the surface of a planar or fibrous sample. It has been used to study the adsorption and desorption of various ingredients on hair [68, 69, 70]. The empirical data for a single DEPA experiment includes the streaming potential (E), conductivity (κ), pH, liquid flow rate, and temperature—all measured as a function of flow volume and time. The zeta potential, ζ, which is an important parameter for gauging the electrostatic attraction or repulsion between an applied rinse-off treatment and hair, can subsequently be derived as a function of time from the DEPA data by applying the Smoluchowski equation (Eq. 9),

$$\zeta = 4\pi\eta\kappa E / P\varepsilon, \tag{9}$$

where P is the drive pressure applied to the flowing liquid, and η and ε are the viscosity and dielectric constant of the streaming polyelectrolyte solution, respectively.

In a DEPA experiment designed to discern the consequences of oxidative damage, the hair sample is wetted in dilute electrolyte solution, chopped into small, compressible pieces (4–6 mm), and then placed in a special flow cell that houses two perforated silver chloride electrodes (**Figure 17**). Dilute electrolyte solution (5×10^{-5} M KCl) is next pulsed through the cell (30 s on, 30 s rest) five times to properly equilibrate the sample [68]. Following equilibration, the dilute KCl test solution is pulsed unidirectionally through the fiber plug for 75 minutes while monitoring the E, κ, flow rate, and ζ. The more highly damaged sample (as assessed by the alkaline solubility test [70]) showed a higher initial solution conductivity and a longer decay to a constant conductivity plateau than the less-damaged tress. Both samples showed a negative decay in ζ and E as a function of rinsing time. Jachowicz et al. suggested that the observed variations in the electrochemical properties of the bleached samples, relative to the untreated control, were more than likely related to residual bleaching components leaching from the hair.

DEPA provides detailed information about the electrokinetical properties of the fluid-fiber interface. By developing a detailed understanding of the dynamics of interactions with the damaged-hair plug, including the kinetics of rinse-out, E, ζ, and κ data may be used to better design intelligent treatments to ameliorate fiber damage.

Figure 17: Schematic of Streaming Potential Cell showing perforated Ag/AgCl electrodes surrounding the compressed hair fiber plug (Adapted from Dynamic Electrokinetic Analyzer manual, Better Cosmetics LLC, US).

5. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) uses a pulsed, finely focused ion beam to bombard the surface of a substrate and release secondary atomic and molecular ions. The desorbed organic or inorganic ions are subsequently accelerated to a Time-of-Flight (ToF) analyzer where their arrival times are correlated with mass (**Figure 18**).

In its simplest mode, ToF-SIMS provides a mass spectrum of the region of interest. However, by rastering the primary ion beam across the surface, two-dimensional images are generated to view the spatial distribution of specific chemical moieties as a function of x-y space. For each pixel in the image, an entire mass spectrum is subsequently obtained, ranging from 1 to approximately 10 kDa. Further, depth profiles are obtained by using a sputter beam technique, where surface layers are removed from an area prior to acquiring images of newly exposed subsurface monolayers (**Figure 19**).

An advantage of ToF-SIMS is that concatenation of images facilitates imaging of fairly large samples (e.g., 9 cm × 9 cm). Further, as the lateral resolution for a surface can be as low as 60 nm [71], ToF-SIMS enables an excellent understanding of the true chemical heterogeneity of a studied surface. A disadvantage is that although ToF-SIMS is a powerful surface analysis technique, quantification of analytes is not straightforward. Typically a matrix with a known amount of analyte must be used as a reference, or comparative differences between two samples may be determined by referencing an internal standard common to both sample matrixes (e.g., protein moiety on the surface of hair). Further, because x-y scanning only examines the first few monolayers of the sample surface, proper care must be taken when preparing samples for ToF-SIMS analysis. For example, touching hair fibers with unwashed fingers may introduce sebum contamination to the fiber surface, thereby confounding the meaning of the results.

Figure 18: ToF-SIMS schematic: A pulsed primary ion beam (orange) is focused on the sample and secondary ions (blue) are desorbed from the surface, focused, and counted by the detector as a function of time (courtesy of ION-TOF GmbH, DE).

Figure 19: The primary ion beam may be rastered on a microarea of the sample to generate a 2-D image. By using a sputter beam to remove successive surface layers, a depth profile of the substrate (3-D) may be generated to view the layers of substrate below the surface monolayer (courtesy of ION-TOF GmbH, DE).

In the literature, ToF-SIMS has been applied to characterization of the detailed chemical structure of the hair surface (e.g., **Figure 20**). Surface and cross-sectional analyses have been used to evaluate a wide range of fiber applications, including evaluation of surfactant deposition [72], conditioning pretreatments [73, 74], oil penetration [75], dye distribution [76], and elemental distribution [77].

Clear examples of applying ToF-SIMS methodology to studying the weathering and chemical strains of the hair's native hydrophobic barrier include the work

of Okamoto et al. [78, 79, 80]. The investigators utilized ToF-SIMS to follow the depletion of 18-MEA as a function of hair length, bleaching, and UV exposure. In the initial work [78], hair fibers were collected from a Japanese woman, aged 30, with no history of cosmetic chemical treatments, such as bleaching, permanent waving, or coloring. Some of the pristine hair fibers were subsequently bleached with H_2O_2, and others were treated with UV light (3500 μW/cm^2) for various exposure times (48–480 hrs). For each studied fiber, a 75 × 75 μm area was scanned and changes in the negative ion spectrum intensity signals, including 18-MEA *thioester*-bound (m/z 341) and 18-MEA *ester*-bound (m/z 325) surface lipids, were monitored. Relative to an internal peak from fiber protein, the results showed that ~80% of the thioester-bound 18-MEA and ~40% of the ester-bound MEA were removed in one bleaching cycle. Further, exposure to 400 hrs of UV radiation (~2–3 months of summer exposure) diminished the relative intensity ratios of thioester-bound 18-MEA and ester-bound 18-MEA by ~85% and ~70%, respectively. Hence, the researchers concluded ester-bound 18-MEA is presumably less susceptible to chemical oxidation and UV exposure than its thioester-bound analogue.

Figure 20: 2-D ToF-SIMS images of (a) CNO^- moiety, from surface protein, (b) $(\text{lauryl sulfate})^-$ from shampoo residue, (c) and $(\text{18-MEA})^-$ distributions on the fiber surface. Pixels with lighter colors represent larger ion counts (courtesy of Physical Electronics Inc., U.S.).

In addition to employing ToF-SIMS to investigate the impact of weathering and chemical insults on 18-MEA depletion, subsequent work by Okamoto et al. [79] discussed resultant changes in surface wettability and potential treatments to mend damage. Hair samples from Japanese women, aged 20–30, with varying exposure to hair-coloring treatments, were analyzed by ToF-SIMS and dynamic contact angle analysis to correlate 18-MEA damage with hydrophobicity data. The results inferred that the 18-MEA thioester content was drastically modified by coloring treatments, especially for exposure to >1 treatments per year. Further,

as the ratio of 18-MEA to fiber protein decreased to <1, the dynamic contact angle dropped from ~100° to ~57°. For reference, the ratio of 18-MEA to fiber protein for *untreated hair* decreased to <1 at a hair length of >40 cm from the root—where the authors suggested that cumulative solar weathering, grooming, and daily shampooing affected the F-layer quality. Finally, ToF-SIMS was used to study formulations of 18-MEA combined with cationic surfactants. Both ToF-SIMS and contact angle measurements showed that the treatments replenished the hydrophobic integrity of the damaged surface, as evidenced in the recovery of the dynamic contact angle (from 65° to ~90°) and the uniform distribution of 18-MEA/surfactant complex on the hair surface.

Although intermolecular interactions within the surface limit the molecular weight of *intact* emission ions, recent technological advances in ToF-SIMS primary ion beam sources, depth profiling, and analyzer technology have increased the applicability of the technique to more complex matrixes, such as biopolymers [81, 82] or biocomposites. In conclusion, ToF-SIMS imaging offers insights into the spatially resolved chemical modifications imparted by applied physicochemical insult; subsequent coupling of ToF-SIMS imaging with data from other performance measurement techniques, such as optical, wetting, or mechanical data, enables a feasible route to designing damage repair treatments.

6. X-Ray Photoelectron Spectroscopy (XPS)

XPS, which is also known as Electron Spectroscopy for Chemical Analysis (ESCA), is a technique employed for the qualitative and quantitative analysis of surface elements within the first 1–10 nm of a solid, such as a hair fiber. The sample is irradiated with photons from a beam of monochromatic X-rays while a hemispherical energy analyzer simultaneously monitors the number and kinetic energy of the emitted electrons [83]. The kinetic energy of the emitted electrons is then normalized into element-specific binding energies (Eq. 10) that can be used to identify and quantify surface elements (± 0.1–1.0 atom-%). As the adjoining chemical environment affects the binding energy of a surface element, XPS may be used to compile information regarding the chemical state of an atom [84]. In addition to resolving the elemental composition of spots as small as 10 μm, XPS may be applied to larger areas comprising a few square millimeters. Further, by combining XPS with ion-induced sputtering, it is feasible to describe the elemental composition as a function of penetration depth.

$$\text{Binding energy} \approx \text{X-ray energy} - \text{Kinetic energy of emitted electron} \quad (10)$$

In combination with SEM, Beard et al. [84] studied the application of surface specific XPS to spectroscopically discern the effects of bleaching and subsequent conditioning on the surface chemistry of hair fibers. The impact of pH and the addition of surfactant on the efficacy of the bleaching process were also investigated.

Because XPS is not suitable for detecting atoms with atomic numbers less than 3, the measurable untreated hair surface is primarily composed of carbon (C), oxygen (O), nitrogen (N), sulfur (S), silicon (Si), and calcium (Ca). C, O, N, and S are components of the surface protein, and the fatty "overlayer" is principally composed of C and O (e.g., 18-MEA). Ca and Si are noted as minor "natural constituents of hair," with Ca levels supplemented by tap water [21, 85].

Bleaching treatment systems for the study included (A) 3% hydrogen peroxide, (B) 3% hydrogen peroxide, sodium bisulfate activator, pH=9, and (C) 3% hydrogen peroxide, sodium bisulfate activator, pH=9, 0.25% SLS. Each treatment was applied to a hair tress for 0, 30, or 45 minutes prior to rinsing. To examine the effect of the rinse-off conditioning treatments on bleached hair, the aforementioned bleached swatches were subsequently treated with a 10% conditioning dispersion (including 2.8% diester quaternary amine, 0.9% dimethicone, 0.7% glyceryl stearate, 0.8% stearamidopropyl dimethylamine, 2.7% fatty alcohols) for 60 seconds and then washed with warm water prior to XPS and SEM analyses. XPS (Al k_α X-ray source) was then used to monitor the surface composition of the bleached tresses, focusing on the C, N, S, and O counts; additionally, fluctuations in the content of Si and Ca were followed for the conditioned samples.

The investigators used the photoelectron transitions from sulfur to monitor changes in the disulfide and sulfonate surface concentrations as a function of the formulation and bleaching time. The data, which are summarized in **Figure 21**, indicate that time, pH, and the presence of surfactant affect the kinetics of oxidation, as indicated by comparative changes in the levels of cysteic acid. The authors rationalized that SLS reduces the induction time for effective oxidation via effective wetting of the formulation with the hair surface; in addition, it was surmised that surfactant facilitates more effective rinsing of the hydrocarbon overlayer during the 30-second rinsing step. Arguments were similarly presented for trends in changes of the C, O, and N chemical states. Clearly, alkaline bleaching removes the protective covalent lipid and subsequently exposes more of the hydrophilic protein surface to the oxidative effects of H_2O_2.

Finally, relative to the bleached sample, XPS analysis of the conditioned tress showed dramatic increases in Si and O, but a decrease in protein-specific N, and the absence of Cl^-. Further, high-resolution XPS was used to confirm the presence of deposited diester quaternary amine. Examining the data, the authors concluded that the conditioner is not deposited homogeneously on the surface, as evidenced by the existence of Ca and N, which are components of the fiber. Additionally, the dimethicone is more than likely associated with the hydrophobic tails of the ester quat, rather than the proteinaceous surface. Conversely, the Cl^- counterion is absent because it is released when the N^+ of the ester quat directly associates with the anionic sulfonic functionalities of the oxidized protein surface.

Figure 21: Total oxidized surface sulfur content (cysteic acid) by XPS for (A) bleached hair, (B) bleached hair, pH=9, (C) bleached hair, pH=9, SLS. Comparing *B* and *C* at 30 min., note that the addition of SLS eliminates the inherent induction period for surface oxidation (adapted from Beard et al. [83]).

In summary, XPS enables the direct spectroscopic measurement of deleterious changes in the hair's proteinaceous surface. Correlation of XPS data with data from other techniques, such as SEM, contact angle analysis, AFM, and ToF-SIMS, facilitates a robust description of the chemically damaged fiber surface while offering a means to match the modified surface chemistry with a properly targeted remedy.

7. Inverse Gas Chromatography (IGC)

Inverse Gas Chromatography (IGC) is a dynamic sorption method used to measure the thermodynamic properties of surfaces. Contrary to traditional gas chromatography, as the name of the technique aptly implies, the analyte is not probed by a known stationary phase. Instead, the analyte is represented as the stationary phase in the custom GC column and helium is used to sweep volatile probes through the solid sample (e.g., hair fibers) to a chromatographic detector, such as a flame ionization detector (FID). Depending on the available surface physicochemistry of the sample in the column, the probe vapors will elute at different retention times (see **Figure 22**). The retention times are subsequently converted to net retention

volumes (V_N), and the V_N values are then used to derive the total surface free energy for the sample—where the total surface energy is a summation of *dispersive* (e.g., van der Waals) and *specific* (e.g., hydrogen bonding, polar, acid-base) surface energy components (86).

Figure 22: Diagram of an IGC experiment showing the interaction of probe vapor with intact hair fibers that have been packed into a custom-sized chromatography column. (a,b) Helium carrier gas and vapor pulse introduced to the column head; (c,d) retention time of the probe molecules delayed by fiber-probe interactions; (e) model chromatogram showing single peak of vapor probe as detected by the FID (adapted from SEA brochure, Surface Measurement Systems, UK).

Further, surface energy heterogeneity may be determined by pulsing precise amounts of the probes that interact with the substrate (87). Surface energy heterogeneity is essentially a map of the probe-sample surface energy distribution as a function of surface coverage. That is, at very low probe gas volumes, or at infinite dilution, probe-probe interactions are minimized and only a small high-energy fraction of the sample surface interacts with the probe gas; however, at higher volumes of probe vapor (i.e., BET region), perhaps all of the active sites on the surface will interact with the probe gas (88, 89). Contrastingly, if a pulsed volume of adsorbate vapor that is equimolar to the spectrum of active sites on the analyte surface is introduced, then the resulting total surface free energy value will be composed of an average of all interactions between the surface and vapor—as is the case in a traditional surface tensiometry measurement done on a single hair fiber (e.g., Wilhelmy balance method).

The cortex of a healthy strand of hair is protected by a very sophisticated 4–5 μm thick cuticular structure. In virgin hair, the protective sheath emerges from the scalp approximately ten cuticle sheets thick (90); however, as mechanical, thermal, ultraviolet radiation, and chemical insults apply daily strains to the

exposed shaft of the complex composite, the damaged sacrificial surface gradually weathers and sheds to spawn a fresh, new surface (91). Consequently, one could argue that most virgin hair samples have been exposed to surfactants (chemicals), a brush or comb (mechanical), and sunlight (UV radiation), and as a result should have varying degrees of history-dependent chemical and morphological micro-heterogeneity at the cuticular surface (**Figure 23**).

Figure 23: Depiction of model probe-surface interaction as a function of the fiber's x-y surface. Cuticle edges and damaged cells have higher specific (e.g., acid-base, polar) surface energy than the untainted, hydrophobic cuticle surface; hence, small volumes, or small coverages, of polar probe vapors will first interact with high-energy polar sites prior to adsorbing to lower-energy lipophilic locations on the cuticular surface.

Because established methods to calculate the surface energy of fiber surfaces, such as contact angle goniometry or Wilhelmy-based dynamic tensiometry, place relatively large droplets of a liquid onto the surface of the hair fiber(s), these methods inherently produce single-point averages that oppress the realistic distribution of energy sites beneath the voluminously applied droplet (87). In addition, as Young's equation applies to contact angle measurements on smooth and homogeneous surfaces, bulk solvent absorption and roughening of the fiber surface by

mechanical, chemical, and aging processes [92] affect the fundamental meaning of the data. In contrast, by varying and strictly controlling the introduction of small volumes of gas/vapor probe molecules, IGC probes the nooks and crannies of the available fiber surface a bit at a time to produce surface energy distribution maps that better describe the breadth of available adsorption sites on the fiber surface.

To better scrutinize the use of IGC in examining damaged hair surfaces, a series of tresses was exposed to a range of mechanical, thermal, environmental, and chemical strains. **Table 6** describes the hair tress preparation details for IGC experiments that were performed on untreated virgin, as well as mechanically, thermally, and chemically strained European Brown hair tresses. Each nontrimmed, full-length hair sample (1.5–2.0 g) was packed into a silanized glass chromatography column (30 cm length, 4 mm ID) and equilibrated for one hour at 25°C/30% RH with a flow of 10 sccm helium carrier gas to normalize the level of physisorbed water. Samples were subsequently run at a series of surface coverages with *n*-alkanes and polar probes to determine the total surface energy (γ_S^{Tot}), which is a sum of the dispersive (nonpolar, γ_S^{D}) and acid-base (polar, γ_S^{AB}) surface energy components, respectively (Eq. 11).

$$\gamma_S^{Tot} = \gamma_S^{D} + \gamma_S^{AB} \tag{11}$$

The initial, low-coverage data in **Figures 24–25** show that pulsing, rather than flooding, the hair samples with limiting moles of *n*-alkane or polar probes results in a treatment-dependent distribution of surface coverages. Introducing incrementally small volumes of probe gas ensures that the full range of the spectrum of surface energies is accurately represented. In contrast, flooding the column with larger volumes of probe vapor causes binding of the probes to high- and low-energy adsorption sites alike; consequently, the coverage data indicate that controlling the extent of the interfacial contact surface ensures that important adsorption contributions from the lower-density, highest-energy adsorption sites of the exposed hair surface are not lost in the statistics.

Table 6: Details of treated European Dark Brown hair tresses later examined by IGC

Sample	Sample Details
Flat-ironed, wet	The tress was equilibrated for 30 min in tap water; the excess water was gently removed followed by immediate exposure to five 12 s sweeps with a 190 °C flat iron; subsequently washed with 3% SLS.
Virgin	The tress was washed with a relatively mild 0.3% SLS solution.

Sample	Sample Details
Heavily abraded	The tress was spread evenly on lab bench (1–2 fibers thick) and was scraped from root to tip 100 times on each side with a stainless-steel razor blade; subsequently washed with 3% SLS.
Bleached	The tress was exposed for 30 minutes to a commercial bleaching formulation followed by 3% SLS wash.
Permed	The tress treated with a 2-step home-perming formulation (ammonium thioglycolate) for 1 hr followed by 3–4 min tap water rinse; in step 2, the tress was neutralized with hydrogen peroxide and treated with conditioner (Quaternium-52); tress subsequently washed with 3% SLS.

Figure 24: Dispersive surface energy profiles overlay for virgin, bleached, permed/conditioned, abraded, and flat-ironed wet hair fiber bundles. Pulsing discrete volumes of nonpolar probes into the column controls the fraction of probe-covered surface, where 100% coverage = 1.00 (i.e., $n = n_m$), where n_m = number of moles of probe to completely cover the sample surface. Results were produced with the iGC Surface Energy Analyzer (Surface Measurement Systems, UK).

Figure 25: Specific (acid-base) surface energy profiles overlay for virgin, bleached, permed/conditioned, abraded, and flat-ironed wet hair fiber bundles. Pulsing discrete volumes of polar probes into the column controls the fraction of probe-covered surface, where 100% coverage = 1.00 (i.e., $n = n_m$), where n_m = number of moles of probe to completely cover the sample surface. Results were produced with the iGC Surface Energy Analyzer (Surface Measurement Systems, UK).

Before speculating on the relationship between fiber damage and the chemistry of the fiber surface, it's useful to gain a visual perspective for the relationship between dispersion, specific, and the total surface energy of the hair surface (refer to Eq. 11). The heterogeneity profile in **Figure 26** shows an overlay of the aforementioned surface free energy distributions for the bleached hair assembly—where the surface energy distributions estimate the percentage of active surface sites at a particular surface energy. Note that the dispersion and acid-base components are shown relative to the total surface energy and suggest that the γ_S^{Tot} distribution more resembles γ_S^{D} rather than γ_S^{AB}. Hence, the contributions to γ_S^{Tot} by induced temporary and complementary shifts in electron density dominate the energetics of the vapor-solid interface.

Figure 26: Dispersive, acid-base, and total surface energy distributions for the bleached hair sample. Results were produced with an iGC Surface Energy Analyzer (Surface Measurement Systems, UK).

The trends in the dispersive and acid-base energy profile/distributions in **Figures 24–28** clearly suggest that abrading, bleaching, perming/conditioning, thermal abuse, and associated grooming and SLS washing processes modify the accessible surface chemistry of the respective cuticles. Heavy abrading likely removes layers of cuticle cells and exposes inordinate levels of subsurface protein, including those in the endocuticle and, perhaps, grants limited access to the fiber cortex [90]. Lengthy bleaching with alkaline hydrogen peroxide causes oxidative cleavage of thioester-bound fatty acids and surface cystine, thereby generating higher levels of cysteic acid on the cuticle and exposed endocuticle [93, 94]. Along with many side reactions [95], permanent waving decreases cystine levels and increases cysteic acid and thiolate anion levels [96]. Further, perming may affect the quality of cystine cross-linking in endocuticle bridging structures, leading to higher protein solubility and subsequent cuticle cell delamination [97]. Heating wet hair above the boiling temperature of water can generate internal pressures from steam rapidly escaping from the matrix, CMC, and cuticles, leading to changes in protein and lipid composition/conformation, and to the production of fragments, lifted cuticles, cracks, and holes in the cuticular surface [98, 99, 100]. Further, the ensuing SLS washing and rinsing steps (see **Table 6**) assuredly strips remaining free surface lipids from the fibers [101, 95].

Figure 27: Dispersive surface energy distribution overlay for virgin, bleached, permed/conditioned, abraded, and flat-ironed wet hair samples. Results were produced with an iGC Surface Energy Analyzer (Surface Measurement Systems, UK).

The IGC experiments ranked the γ_S^D *surface heterogeneity*, which is gauged from the breadth (i.e., highest γ_S^D - lowest γ_S^D) in each dispersive surface energy profile in the overlay (**Figure 24**), as follows: wet flat-ironed > abraded ≈ **virgin** > bleached > permed. The γ_S^D *peak maxima* trends, which are captured from the maxima in the surface energy distributions (**Figure 27**), ranked as follows: wet flat-ironed (54.33 mJ/m^2) > abraded (52.52 mJ/m^2) > bleached (51.51 mJ/m^2) > **virgin** (50.07 mJ/m^2) > permed (46.68 mJ/m^2). The results suggest that the heterogeneity in dispersive surface energy on the fiber surface is increased, relative to virgin assemblies, by wet flat-ironing and heavily abrading processes; whereas the damaging consequences of oxidative bleaching and chemical perming/conditioning appear to modify the surfaces more homogeneously. Additionally, the frequency maxima trends specify that the applied thermal and mechanical processes resulted in the largest γ_S^D peak maxima—indicating that the applied non-chemical strains impart surface stresses that increase the interaction between nonpolar probes and the highest energy sites on the fiber surface. Although changes in surface chemistry are no doubt

involved, treatments involving heat, abrasion, perming, and bleaching may add uniform submicron-level surface roughness to the cuticle surface; hence, it is likely that the number of active adsorption sites increased accordingly. Interestingly, bleaching and the lengthy perming process produced the narrowest γ_S^D heterogeneity and lowest γ_S^D peak maxima. Why? Both treatments diminish the interaction of the nonpolar fractions of the surface with dispersion probes. Oxidation increases the number of cysteic acid residues and possibly results in some loss of surface-bound lipids. Perming should also result in increases in cysteic acid surface moieties. However, the γ_S^D trends may be more complex for the perming-conditioning process, as the pH of the combined acidic neutralization and conditioning step may affect the ensuing binding conformation and deposition pattern of quaternary surfactant on the hair surface. More explicitly, at low pH the adsorption mechanism of cationic surfactant may be hydrophobically, rather than electrostatically, driven; therefore, the lower γ_S^D may be due to hair damage as well as the additive influence of accessible polar groups on the deposited cationic surfactant [102].

Figure 28: Polar (acid-base) surface energy distribution overlay for virgin, bleached, permed/conditioned, abraded, and flat-ironed wet hair samples. Results were produced with the iGC Surface Energy Analyzer (Surface Measurement Systems, UK).

Figure 28 depicts the specific (i.e., polar, acid-base) surface energy distributions for the five hair samples. The γ_S^{AB} *heterogeneity* ranked in the order: abraded ≈ wet flat-ironed > permed > **virgin** ≈ bleached; whereas the trend in γ_S^{AB} *peak maxima* positioned in the following way: abraded (8.10 mJ/m^2) > bleached (7.42 mJ/m^2) > permed (5.68 mJ/m^2) ≈ **virgin** (5.53 mJ/m^2) ≈ wet flat-ironed (5.39 mJ/m^2). The virgin and bleached samples had the narrowest γ_S^{AB} distribution profiles, indicating that the surfaces had the narrowest allotment of polar sites on their respective surfaces. Because the polar sites on healthy virgin hair are mainly at the cuticle edges and aged fiber tips, and oxidation generates widespread cysteic acid on a bleached hair surface, one can surmise that the γ_S^{AB} distributions should be well defined. In comparison, abraded and wet flat-ironed samples have damaged and missing cuticles, including an augmentation in the available surface area, as well as exposed cuticular and cortical proteins. Therefore, one would expect to see larger breadth in γ_S^{AB} surface energy heterogeneity. The γ_S^{AB} maxima rankings imply that the abraded and bleached samples have elevated fractions of accessible higher energy γ_S^{AB} adsorption sites—indicating that these fibers possess higher fractions of acid-base interactions with the polar probes (e.g., more probe-protein interactions). The data for the wet flat-ironed sample infers that heating the tress may have melted and redistributed the lipids on the fiber surface, thus preventing considerable changes in the number of accessible protein-polar probe interactions. In combination with the observation that the treated assembly was shiny and healthy-looking, the virgin-like γ_S^{AB} data for the permed sample suggest that the conditioning step may have masked some of the chemical damage provoked by the perm treatment. Further evidence is evident in the γ_S^{AB} to γ_S^{Tot} ratio, which is indicative of trends in fractional surface polarity, or surface wettability. **Figure 29** shows that the abraded and bleached samples have the highest fraction of acid-base surface free energy component; whereas the wet flat-ironed, UV-treated, and permed/conditioned samples appear to maintain similar hydrophobicity to that of untreated virgin hair.

In conclusion, IGC has proven useful in gaining insight into changes caused by the damaging effects of environmental, chemical, and thermal strains on the fiber surface. Parameters such as surface energies and enthalpies, surface heterogeneity, and acid-base interactions can be measured at controlled temperature and humidity. As evidenced by the impact of mechanical, thermal, environmental, and chemical strains, as well as the impact of a conditioning step in the perming process, appreciating the interactions between known vapor probes and the hair surface enables an improved understanding of externally induced stresses within the surface structure. Moreover, with the assistance of complimentary surface analytical techniques, such as SEM and IR imaging, IGC may enable a deeper and fundamental understanding of important hair care properties, such as the principles of adhesion, deposition, conditioning, and structure repair.

Figure 29: Fractional polarity ($\gamma_S^{AB}/\gamma_S^{Tot}$) comparisons for thermally, environmentally, chemically, and mechanically strained European Dark Brown hair fiber assemblies. The error bars indicate slight variation in polarity as a function of surface coverage. Results were produced with the iGC Surface Energy Analyzer and SEA Analysis software (Surface Measurement Systems, UK).

b. Spectroscopic

1. Infrared Imaging (IR imaging)

Infrared (IR) light is radiation that resides between the visible and microwave regions of the electromagnetic continuum. The IR spectrum is arbitrarily divided into the near, mid, and far infrared spectral regions. The wavelength (λ) of near IR (0.8–3.0 μm) is closest to red visible light, whereas the wavelength range of far IR (30–300 μm) is nearest to the microwave region. Since mid IR (3.0–300 μm) has applicability to functional group analysis, it has arguably been the spectroscopic region of choice for chemical investigators.

Not all bonds are active to IR energy. The ability of a particular bond to absorb IR radiation (i.e., IR active) depends on the presence of a net change in

dipole moment as the interatomic distance of the bond fluctuates. If a bond is IR active, the imposed IR radiation couples, or resonates, with the electrical field of the vibrating charge, and the absorbed radiation is subsequently transformed into larger-amplitude bond oscillations (excited state) that have increased rotational-vibrational energy. Fortunately, each functional group has an unambiguous, intrinsic resonant frequency as the magnitude of the discrete, or quantized, absorption energy depends on the native characteristics of the vibrating bond. More specifically, the characteristic absorption frequency for a bond is related to its spatial distribution, force constant, and to the relative masses of the atoms within the vibrating bond [103]. Additional factors, such as solvation (e.g., hydrogen bonding) and coupling of fundamental vibrations with neighboring fundamental vibrations (or with higher overtones), add interpretive power and complexity to the technique. In IR spectroscopy, absorption bands, or absorption frequencies, are typically conveyed in terms of wavenumbers ($\bar{\nu}$), which are in units of cm^{-1} (i.e., λ^{-1}). Note that the term "absorption frequency" is synonymously interchanged with "wavenumber" because the two magnitudes are both proportional to the imposed IR energy.

A modern FT-IR instrument uses an interferometer to produce a superimposed interference spectrum called an interferogram that contains a complete description of all of the IR frequencies produced by the IR source. The produced interferogram is then passed through the sample, which subsequently absorbs IR energy corresponding to the specific resonant frequencies of its IR active functional groups. The transmitted (or reflected) beam finally passes to the detector. The resulting interferogram is subsequently digitized and sent to the computer for Fast Fourier Transform (FFT) analysis, which basically converts the interferogram, which is in the time domain, to a frequency-based spectrum that can be interpreted by the scientist.

IR Imaging is an extension of FT-IR spectroscopy, and was initially developed to automate the surface composition of larger samples. The system consists of an automated, high-precision x-y stage that sequentially rasters the focus of the IR microscope from spot to spot on the sample. At each position on the surface, complete FT-IR scans are collected. The powerful, nondestructive technique produces high-resolution images that visually illustrate the 2-D distribution of chemistry as a function of x-y sample space [4, 105, 106, 107]. A schematic of the process is portrayed in **Figure 30**.

Figure 30: Schematic portraying the assembly of spatially resolved, high-resolution IR images. The spectrometer produces mid-IR radiation that is focused through the optical microscope. The automated stage rasters the sample to reposition the focus of the spectrometer (IR beam) to the new x-y space. The resulting interferograms are generated and processed by the software (FFT) to FT Images for further analysis. Each pixel in the image represents a 6.25 μm spot (Courtesy of Y. Zhou, ASI, Wayne, NJ).

Zhou et al. (4) applied IR Imaging to hot flat-ironed hair tresses to investigate the impact of excessive heat on European dark brown hair. Prior to flat-ironing, tresses *B* and *D* (**Figure 31**) were treated with polymer (0.5 g w/w of 1% solution) and then blow-dried. Subsequently, all four tresses were hot flat-ironed (232°C) with the same treatment protocol: 12 minutes total thermal insult, using 12 s exposure periods and intermittent 10% SLS washing cycles. Small bundles of the tresses were then microtomed to produce very thin 4–5 μm thick cross-sections that were collected on CaF_2 windows for conducting FT-IR image analyses. An MCT linear diode array detector and optical microscope equipped with a high-precision XY sample stage were used for the image mapping process. The individual scans were analyzed and concatenated to produce images (**Figure 31**).

Figure 31: –CH asymmetric stretching band (2960 cm^{-1}) from $–CH_3$ terminal residues of fiber protein, with a minor contribution from the hair lipids. The trends indicate changes in the protein and lipid concentration due to thermal insult, especially for cross-section *C*; where A=Untreated virgin, B=cationic polymer treated virgin; C=thermally treated virgin, and D=pseudo-quat treated virgin fiber cross-sections (courtesy of G. Zhang, ASI, Wayne, NJ).

Each pixel on the image represents the results of a separate scan and corresponds to a specific 6.25 µm × 6.25 µm x-y area coordinate on the sample surface. More specifically, the pixels correspond to the relative concentration of terminal CH_3 residues in the fiber protein (with a minor contribution from the integral lipid), which absorb mid-IR energy at 2960 cm^{-1}. The intensity bar, on the right of **Figure 31**, is used to visually gauge the relative surface concentrations of the chemical species—where more red represents higher levels of intact protein/lipid, and more blue represents a change, truncation, or absence of the species described by the 2960 cm^{-1} asymmetric C-H stretching frequency. As indicated by the marked decrease in the band, the results suggest that the pretreated and thermally exposed fibers (**Figures 31B and 31D**), which were pretreated with quaternized and pseudo-quaternized styling polymers, respectively, fared better in the excessive heat than the untreated hair assembly (**Figure 31C**). The investigators developed similar spectroscopic trends to visualize fiber-protein damage at the molecular level, including changes in α-helix cortical hydrogen bonding, which affected the quality and sustainability of the secondary structure (e.g., irreversible α-helix conversion to β-sheet).

IR Imaging is an indispensible tool for viewing molecular changes to materials such as keratinous substrates. By coupling the technique with other analytical tools, such as DSC, DVS, and sensorial appeal, it may be possible to directly relate claims and performance attributes to trends and variations in the molecular structure.

2. Spectrofluorimetry

The physical basis of fluorescence that makes up the basis of spectrofluorimetry, also known as fluorescence spectroscopy, entails the electronic state of a molecule where a photon is first absorbed by the molecule, which elevates an electron from a ground to a more excited state. This initial phase is termed excitation and has a characteristic wavelength for a particular molecule. Photons are then emitted by the molecule when the excited electron drops down to lower energy levels; this is termed its emission spectrum. The spectrofluorimeter utilizes this physical phenomenon to identify and quantify the amount of the fluorophore present in a system. There are certain amino acids that make up the protein of hair that will fluoresce when they are exposed to a certain wavelength of light. One such compound is tryptophan which, being a heterocyclic amino acid, has an emission spectrum when excited by a certain wavelength of light. Since tryptophan is part of the structure of the various types of proteins of hair, its quantification can be used as an indicator of the level of damage to the structure of these proteins so that conclusions can be made as to the effects of stressful treatments such as UV light and thermal styling as well as the alleviation of this damage through cosmetic pretreatments.

The fundamental research performed by Chandra and Jachowicz formed the basis of using the degradation of tryptophan in hair as a marker of hair photodamage [108]. Their initial experiment consisted of taking sections of hair from a person that had hair previously oxidized by the sun. They exposed the undamaged root section and sunbleached tip sections of the hair to light that had a wavelength of 295 nm, resulting in a strong emission at approximately 340 nm. Thinking that tryptophan was responsible for this response, they subjected L-tryptophan powder and the undamaged brown hair to the same excitation wavelength. The same emission spectrum was produced, proving that they were indeed measuring the amount of tryptophan in the hair of the subject. To further explore this phenomenon, they irradiated hair in a controlled fashion and found that the degree of tryptophan degradation was directly correlated to the time of irradiation. Later, McMullen and Jachowicz performed experiments showing the utility of quantifying tryptophan degradation as applied to the thermal degradation of hair. Controlled experiments using specified treatment regimens with the heat from a hot curling iron indicated that the hair suffered degradation of tryptophan that could be quantified spectrofluorimetrically [109].

Those molecules that are part of the chemical makeup of hair that are considered chromophores will exhibit fluorescence when exposed to a particular wavelength of light. The amino acid tryptophan is one such compound. This heterocyclic compound is illustrated in **Figure 32** with its associated excitation and emission wavelengths. The intensity of the decrease in the emission of tryptophan is an indicator for hair photo or thermal degradation. Typical equations utilized for calculating percent protection from a photofilter or pretreatment to thermal exposure follow (Eqs. 12, 13, and 14).

Tryptophan
Excitation: 290 nm
Emission: 336 nm

Figure 32: Heterocyclic structure of tryptophan

$$\text{Ratio of tryptophan intensity} = \frac{\text{Emission intensity at 336 nm of experimental}}{\text{Emission intensity at 336 nm of reference}} \quad (12)$$

The fluorescence emission intensity for an unheated and untreated hair tress is also included in this study as a reference response factor to standardize the tryptophan emission intensity of the experimental hair tresses.

% Degradation is calculated as follows:

$$\left(\frac{\text{ratio tryptophan intensity before treatment - ratio tryptophan intensity after treatment}}{\text{ratio trytophan intensity before treatment}}\right)100\% \quad (13)$$

% Protection is calculated as:

$$\left(\frac{\text{\% degradation of control} - \text{\% degradation of experimental}}{\text{\% degradation of control}}\right)\times 100\% \quad (14)$$

Figures 33 and 34 provide examples of fluorescent spectrums of hair tresses. They consist of with and without protective polymer pretreatments, and before and after exposure to 12 minutes of hot flat-ironing. The spectrum before exposure is indicated by the blue line in **Figures 33 and 34**. The initial amount of tryptophan is measured at the emission peak at 336 nm. After exposure both tresses suffer tryptophan loss as indicated by the lower red spectrum; however, there is also a decrease in the amount of tryptophan lost in the tress with the polymer pretreatment. Percent protection can then be calculated by the difference in degradation from control versus experimental.

Figure 33: Fluorescent spectrum of control tress before and after thermal exposure

Figure 34: Fluorescent spectrum of tress treated with a polymeric pretreatment

In practice, since tryptophan levels usually decrease from root to tip from normal weathering processes, the hair tress is usually placed into a frame so that measurements are made in one specified location window. Multiple readings are then taken in this one location so as to form a statistical basis for the results.

As mentioned before, fluorescence measurements of hair chromophores were utilized to show hair photodegradation and damage from thermal styling appliances. The utility of this method was also utilized in demonstrating the protective effects of particular ingredients. It was shown, for example, that dodecyl dimethylaminobenzamidopropyl-dimethyl ammonium tosylate, a substantive photofilter, decreased tryptophan degradation, especially when applied from a hair conditioner. This was supported by other methods such as mechanical combing analysis [110, 111]. The photofiltering effect was due to this compound absorbing primarily in the UVB region of the spectrum, thus protecting the hair protein from degradation.

Fluorescent measurements were also utilized to demonstrate the thermal protective effects of VP/DMAPA Acrylates Copolymer and Quaternium-70, which were also supported with combing and textural analysis measurements [112]. VP/DMAPA Acrylates Copolymer and Polyquaternium-55 were shown to protect the hair from the more excessive heat of hot flat irons (**Figure 34**). These were additionally supported by SEM, DSC, and the quantification of fiber fragmentation using a controlled combing method [113].

Besides these techniques, further investigation for thermal protection from hot irons performed by Zhou et al. utilized Atomic Force Microscopy, Differential Scanning Calorimetry, FTIR image analysis, Dynamic Vapor Sorption, and thermal image analysis. Analyzing the date from all these methods provided evidence for the thermal-protective effects of the polymer pretreatments, as well as contributing to the formation of a proposed mechanistic explanation for the thermal degradation and protection processes [4]. The variety of methods are mentioned here as an example of how researchers utilize multiple methods to provide evidence for a particular result or theory, as well as substantiating the validity of measuring tryptophan as a viable method for assessing the alleviation of hair protein degradation with polymeric pretreatments.

To expand the utility of using fluorescent measurements even further, three-dimensional graphs, or matrices, were produced by plotting intensity of emission as a function of excitation wavelength. By so doing, the progressive increase or decrease of chromophores contained in the hair could be monitored. From the results, conclusions could be made as to the chemical changes taking place during a variety of damaging treatments on several different hair types [114].

c. Microscopy

The term "microscope" is derived from the ancient Greek words meaning "small" and "to look." It was developed in the late 16th and early 17th centuries in the era of Galileo. Because of the utility of this instrument for studying what the eye cannot see, the technology continues to advance. Instruments are now available to see beyond what visible light can discern by its refraction through convex lenses. Consequently, we can now observe objects on a nanoscale level as well as the micron and submicron level. This is an ideal situation for studying hair and its hierarchical structure of components. For example, not only can we observe cuticles on the hair surface, but also the deposition of polymers and the effects of damage, such as cracking and pore formation. These observations allow for the diagnosis of the effects of stresses on the hair as well as providing essential information on the protective and cosmetic effects of deposition agents. As a consequence, the literature abounds with photomicrographs of many of the different states of the hair to support scientific hypotheses and conclusions. Some of the results of microscopy, as they relate to hair science and specifically hair damage and its protection and repair, will serve as examples of this most important field.

1. Optical Microscopy

The optical microscope is indispensible in many disciplines in the physical and biological sciences. A few examples of its use in cosmetic chemistry alone include assessing emulsion stability, interaction between polymers and surfactants, and identification of microbes. However, one particular drawback in studying a hair fiber with conventional optical microscopy is the narrow depth of field, where the plane of focus perpendicular to the direction of the vertical line of view does not encompass the whole specimen. This prevents the entire fiber from being viewed with total clarity. As the microscope focus is adjusted, the plane of focus is adjusted through different parts of the hair fiber so that different parts of the fiber come into clear view. Also, light that is coming from under the stage is transmitted through the fiber and prevents the observation of details on the surface.

However, this narrow depth of focus can be advantageous in assessing the degree of thermal damage inside the cortex. A hair fiber taken from a tress that was exposed to the heat from a hot iron was shown to have micropores localized within the cortex of the hair. These were observed as the plane of focus was shifted towards the center of the hair fiber. The micropores were actually seen as white dots due to diffuse light scattering contrasted with the consistency of the cortex. Through digital photography and image analysis, the pore count was quantified with respect to their distance from the surface to the center of the fiber [115]. Using this technique, Gamez-Garcia was able to demonstrate that a corresponding increase in pore count was observed as the number of hot iron cycles and the

temperature of the iron were increased. This result suggests an increase in thermally induced cortical hair damage. Also, polymeric pretreatments, such as applying a solution of vinylpyrrolidone methacrylamide/vinyl imidazole copolymer, were able to reduce pore count substantially in both Asian and Caucasian hair.

Gamez-Garcia also utilized optical microscopy to detect damage to the cuticle. This was accomplished by observing light interference patterns using a Hi-Scope digital optical microscope. When a stress was applied to the hair, such as tensile stress, it was observed that iridescent color patterns of light were observed where the cuticle had lifted. The theory of why this occurs entails the phenomenon of thin film interference, such as the phenomenon observed in soap bubbles [116]. The degree of cuticle lifting was quantified by digital image analysis. Observations on thermally damaged hair indicated that there was an increase in cuticle lifting. In contrast to the control, when a protective polymer pretreatment was applied prior to hot ironing there was a reduction in cuticle lifting [115].

Other advancements in optical microscopy include the use of a mechanical z-stage to make a composite of multiple transverse layers (multiple images) of the fiber based on the plane of focus to produce a composite image in which all of the elements are in focus (this eliminates depth of field effects). To overcome the drawbacks of a standard transmission microscope, where the sample is between the light source and the optics, a light source above the image (epi-reflectance) allows light to be reflected off of the surface of the specimen to provide more surface detail as is naturally found in a hair fiber's cuticle.

2. Microfluorimetry

When a material absorbs and subsequently re-radiates light, fluorescence or phosphorescence occurs. Fluorescence, as was noted in the section on Spectrofluorimetry, occurs almost instantaneously (in the time frame of a microsecond), whereas phosphorescence consists of a longer lifetime emission after the excitation light has been cut off. If a material has the natural ability to fluoresce, termed primary or autofluorescence, or can be made to fluoresce when treated with a fluorophore (a chemical capable of fluorescing), then the specimen can be studied with fluorescent microscopy, or commonly termed microfluorimetry.

From an optical standpoint, a fluorescent microscope works the same as the optical microscope, with the exception that a fluorescent microscope is augmented with a series of light filters and an excitation lamp (e.g., Hg lamp). With the exception of a very narrow band corresponding to the absorption wavelength, the incident radiation used to illuminate the specimen is blocked. The wavelength band that is transmitted through this filter is selected depending on the excitation wavelength of the molecule in the specimen under study. Various filters can be chosen and the selection depends on the wavelength under

consideration. The electrons in the fluorescent molecule of the specimen jump to a higher orbital and release a photon when relaxed to its ground state, with the resultant emission of light typically shifted to a lower wavelength. There is then another filter that excludes the wavelengths used for excitation and transmits the emission spectrum of the fluorescent molecule of the specimen so that this fluorescent effect can be observed either by eye or by a digital camera. An example of a filter system consists of blocking light except in the excitation band of 545 +/– 15 nanometers (green part of the visible spectrum). The filter system also only allows a characteristic emission spectrum of 620 +/– 22.5 nanometers (orange part of the spectrum). In this case, a fluorescent molecule that either makes up the structure of the specimen, or has a compound added chemically as a tag that has an excitation and emission in the specifications of this filter system can be quantified by the intensity of the fluorescent effect which in this emission range is orange.

Human hair has the property of autofluorescence since it contains within its protein structure aromatic and heterocyclic amino acids. This property of hair was utilized by Ruetsch and Weigmann [117] to study the effect that tensile stress has on hair. In their method an individual hair fiber is mounted on a frame that is adapted with a device that can stretch the fiber in a longitudinal fashion (controlled strain). The frame is placed under a fluorescent microscope and the autofluorescence of the hair as observed by the presence of a diffuse blue-white light. The fluorescence in the hair is produced by illuminating the specimen by filtering all light except in the UV region of 340–380 nm. It was observed that as the hair was stretched more fluorescence was observed, especially at the cuticle scale edges. The authors hypothesized that as the scales of the cuticle moved by each other during stretching, the endocuticle would no longer be protected by the upper layers of the cuticle cell where the higher disulfide cross-linking would quench the fluorescent effect. As the hair is continually stretched, the degree of scale lifting was categorized into random scale lifting, common scale lifting, and extreme scaling lifting, which is close to the point of fiber failure. It was found that it took less deformation of the fiber to cause scale lifting at the tip versus the root sections of the hair fiber, indicating that the hair was more damaged as you proceed down the length of the fiber. From the results of this fluorescent technique, in combination with Scanning Electron Microscopy (SEM), the authors proposed a mechanism of scale edge lifting that consists of stresses in the stretch-induced endocuticle and results in structural failure of the cuticle layers. Alternatively, increased fluorescence intensity upon stretching could also be due to a change in the fluorophore's immediate environment (e.g., if it had more degrees of motion). This is a known effect in the study of other substrates.

Rhodamine B has been used as a fluorescent tag in many studies to show the effect of damaging treatments to hair. Since it is cationic (see structure in **Figure 35**), it will preferentially bind to anionic sites of hair. Since damaged hair has more cysteic acid, the anionic byproduct of disulfide bond cleavage, it is expected that there will be a higher deposition of Rhodamine B to hair as indicated by a higher level of fluorescence. This was observed experimentally where it was found that a higher level of fluorescence intensity was directly related to the time of exposure to 6% hydrogen peroxide during a bleaching treatment [118, 119]. It was also shown that fluorescence was evident on untreated fibers mostly along the scale edges. This would suggest that this part of the hair morphology carries an anionic charge, which relates to the behavior of cationic polymer deposition [118].

Figure 35: Structure of Rhodamine B

Microfluorimetry has been used to provide evidence of the deposition of compounds on the hair surface. There are two ways a fluorescent tracer can be utilized for this. The first is a non-covalent binding, where the tracer has an ionic attraction for the compound in question, such as an anionic fluorophore being attracted to a cationic polymer. The other is when the molecule being studied can be modified chemically such that it will have an intrinsic ability to fluoresce when exposed to a certain wavelength of light. In the case of polymers, there are two synthetic routes to do this. Polymerization can be performed where one of the constituent monomers has a fluorescent tracer bound to it. Also, a fluorophore can be grafted onto a preformed polymer as long as one of the polymer's functional groups is reactive with the fluorophore. It is important to consider whether the addition of the fluorophore to the polymer affects the fluorescent effect of the molecule, or the substantivity of the polymer.

An example of a non-covalently bound tracer is found in studies by Weigmann et al. in which the sodium salt of fluorescein, also known as uranine, an anionic fluorophore, was added to solutions of cationic cellulosic ethers. By experimenting

with various treatment schedules, the deposition behavior of this cationic polymer to the hair was characterized [120].

Polymer deposition was also characterized by fluorescein-labeled polyquaternium-10. Here the fluorophore was covalently linked to the reactive sites on the parent polymer. The synthetic pathway and initial deposition results are described by Goddard and Winnik [121]. As indicated by microspectrophotometry, fluorescent deposits could be seen on a hair fiber, as indicated by the presence of a bright green color on top of a dark background after it was immersed in a 0.1% solution of the labeled polyquaternium-10. Deposition was especially concentrated near the cuticle edges. Since this polymer is applied to hair from a shampoo system, where the mechanism of deposition is quite different from that of the solution, Gruber utilized the same labeling technique for polyquaternium-10 and studied deposition from a model surfactant system [122]. Different molecular weight and charge density variants of tagged polyquaternium-10 were investigated. The study was done quantitatively, by measuring the amount of fluorescence from digested hair samples, as well as by observing the qualitative fluorescent photomicrographs. Quantitative results indicated that polymer molecular weight, polymer charge density, number of washes with the shampoo, and the location of the sample of hair taken on the tress were important variables in polymer deposition. Qualitative photomicrographs were similar to those taken by Goddard, where the green fluorescence on the hair especially along the cuticle edges indicated polymer deposition.

Additional details concerning optical microscopic techniques can be found online on the Nikon and Olympus websites [123, 124]. Despite recent advancements in optical microscopy, it still has limitations in magnification, for example, and the lack of sub-microscopic detail limits the understanding of the mechanism of a damaging process or the effect of compounds frequently formulated for the hair's protection or repair. In hair damage research, efforts focus on obtaining high-resolution images on the nanoscale as opposed to the micron scale. This is done with Scanning Electron Microscopy (SEM), and also Atomic Force Microscopy (AFM), which are discussed in the following two sections.

3. Scanning Electron Microscopy (SEM)

This technique has been utilized extensively in hair research. Technical articles abound with high-resolution SEM photomicrographs of the surface of hair. These have illustrated, for example, characteristics of surface morphology for forensic evidence, surface damage through the effects of chemical treatments, and demonstrating the deposition of polymers through rinse-off conditioning products. The basic operation of the SEM consists of focusing an electron beam on the specimen surface. As this incident beam rasters back and forth over a

particular area of the surface, it results in the emission of low-energy secondary electrons from the atomic elements on the surface. With the use of a detector and other components of the SEM, the emitted electrons are translated into a three-dimensional topographical image of the surface. Besides the high resolution, another advantage of the SEM is the high depth of focus, which gives the three-dimensional aspect to the photomicrographs. This is exemplified in the SEM image in **Figure 36**, where a hair fiber is magnified 11,000X to show the morphological character of the cuticle.

Figure 36: High magnification of the cuticle using SEM (B. Thompson, Ashland Analytical Dept.)

SEM has shown great utility in capturing various types of hair damage. Brown and Swift have used it as a diagnostic tool to show hair damage from various straining experiments (39). Examples of some of the different types of hair damage are portrayed in **Figures 37** through **40**. Protein denaturation is shown in the cuticula after exposing to the heat from a hot flat iron (**Figure 37b**). Cuticular lifting, transverse fractures, and a split end fiber in **Figures 38, 39,** and **40**, respectively were produced from mechanical or a combination of thermal and mechanical stress. An undamaged fiber in **Figure 37a** can be used for comparison.

Figure 37: SEM of a) undamaged fiber; b) heat treated fiber (B. Thompson, Ashland Analytical Dept.)

Figure 38: SEM of cuticular lifting (B. Thompson, Ashland Analytical Dept.)

Figure 39: SEM of a transverse fracture (B. Thompson, Ashland Analytical Dept.)

Figure 40: SEM of a split end fiber (B. Thompson, Ashland Analytical Dept.)

Despite the quality of detail that the SEM affords, the experimenter must proceed with caution in making overriding conclusions. It is important for the microscopist to take many pictures along the length of the fiber and to choose those that are most statistically representative of the damaged state. Swift put it very precisely: "To put it into context, a photograph of a single hair, even at the relatively low magnification of 1000X from the average SEM, samples only a mere one hundred millionth of the total surface of the hairs on the average scalp" [125]. Therefore, SEM pictures provide only partial evidence for a particular damaging or protective effect. Typically, as can be seen in the technical literature, it is substantiated by results from other methods such as those portrayed in this chapter.

4. Atomic Force Microscopy (AFM)

Unlike other microscopic methods, this technique does not employ any part of the electromagnetic spectrum. Instead, the AFM consists of a conical or pyramidal shaped probe that has a very sharp submicron diameter tip. The probe is scanned back and forth over an area that is in the realm of square nanometers or microns. As the probe rasters across the surface it analyzes the surface for spatial characteristics that can produce topographical images of the surface as well as provide a host of mechanical characteristics. The method has distinct advantages in that the specimen is not usually sacrificed in its analysis so that treatments can be assessed both before and after their application. This makes the technique ideal for hair protection and repair studies. Another advantage of AFM is in studying the surface when exposed to different humidity levels. In fact the surface can be analyzed in water, which is an obvious advantage for certain hair treatments. The capability of AFM

and its utility in the personal care industry has advanced over the last two decades and it is anticipated that this trend will not change.

As the sharp tip traverses and interacts with the surface of the specimen there is a mechanism that the equipment employs for data collection. This is accomplished by having the probe attached to a cantilever. As the tip interacts with the surface it deflects the cantilever and the deflection is followed by a laser beam focused on the surface of the cantilever. This deflection is converted to an electrical signal that then moves the location of the mounting stage to keep the cantilever in its normal position. The movement of the stage in the vertical direction is combined with the coordinates of the back-and-forth movement of the stage as the probe rasters across the surface. This data are then converted with the help of a computer to produce a topographic map of the surface. If the sample contains a soft surface, such as a polymeric film, then tapping instead of a contact mode is used [126]. Other modes are available to detect the adhesion properties of the surface through torsional deflection of the probe on the surface, as in Lateral Force Microscopy (LFM) [127]. Another capability is nano-indentation that examines the surface for its micromechanical properties such as hardness and elasticity [128]. Following is an introduction on some of the major areas of the utility of AFM techniques in hair care.

Structural characterization—In some of the early work by Goddard and Schmitt, polymer and polymer-surfactant complexes were identified on mica surfaces. This substrate was chosen since it presented the probe with a flat and anionic surface to study deposition of cationic polymers from rinse-out systems. A preliminary study showed that contact of the sharp edge of the cantilever as it moved across the surface would tend to perturb the polymer coating as exhibited by a loopy structure. This was later rectified by utilizing the tapping mode of the instrument. J. Smith utilized AFM to demonstrate the high-resolution topography of the hair surface [129]. A 20 × 20 um area was scanned and the resultant profile not only showed the cuticle cells but also the subcellular endocuticle, exocuticle, and A layers of the cuticle cells. In another series of experiments this author was able to identify surface deposits such as the microgranular texture of zinc pyrrithione (anti-dandruff active) on the cuticle surface. When analyzed in the wet state with a specialized environmental chamber, the particles were removed. Also, rough surface deposits in unwashed hair were removed by washing with a conditioning shampoo. McMullen et al. showed that damaged hair, especially hair that has gone through the process of solvent extraction, exhibited nanometer-sized micropores, which the author believed was due to the dissolution of surface lipids and subsequent exposure of the proteinaceous fiber surface. He also found that damaged hair treated with the cationic polymer, VP/MAPTAC copolymer, contained surface deposits. Evidence suggests that this polymer binds to the micropores, the

affinity being based on the negative charges of the micropore having a higher affinity for the cationic charges of the polymer. To prove that these micro-structural effects impact the cosmetic behavior of hair, combing work done with a Dia-Stron miniature tensile tester indicated the level of damage and was reduced almost to the level of undamaged hair when treated with a cationic polymer [127].

An example of the surface characterization of the hair by AFM is shown in **Figure 41**. On the left is an illustration of hair in its undamaged state. It can be observed that there is an intact undamaged cuticle. The picture on the right is taken from a fiber that has suffered from a damaging heat treatment. In this case, the cuticle appears less smooth and the cuticles edges are more jagged. Another indication of damage is the presence of small holes or cavities, which may affect the water management of hair as well as its color wash-fastness.

Figure 41: AFM images a) undamaged hair, and b) thermally damaged hair (G. Zhang, Ashland Material Science Dept.)

Tribology—Friction is an important attribute in characterizing the cosmetic behavior of hair. Friction is not just evident between the sensory aspects of the skin and hair, as in the case of the conditioned feel of hair, but friction between individual fibers contributes to the cosmetic behavior of hair and negatively affects combing properties. There are three factors that characterize the frictional properties of hair. These include the surface roughness, frictional force, as measured by the coefficient of friction (COF), and adhesive forces. All three of these micro-tribological characteristics can be measured by AFM and can be useful in making conclusions, along with more macro-tribological tests, in assessing fiber damage and the efficacy of conditioning and protection treatments.

Friction is measured synchronously with surface roughness using contact mode AFM (i.e., LFM). The torsion that the cantilever experiences due to friction as it scans the surface is monitored by the angle of deflection of the laser beam as it is reflected off of the cantilever surface [127]. As with topography, frictional force maps can be constructed to show areas on the hair surface of varying friction. COF of the surface is derived by measuring frictional forces as the probe rasters across

the hair surface. A different mode of measurement is used for adhesion. Here the stage that the hair is mounted on is lowered and the force that is required to separate the probe from the surface is measured. Many measurements are made in a nanoscaled region of the hair surface to produce an adhesion map of the area [130].

From these three types of measurements, La Torre et al. were able to study the effects of various conditioning treatments. It was found that cationic surfactants in a fatty alcohol base preferentially deposited at the cuticle edge as revealed by the lower frictional forces and higher adhesion forces. The increase in adhesion was due to the meniscus effect between the probe and the deposits on the hair. Adding PDMS to the conditioner system does not show an increase in adhesion to the same extent due to both a lower coefficient of friction, as well as a decreasing meniscus effect due to its higher mobility on the hair surface. Amodimethicone was also shown to not deposit uniformly on the hair surface [130].

Mechanical Properties—This technique can determine the nanomechanical properties of a surface. The deflection of the probe is monitored as it is pressed into the surface with a set amount of load, usually in the mN range of force. From these data, hardness, elastic modulus, and creep of the surface against a particular load can be measured. The indentations are usually performed adjacent to each other on the same fiber. Again, since this is not a destructive technique, the indents can be done both before and after various treatments on the same fiber.

Nano-indentation was utilized by Wei et al. to study the different micromechanical properties of Asian, Caucasian, and African hair and the response to damaging and conditioning treatments. Some important findings as outlined in the conclusion are:

- The hair cuticle was found to have a higher hardness and elastic modulus than the cortex.
- The hardness of the different layers of a particular cuticle cell decreases and correlates well with cross-link density.
- Asian hair has higher mechanical properties than both Caucasian and African hair.
- The depth to which the damaging or conditioning treatment is effective can be determined by measuring the mechanical properties of the surface at various depths. In this study, the treatments had an effect to a depth of 1.5 um, which equates to 3–4 cuticles thick. The conditioning effects were not uniform on the surface.

These conclusions show that morphological differences can be observed with different hair types, in addition to their response to different treatments. This suggests that AFM can be utilized to further study the nature of hair damage and the effects of protective and repair treatments.

Reutsch found that multiple treatments of hair fibers with a cationic conditioning polymer, Polyquaternium-10, reduce the surface hardness of the cuticle face.

The reduced surface hardness was indicated by an increase in indentation from 49.4 ± 5.6 nm to 61.9 ± 8.8 nm for unconditioned and conditioned measurements respectively. The mechanism behind the softening effect of the conditioning agent may be due to the hydrophilicity of the polymer [131].

d. Mechanical Properties

1. Tensile strength

The historical method for evaluating the mechanical properties of hair is tensile strength testing, as indicated by the early publication dates of some of the technical articles in the personal care literature and wool industry [132, 133]. Tensile strength is defined as force per cross-sectional area (e.g., g/cm^2) and requires two key measurements in its calculation. First, an average diameter of the fiber is measured so that cross-sectional area (πr^2) can be calculated. Then the force in grams is measured as the hair fiber is strained in a longitudinal fashion; see insert in **Figure 42a**. Here we are controlling the rate of strain (deformation) and measuring the stress. The resultant stress-strain curve for hair is very characteristic compared to other fibers. The profile shows an elastic, yield, and post-yield region prior to breakage and is due to the composite nature of fibrous proteins and the morphological components residing in the cortex. **Figure 42b** depicts a typical stress-strain curve of human hair. The tensile properties are affected by humidity since the cortex of the hair imbibes water and swells. As can be seen in **Figure 42b**, hair at lower humidity has a lower tensile strength and higher elongation before breakage. Clearly, the contrasting results of the water content on the mechanical properties of the fiber convey the impact.

Figure 42: a) Instron imparting a controlled strain on a hair fiber and a description of units in the stress-strain curve. b) Stress-strain-behavior of human hair at high and low humidity as well as the nomenclature of the various regions in the stress-strain curve.

Tensile strength is a destructive method in that after the hair breaks it cannot be used again to see the effect of a damaging treatment or a protective pretreatment. Therefore, a population of fibers is measured from a hair tress or series of hair tresses. These data are then compared statistically to a population of fibers taken from a second experimental series of tresses, or a control. Usually, on approximately one hundred fibers are used for each experimental campaign to form a statistical basis for the conclusions.

It has been argued by several researchers that the tensile strength test does not provide a fair assessment of the state of the hair with respect to its strength or weakening since the strain on hair is in a one-dimensional longitudinal direction [134, 135, 136]. There are many other factors that contribute to mechanical stress, especially during the combing process, which have been mentioned in the previous section on simulating the damaging effects to hair. Other methods have been developed to better understand the effects of these strains on hair and include Impact Loading, Flexabrasion, Fatigue Analysis, Torsion, Texture Analysis, and Dynamic Mechanical Analysis (DMA).

2. Impact Loading

One proponent of the theory that hair breakage during the mechanical action of combing is due to more than just a one-dimensional linear deformation of the fiber was the late Clarence Robbins. Providing evidence for this is a four-part series of articles where he formulates conclusions from the results of a series of simple but convincing tests [135, 138]. One experiment included what he termed impact loading where he simulates one hair fiber impacting and being compressed against another during the combing process. This impacting event during combing is most prevalent when the teeth of the comb hit a snag so that fibers not only hit each other with a high rate of velocity, but also hit the comb tooth, which is an event that cannot be simulated by the limitations of tensile load experiments. To more realistically simulate this strain on hair, Robbins designed a test that consisted of making a circular loop with one hair fiber that has a weight attached to one end. Another hair fiber is threaded through the loop and held in a taut horizontal position. The weight of the fiber loop is lifted and then dropped in such a fashion that the fiber of the loop impacts the fixed horizontal fiber. In another test, the fiber loop is also made to impact in the same way on a fixed comb tooth. The number of impacts that the fiber can withstand is then quantified. One conclusion from this study was that hair fiber loops break more readily over other fibers as opposed to the comb tooth. From a physical standpoint there is more stress per unit area on a single fiber than on a comb tooth due to the difference in diameter (Stress=F/A).

In another series of experiments, Robbins conducted fiber fragmentation during combing. Quantification of broken fibers was assessed by classifying them by size. From the fiber fragmentation and impact loading results, Robbins concluded

that short fiber fragmentation, those fibers that are less than 2.5 cm in length, and long fiber fragments break during combing by different mechanisms. The shorter fiber fragments are formed from the ends of the fibers, which is the weakest part of the hair. These hair ends wrap around themselves and around the comb tooth, which produces multiple stresses resulting in breakage. Longer fiber fragments are a result of impact loading of hair fibers against each other and against the comb tooth, especially when hitting a snag.

Although stress response behavior of hair fibers as they are deformed in a longitudinal direction, as in classical tensile strength studies, has its place in studying hair damage, it is obvious that the stresses that hair fibers are subjected to is more complex during the combing process. Although extension is one strain that hair is subjected to, the experiments by Robbins' show that impacting one hair fiber over another or over the teeth of the comb as well as compression and abrasion are other factors in the stress of combing as well.

3. Flexabrasion

Another proponent of the theory that hair breakage during combing occurs by more than just extensional strain is Alan Swift. He designed a method where a single hair fiber with a weight on one end is draped over a horizontal wire. The fiber is then moved back and forth over the wire that simulates the dynamic bending that occurs especially during combing. The number of cycles required to break the fiber is a measure of evaluating hair strength [139, 140]. Again, this method requires a statistical analysis of populations. Swift has argued that this is an improved method to assess the strength of a hair fiber since the hair fiber is never subjected to an excessive strain in a longitudinal direction that will subject the hair to enough stress that it will break. In fact the hair will most likely be removed from the follicle before breaking. Breakage occurs when hair is bent in a dynamic fashion; that is, the bend propagates from root to tip, and it is the shear stresses taking place in the interior of the hair that are responsible for breakage [141].

4. Fatigue analysis

In most cases, the force required to break a fiber is higher than the force that it takes to extract it from the hair follicle in the scalp [142]. Therefore, during the styling process, hair fibers will actually experience a variety of stresses below those that produce total fiber failure. Based on the magnitude of the deformation, hair fibers could experience stresses in various parts of the stress-strain curve that result in different levels of permanent damage. When hair is deformed in a longitudinal direction, as in tensile strength testing prior to the onset of the yield region, it will typically recover to its normal state since deformation is within the Hookean or elastic region of deformation. Here chemical bonds or morphological structures are not broken. However, when hair is deformed beyond the yield region, a permanent structural change occurs

that tends to weaken the hair and to lower its resistance to withstanding successive deformations. The fiber will ultimately break, depending on the number and severity of these successive deformations. During grooming, especially during combing or brushing, the strains on individual hair fibers are random and are based on the nature of their interactions as they weave in a complex fashion with each other. The purpose of the fatigue experiment is to explore the effects of repeated mechanical insults to fibers in a more controlled fashion by standardizing the cyclical level of deformation or stress imposed on the fiber.

To assist in this type of experiment, an instrument normally used for tensile strength testing can be utilized. Instead of having the equipment set up to deform or stretch the fiber and monitor stresses through the point of break, it is instead used to deform the fiber a certain percentage of its total fiber length, or to a certain degree of stress. These controlled deformations or stresses are repeated and the number of cycles is monitored until the fiber fails. A population of fibers from a given tress with a particular treatment serving as a control can then be compared to a population with an experimental treatment to see if there is an effect on its alleviation of the weakening of hair due to the repeated cycles. Instruments have been designed with adaptations to specifically test for fatigue behavior. Dia-stron has developed the Cyclic Tester, which repeatedly applies a user-defined cycling force at a particular rate of deformation [143]. Since the fiber cross-sectional area is measured before cyclical testing, the stress that the fiber is subjected to is also known. The number of cycles to break is recorded, after which the equipment automatically installs a new fiber for the next trial. Further, stress-strain curves can be generated for each measured fiber so that the experimenter knows what region of the stress-strain curve the fiber is being subjected to.

Using the Dia-stron CYC800 equipment, Evans treated his resultant fatigue data in a statistical manner to produce survival probability plots that could be used to predict the number of cycles to break a fiber based on a controlled and repeatable applied stress [144]. He first produced what he termed S-N curves, which are constructed by plotting applied stress (g/um2) versus cycles to failure. The trend showed that the higher the imposed stress on the fibers, the lower the number of cycles it took before the fibers broke. Based on regression analysis of the data, it was possible to determine the average number of cycles to break a fiber as a result of a specifically applied stress. Exponential distributions of survival probability versus cycles, or survival probability plots, are constructed from mathematical treatment of the data using the Weibull function. The analysis using this equation includes the shape factor that indicates whether the failure rate increases or decreases with the number of cycles. One result the author used to demonstrate the utility of these survival probability plots was to show the lower survivability of hair fibers stressed at 60% versus 20% relative humidity, confirming that water plasticized and weakened the fibers due to the humidity in the atmosphere.

The implications of the fatigue experiments, where greater survivability is shown at lower levels of stress, are clear when the mechanism of how hair conditioning agents work is considered. These ingredients act primarily as lubricating agents, such that there is less friction of the comb or brush traversing the assembly of fibers during grooming. Since the fibers are subjected to less cumulative stress, their survivability is greater.

5. Torsional strain

It can clearly be envisioned how combing or brushing leads to fracture and breakage through both tensile and bending stresses. The tensile stress imparted by a one-dimensional longitudinal strain caused by combing cannot be argued. Neither can the two-dimensional strain of bending in an x-y plane. However, hair shape is in three-dimensions and stresses need to be considered in the x, y, and z planes. To accomplish 3-D analysis, the factor of torsional strain has to be considered. A model for visualizing this is the mechanical behavior of a helical coil or spring that is subjected to an axial load as per equation 15 [145]. An illustration of the torsional components applied to hair tresses is illustrated in **Figure 43**.

Obviously the deflection of the coil is directly dependent on such factors as the load (P), but it is inversely related to the torsional modulus of the fiber itself. That is, the harder it is to deform the fiber through twisting, the less the coil will be deflected. It is also interesting to note that deflection is very much dependent on the diameter of the fiber, considering the d^4 in the denominator of the equation, which partly explains why hair morphology has an influence in hair styling; see equation 15 in **Figure 43**. As curls are brushed and consequently stretched they are subjected to torsional strain. Torsional stresses are also involved in combing straight hair since the fiber entanglements that are present in the advancing front of the comb are also three-dimensional.

$$\Delta = \frac{KFr^2n}{Gd^4} \quad (15)$$

where:
Δ = deflection
K = constant
F = axial load
r = radius of spring
n = number of coils
G = shear or torsional modulus
d = diameter of spring

Figure 43: Deflection is inversely related to the fourth power of the spring diameter.

Hence, measuring the fiber torsion modulus (G) may prove to be as useful as tensile and bending strains. How do the various damaging effects as well as environmental and cosmetic factors affect G? Bogarty mentions several references in the textile industry for the use of the torsion pendulum method and was one of the first to apply it to study of hair fibers. The torsion pendulum method in essence is based on suspending a hair fiber using a weight suspended from its free end. The mass is then put into rotational oscillation. The fiber torsional modulus is then calculated from Eq. 16

$$G = \frac{128\pi Il}{T^2 d^4} \tag{16}$$

Where:
I = moment of inertia of the suspended weight
l = fiber length
T = period of oscillation
d = fiber diameter

Torsion pendulums designed by Bogarty as described in his paper in 1967 were effective in measuring the fundamental relationships in this physical equation. The first apparatus allowed the operator to measure the amplitude of the fiber oscillations through a reflective device on the suspended weight. This permitted calculation of the dampening effect due to the state of the hair fiber. This is described by Eq. 17.

$$S = \frac{2.3}{n} log_{10} \frac{a_1}{a_n} \tag{17}$$

Where:
S = logarithmic decrement
a_1 = displacement on the first cycle
a_n = displacement on the nth cycle

Another adaptation by Bogarty was to house the fiber in a bell jar so that the torsion modulus and dampening could be measured as a function of humidity. Since then, more advanced torsion pendulums have been designed, including an apparatus built at TRI, where the oscillations of the pendulum weight are monitored by the reflection of a white strip by a light detector [146]. Recently, high-throughput measurements of hair fiber torsional properties were made possible by the use of an automated device designed and manufactured by Dia-Stron Ltd. [147].

There are various morphological features of hair that affect the torsional modulus of the fiber. When a fiber is twisted one can visualize that the section of hair that suffers the most from the torsional deformation is the outer circumference of the fiber and that this stress progressively decreases towards the middle of the fiber. Ultimately, in the exact middle of the fiber, the stress is negligible. This would

be a simple physical situation if the fiber were a monofilament that does not react with water, such as a fishing line or an electrical copper wire. However, hair has a complex structure, both on the micro- as well as the nanoscale level. Since the circumference of a fiber suffers from the most stress during torsion, it is reasonable that the cuticle plays a large role in the torsional properties of hair. There are several findings that prove this. When torsional modulus of hair is measured at 65% relative humidity, there is an inverse relationship between the torsional modulus and diameter. As the diameter of the fiber decreases, the torsional modulus goes up. This is due to the fact that as hair diameter decreases, the cuticle to cortex ratio increases; see illustration of cuticle to cortex ratio in **Figure 44**. In larger-diameter hair that has the same approximate thickness of cuticle as fine hair, the cuticle to cortex ratio goes down with a corresponding decrease in torsional modulus [146]. To prove the role of the cuticle, Harper and Kamath [148] decuticled hair and found a significant reduction in the torsional modulus over the untreated control that had an intact cuticle.

Figure 44: Cross-section of hair fibers from Caucasian and Asian hair types illustrating the relative differences in cuticle width to cortex diameter ratio. The ratio is higher for Fine Caucasian hair compared to Asian hair, a difference that influences torsional properties.

When measurements are taken with wet hair, the torsional modulus decreases compared to hair measured at 65% relative humidity [146]. Hair tends to absorb water, especially the lower cross-linked endocuticle layer of the cuticle. This tends to plasticize the cuticle and to reduce its torsional modulus. The bulk of the hair is also weakened when hair gets wet since water penetrates into the cortex easily and reacts with the intermediate filament associated proteins (IFAPs).

This situation is aggravated by hair damage, especially through chemical treatments such as bleaching. Here, disulfide bonds that are important for the integrity and strength of the hair are broken and in their place cysteic acid groups are formed that increase the hydrophilicity, or increase hair's ability to interact with water. Consequently, when bleached hair is wet or is subjected to high humidity, the torsional modulus is severely reduced. Interestingly, however, the shear modulus increases with decreasing humidity since the predominance of cysteic acid in the absence of water will form intermolecular salt linkages that increase torsional modulus higher than that of the unbleached state [148]. It can be concluded that torsional measurements can be utilized to assess the damaged state of hair, and whether a treatment composition can alleviate the extent of damage. One such compound found to fortify hair, as measured by torsional analysis, is cetrimonium bromide (CETAB) [148]. A proposed mechanism involves penetration of the low-molecular-weight compound into the endocuticle and the keratin associated proteins of the cortex. As Harper and Kamath describe, the penetrated CETAB molecules form salt linkages through the association of the cationic charge of the quaternary nitrogen and the negative charges of the hair's protein, while the hydrophobic portions of the CETAB line up in parallel. The whole structure forms a physical cross-link between the proteins of the hair that strengthen the cuticle and cortex. The result is a fortification of the hair as measured by a higher torsional modulus.

6. Texture Analysis

Hair-styling polymers are designed to provide certain mechanical properties to the hair to help set the hair in place and to contribute certain cosmetic effects to the behavior of hair while maintaining the desired properties under the stress of humidity and mechanical deformation. The initial set is indicated by the hair's stiffness and its cosmetic effects are exhibited by more advanced hair attributes such as volume, body, fiber alignment, shine, smoothness, and the like. Many of these attributes are evaluated from a sensory point of view, where panelists judge the stiffness of the applied resin on a hair tress; or by a trained cosmetologist who can assess more subtle performance effects, such as hair body, that can only be adequately perceived on a live subject. Although the sensory aspects of quantifying hair styling are important, scientists have attempted to measure these effects instrumentally. For this, texture analysis has been employed [149, 150]. Besides styling, the texture analyzer has also been used to measure the change in the mechanical properties of hair after the damaging effects of styling regimens, as well as how ingredients can affect these mechanical properties. These mechanical property changes are driven by changes in the fine structure of the hair, such as the conformation of the fibrous proteins in the microfibrilar region of the cortex, as well as other subcellular structures of the cortex and cuticula.

The texture analyzer is a force-measuring device. As with the tensile tester, it has a load cell that is designed to measure the force required to deform an object, such as a hair tress, and to measure the resultant forces during the compression cycle. As can be seen in **Figure 45**, a probe attached to the device slowly compresses a hair tress. When a threshold force is reached, the instrument will measure the force as a function of compression to produce a stress-strain curve, **Figure 46**. The two types of tress configurations depicted in the figure are the omega loop and three-point bending ribbon. These configurations have been used to assess the mechanical properties of polymer-treated hair such as initial stiffness, stiffness durability, elasticity, and plasticity [151].

Figure 45: The texture analyzer measuring the mechanical properties of polymer-treated hair using a) the omega loop method; b) three-point or cantilever bending method. The whole apparatus is housed in a humidity-controlled box and is used to measure stiffness, flexibility, elasticity, hold, work of adhesion, and dry times of products on hair. Products may be tested at various humidity conditions to understand the product's response to humidity.

An example of how hair damage affects the mechanical properties of polymer-treated hair using the texture analyzer is provided in a study by Jachowicz and Yao [150]. Several hair spray polymers were applied to omega loops made from both virgin undamaged dark brown hair and triple-bleached hair. Mechanical properties were then measured and the results reported as the stiffness ratio, which is defined as the ratio of the maximum force measured during compression of polymer-treated hair to untreated hair. A stiffness ratio above one indicates that there is an increase in the stiffness of the fiber assembly. For each polymer tested, the stiffness ratios for bleached hair were significantly higher than for virgin brown hair. The authors hypothesized this behavior was due to the altered state of the bleached damaged hair. Since this type of hair is more hydrophilic, the wettability of the surface is higher than undamaged hair, which is more hydrophobic. Consequently,

polymer solutions interpenetrate the fiber assembly to a greater extent and form more inter-fiber bonds resulting in enhanced stiffness [150, 152]. Also, because of the higher surface energy of the more hydrophilic damaged hair, the hydrophilic polymers wet more easily and adhere better to the substrate.

Figure 46: Typical stress-strain curve produced by a Texture Analyzer

Texture analysis was also utilized to demonstrate the protective effect of polymer pretreatments after exposure to heat from hot curling irons [112]. Hair tresses were exposed to a regimen of shampooing, application of polymer, and then heating with a hot curling iron such that the hair was given a total of 4, 8, and 12 minutes of total heat exposure. Besides mechanical combing and spectroscopic analysis, the textural properties of the hair tresses were assessed using the three-point cantilever bending test. It was found that after the hair was shampooed and dried to remove the residual polymer pretreatment, hair tresses that did not contain a protective ingredient had a stiffness ratio above 1, indicating that the unprotected hair exhibited damage. In contrast, hair that contained a thermal protective pretreatment had a stiffness ratio of one, indicating that stiffness did not change compared to the initial state of the hair. McMullen infers that the increase in stiffness in unprotected hair is due to an increase in the hair's crystallinity as a result of the exposure to high temperature.

7. Dynamic Mechanical Analysis (DMA)

Perhaps the basis for the perception that rheology is an esoteric discipline is related to the unavoidable link between rheological jargon and higher-level mathematics. In simplest terminology, however, rheology is the study of how materials flow. But, perhaps more appropriate to investigating the flow properties of treated hair fibers, rheology has also been described as the study of the flow of materials that behave in an unusual or interesting manner [153]. In extension, and pardon the pun, envisioning the prospects of *solid-like* components of a stretched hair fiber flowing is not very intuitive; however, faith in the philosophy that preaches that sufficiently coaxed solids will ultimately flow, may add clarity to the seemingly odd mental image.

Dynamic Mechanical Analysis (DMA) entails the application of a sinusoidally applied force to a sample of known dimensions, and subsequent evaluation of the material's oscillatory response [154]. For a solid material, properties such as stiffness and energy dissipation are calculated from the sample recovery and phase lag responses (**Figure 47**).

Figure 47: Response of a viscoelastic material to an applied stress. In the linear viscoelastic range the material responds to the sinusoidal stress (σ) oscillation by yielding, or straining (γ), sinusoidally. The phase angle (δ) is the difference in phase between the dynamic stress and dynamic strain and relates to the level of elasticity in the sample; and σ_{amp}, and γ_{amp} refer to the applied stress and resultant strain amplitudes, respectively. The measurement response is a function of applied stress, time, frequency, temperature, and humidity.

The stiffness is reported as the *elastic (or storage) modulus (E')*, and the energy dissipated as heat is described by *tan δ*; where tan δ is the ratio of the *viscous (or loss) modulus (E")* to the elastic modulus (see **Figure 48**). A perfectly elastic response will have no phase lag (i.e., $\delta = 0$) between the response and the applied sinusoidal stress, or strain; whereas a solely viscous response will be out of phase ($\delta = 90°$). The phase lag for *viscoelastic* materials lies between the two extremes (i.e., $0° < \delta < 90°$). Steel is an example of a perfectly elastic material. Honey is a completely viscous fluid, and dried hair-styling polymer films are typically viscoelastic—meaning that hairspray resins dissipate some of the initially applied strain as heat rather than storing the energy for a completely elastic response. In other words, after removing the deformation force applied to a viscoelastic material, the material will not immediately spring back to its original position. Important to all analogies and to all definitions is the following: all of the fancy rheological measurements are related to *reversible* deformations. That is, all applied strains and forces must be small enough to retain structure memory. Adequate "back forces," which are related to the integrity of the chemical structure and its wish to return to a lower energy state, must be preserved to enable restoration of the original structure. In analogy, imagine two styled hair tresses composed of polymers with differing moisture-dependent glass transition temperatures. Tress 1 is set using a hydrophilic, stiff and brittle polymer, whereas Tress 2 is styled with a hydrophobic and flexible polymeric resin. On application of a small sinusoidal force at low humidity, Tress 1 responds elastically and the stress response wave immediately follows the shape of the applied strain wave ($\delta \approx 0$). However, Tress 2 yields slightly as the deformation energy incites molecular rearrangements in the softer, flexible film that releases energy as heat. The lost energy, in the form of thermal dissipation, delays the inevitable return of the material to its original position—a delay that can be visualized by watching upward trends in *tan δ*. Fear not—not all of this is bad news! By *damping* the applied energy, Tress 2 may ultimately be able to survive greater deformation strains than the stiffer Tress 1. Now, imagine that low-pressure system suddenly moved into your region and brings sufficient precipitation. At higher ambient humidity, the more water-loving Tress 1 absorbs water vapor and the polymer film is subsequently plasticized; the moisture-induced plasticity of the film then renders a delay in the expected stress response, which can be viewed in a decrease in *E'*, and associated increases in *E"* and *tan δ*. In contrast, at higher humidity, the performance of Tress 2 is less influenced as its chemical structure is relatively more hydrophobic. Hence, by analogy, trends in *tan δ* may be used to monitor the assault of external strains, such as temperature, humidity, and chemical treatment, on the energy-absorbing physicochemistry tied to the stiffness and softness of treated hair composites.

Figure 48: Argand diagram relating the real axis (E') to the imaginary axis (E"). Hence, the complex modulus (E*) has both elastic and viscous components, meaning E* is a mechanical blend of a Hookean spring and a viscous fluid.

Using DMA techniques, Gao et al. investigated the viscoelastic response of single fibers as a function of relative humidity and applied cosmetic treatment [155, 156]. Single fibers were challenged by DMA in tensile mode and compared to Young's modulus data acquired from traditional stress-strain testing. **Figure 49** shows the correlation of the Young's modulus with *E'* data acquired from DMA testing. The profiles suggest that the two modulus methods produce proportional magnitudes of stiffness data; however, the virgin sample has a different sensitivity (i.e., slope) and a lower R^2 than the bleached samples. Because the stiffness data were acquired at different isohumes, it is probable that the noted slope differences are related to oxidative fiber damage. At higher humidity levels, **Figure 50** echoes the same trend. Higher *tan δ* levels are related to the ability of a material to dampen the stressed structure via molecular rearrangements and internal friction—that is, the effects of chemical oxidation reduce the covalent cross-link density and introduce more molecular freedom. At higher humidity levels, a more rapid influx of water vapor interferes with the electrostatic attraction of the new ionic cross-links and allows the chemically compromised hair structure to dissipate the tensile strains by thermal means.

Figure 49: Correlation of Elastic Modulus with Young's Modulus data for virgin European brown, 15 min bleached European brown, and 30 min bleached European brown single hair fibers (adapted from Gao et al. [155, 156]).

Figure 50: Damping data (tan δ) for virgin and bleached single fibers as a function of relative humidity (adapted from Gao et al. [155, 156]).

e. Image Analysis

The process of seeing by eye consists of the excitation of millions of nerve endings in the retina housed inside of the eyeball. These nerve impulses then travel by way of the optic nerve to the brain where this information is processed into a visual image. The visual image perceived by the brain is a representation of what exists in the outside world. The fundamental unit of seeing is a nerve impulse. Although this process is still being worked on by neuroscientists, the point here is that an image in the mind is not produced by one nerve impulse, but rather by millions taken collectively. Using this as an analogy to describe the nature of digital photography, the fundamental unit is the pixel and when hundreds of thousands of them are put together, which is not unreasonable given the advancements in digital cameras, they produce an image resolution that is only limited by the number of pixels making up the picture. With the help of computers and appropriate software, these pixels can be analyzed to provide information about the image. Quantification is performed with respect to the distribution of light and dark pixels, or the color distribution of pixels. Other applications exist, with the help of image analysis software, including counting particles and analyzing light and dark patterns. Based on the fact that digital cameras, as well as computer hardware and software, are continually advancing, this trend will surely continue.

There are three basic steps involved in image analysis [157]. These include the capture of the image with a digital camera, segmentation of the part of the image that needs to be analyzed, and finally with the help of appropriate software, the analysis of the digital image. Digital image analysis has been used for such things as quantifying hair luster, volume, fiber orientation, curliness, and color such as color fading or wash-fastness [159]. Although the human eye is a very good judge of these cosmetic qualities, there are times when the qualitative assessments generated from, for example, panel studies need to have the quantitative backing of instrumental data given by the image analysis technique. Following are examples of the use of this technique to illustrate its ever-increasing utility for hair damage and protection studies.

Hair shine or luster is one attribute of undamaged hair, as it provides the hair a healthy appearance. When hair becomes damaged there are two factors that contribute to its dullness or reduction in shine. The surface topography of damaged hair is rougher and accounts for a greater degree of diffuse, as opposed to specular, or mirror like reflection. Also, the way individual fibers of the assembly align themselves accounts for the degree of shine on hair. Usually damaged hair, especially at the ends, has more of an unmanageable appearance and looks dull irrespective of how shiny the individual fibers are.

Quantification of the level of shine has been achieved through image analysis [159, 160]. In this method, hair is positioned on a semi-cyclindrical barrel. A specular band of light is observed when a polarized beam of light is directed at the hair tress at a particular angle. A digital picture is then obtained. The apparatus is entirely housed in a dark box. With proper image analysis software, a plot of luminance versus distance along the hair tress is produced. Inspection and calculation of various parameters of the resultant plot will indicate if a particular treatment of the tress has resulted in either an increase or decrease in shine. Hair that has an increase in shine will be portrayed by an increase in intensity of the specular band, and a decrease in the level of diffuse light. From this apparatus, McMullen was able to show different factors that were responsible for hair luster and dullness. It was found that hair luster is proportional to the level of pigment in the hair such that dark brown hair for example was shinier than blonde hair. Addition of oils to the hair, especially phenyltrimethicone, as well as certain hair spray and styling polymers increased shine. Also, reduction in hair luster was evident in the deposition to the hair of either micronized ZnO or sebum [159].

Image analysis can also be used to assess the fiber configuration of the hair assembly. For example, an assembly of hair that has proper fiber alignment will be perceived as shiny due to the exhibition of specular bands. Frizzy or unmanageable hair will be perceived as dull due to the absence of these bands. Therefore, analyzing luminance along the length of the fiber assembly for the intensity of the specular bands will be not only an assessment of an increase in hair shininess, but also how a styling polymer can control unmanageable hair. More sophisticated techniques can be used to assess very curly hair such as hair that comes from African descent [160].

Image analysis can be used to assess the color changes in hair. This could be applicable in such areas as how dyed hair changes color due to shampooing, heat treatments, or UV exposure, as well as measuring effects of protective ingredients. One way to accomplish this is to take digital pictures during the treatment schedule. The hair tress or surface has to be held flat to present a uniform surface to the camera lest there be interfering effects of shadows due to external effects. Sections of the pictures are cropped to the same coordinates and analyzed with appropriate image analysis software. Data analysis is performed with the use of histograms. Since a digital picture is made up of pixels, there are populations of pixels of either a particular color (red, green, or blue, commonly referred to as RGB) or shade in the black-and-white or gray scale. How the pixel density and distribution changes during the treatment schedule provides an indication of the effect of either the damaging or protective treatment. For example, if hair is dyed red with a permanent hair dye and subjected to multiple stripping shampoo cycles, the tress will exhibit fading of its original color. A histogram can be produced in the red

scale to show how the population of dark red pixels shifts to a higher proportion of lighter red pixels. Also, the pictures can be converted to black and white with, for example, Adobe Photoshop and a histogram produced to show the population of light and dark pixels. Hair color fading would be indicated in this case by a higher population of lighter pixels.

An example of this is shown in **Figure 51**. A digital picture of three hair tresses is taken and is represented in **Figure 51a**. The hair tresses were treated in the following way. Dark brown hair tresses were bleached and then dyed red with a commercial oxidative hair dye. The tress on the left is the control or reference tress that did not go through any subsequent treatment. The middle tress was shampooed ten times with a nonconditioning system and a leave-in color protection treatment was added after every shampoo prior to drying. The tress on the right was treated with ten cycles of shampooing and drying. A rectangular section of the digital image is segmented from each of the images of the tresses as shown in **Figure 51b**. These sections are used to perform image analysis. A histogram is produced of the distribution of pixels in each of the sections. It can be seen in **Figure 51c** that the tress treated with just the shampoo has faded relative to the reference tress since the majority of pixels are lighter (it is more to the left in the histogram). The protected tress, although more to the left than the reference tress, demonstrated shown color protection since there is less fading compared to the negative control. The images can be analyzed in other ways such as for color shift or luminance with respect to distribution along the length of the tress.

Figure 51: a) image capture; b) segmentation; c) image analysis

f. Infrared Thermography (IRT)

IRT uses thermal imaging cameras that detect emitted radiation in the infrared region of the electromagnetic spectrum. The usefulness of IRT in quantifying heat depends on the quasi-relationship between the emitted infrared radiation (λ=3–15 μm) and the magnitude of the surface temperature. Subsequently produced digital images, or *thermograms*, consist of two-dimensional image grids with temperature

measurements plotted on a third axis using a relative color image scale. In the human body, heat is lost from the surface by conduction, convection, evaporation (e.g., respiration, perspiration), or radiative processes [161]. Convection transfers heat between the body and the air or liquid interface. Conduction occurs between two solid surfaces in direct contact, whereas radiative heat transfer takes place between two solid surfaces that are not physically touching.

Figure 52 invokes the Stefan-Boltzmann law, which is the mathematical connection between the maximum attainable emissive radiation across all wavelengths, Q, and the fourth power of the absolute surface temperature, T, of a material. For a solid black body radiator, which is a nonrealizable ideal, Q reaches its maximum because the *emissivity* (ε) equals unity. The emissivity, which is a measure of how much radiation a material emits relative to a black body radiator, ranges between 0 and 1, where higher magnitudes are indicative of more efficient radiators. Explicitly, if you placed a thermocouple on the surface of a black body and compared the value to the radiative energy emitted by the black body across all emitted wavelengths, the power of the radiation (Q) would correlate to the fourth power of the measured absolute surface temperature of the solid (see **Figure 52**). In effect, the human body has an emissivity similar to that of a black body—meaning that ε approaches unity and Q is nearly proportional to T^4; for example, the ε of water and human skin are 0.96–0.98 [162], and the ε for human hair is 0.91 [163]. Hence, at thermal equilibrium, human hair and the hair-water composite are intrinsically available for very efficient absorption and emission of thermal radiation.

Figure 52: The Stefan-Boltzmann law describes the relationship between the observed radiation intensity (Q) and the absolute temperature (T), where σ is the proportionality, or Stefan-Boltzmann constant. Black body radiators have emissivity (ε) values equal to unity, whereas real materials radiate less than perfectly and $\varepsilon < 1$.

To gauge the impact of thermal insult on the radiative decay properties of human hair, a virgin hair tress and a previously flat-ironed hair tress were simultaneously evaluated after exposure to a convective heat source. A dark brown virgin hair tress was thermally treated with a 175°C flat iron for an exorbitantly long 5-minute period. During treatment the flat iron was repeatedly swept from root to tip at a rate of 0.6 in/min. After the 5-minute treatment protocol, the untreated virgin and thermally insulted tresses were subsequently set aside for 24 hours (20–22°C, 32–35%RH) to facilitate proper equilibration with ambient water vapor—which should impart approximately 6% water to the untreated virgin tress [95]. One end of a length of adhesive tape was applied to the PMMA tabs on the tress, and the other end of the tape was looped around a 12-inch wooden rod. The wooden rod was then fastened to the apex of two 10-inch glass bottles such that the two tresses were freely hanging from the horizontal rod. To maintain fiber alignment and control the thickness of the fiber bundles, large binder clips were then clamped to the ends of the dangling tresses. Next, a portable space heater was positioned behind the tresses and powered on high for 5 minutes. The heater was then removed and an IR camera was used to immediately monitor the radiative emission of heat from the tresses as they cooled from ~70°C to ambient. **Figure 53** captures the decay as a series of sequential thermograms and clearly suggests that the virgin tress (left) cools to room temperature more slowly than the previously flat-ironed tress (right).

Figure 53: Thermograms showing the radiative dissipation of heat from virgin (left) and hot flat-ironed (right) hair tresses as a function of time. Images were sequentially collected 5 s (A), 15 s (B), 25 s (C), and 35 s (D) after removing the heat source from the tresses. Prior to experimentation, the thermally insulted tress was equilibrated for 24 hours at ambient humidity and temperature (32–35%RH; 20–22°C). Note that the glass supports (ε=0.93, internal control) next to each tress each reached maximum temperature, suggesting even heating across both samples.

Although it is not possible to postulate an accurate structure-property relationship for the data in **Figure 53** without supporting measurements, it is clear that excessive thermal treatment impacts the rate at which hair ultimately dissipates applied heat. Using DVS experiments, Zhou et al. [4] acknowledged the impact of excessive heat on the moisture regain of hot flat-ironed (232°C) virgin hair and indicated that the thermal response and water management properties of virgin hair are quite different than those of thermally treated fiber bundles [4]. One chemical change may be attributable to the formation of diisopeptide (amide) crosslinks, which have been reported to occur at approximately 165°C in keratin [164, 109]. Extensive amide cross-linking may lead to a reduction in swell volume, thereby limiting the maximum water regain of thermally damaged fibers. Hence, one possible explanation for differences in the radiative decay rates for virgin and thermally damaged hair is that excessive thermal treatments affect the core fiber structure and, subsequently, the intrinsic water-binding, hydrogen bonding, and thermal capacity systems of the hair.

11.4.5 COLOR PROTECTION

a. Color Wash-Fastness of Oxidative Hair Color from Shampoo Stripping

A common practice for consumers is to permanently color their hair with the use of oxidative hair colorants. This is done to cover gray or to change the color for cosmetic reasons. Many consumers realize that permanent hair color is not so permanent, and not just because the hair grows out from the roots, requiring periodic touch-ups. Rather, the initial color changes due to washing, putting the hair through various styling regimens, and weathering through exposure to the elements. From this stress, the hair becomes lighter and the hue and chroma, or saturation of the color, changes many times for the worse. For example, a bright vibrant red color will slowly change to an unnaturally looking shade of orange-red. There have been many attempts to help preserve this color. One strategy is to prevent leaching of hair color with continued exposure to washing, or to improve color wash-fastness. Another idea is to protect the hair from UV damage, which is an indirect way of preserving the color from wash-out; after the hair's integrity has been compromised, there is less of a barrier to prevent the dyes from being leached during the repeated washing cycles. Whatever the case may be, methods need to be employed to assess the original color of the hair and to monitor subsequent changes.

The human eye is an excellent instrument in detecting small differences in hair color changes both with respect to intensity and hue. An individual's opinion is very important even if instrumental methods show more precision in their discrimination of color shifts. Of course, the consumer is the final judge in deciding whether the change in color intensity or hue is acceptable. Therefore, panel testing is a necessity due to the importance of consumer perception. Nevertheless,

instrumental techniques have their place in quantifying color changes in that they provide quick feedback to researchers in determining the efficacy of their raw material or formulation development efforts. However, data generated from instrumental measurements have to be correlated to real-life situations; therefore, questions in color change include determining the minimum difference measured by the instrument that can be detected by the human eye. If this correlation is not well established then panel testing results are required.

1. Colorimetry

The most common way to monitor color changes is by measuring Hunter L, a, and b values with a HunterLab colorimeter (HunterLab Ultra Scan). In this system, various aspects of color are reported, namely lightness (L), and chroma (a and b). These components come from a three-dimensional color space model created by the International Commission on Illumination (CIE) and can be monitored alone, but more commonly ΔE and ΔC are utilized to follow color changes. These are defined as follows (Eqs. 18 and 19):

$$\Delta E = ((L_t\text{-}L_o)^2 + (a_t\text{-}a_0)^2 + (b_t\text{-}b_o)^2)^{1/2} \tag{18}$$

$$\Delta C = ((a_t\text{-}a_0)^2 + (b_t\text{-}b_o)^2)^{1/2} \tag{19}$$

Where:

L = luminance
a = red to green shift
b = blue to yellow shift
ΔE = Total change in color including lightness
ΔC = Change in color

L_o, a_o, b_o ; and L_t, a_t, b_t are measured Hunter L, a, b color parameters before and after treatment, respectively. The reader is encouraged to explore this more in depth by referring to www.hunderlab.com.

Using the values of E and C in comparison to an appropriate control can be calculated from equation 20.

$$\%\ Color\ Retention = \%\Delta E_{improvement} = \frac{\Delta E_{treatment} - \Delta E_{control}}{\Delta E_{control}} \times 100 \tag{20}$$

Attempts have been made to prevent color fading in hair by investigators working in the personal care industry. For example, by utilizing ΔE and to a lesser extent, ΔC, it was shown that products containing Polyquaternium-55 reduced the color change due to shampoo stripping [165, 166]. The Polyquaternium-55 was tested in different regimens of shampoo, conditioner, and leave-in treatment, with favorable results compared to appropriate controls. It was theorized by Zhou et al.

that the color wash-fastness was due to both the cationic nature of this polymer as well as its degree of hydrophobic modification, which forms biocompatible films on the hair and prevents the leaching of color during subsequent shampooing.

Another example where color indexes were utilized to show color effects entailed the use of Multi Lamellar Vesicles (MLV) made up of phosphate esters. When these were added to the dye base, the lamellar structure produced by these types of surfactants could be observed using optical microscopy equipped with cross-polarizers. The birefringence was used as an indicator of lamellar structure formation. It was theorized that the dye intermediates would be entrapped by the lamellar structure and slow down its reaction with hydrogen peroxide. This would give the intermediates a chance to absorb into the cortex of the hair before reacting with hydrogen peroxide. From this it was found that less dyeing time was necessary to achieve the same color index compared to longer dyeing times with lower MLV structures. Also, color wash-fastness through shampoo cycling was increased as well. Most of the work to show efficacy was through the monitoring of color indexes and their change with different dyeing times and shampoo cycles [167].

One must be careful in making conclusions in regard to ΔE and ΔC, as well as percent protection. By considering the equations to determine ΔE and ΔC, one may take note that the color difference takes into consideration multiple color indices. If, for example, ΔE and/or ΔC do not change, that does not necessarily mean that there is no change in color. It could be that the increase of one color index may increase and may be nullified by the decrease in another. Therefore, it is important to separately look at the data for L, a, and b both before and after the treatment regimen to see exactly which indices are changing most dramatically. From this perspective, it may make more sense to indicate the results with Δa, Δb, or ΔL individually. For example, Gao monitored Δa, which is a change in the saturation of red, for different red hair colors containing different levels of phosphate esters.

It should be mentioned that percent protection needs to be used with caution when claiming color protection. For example, a large percent protection can be calculated from very small changes of ΔE of the experimental treatment versus the control, which may not be consumer perceivable.

2. Image Analysis of Digital Photographs

Determining the color wash-fastness of a hair dye on a human subject is quite difficult. To compare two treatments, one product with a protective ingredient needs to be on one side of the head and a placebo on the other. This may be practical in the salon, but becomes difficult when the panelist is asked to do this at home, especially with rinse-off products. Usually, these types of tests are performed on tresses and mannequin heads made with natural human hair. Hair tresses can be treated with a protective ingredient and then subjected to a series of wash and dry cycles to see the change in color compared to the color change of a tress serving as

a control. Of course the regimen can be designed by the experimenter to simulate practical conditions. Measurements of color changes are monitored after washing cycles using techniques such as colorimetry or image analysis. Instrumental measurements should be supported by panel studies.

Utilizing a mannequin head, the color wash-fastness effect of a hydrophobically modified cationic polymer was demonstrated by incorporating it into a regimen of products consisting of shampoo, conditioner, and leave-in treatment. The mannequin head was dyed red with a commercial hair color. One side of the head was treated with a commercial shampoo and conditioner containing a color-protective claim. The other side was treated with the color-protective regimen. After ten cycles the hair was dried in a straight style so the fibers would present a flat surface for taking digital pictures. This is an important step since, if the style of the hair has texture, it will present to the camera dark and light patches (shadows and highlights) that will interfere with true color measurements of the fiber assembly. Photographs of both sides of the head were taken in strategic areas and cropped to specific proportions as can be seen in **Figure 54**. The image analysis data can then be compared as shown in the histogram of luminance. The quantity of pixels is plotted as a function of luminosity with low luminosity values corresponding to dark shades and higher values to lighter colors. In the example, the data indicate that the side of the head treated with products with the protective polymer has a population of pixels that are darker than the control side, indicating less color loss or more color wash-fastness [165]. The mannequin head also facilitates visualization of the color differences of the two sides of the head by a panel to confirm the data generated from the digital image analysis. Also, panelists viewing a mannequin head can perceive the three-dimensional aspect of the head where specular bands due to curvature are evident and have an effect on the perception of color.

Figure 54: Methodology of utilizing image analysis of digital photographs taken from a half head evaluation on a mannequin head

b. Color Protection from UV-Induced Fading

Besides the continual washing that hair is normally subjected to, hair also fades from exposure to solar radiation. There are many factors that put a level of complexity in its study. Sunlight is composed of a spectrum of wavelengths, where the shorter the wavelength the higher its energy and level of stress that it imparts to the hair. From highest to lowest energy, the electromagnetic spectrum incident on hair from solar radiation is UVB (280–320 nm), UVA (320–400 nm), visible (370–780 nm), and IR (750–2800 nm). Besides wavelength, the length of time and intensity of the radiation has to be considered as well, usually reported as J/hr-cm^2. Another factor that makes this area complex to study is the nature of the substrate. Some hair types have a different type and level of melanin, which is hair's natural defense against photodegradation. Hair is also treated with permanent artificial hair colors that have their own sensitivity to light exposure. These oxidative hair colors are made up of a complex blend of dye intermediates that react inside the hair shaft with hydrogen peroxide to form the desired color. Therefore, in this area of study, the experimental design with respect to the treatment regimen (e.g., shampooing and light exposure cycles), exposure conditions, and hair type, need to be carefully controlled and specified in the material and methods section of the study.

Melanin, which is present in the cortex of the hair as fine granules, provides natural protection to hair from sunlight as it surrounds the keratin intermediate filaments. This level of protection depends on the type of hair with respect to the level and type of melanin. For example, dark brown hair with a high level of eumelanin affords more protection to the proteins of the hair as opposed to natural blonde or bleached hair. Artificially colored hair affords less protection to the hair since these compounds are more sensitive to decomposition by sunlight. Hence, naturally colored hair retains its color longer than artificially colored hair. It has been shown that UVA and visible light predominantly fade artificially colored hair [168]. What aggravates fading of artificially colored hair is a combination of both irradiation and shampooing [169]. The breakdown of protein or lipids on the hair surface from UV irradiation makes hair more porous; and because hair dyes are water soluble, especially those based on pyrazole dyes contained in some red colors, the dye leaches out more readily during shampooing and rinsing. Therefore, it would be beneficial to know the nature of the solar breakdown of hair proteins in order to select the correct UV absorber system to provide maximum protection.

As studied by Hoting and Zimmerman, each part of the solar spectrum has a different propensity to fade dyed hair. UVB radiation is the most damaging to hair followed by UVA, visible, and finally infrared light. These relative frequencies are illustrated in **Figure 55**. However, it should be reemphasized that the visible and UVA region of the spectrum are most degradative with respect to hair pigment. The reason is that the relative intensities of different parts of the solar spectrum are different. As measured by Locke, the ΔE values of photo-faded hair were 8.5,

3.8, 0.95, and 0.84 for visible, UVA, UVB, and IR light irradiation, respectively [169]. These relative intensities are also illustrated in **Figure 55a** for comparison. The visible and UVA portions of the spectrum account for a major share of the total photo-fading effect with UVB having a more minor role. Based on theoretical calculations, Locke and Jachowicz calculated the fraction of light absorbed at each wavelength by a given quantity of UV absorber on the hair. As expected, UVB absorbers only absorbed a fraction of the UV absorbed on the hair since the UVB only accounts for a fraction of the total amount of UV radiation. In choosing a UV filter for photoprotection, the wavelength of light that the ingredient is designed to absorb is most important. Although filters in the visible range may have a negative cosmetic effect on hair (i.e., these filters are also visible by eye), increased photoprotection can be realized by using a filter that has UVA as well as UVB properties. Other factors that the formulator must keep in mind are the extinction coefficient or the amount of radiation absorbed by the filter at a particular range of wavelengths, the photostability of the absorber after a certain degree of radiation, and the cosmetic effects when added to a hair treatment and ultimately on hair. If the product is used in a rinse-off application, such as a shampoo or cream rinse, then a mechanism must be considered to deliver the UV absorber onto the surface of the hair. Examples to consider are dilution deposition as is found in conditioning shampoos or, intrinsic features of the ingredient such as having a quaternary group that would allow substantivity of the absorber during rinse-off.

Figure 55: Photodegradation of hair as a function of wavelength, exposure to hair, and effect of UV absorbers. Length of arrows shows relative effect. a) relative effectiveness of photodegradation based on wavelength—UVB is the most degradative; b) relative actual degradation based on intensity—visible and UVA have the most effect; c) effect of UV absorbers—reflected vector represents blockage of UV by absorbers.

11.4.6 REPAIR TECHNIQUES

Thus far, we have described hair damage and various ways it can be protected from the various stresses that it is subjected to. What is more challenging is its repair. Claims abound in the market for products that strengthen and repair damaged hair. Since hair is a complex structure, there are different aspects of repair and, as a consequence, the term needs to be defined appropriately. The definition can be explained by analogy. Consider a brick wall that has a crack as by some uneven distribution of the weight of the structure that it supports. The process of repair is simply to excavate the crack so that it can accommodate mortar to fill in the crack; the chemical nature of the brick is still the same. On the other hand, if you observe a brick that has been weathered by the elements, the actual molecular structure of the brick has been compromised to the point where it will not bear a load without crumbling. At this point the brick can no longer be repaired. These two aspects of damage and the repair process are included in the term "hair repair." The first aspect of hair repair includes repairing the broken subassemblies of the fiber through a physical-chemical process. The chemical interactions associated with this would be salt linkages, hydrogen bonding, and hydrophobic effects. Since the energy to break these bonds is low, the resultant repair is temporary or at best, semi-permanent. The more intimate bonding of the molecules in hair for a permanent repair would entail covalent bonds. There are examples of each of these aspects of repair in the literature.

Repair can also be considered from a morphological point of view by observing how treatments repair the subassemblies of the fiber. Compared to synthetic fibers, such as is present in polyester or nylon fabric, human and animal hair fibers have a complex structure. Swift coined it appropriately as a hierarchical composite [125]. There are many book chapters [3, 125, 170] as well as detailed technical articles [45] on the subject that the reader is encouraged to refer to for an explanation of the complex structure evident in human hair. It should be mentioned that in going from low to high magnification in the description of the fine structures found in hair, we start with the cortex being built by smaller longitudinally shaped cortical cells which house macrofilaments. These then are composed of well-organized intermediate filaments (IF) surrounded by intermediate filament associated proteins (IFAPs) embedded in an amorphous matrix. These proteins have a specific primary, secondary, and tertiary conformation and association with each other that gives hair its physical properties, such as strength and elasticity, which then translate into cosmetic attributes such as hair body. When hair is under stress, it is damaged at its various hierarchical levels, namely from the molecular level to the subcellular level, and ultimately to the cellular level present between and amongst cortical and cuticle cells. True hair repair would bring these structures back into their normal states such that: 1) intermediate filaments that have been

severed have been re-fused together, that is, broken peptide bonds that make up the backbone of the molecule are re-formed; 2) also, this would include restoration of the organized structure of the complex lipid structures found in the cell membrane complexes between cortical and cuticle cells; 3) the complex three-dimensional conformations of the IF and IFAPs are restored to their pre-denatured state; 4) damage to the cells such as micropores formed in the surface of the cuticle cells, and fibrillation of cortical cells. It is indeed a high calling to have this definition of hair repair since not only chemical bonds need to be restored, but also the complex three-dimensional structures prior to their denaturation as well. The effect is the same as descrambling an egg. In this respect there are very few treatments that will achieve such results. Rather, the effects either are cosmetic or at best have a certain durability of effect to retain the cosmetic effect of hiding the damage by keeping intact important hair attributes. However, there have been attempts to repair hair on a more molecular level. In this section examples are given on those hair repair techniques that range from temporary effects to those are more permanent.

a. Protein Hydrolysates

One realizes very quickly when studying compendiums on the chemical composition of hair that it is primarily made up of proteins [3]. Some proteins are fibrous, such as the intermediate filaments, and contribute to the supporting structures of hair. Others are more amorphous, such as those found surrounding the microfibrils. The structure of these proteins on a primary conformational level is made up of the various amino acids characterized by different functional groups. These functional groups consist of acid, base, aromatic, heterocyclic, aliphatic, and sulfur containing proteins. The higher-level secondary and tertiary structures are dependent on the sequence of amino acids in the protein's primary structure [171]. Also, the reactivity of the protein is dependent on the nature of the functional groups of the protein. In hair the sulfur-containing proteins are most important in that it is the nature of the disulfide bond to give hair its strength through molecular cross-linking. Other bonds, again dependent on the side groups, take part in hydrogen bonds, salt linkages, and hydrophobic interactions both on an intra- as well as intermolecular basis. From this standpoint, it would make sense that the addition of proteins to hair would allow them to interact with the proteins of the hair through similar chemical bonds. The end result is to provide a restructuring of the damaged proteins and to help bring back the condition of the hair to its natural state.

Since protein is a result of biosynthesis in a living organism, it can be derived from either plant or animal sources. Based on consumer demand, vegetable-derived proteins are more popular today due to the shift away from animal-derived products and towards more green and environmentally friendly sources. Although these proteins are naturally derived, they do experience processing and chemical

modification prior to their addition to a hair or skin care product. One reaction includes the hydrolysis of the peptide bond with either an alkali or enzyme in order to reduce molecular weight, primarily done to increase water solubility. Functional groups are also added to proteins to increase their interaction with hair as well as their performance. Examples of this include quaternization for hair conditioning, and reaction with a silanol group. Therefore, the conformational and chemical variations that proteins can assume are quite large.

There is evidence that proteins that have been hydrolyzed by various means to lower molecular weight can absorb into the interior of the hair as well as on its surface. As mentioned above, they then can interact with the fibrous proteins of the hair with the same bonds that are found both on an intra- as well as intermolecular basis of the native proteins. Various methods have been utilized to show their substantivity to hair.[125] I-labelled collagen was used to determine substantivity from a surfactant system. This study also revealed the important factors governing substantivity such as molecular weight, isoelectric point of the protein, and formulation variables [172]. Radio-labeling techniques using ^{14}C-labeled amino acids produced from the complete hydrolysis of wheat proteins showed penetration from shampoo and conditioner [173]. Another method consisted of removing protein from treated hair using a high-temperature salt solution and then analyzing the resultant extract using gel filtration and fluorescent techniques [174]. Diffusion of hydrolyzed wheat proteins into hair was detected using fluorescently labeled peptides, analyzing the hair using laser-scanning fluorescent microscopy [175]. The details of the methods and results will not be described here, but rather serve as examples of the substantivity of proteins.

Proteins have been formulated into rinse-off and leave-in hair products for many years based upon their interactions with hair protein and their consequent cosmetic effects. Although some claims may be sometimes exaggerated, there is documented information that substantiates their benefits. Teglia and Secchi [176] state explicitly that proteins are proven to increase the hair's tensile strength, elasticity, body, softness, repair of split ends and cuticular damage, and protect against insulting treatments such as detergents and chemical treatments. References are also cited by the authors stating that protein hydrolysates and those that are derivatized such as with quaternization, reduce the loss of tensile strength caused by anionic surfactants. It would be worthwhile here to mention that these compounds may have a resurgence of use due to their natural origin and appeal for sustainability in addition to its functional attributes.

b. Cuticle Decementation and its Repair

There is ample evidence showing that the tensile properties of hair are due primarily to the cortex and not to the cuticle. Robbins proved this with the use of

diperisophthalic acid, which oxidized the cuticles of the hair. It was shown that despite the damage incurred to the surface of hair as assessed microscopically, the tensile properties of hair were not affected [177]. Another important conclusion from this work was that tensile properties alone could not totally assess hair damage since cuticular damage can occur without being detected by changes in tensile strength. This was indeed the case, as demonstrated by Ruetsch and Weigmann, where cuticular damage was shown using both SEM and microfluorescence. Here, hair was extended in a longitudinal fashion. It was observed that the degree of cuticular lifting was greater for a given extension percent as hair samples were tested from root to tip. The weathering of the tip sections of hair resulted in a higher degree of cuticular lifting and a lower percent extension. Although the mechanical properties would be reversible as detected by tensile forces in the yield region, the reversion of the cuticulae was not. It was theorized that as the hair was being stretched, shear forces in the endocuticle, the least cross-linked area of the cuticle, would suffer the most stress and fracture. The cuticle would then lift and expose the fractured endocuticle.

Gamez-Garcia studied this phenomenon of cuticle decementation further and found that cuticle lifting increased with the number of applied tensile cycles and was sensitive to moisture, whereas at lower humidity levels it took less strain and/or fewer cycles to initiate cuticle lifting [178]. The mechanistic explanation for this is twofold. At high humidity the endocuticular region of the cuticle is plasticized and is more pliable. It is able to hold more moisture due to its lower cross-link density. Also, when the cortex is swollen with water there will be less of a Poisson contraction, which normally puts stress on the cuticular envelop during strain. By putting certain polysiloxane-modified compositions on hair with lifted cuticles, a recementing would take place in that the cuticles would not lift with subsequent strain. This was not the case with several other ingredients. The phenomenon could be easily observed by knotting a hair fiber and observing the lack of cuticular lifting as seen by SEM. The recementing of the cuticle by these compounds was shown to entail cross-linking as shown by measuring the rate of solubility of dried films. The dried films of these compounds formed insoluble films despite the drying conditions, providing evidence for cross-linking [179, 180].

c. Repair of Split Ends

One of the manifestations of damaged hair is the presence of split ends, which are perceived as longitudinal splits in the end of the fiber. They form through the shear stresses at work as a comb or brush is pulled through the hair and eventually produces the longitudinal fracture [134, 141]. Once enough of them form they become noticeable. This is usually accompanied by other manifestations of damage such as ends being unmanageable during styling, hard to comb through, and lacking shine.

To repair split ends and restore hair to its normal state, a technology is available that consists of a polyelectrolyte complex that is able to semi-permanently mend the damaged ends. The semi-permanence here is defined as having the durability to further mechanical action such as combing [181, 182].

The method consists of tagging fibers at their root end in a typical hair tress to determine their fate during a treatment and/or styling regimen. The tress is first damaged mechanically by exposing it to a combing device, where it is continuously combed to produce split ends. Split end fibers are then located in the tress and tagged appropriately. With the use of a stereomicroscope, a small red dot is added right before the split end to act as a landmark to make sure through subsequent treatments that the breakage of the split end off of the fiber is not taken as a false positive. A picture of the damage is captured and then compared and quantified after addition of the repair ingredient. Combing after the repair ingredient has been applied tests the durability of the mend from the shear forces at work during the combing process (see **Figure 56**). The advantage of the method, although time consuming, allows the tagged split end fibers to experience typical combing forces or the stress of other designed treatment regimens. From this method it was shown that the polyelectrolyte complex mended the split end after treatment, but more importantly, was able to show durability after the post-combing process, thereby simulating normal grooming and mechanical stress for consumer relevance [181, 182].

Figure 56: Spit end mending as observed under a stereomicroscope (20X), a): before treatment; b) post-treatment after the stress of combing

Mending of the split ends occurs based on the morphology and physical-chemical properties of the resultant microgel that forms as a result of the complexation. A proposed mechanism consists of the following. The microgels are characterized microscopically and are dispersed particles on the scale of one to three microns. These tend to infiltrate the subassemblies of the broken fiber. Cationically charged domains of the microgel, which are based on unassociated moieties of the cationic polymer, interact electrostatically with the cortical proteins, which are

predominantly anionic. Also, anionic domains of the microgel work as an adhesive through hydrogen bonding. Based on the physical chemistry of these microgels, they act as cross-linking structures to draw together and bind the broken parts of the fiber in such a fashion that the split ends will not open up with the stress of subsequent combing.

d. Repair of the Surface Lipid Layer

The F layer located on the periphery of the hair surface is an important morphological component that is important for many cosmetic attributes such as shine, combing, feel, and overall manageability. The appearance and behavior of hair's damaged state is evident once this lipid layer is compromised by either an aggressive treatment such as bleaching or the persistent weathering of hair through UV or daily grooming practices. Rinse-out conditioners are then utilized that deposit a layer of cationic surfactant, such as behentrimethyl ammonium chloride in combination with fatty alcohols, to restore this hydrophobic layer and its resultant cosmetic effects. However, this is temporary since this layer is easily removed during the shampooing process, with the result that the hair reverts back to its damaged state. There is a need, therefore, to provide a more permanent hydrophobic layer on the surface of hair that will maintain hair health despite the weathering and damaging treatments that hair is subjected to.

A technology that provides a persistent hydrophobicity to the surface of damaged hair has been achieved by depositing a complex of 18-MEA in combination with stearoxypropyldimethylamine (SPDA) [183]. The persistence was shown over the course of one shampoo cycle with an anionic surfactant system. Controlled experiments were performed using 18-MEA in combination with a series of tertiary amines and fatty acids that varied in alkyl chain length, degree of branching, and functional substitution. A proposed mechanism was formulated through data generated with a variety of techniques. These included measuring the hydrophobicity of the surface through contact angle measurements, determining surface roughness and mechanical properties with AFM, chemical state versus depth on the hair surface with angle-resolved X-ray photoelectron spectroscopy (ARXPS), and fatty acid absorption with liquid chromatography/mass spectrometry. The model of surface lipid restoration consists of 18-MEA and SPDA being packed on the surface of the hair with the carbonyl and amide groups attaching to the surface of hair. The orientation of the lipid molecules are at an approximate angle of 35 degrees, which translates into a layer being 1.4 nm in thickness. Another factor in producing a persistent hydrophobicity is the fluid-like nature at the upper region of the lipid film caused by the anteiso branching of the 18 MEA molecule. This fluid character imparts a greater molecular mobility that contributes to the persistent nature of the film after shampoo washing [184].

e. Strategies for Permanent Mending of Hair

The major constraint in using chemical means to achieve a permanent repair to damaged human hair is the fact that it is attached to a living person. If hair was like wool sheared from its owner, or synthetic or cotton fabric that can be manipulated at extreme conditions of pH, temperature, or strength of chemical treatments, then products would be on the market that would deliver one hundred percent on its claims. As Jachowicz states in his article on hair damage and attempts at its repair, there are no methods of practical utility to achieve this; however, there are several that he cites in the literature from a general research strategy point of view [67]. The first includes reactive low-molecular-weight compounds that absorb into the hair and react with the cortical proteins.

Examples include compounds that contain alkyl groups that have the effect of reducing water swellability, resulting in a positive effect on mechanical strength. Other compounds can react with hair proteins through covalent bonds, which act as cross-linking agents. One such compound is formaldehyde, which is used in products designed to straighten the hair. This ingredient, however, has come under severe scrutiny since it is now considered a carcinogen and requires appropriate warning labels for its proper use [185]. Another category includes compounds that polymerize within the hair. Just as in an organic synthesis reaction, small monomeric intermediates such as methacrylic acid in the presence of appropriate initiating agents will react *in situ* in the hair. Although these reactions can take place *in vitro*, their practicality is quite limited. However, research may uncover someday a reactive system that can repair and strengthen hair *in vivo* without negative consequences to the consumer.

The consumer deems hair repair products efficacious from either a product-perception point of view or its actual performance. In the technical community, these products, and the key ingredients used for hair repair claims, need to be substantiated by both a sound theoretical mechanism of action as well as data from appropriate test methods. Based on this latter consideration there are not many hair repair systems currently available that provide permanent repair. Hence, there is the opportunity for innovation for new technologies as well as the accompanying test methods to show their benefits.

11.4.7 TESTS TO STUDY WHOLE HAIR ATTRIBUTES FROM DAMAGING EFFECTS AND IMPROVEMENTS WITH COSMETIC TREATMENTS

There is a data continuum inclusive of the most analytically based techniques to the more subjective, with the latter involving perception of the human senses in consumer evaluations. Instrumental techniques, although of great value in themselves for substantiation of effects, have to be correlated to sensorial attributes since this

is the ultimate criterion for the success of a technology or finished product. For example, a product such as a conditioning shampoo can be shown to deposit ppm levels of silicone on the surface of hair; but if this amount of silicone does not surpass the threshold of what can be felt by the consumer with respect to combing and feel properties, then a statistical increase in silicone deposition is without merit. Showing a statistical difference in data between a control and experimental treatment by an instrumental technique has to take into account the minimum difference perceived by the senses before a conclusion is made. If you cannot see it by eye, then a statistical difference is purely a mathematical one. Another factor is working solely with hair tresses to assess the efficacy of ingredients. Although this comes closer to real-life consumer usage, it will be mentioned in more detail later that some hair attributes are only revealed by evaluation on a whole head of hair assisted by the trained eye of a cosmetologist. This section then goes into those techniques that are closer to what can be perceived in the more macroscopic view of product evaluation.

a. Panel Testing

The only industry standard utilized in panel testing with hair tresses is to use properly controlled designed experiments so that conclusions are based on a deductive reasoning of the results. Hair tresses are made to a set configuration of length, width, and net mass of hair as well as hair type that will be appropriate for testing the hair attributes under study. For example, damaged hair such as from bleaching that is eight inches in length would be most appropriate to test wet and dry combing properties from a conditioning shampoo and/or conditioning treatment. Fine hair would be most appropriate for testing volumization effects of, for example, polymers from a leave-in styling treatment. A selection of hair attributes can be evaluated that will provide information for conditioning, protection, repair, or styling type of products. It is up to the discretion of the scientist to choose the right substrate for the test that he or she has in mind for the particular hair attribute(s) that are important to the study.

There are essentially two types of designs for panel tests. One is based on an absolute and another on a relative scoring system. In an absolute scoring system, the panelist judges a particular hair characteristic against set criteria. For example, if the hair attribute is ease of combing and if the scoring consists of values between 1 through 5, where 1 represents poor combing and 5 is excellent combing, then each score value would have a particular standard that the panelist can be calibrated against. This may be done by treating a tress with a highly conditioning system that would provide excellent comb-through and having the panelist evaluate this as a standard for a score of 5. Standards can also be designed for scores of 1 though 4 in the same manner. Once the panel is calibrated against these standards they can

be considered "expert" panelists and can then be human instruments to provide data on the effect of experimental treatments on tresses. This type of scoring system was utilized to show the contribution of the Silicone Quaternium-22 (INCI) for improving detangling, wet-combing, and wet-feel properties in a shampoo and conditioner formulation [186]. Here a panel consisted of ten experts that combed tresses damaged through bleaching after being conditioned by various treatments with the Silicone Quaternium-22. Bar charts are utilized to portray the positive effects over the control systems, where the individual bars represent an average of multiple measurements. Using the absolute scoring system, other hair attributes can be handled in the same way depending on the objectives of the test.

In a relative scoring system, tresses with various treatments are compared to each other without reference to a particular standard. In a simple case, two tresses are used for comparison and a group of panelists are asked to simply evaluate which tress is preferred with respect to a particular hair attribute. Although these panelists do not have to be expert in the sense that they need to be calibrated, as with absolute scoring, they do need to be trained on the proper technique of evaluating the particular hair attribute being considered. For example, it seems simple enough to comb through hair to get the perception of friction of the comb going through the hair; but results will be varied if each panelist holds the comb at a different angle. After the panelists provide their scores on the paired tresses, they are tallied to indicate which treatment is preferred for that particular hair attribute. This method was utilized to compare the conditioning properties of a hydrophobically modified cationic cellulosic polymer, Polyquaternium-67, to quaternized guar gum when incorporated into a silicone containing shampoo. A group of ten panelists were asked to judge wet comb and feel, dry comb and feel, as well as volume properties on tresses conditioned with the two treatment types, one treated with the Polyquaternium-67 shampoo and the other with the cationic guar shampoo. Results indicated that wet combing was equivalent despite the fact that the Polyquaternium-67 shampoo had one-fourth the amount of silicone, indicating the increased silicone deposition to the hair surface. These results were correlated with quantification of the silicone deposition on the hair surface using atomic absorption spectroscopy. Preferences were also seen with other hair attributes for the Polyquaternium-67 shampoo treated tresses. From a structure-performance point of view, the authors indicated that hydrophobic modification is one more intrinsic variable of the polymer that can be modified, in addition to molecular weight and charge density, to affect improved properties over its parent compound, Polyquaternium-10 [187].

Besides combing and hair feel properties, panel tests can also be used to test the color and shine of hair tresses and again can be judged by panelists in either an absolute or relative fashion. The difference is that one must control lighting

conditions. Light boxes can be used and are equipped with different light sources such as incandescent and fluorescent light. A typical setup is illustrated in **Figure 57.**

Figure 57: Light box to conduct panel studies to assess such hair attributes as color and shine.

b. Fiber Fragmentation Techniques

Combing methods—Breakage through combing imparts many mechanical stresses to hair. Test methods have been employed where hair is combed by hand in a controlled fashion or combed with a mechanical device and the number of fibers that have broken from the hair are quantified. The number of fibers, when compared against appropriate controls, is an assessment of the degree of damage or the alleviation of weakening by a pretreatment [4, 113, 137, 188]. Normally, these tests are conducted so that there is a statistical basis to the conclusions from the test due to the very nature of the variables inherent in the combing process.

c. Mechanical Combing

As a comb is passed through hair it can be observed in the advancing end of the comb that the fibers appear to be weaving among each other in a random fashion. Hair that is undamaged is relatively easy to comb through because of the intact lipid layer that is present on the surface of the cuticles. This provides a lubricating action so that fibers can pass over each other as well as through the teeth of the comb easily during the combing process. Another factor is the greater pliability of undamaged hair. The undamaged proteins in the cortex have more elasticity and can take the stress of bending with more ease and without the propensity to fracture and break. Damaged hair does not have these advantages and as a result, passing a comb through hair is a lot harder due primarily to higher frictional forces. A measure of hair damage and the effect of treatments on alleviating damage is through measuring combing forces and the work it takes to pass a comb through the hair. This can be done by hand, and in fact is utilized in sensory testing techniques. Instruments are available that can quantify combing forces in particular the Dia-Stron, Instron, or texture analyzer, which are adapted with a mechanical comb device. Because the comb traverses through the hair at a constant rate and the resultant stress is measured by force in grams, the testing technique is then a strain-controlling device. The data are portrayed as a combing curve and with the help of computer software, the work involved in passing the comb through the hair is calculated (e.g., in grams/cm). Comparing the combing work of a tress treated with a conditioning treatment will result in a reduction in combing work, and usually a percent reduction in combing is reported [189, 190].

Combing work, as measured by mechanical combing devices, has been used to not only determine the conditioning effects of shampoos and conditioners, but also with respect to this chapter, the protective effects of pretreatments to factors that damage the hair. Locke studied the reduction in combing work observed by applying a substantive photofilter to hair prior to a controlled solar irradiation and treatment regimen [111]. McMullen utilized combing work measurements to show the protection of hair from curling irons with the use polymeric pretreatments in the treatment regimen [107, 112]. Comparing polymeric treatments to appropriate controls, there was found to be less of an increase in combing after successive applications of the heat from a curling iron. In this case the iron was applied to one particular spot for a set period of time creating an area of the tress that would suffer damage as indicated in an increase in combing work. As seen in **Figure 58**, the section of hair that was exposed to eight one-minute cycles of the curling iron, the window of exposure, had higher combing work values as compared to the area after four one-minute cycles. This increase in combing work appears in this

window of the full profile of the combing curve, where the area under the curve in the window is a measure of the combing work to pass the comb through that part of the tress.

Figure 58: Increase in damage from heat damage of hair as evident from higher combing forces in the window of damage.

The advantage of the window technique in combing tests is that the combing work can be compared directly both before and after treatment on the same tress. Comparing combing work from different tresses requires a statistical base of samples since there is a high variability of combing forces from one tress to another. Comparisons with the windows technique can be done using one tress since it serves as its own control. This technique was developed further by Jachowicz and Helioff in a method they termed spatially resolved combing analysis [110]. In this method a tress is enclosed by a frame that allows only a certain part of the tress to be exposed; the other parts of the tress are covered by the housing of the frame (see **Figure 59**). Two variants of the procedure can then be employed. In the first procedure the tress is contained in the frame and only the window section(s) of the tress is damaged, such as can be done with a bleach or permanent wave. After rinsing, the tress is then removed from the frame and a combing curve is generated showing an increase in combing work in the windows from the damaging treatment. The tress is then put through a conditioning regimen to show the reduction in combing work, especially in the windows section. In a modified version of the

method, the whole tress is exposed to a damaging treatment and combing forces then measured. The tress is then enclosed in the frame and a conditioning treatment added to the window section. The resultant combing curve will show a reduction in combing work just in the area of the window showing the conditioning effect. Besides conditioning of damaged hair, this method has also been employed in showing the protection of hair from UV light with a photofilter [191] and polymeric pretreatment on thermal protection [107, 112].

Figure 59: Spatially Resolved Combing Analysis; treated tress where exposed areas of tress were bleached and specialized frame for treating tress

Continual brushing and combing of hair imparts various levels of stress to individual fibers of the hair assembly. For the most part, these stresses are not strong enough to break the hair; but with repeated application of smaller stresses, eventually fibers become fatigued and break. Here the stresses are applied over hundreds of fibers during a stroke of a comb. Evans saw that this was similar to his fatiguing experiments using single fiber studies and applied the same type of statistics to analyze breakage data to construct survival probability plots [192].

A mechanical combing device is constructed to help understand the variables involved with hair breakage or prevention of breakage and consists of a hollow rotating drum containing four brushes or combs attached on its outer circumference. As the drum rotates, the combs traverse a hair tress so that it experiences 50 strokes per minute. Broken fibers begin to fall out on a plate on the bottom of the apparatus after many strokes of the comb. In experiments conducted by Evans [192], fibers were collected from eight tresses after every 1,000 strokes up to 10,000 strokes. The resultant data were then subjected to Weibull statistical analysis to determine survival probability plots, and its converse, failure prediction plots. Major findings were reported as predicted failure vs. the number of strokes. Results indicate that the failure prediction vs. the number of strokes were lower for conditioned vs. unconditioned hair, virgin undamaged hair vs. bleached, bleached conditioned vs. bleached unconditioned, bleached vs. 3X bleached, and lower vs. higher humidity. One of the interesting features of running the Weibull statistics on the breakage data is the characteristic shape parameter, which indicates whether the majority of breakage occurs early or late in the fatigue experiment. By quantifying not only the number of breaks but also the size of the broken fibers, Evans was able to theorize the breakage mechanism using the shape parameter. Here he concluded that small fiber fragments broken from the tip end of the fiber form from a wear-out mechanism, whereas longer fragments form from a premature failure most probably caused by the comb hitting entanglements [192].

d. Salon Testing

Salon testing is not an incidental process in the testing of products for hair damage and repair. Albanese makes a good case for the value of salon testing in product research and development [193]. Many advanced hair attributes, which only a trained cosmetologist can pick up in his or her evaluation, will be missed. These include such styling attributes as hair body and manageability, stylability during brushing and blow drying, and subtle auxiliary attributes such as fiber alignment and curl definition. Therefore, the human sensory aspect of results on the whole hair attributes on panelists have to be included with subjective panel testing on tresses and in objective and detailed instrumental analysis.

Advice from an experienced cosmetologist is important for the experimenter in designing the treatment scheduled as simulated on hair tresses in the laboratory. For example, in exposing hair tresses to the heat of a hot flat iron, the rate of descent of the iron along the hair tress, the amount of heat applied as controlled by the thermostat, as well as the pressure of the jaws of the iron on the hair are all important variables to consider. Other damaging regimens such as relaxing, perming, oxidative hair coloring, and the like also have their own subtle, yet important variables to consider.

Unlike conditioning and styling products, hair protection and repair product testing in the salon is somewhat limited. This is due to the simple fact that no one would like their hair purposely damaged, whether it be a whole-head study where candidates have their hair damaged, where half of the panelists have a protective pretreatment, or half-head study where one side of the head has the control formula and the other the product with the experimental protective ingredient. The best course is simulating the damage in the lab on tresses or possibly a mannequin head that are based on human hair that have a treatment schedule as realistic as possible.

CONCLUSION

If cannot be stressed too much that hair has a complex structure and that because of this it responds to stresses induced by external factors in various ways. Changes occur at different levels consisting of the molecular, the nanoscale, subcellular, and the micron-size cellular level. Damage on each of these levels produces effects that contribute to the decrease in the cosmetic behavior of hair and ultimately can be perceived by a person during their normal grooming routine. To help study this multifaceted array of variables and effects, there are many instrumental techniques, each designed to give a clear picture of the changes incurred to the different hierarchical components of hair. One must, therefore, know exactly what the instrument measures and the consequent conclusions that can safely be made. When a damaging process has multiple effects on hair, such as surface as well as cortical damage, then various techniques need to be employed, where one reinforces the conclusion of the other. As in a legal argument, one must make a case to prove through appropriate evidence that the data link cause-and-effect relationships. The planning and execution of the experimental design, such as the practicality of the treatment schedule and employing the proper measuring techniques, are part of this process as well.

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CLINICAL TESTING OF COSMETICS AND SKIN CARE PRODUCTS: METHODS AND INSTRUMENTATIONS

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ABSTRACT

In the field of skin research, visual examination and touch are no longer solely employed to assess skin conditions. The field has evolved to include instrumental methodologies to quantitatively and qualitatively assess skin properties. Principles of physics, chemistry, biology, and engineering are combined to study skin both at the surface and in-depth.

Cosmetics and Skincare products are used to enhance the look and feel of the skin and to protect the body from the elements of nature. To study the effects of cosmetics and skin care products on skin, biophysical measuring techniques can be employed in a variety of experimental models. Both dose-response and time-response studies can be performed with greater precision. For example, topical products may affect skin in a variety of ways (e.g., changes in *stratum corneum* hydration, trans-epidermal water loss, blood flow, pigment, morphology and elasticity, etc. These changes are measured by different instruments.

This chapter presents an overview of some of the commonly used, powerful techniques currently in use. The electrical impedance of skin has been shown to change with *stratum corneum* hydration and, based on this principle, a number of devices are available that are routinely used in studying *stratum corneum* hydration changes. The skin barrier can be studied by trans-epidermal water loss measuring devices. To quantify erythema on skin a number of devices based on various physical principles are in use (e.g., colorimetry, spectrophotometry, and laser doppler flowmetry).

Many imaging techniques have been developed to reveal subtle changes in skin on the surface and in depth. High-resolution digital photography with options of polarized and fluorescence imaging and videomicroscopy are useful to explore the microstructure. Some more advanced techniques such as *in vivo* laser confocal microscopy and optical coherence tomography reveal the horizontal and vertical sections of skin at various depths. Measuring skin topography parameters is useful to show smoothing of skin surface after a product application. We can now study viscoelastic properties of skin in a variety of ways using twisting, impacting, suction, and shearing devices. The list of skin measuring tools is growing . . .

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11.5.1 INTRODUCTION

The effect of skin care products that improve the structure and function of skin, as well as enhance the appearance of skin, can be studied using a number of bioengineering techniques. We no longer depend solely on clinical gradings. The idea is to get a complete picture of improvements, not just look at one or two parameters. Traditionally, visual examination and some simple evaluations have been used on large pools of subjects to show the efficacy of these products. Often the difference between the treatment and control is subtle, requiring advanced methodologies to be employed. A good collection of these skin-measuring techniques are given in *Handbook of NON-INVASIVE METHODS and the SKIN*, second edition [1]. Some skin-imaging techniques are given in *BIOENGINEERING OF THE SKIN, Skin Imaging and Analysis*, second edition [2].

Measuring *stratum corneum* hydration using electrical devices is a commonly used method. Often there is a need to use advanced methods like measuring skin hydration kinetics which are based on the property of *stratum corneum* to hold, release or accumulate water. These include sorption-desorption (S-D) and moisture accumulation test (MAT). The use of sorption-desorption method is described by Tagami et al. [3] and moisture accumulation method by Pellacani and Seidenari [4].

The barrier function of SC can be assessed by measuring the trans-epidermal water loss (TEWL) as well as by using a number of chemical probes, e.g., methyl-nicotinate, lactic acid, and balsum of Peru. Furthermore, penetration of fluorescent dyes, e.g., pyranine, can also be utilized.

Erythema can be assessed in a variety of ways. The erythema may be graded visually; however, it will depend on the particular observer, ambient light, previously seen colors as well as the mood and prejudices of the observer. Human eye is good in discriminating colors and brightness of objects when they are adjacent, but poor in grading single color or intensity. It also cannot remember a previously seen color or intensity quantitatively. The search for an objective method to assess erythema, over the last 100 years, produced a range of techniques. Topical drugs and skin care products are tested by either comparing post-treatment erythema values to pre-treatment or comparing the erythema value on the test site to the adjacent normal skin. Conversely, some products cause vasoconstriction, resulting in an apparent blanching. The pallor is caused by relatively lower amount of blood at the test site. Both redness and blanching can be graded visually as well as measured by various instrumental modalities, e.g., visual examination, various scales, graded colored paper, photoplethysmography, colorimetry, diffuse reflectance spectrophotometry, blood flow by laser doppler imager, digital color photography, thermography, etc. Subclinical erythema can be detected by reflectance spectrophotometry. Laser doppler imager is not useful for blanching assays. Blanching (by corticosteroids) can be detected very well by reflectance spectrophotometry.

Skin pigmentation can be measured by a variety of techniques, some of them common to erythema measurement, e.g., visual examination, various scales, colorimetry, reflectance spectrophotometry, optically filtered digital photography, etc. For melanin pigmentation we can also add controlled UV-photography.

Optics and advanced imaging techniques are important in showing the effects of products on skin. Filtered digital photographic techniques (cross-polarized, parallel-polarized, and fluorescence) can reveal features of skin that are not shown in standard photography. Ultraviolet light photography dramatically reveal the pigmented lesions of skin. Side-lighted videomicroscopy dramatically enhances the view of scaliness of skin, especially if blue-UV light is used for illumination. This method also shows an enhanced view of the pigmented lesion. Ultrasonography shows the skin structure (cross-section) and reveals the presence of edema. Optical Coherence Tomography (OCT) shows cross-section of skin at a much higher resolution than ultrasound. Thickness changes in SC and epidermis can be measured by Optical Coherence Tomography (OCT). Some applications of OCT on skin are given by Pagnoni et al. [5]. Confocal Scanning Laser Microscopy reveals cellular structure in various strata of skin as horizontal sections, complementing OCT's cross-sectional view. The confocal microscope can also be used to measure

thickness of *stratum corneum* and epidermis. Confocal microscope images show melanin-rich areas as bright structures, particularly near basal layer. Rajadhyaksha et al. describe a confocal microscope in detail [6].

An important parameter in studying skin is topography. In the past, replicas of skin have been used to assess the surface for roughness and glyphic line structure. The replica samples have been imaged using many different angles of illumination. In some experiments we have used a narrow-angle (~ 10°) illumination by a ring light to enhance the fine surface structure. The image is digitally captured and analyzed using computer programs developed in our laboratory. The line structure is analyzed for lengths and areas. Also surface roughness is calculated. The fringe projection device has, by and large, replaced the replica method. Fringe-projection device with phase-shift procedures can reveal ultra-fine changes in skin surface roughness. This device images skin surface directly and the calculation of topography parameters are much more precise. Skin roughness and smoothness values can be calculated in a variety of ways. We have shown the disruption of surface in the photo-damaged skin compared to relatively normal skin. Detailed descriptions of the fringe projection device are given by Jasper et al. [7] and Frankowski et al. [8].

Viscoelastic properties of skin are important for a healthy skin. Skin is a complex composite made up of elastic and viscous parts. The bulk of elasticity in skin resides in the dermis with its collagen and elastic fiber matrix. The epidermis and *stratum corneum*, however, also influence the elastic properties somewhat. In general, any product that affects the hydration levels of skin or influences the dermal fiber network would show change in the viscoelastic properties of skin. To measure the elastic properties, a force (stress) is applied to the skin and then released while deformation (strain) is measured during this cycle. The *in vivo* elastic properties of the skin can be measured by several methods like ballistometry, dynamometry, torque, and suction devices.

Some *ex vivo* techniques are available to quantify features of skin, e.g., sebum output, number and size of follicular casts, and desquamation. Samples are first collected off the skin by an appropriate method and then an image is obtained under a microscope. Image analysis programs are then used for quantification.

The desquamation property of skin is commonly studied by collecting the surface scales on an adhesive disc. A more advanced method is to harvest 3D squame samples sequentially from the same site and look at the dynamics of scale-collection.

Many of the methods described above have been used to study sun-damaged skin, also referred to as photo-damage. There is great interest in treating the signs and symptoms of sun-damage. The basic science of skin intrinsic aging and photo-aging have been described by many authors [9], [10], [11], [12].

11.5.2 COSMETICS AND SKIN CARE PRODUCTS FOR HUMAN USE

Cosmetics and skin care products are used to enhance the look and feel of the skin and to protect the body from the elements of nature. Topical drugs, on the other hand, are used to prevent or treat skin diseases. Considerable overlap, however, occurs between these two fields; e.g., control and treatment of dry skin are tackled by both skin care products and skin pharmaceuticals.

The development of a skin care product starts with market research and current fashion trends; a product is then designed based on technical research and development. Before the product reaches the consumer, a number of testing and research procedures are followed at various steps. First, chemical and microbiological stability is tested. Safety tests are then performed followed by efficacy testing. Finally, usage testing is done, which is based on sensory, tactile, and visual appeal of the product.

The efficacy testing of a product on human subjects starts with hypotheses. A good hypothesis is based on current knowledge of the properties of a product and its component chemicals, and the potential effect on human skin. The experiment is designed by choosing appropriate measuring techniques and using currently acceptable principles of statistics. The size of the test panel is based on the precision and resolution of measurements. Proper controls should be used to show that the active ingredient, indeed, have produced the change.

The test product should be blinded from the subjects as well as from the evaluators to rule out any bias that may creep in. In general, a good test would have double-blinded, randomized, and placebo-controlled design. Negative and positive controls may also be added.

11.5.3 SKIN STRATA

Human skin covers the whole body, an approximate surface area of 2 m^2, varying in thickness with location as well as with age, sex, ethnicity, and dermatological conditions of health and disease.

The skin is divided into two main compartments: the outer epidermis and the inner, and much thicker, dermis. As we journey from the outermost layer of skin to the innermost, we pass through various strata of epidermis and dermis. A sketch of skin strata is shown in Figure 1.

The outermost surface is the *stratum corneum* (SC), a multilayered, keratinized surface with flattened and non-nucleated cells, post mortem. This is followed by *stratum granulosum* (SG), which is composed of one or more layers of granular cells that contain keratin fibers and either has shriveled nuclei or no nuclei. Below the SG there is a thick layer, *stratum spinosum* (SS), composed of several layers of

cells with large oval nuclei and spiny processes. This is followed by *stratum basale* (SB), which is the last epidermal layer at the dermal-epidermal junction. The SB is composed of a single layer of columnar cells (basal cells), which include melanocytes and Merkel cells. The melanocytes are dendritic cells that perform melanogenesis through melanosomes. The melanin pigment is finally transferred to the keratinocytes. At the basal layer, cells divide continuously and move outwards towards the surface of the epidermis.

Figure 1: A general sketch showing the various strata of skin.

The dermal-epidermal junction is a wavy surface forming peaks and valleys (rete ridges). On the dermal side of this papillary surface, is the skin blood microvasculature.

The dermis forms the bulk of the skin, protecting the body from physical injury, providing pliability, elasticity, and tensile strength. The dermis is composed of bundles of fibers of various types (e.g., collagen and elastic fibers), distributed among which are blood vessels and nerves. Dispersed among these are hair follicles, sebaceous and sweat glands. A variety of cells can be found in the dermis,

e.g., fibroblasts, macrophages, and mast cells. The dermis is divided into two layers, papillary dermis and reticular dermis. The cutaneous blood supply is arranged as two horizontal vascular networks, one at the dermal-subcutaneous junction and the other at the subpapillary level. From the subpapillary horizontal plexus capillary loops rise up into dermal papillae, usually one per papilla. The direction of blood flow in this network is from the arterioles to venules. Figure 2 shows the general layout of the vasculature.

Figure 2: Microvasculature of the skin.

The main barrier to the skin is provided by *stratum corneum*. It protects skin against outside chemical insults and controls the trans-epidermal water loss (TEWL). The flattened cells of the SC, corneocytes, are continuously being renewed as a result of a process of epidermal maturation and terminal differentiation. The corneocytes are embedded in a matrix of intercellular lipids (brick and mortar model). They are also riveted together by intercellular protein structures called corneodesmosomes. There may also be ionic bonding between the corneocytes. Each corneocyte is a protein complex encapsulated within a protein shell called the cornified cell envelope. Within the corneocytes reside a number of water-soluble compounds collectively called natural moisturizing factor (NMF), which have the ability to bind water. Both the intercellular lamelar lipids and the natural moisturizing factor maintain the balance of water in a healthy *stratum corneum*. Lack of water can result in dry, brittle, cracking, and rough skin.

11.5.4 BIO-INSTRUMENTATION

a. General

in most clinical studies, evaluations are performed combining visual grading of skin attributes as well as measurements by a variety of bio-instrumentations.

Following each laboratory's "standard operating procedure" (SOP), the instruments are calibrated and tested on regular basis.

For some of the instrumental measurements, keeping the room temperature and humidity constant is critical—for example, measurement of *stratum corneum* hydration and trans-epidermal water loss. Generally, a temperature of 21° ± 1° Celsius (70° ± 1° Fahrenheit) and relative humidity of either 40% ± 5% or 50% ± 5% are required for some types of skin evaluations.

An environmental chamber is the best way to control the temperature and humidity precisely.

b. Environmental Chamber

A variety of types and sizes of environmental chambers are available. We use a closed chamber with a single entry door and with thick wall insulation for controlling the temperature and humidity. A highly precise process control unit is used for reading the values of temperature and humidity from the sensors; it also controls the ambient condition by sending pulses of hot, cold and humid air, along with cycles of dehumidification.

The mixing of hot, cold, and humid air is done outside the chamber, in a sub-chamber above the ceiling. The ceiling has 56,800 micro-holes, 3-mm diameter each and distributed 12 mm apart. The mixed air from above continuously filters through these micro-holes down into the chamber to provide a uniform atmosphere inside, and holds the temperature and humidity constant. In this system there is no air draft or substantial air movement inside the chamber, which is very different from an air-conditioned room where the fans blow the air into the room.

The inner dimensions of the chamber are: width 301 cm, length 355 cm, and height 213 cm. Outside the chamber there are refrigeration and heating units as well as humidity and dehumidifying units. The humidifying system consists of water mist producer (vapor injection). The water supplied to this unit comes through a two-stage laboratory water filter. The dehumidifier system consists of a large freezer coil with condenser fins, placed just outside the wall, across a screened opening.

The temperature and humidity are continuously recorded on a chart recorder. The recommended time for acclimation of subjects is between 15 and 30 minutes, but could be longer for some experiments.

11.5.5 SKIN HYDRATION

Proper level of *stratum corneum* (SC) hydration is essential for healthy skin. Lack of moisture leads to rough, dry skin and can cause a number of skin ailments.

Skin moisture can be measured in many ways but by far the most common method is to measure the electrical impedance of skin. Hydration changes in the *stratum corneum* influence the electrical capacitance and conductance of skin. Three of the most common instruments these days are: novameter, skicon, and corneometer. These are simple and easy to use instruments utilized by dermatologists to follow skin disease during treatment as well as by industry to test moisturizers and topical drugs. Figure 3 shows the three hydration probes, which are placed in contact with skin surface.

Figure 3: The hydration measuring probes of Skicon, Novameter, and Corneometer. The electrode dimensions are also shown.

a. Skicon

The Skicon (Skicon-200, I.B.S. Co. Ltd. Japan) is a conductance-measuring device with concentric circular electrodes. It uses a fixed electrical frequency of 3.5 megahertz. The measurements are given in microsiemen (μS) units and have a range of 5 to 1999.

b. Novameter

The Novameter (Model DPM9003, Nova Technologies, NH) is an impedance-measuring device with concentric circular electrodes separated by a gap of 1 mm. It uses varying electrical frequencies over a range up to 1 MHz, and the capacitance

is derived from the signal-phase delay. The measurements are given in arbitrary units referred to as DPM units (DPM stands for Dermal Phase Meter). The range is 90 to 999.

c. Corneometer

Corneometer (model CM 825, Courage + Khazaka electronic GMBH, Germany) is a capacitance device, which consists of a pair of electrodes formed as an interdigital grid with small spacing between the elements. Readings are given in arbitrary units.

d. Sorption-Desorption Test

In a more sophisticated mode these instruments can be used to determine the ability of skin to be moisturized and its capacity to hold water. This is called the sorption-desorption test, in which a drop of water is applied to skin for a brief period and the dynamics of hydration is followed through a series of moisture measurements. Using this test we find the water-holding capacity, hygroscopicity, and desorption rate constant of skin in health and disease.

e. Moisture Accumulation Test

Another test is the moisture accumulation test (MAT), in which the skin is occluded with the measuring probe for a period of time and the increase in hydration is continuously recorded. Using these instruments, efficacy of moisturizers can be tested.

11.5.6 TRANS-EPIDERMAL WATER LOSS

Another important phenomenon in skin is the loss of water through epidermis, which essentially relates to the barrier function of skin. This is measured by an instrument that measures the water evaporation from a surface by detecting the partial pressure gradient of water vapors. Two pairs of sensors, measuring humidity and temperature, are arranged in a cylindrical well in such a way that one pair is farther than the other from the surface whose evaporation rate is to be measured. The schematics of this arrangement are shown in Figure 4. The reading displayed is called trans-epidermal water loss (TEWL) and is given in units of g/m^2h. In general, skin irritation, barrier disruption, and sweat output increase the TEWL. Effects of various cosmetics, toiletries, and topical drugs can be studied with this instrument. Various dermatoses can also be followed.

Figure 4: Measurement of Trans-Epidermal Water Loss using two pairs of humidity and temperature sensors.

a. Evaporimeter

Evaporimeter (Model EP2, Servomed, Sweden) measures TEWL in an area of 12-mm diameter circle. Measurements are done in an environmental chamber at a temperature of 70° ± 0.5°F and a relative humidity of 40 ± 5%. Readings are recorded between 45 and 60 seconds after placing the probe on the skin surface. The data can also be collected through an EP2 program. The TEWL is given in g/m^2h.

b. Dermalab

The Dermalab TEWL instrument (Dermalab, Cortex Technology, Denmark) has a probe diameter of 11 mm. Measurements are done in an environmental chamber at a temperature of 70° ± 0.5°F and a relative humidity of 40 ± 5%. Readings are recorded at 60 seconds post-probe contact with the skin surface. The data can also be collected through a program. The TEWL is given in g/m^2h.

11.5.7 SKIN BLOOD FLOW, COLOR, ERYTHEMA

The redness is caused by increased blood flow in the superficial capillaries in skin. It is important to measure erythema during treatment of skin diseases as well as during safety testing of topical products. It is also useful to test skin by provoking erythema reaction (irritation model) and then test the ability of a topical product in reducing erythema. Various instruments have been devised to measure erythema. Some are based on measuring the movement of erythrocytes and others are based on measuring the color or spectra of the light reflected off the skin. Some of the

common instruments are: Laser Doppler flowmeter, colorimeter, and diffuse reflectance spectrophotometer. The inflammatory reactions of skin can be studied, e.g., due to a variety of stimuli, e.g., irritants, vasodilators, UV exposure, mechanical abrasion, topical application of products, and drugs.

a. Laser Doppler Flowmetry

The Laser Doppler Flowmeter works on the principle that light reflected off the moving erythrocytes experience a Doppler shift in frequency. The interaction of the frequency-shifted light waves with the nonshifted light waves produces a signal that is proportional to the blood flow in the capillaries. The laser Doppler imager (MoorLDI, Moor Instruments, Devon, UK), scans a laser beam on a predetermined rectangular area on the skin surface by a moving mirror that produces a raster pattern. The Doppler-shifted signal is recorded as an image of 250 × 250 pixel. The blood-flow values at each pixel are given in an arbitrary "Perfusion Unit (PU)," which is proportional to the product of "number of erythrocytes" and their "mean velocity" in a volume of tissue. Figure 5 shows the schematics of the Laser Doppler Imager. A Helium-Neon (He-Ne) Laser is used with a wavelength of 632.8 nm.

Figure 5: Schematics of the Laser Doppler Scanner.

b. Colorimetry

Chromameter measures the color of skin quantitatively. Light from a xenon flash lamp illuminates a 1-cm diameter circular area of the skin, and the reflected light is detected and analyzed for its color components and intensity. The measurement is given in L* a* b* system of colorimetric notation, where L* stands for brightness from black to white, a* describes color from green to red, and b* gives color from blue to yellow. Thus 1/L* is a measure of darkness, a* a measure of redness, and

b* a measure of yellowness in relative terms. Erythema on skin is measured on a* scale and pigment on 1/L* scale. The schematics of the chromameter are shown in Figure 6.

Figure 6: The probe head of the chromameter.

c. Reflectance Spectrophotometry

The spectrophotometric measurement of erythema is based on assessing the hemoglobin content of skin by measuring the specific absorbance peaks. Oxy-hemoglobin have absorbance peaks at 415, 542, and 577 nm, and deoxy-hemoglobin have peaks at 430 and 555 nm. The common end of a bifurcated fiber optic cable is placed on the skin surface, one arm is connected to a light source, and the other arm is connected to spectroscopic detector (see Figure 7). Using a tungsten-halogen light source and a spectrograph with a diode array of 1024 elements, an absorbance spectrum in the wavelength range of 400 to 800 nm is obtained.

The spectra are always referenced to a white standard. Barium sulfate and aluminum oxide are common white standards. White ceramic tile can also be used as a reference. The apparent absorbance is calculated as:

$$A(\lambda) = \mathrm{Log}_{10}\{ W(\lambda)/S(\lambda) \}$$

Where $A(\lambda)$ is the apparent absorbance, $W(\lambda)$ is the white reference spectra, and $S(\lambda)$ is the spectra from skin.

Figure 7: Reflectance Spectrophotometer with a bifurcated fiber optics cable.

Often a "dark current" is associated with the detector; a dark reference is measured as well. Placing the probe on a mat black surface and switching the light source off and then recording spectra can easily do this. In general the white reference and the dark reference is measured at the start of a measurement session. The apparent absorbance is calculated as:

$$A(\lambda) = \mathrm{Log}_{10}\{ [W(\lambda) - D(\lambda)] / [S(\lambda) - D(\lambda)] \}$$

Where $D(\lambda)$ is the dark reference.

To calculate the apparent hemoglobin content, first a straight line is mathematically fitted on the points in the wavelength range of 620 to 720 nm. This straight line is subtracted from the absorbance values of the spectra. This is done to remove the effects of melanin and scattering by other structures in the skin. An easier, but less accurate routine is to subtract the value at 630 nm. Absorbance values at 542 nm and 577 nm for oxyhemoglobin and 555 nm for deoxyhemoglobin are reported. Other quantities like total hemoglobin and melanin level can also be derived.

The choice of methodology depends on type of reaction, design of experiment, and required precision of measurement. The Laser Doppler Flowmeter measures very well the increased blood flow by irritants but gives poor results

in vasoconstrictive assays using corticosteroids. Laser Doppler flowmetry can be used for continuously monitoring blood flow as well as scan large areas of skin to see the spatial distribution. The colorimeter shows the changes in red color but would not give information about specific chromophores in the skin. The diffuse reflectance spectrophotometer on the other hand shows the absorption peaks of particular chromophores, e.g., oxy-hemoglobin, deoxy-hemoglobin, and bilirubin.

11.5.8 IMAGING TECHNIQUES

a. Digital Photography

Digital photography is used in a variety of ways to assess the changes in the appearance of skin after a specific treatment regimen. Facial photography is particularly very popular.

Many photography systems are available, using different cameras, geometric arrangements, lighting systems, and optical filter systems. In general, the most frequently used photography systems are: 1) Digital Flash Photography, 2) Polarized Light Photography, 3) Fluorescence Photography, and 4) Ultraviolet Light Photography.

In case of facial photography, the camera is moved around the face to capture left face, front face and right face. Conversely, the camera position is fixed and the face is turned left or right. Generally photographs are obtained at –45, 0, +45 degree angles to the front of the face.

One of the commonly used systems is the Canfield Clinical Photography System (Canfield Imaging System, Fairfield, NJ, USA).

1. Face Photography

The photos of the left, front, and right sides of the face are obtained using a computerized photography system with a 21.1-Megapixel (5616 × 3744 pixels) Canon EOS 5D Mark II digital camera.

The camera, along with the twin flash lamps (Canfield Intelliflash), is mounted on a base that rides over an arc-movement track on the Photography-Table, see Figure 8. The camera is positioned, relative to the face, at angles: 0° (Front face), –45° (Right face), and +45° (Left face). Two strobe lights are used at the neck level to reduce the shadows under the cheeks.

The face of the subject is first positioned in front of the camera, using a chin-rest and a nose-bridge device. After the alignment, the nose-bridge device is removed. A photo of front of face (0°) is obtained. The photos of the right and left sides of the face are obtained sequentially after moving the camera –45° (Right face) and +45° (Left face).

Figure 8: Top-view of the Facial Photography System.

Mirror software (Canfield Imaging Systems, NJ, USA) is used for controlling the camera and saving the captured photos in a database. Mirror "PhotoTools" is a computer program that is used for tethered capture of images from the digital camera with "Live-Preview" function. The Mirror "PhotoFile Image Management" is a database program which helps in storing, retrieving and exporting captured images, as well as applying attributes to distinguish between different sets of images. In the "Live-Preview" mode, the picture of the face is displayed on the computer screen in real time within a rectangular frame with vertical and horizontal grid lines. The face is aligned to the grid lines. After removal of the nose-bridge device, the photo is captured by clicking the "Capture" bar. The image is stored in the database. The first set of photos, e.g., at baseline, are obtained using the function called "Pre-Op." For post-treatment photos, first the appropriate baseline photo is selected, and then the "Post Op" function is selected to display the live image of the face on top of the original (baseline) image, each image with 50% transparency. The face is aligned to match the baseline image as closely as possible, and the photo is captured.

A laptop computer, DELL Latitude E6410 14, is interfaced with the camera through a USB cable. All functions of the camera are controlled by the computer.

An external flat-panel monitor is connected to the laptop computer using a VGA cable. This monitor helps during physical alignment of the face in the "live-preview" mode. Another computer is used to display baseline photos of subjects so that the position of headband and hairs can be matched, as closely as possible, in the current photo session.

2. Polarized Light Photography

The Polarized Light Photography is done using another Canfield Photography System, which has table and arch movement similar to the one described above, but different camera and flash lights. Linear polarizers are positioned on the two flash heads and on the camera lens. The lens polarizer can be rotated by 90° to achieve either cross-polarization for subsurface details or parallel polarization for enhanced surface details.

3. Fluorescence Photography

Fluorescence Photography is achieved using yet another photography system that allows visualization of porphyrin and comedonal fluorescence as well as fluorescent stains. Two filtered flash lamps emitting at a center wavelength of 417nm (visible-violet) are positioned symmetrically at the side of the camera body. A UVA-Blue cutting filter GG475 (Schott Glass) is placed in front of the camera lens. The sensitivity is set at STD (400 ISO) and two flash units are used at full power (400 watts each). F-stop is set between 22 and 8.

4. Ultraviolet Light Photography

For enhanced visualization of pigmented lesions and melanin content of skin, photography with light in the ultraviolet wavelength range (320 to 400 nm) is used. A 35-mm film camera with a 60 mm lens and a 400 W twin-flash lamp is used. Optical filters, passing Ultraviolet-A light (UVA, peak at 365 nm) and blocking visible light, are mounted on the flash lamps and in front of the camera lens. An ISO-400 black-and-white film is used to collect the image.

b. Ultrasound

A 20 MHz ultrasound machine (Cortex Tech., Denmark) is used to image a cross-section of skin. A hydrogel is applied between the skin and the ultrasound probe for coupling. The image is pseudocolored to show the ecogenic density distribution. The skin thickness is measured by the ultrasound image analysis software (Cortex Tech., Denmark).

c. Videomicroscopy

Videomicroscopy is commonly used to look at the various skin features and lesions. Two types of videomicroscopes have been utilized, one a hand-held visible light videomicroscope with changeable vertical or horizontal lighting and various magnifications, and the other a UVA-light videomicroscope with side lighting. The illumination systems are shown in Figure 9.

Figure 9: Visible light and UV-light videomicroscopy systems.

In-vivo microscopy of skin is done using a videomicroscope which consists of a hand-held probe, fed with light from a fiber-optic cable and connected to an image processor and a computer that displays the image live on the monitor (Hiscope, Model KH2200 MD, Hirox, Japan). The magnifications can be selected 20-100 X (variable) and 400 X (fixed). It has the capability of varying the illumination from almost vertical to almost horizontal to the skin surface. This has made it possible to view skin at surface as well as deeper layers. In general, light falling

on the skin is diffused and reflected in various proportions. Reflected light shows the surface structures; the diffused light shows color structures, e.g., pigment and erythema lesions.

Vertical illumination shows surface reflections very well, revealing the topography of skin, hairs, follicular lesions, and acne. Effects of topically applied lotions and creams on the skin can be studied in this way.

The horizontal lighting reveals the deeper levels such as pigment and capillaries in the skin, as well as the diffusing objects on the surface such as dry skin flakes. This method for illumination can be used for moisturizer efficacy studies or mildness of surfactant studies.

The theory of imaging of deeper levels by horizontal lighting is different than cross-polarized light imaging. Since for specular reflection, the angle of incidence is equal to angle of reflection, light striking a surface at a narrow angle is mainly reflected at a narrow angle; hence most of the light is reflected off the field of view. Only the portion diffused into the skin affects the image as viewed by the video sensor. Therefore, the oily shine of the skin surface is reduced, giving way to visualization of deeper levels. It may be noted that if the skin surface is too scaly, it is difficult to see the deeper layers, since the horizontal light also strongly illuminates the surface scales.

Using the videomicroscope with an appropriate illuminating system, one may follow various dermatoses and treatment regimens over a period of time, recording images along the way on the computer disk. In many cases the skin lesions can be located reasonably accurately by comparing current image with the previous one and adjusting the position and orientation of the video probe.

d. Confocal Microscopy

The confocal microscope is gaining prominence in biology, particularly the *in vivo* video rate microscope. It has great potential to complement conventional histology. In some instances the optical sectioning of confocal microscopy can replace the physical cutting of tissue for histology. It has an added advantage of looking into the skin in real time, e.g., observing the movement of erythrocytes and leukocytes through the capillaries.

Living skin can be optically sectioned by confocal microscopy from the surface downwards creating a series of horizontal images in real time without physically invading the skin. It produces high-contrast images of the cellular structure with very small depth of field (thin section), by rejecting the out-of-focus parts of the image. The optical schematics are shown in Figure 10.

Figure 10: The Optics of the Confocal Microscope.

The confocal scanning laser microscope Vivascope1000 (Lucid, Inc. Rochester, NY) with an objective lens of 30× and 0.9 NA, scanning a field of 155 × 155 μm is used. The light source is a Gallium Arsenide (GaAs) Laser diode emitting at 830 nm. The light is detected by a Silicon Avalanche Photodiode through a pinhole of 100 μm. The scanning is achieved by a rotating mirror polygon and an oscillating mirror. The laser power is computer selectable between 0 and 20 mW. A polarization stage with adjustable angle further enhances the images. The imaging depth (z-axis) is controlled by computer in 3.6 μm steps. A horizontal resolution of 1.3 μm and a vertical resolution of 5 μm can be achieved. The objective lens is coupled to the skin surface through a circular cover glass (18 mm dia) attached to a circular ring that was affixed to the skin by a double-sided sticky tape. A variety of coupling media can be used, the most common being water. Sucrose 40%, hydrogel, and solutions of hydrogel in water (20, 25, and 50% v/v) have been used in the past for refractive index matching in a variety of situations.

A more advanced confocal microscope, Vivascope 1500 (Lucid, Inc. Rochester, NY, now called caliber I.D.), was later introduced with Z-steps of 1.6 microns, but with somewhat lower magnification. The new software can capture multiple images, as a block by x-y mapping as well as a stack in depth by z-mapping. For the z-stack the depth profile, i.e., the depth of each image of the stack, can be selected in advance. A gallium arsenide (GaAs) laser diode (830 nm) scans a field of 500 × 500 μm. The depth profiling is achieved by a computer-controlled stepper motor. The objective lens is coupled to the skin surface through a circular cover glass (18 mm dia) attached to a circular ring that affixed to the skin by a double-sided sticky tape.

e. Optical Coherence Tomography (OCT)

Optical Coherence Tomography (OCT) is a technique to record cross-sectional structures of skin. OCT is based on an optical interferometry principle, where a broadband light beam of short coherence length is split into sample and reference beams; the reflected light is coupled to produce an interference signal. The schematics of the OCT principle are shown in Figure 11.

Dermal and epidermal structures are clearly outlined. *Stratum corneum* (SC) is visualized as a bright line, sometimes thick and sometimes broken. The epidermis appears as a band with a wavy lower edge, and its thickness can be measured using the software. The geometry of isolated legions such as comedones can be visualized. The emergence of OCT as a skin-imaging tool fills the gap between the high-resolution (~ 1 micron) confocal microscopy and lower-resolution techniques like ultrasonography (~ 100 microns) and Magnetic Resonance Imaging (resolutions 50 to 300 microns).

The OCT device, SkinDex300 (GS-1 series, ISIS optronics, Mannheim, Germany), consists of an eight-channel OCT scanner and uses a 1300 nm broadband light source. It views skin 1 mm in horizontal direction and 1 mm in depth. The scanning head is optically coupled to the skin by a thin layer of gel.

Figure 11: Schematic showing the basic principle of optical coherence tomography.

11.5.9 SKIN TOPOGRAPHY

Reduction of fine lines, wrinkles, and surface roughness, following various treatments, has been studied in the past using two main techniques: 1) Obtaining silicon rubber replica of skin, and 2) fringe projection devices. The replicas are analyzed by a number of methods, e.g., stylus surfometry, laser depth profiling, or optical shadow method. The fringe projection device projects a fringe pattern directly on the skin, and the reflected image is analyzed by the computer software.

a. Replica of Skin

The surface structure of skin has been studied extensively by many groups by obtaining a replica of skin, using a silicon rubber type compound. Skin surface texture

is transferred permanently onto the replica as a negative impression, i.e., furrows will be ridges and cristae will be troughs. A number of techniques are available to analyze the replica. The image of the replica is captured through a stereomicroscope with specialized illumination, and image analysis is done by computer software. Using a narrow-angle ring light, the surface line structure is analyzed and the line lengths and areas are reported. Using a fiber-optics side lighting, roughness parameters, e.g., Rz, Ra, are calculated. Figure 12 shows the setup for microscopic imaging of replica using a side light and a ring light. Skin topography changes with age, body sites, various dermatoses, and use of topical products.

Figure 12: Imaging of Replica by Stereomicroscope with a side-light (left) and a ring-light (right).

b. Phase-shift fringe projection device

A digital micro-mirror projection device PRIMOS (Phaseshift Rapid *in vivo* Measurement of Skin, GF Messtechnik Gmbh, Teltow-Berlin, Germany) is used. This device directly measures the 3-D surface structure of skin without having to obtain a replica. Light from a tungsten-halogen lamp reflected off a digital micro-mirror projects stripes of Cos^2 type in a rectangular area of 13 mm × 18 mm on the skin surface (see Figure 13). The distortion of these stripes, due to the topography of skin surface, is captured by a videomicroscope camera. After application of phase-shift procedure and mathematical manipulation of the data by the computer, a color-coded height image of the skin surface is displayed on the computer screen. The surface height at each point of the image is precisely calculated by the software and displayed on the computer monitor on a color scale, e.g., blue is the lowest point, green is the medium height, and red a high point.

Figure 13: Schematic showing the basic design of the Fringe Projection Device.

c. Calculation of Roughness Values

First the hairs from the image are removed by a mathematical filter, then a fifth-order polynomial (n=5) is applied to the 3-D image to remove the gross contours of the skin surface. This flattening of the surface is done using the following equation:

$$Z(x,y) = \sum_{i=0}^{n} \cdot \sum_{j=0}^{n} a_{ij} \cdot X \cdot \left(X - \frac{X_{max}}{2}\right)^{i} \left(Y - \frac{Y_{max}}{2}\right)^{j}$$

On this processed image, 16 radial lines are marked inside a circle, each consecutive line subtending an angle of 22.5°. This is commonly called the "Star" roughness method. The roughness parameters, Rz and Ra, based on DIN4762 and DIN4788, are calculated for each of the 16 line profiles. The average value of Rz and Ra from this star formation is reported. The Rz values from baseline are compared to those obtained after treatment. Roughness parameters are derived for each line profile according to the following equations:

$$Rz = \frac{1}{n} z \sum_{i=1}^{i=n} Z_{max}(i) - Z_m \; l \quad \ldots \; [n = 1, 2, \ldots 5]$$

$$Ra = \frac{1}{\ell} \int_0^{\ell} (x).dx$$

d. Surface Area Calculation

In addition to the roughness parameters, Rz and Ra, the ratio of the real surface area to the area of the field of view, sometimes referred to as Rugosity, is also derived for each image. The field of view of each image is a rectangle of dimension 13mm × 18mm. The area of the theoretical plane covering the field of view is 13 mm × 18mm = 234 mm^2. Due to rough topography of the skin surface, the actual area would always be larger than 234 mm^2. The rougher the surface, the larger the area. Therefore, change in the surface area would indirectly indicate change in surface roughness.

11.5.10 VISCOELASTIC MEASUREMENTS

Skin is a complex composite made up of elastic and viscous components. To measure the elastic properties, a force (stress) is applied to the skin and then released while deformation (strain) is measured during this cycle. Some components of skin are mainly viscous and recover slowly while others recover quickly. The *in vivo* elastic properties of the skin can be measured by several methods like ballistometry, dynamometry, torque and suction devices (Figure 14).

a. Suction Device

Suction devices, e.g., Cutometer (Courage Khazaka, Germany) produces a small vacuum on the skin surface, making it rise in a small cavity. The elevation is optically detected. During a number of cycles of vacuum and release, the elevation and return of skin is measured and presented as a graph. The following elastic parameters

are calculated: Extensibility, Gross Elasticity, Elastic Function, and Viscoelastic Ratio. The suction and release of skin in a single cycle is shown in Figure 15.

Figure 14: Sketches of some viscoelasticity measuring devices. The Ballistometer, GBE, Torquemeter, and Cutometer are based on applying force by impaction, translation, twist, and suction respectively.

Figure 15: The parameters evaluated during the suction (3 seconds) and release (3 seconds) of skin in a single cycle by the Cutometer.

Calculation of some of the most common parameters are as follows:

1. R0 = e(a) = [Uf] Maximum Amplitude of the first cycle (Maximum Deformation). Value of extension "e" at the end of the first suction period "a."
2. R1 = e(a+b) Minimum Amplitude of the first cycle (Residual Deformation or return). Value of extension "e" at the end of first cycle (suction "a" + Release "b").
3. R2 = (R0 – R1)/R0 = [Ua/Uf] Gross Elasticity: Ratio of return to maximum extension in the first cycle.
4. R5 = [Ur / Ue] Elastic Function: Ratio of Immediate Elastic Retraction (0.1 sec. after removal of suction) to the Immediate Elastic Extension (0.1 sec. at the start of vacuum period). This refers to the Elastic component of the Gross Elasticity.
5. R6 = [Uv / Ue] Viscoelastic Ratio: Ratio of the viscous extension to the elastic extension.

There are many other ratios calculated, particularly when multiple cycles are used. Generally, three cycles of suction and release are recorded and some parameters comparing the first cycle to the third cycle are evaluated. In some experiments large number of cycles are used, e.g., 20 or 30 cycles, to show the elastic fatigue of skin.

b. Ballistometry

The ballistometer is an impacting device that lets a small spherical load fall on skin under gravity and bounce until it comes to rest. The movement is recorded electronically and displayed as a graph of diminishing peaks. The data is collected on the computer through an Analog to Digital Board and analyzed by a software program. The program detects the peaks and calculates the coefficient of restitution, which is the square root of the ratio of two adjacent peak heights. An example of ballistometry on the volar forearm vs. silicon rubber pad is shown in Figure 16.

c. Torsional Ballistometry

The torsional ballistometer (Dia-Stron, UK) is an impacting device that lets a small spherical load bounce on skin surface until it comes to rest. The movement of the ballistometer arm, which is mounted on a stainless-steel torsional wire, is recorded electronically and displayed as a graph of diminishing peaks. The data are collected on the computer. The program detects the peaks and calculates the coefficient of restitution, which is the square root of the ratio of two adjacent peak heights. The program also calculates the depth of the first indentation, and the rate of decay of the bounce peaks "Alpha" and "Area" of the bounce profile.

Figure 16: Graphs of diminishing peaks recorded for silicon rubber pad (left) and volar forearm of a volunteer (right).

11.5.11 SOME *EX VIVO* TECHNIQUES

a. Desquammation Measurements

A 22 mm diameter adhesive disc, D'Squame (CuDerm Corp. Dallas, TX) is used to remove a layer of *stratum corneum*. The scales collected on the disc shows the skin desquamation property and corneocyte morphology (Figure 17). The *stratum corneum* is first delipidized with a mixture of ether and acetone (1:1) for one minute. The disc is pressed with a spring-loaded pad for 30 seconds and removed.

Figure 17: D-Squame sample showing scales removed from the skin.

The disc is placed on the standard black card (CuDerm Corp. TX), which is mounted under the stereomicroscope and illuminated by standardized fiber-optics light. The image is captured by a video camera and analyzed by a special computer program using Image Analysis software. Desquamation Index and percent of area covered by the scales are reported. A second D'Squame may be obtained from the same site for cytology and cell morphology.

b. Cyanoacrylate Surface Biopsy

Cyanoacrylate, a fast-setting glue, is used to harvest a layer of skin along with microcomedones and vellus hairs. One drop of cyanoacrylate adhesive (Krazy Glue) is applied to the skin within a circular paper ring of 1 cm^2 area and covered with plastic slide. After complete polymerization of the glue, which occurs in approximately five minutes, the slide is gently lifted from the surface carrying with it vellus hairs and enveloping horny material, termed "horny casts." Figure 18 shows a sample from the cheek.

Figure 18: Cyanoacrylate surface biopsy from face showing fluorescent follicular casts.

The slide is imaged using fluorescent photographic techniques. Two filtered light sources emitting at a center wavelength of 417nm (visible-violet) are positioned symmetrically at the side of the camera body. A UVA-cutting filter (GG475) is placed in front of the camera lens. The images are then downloaded into a computer and analyzed. The number of casts and total area of casts (mm^2) are calculated.

c. Sebum Collection Assay

A porous translucent plastic film, Sebutape (CuDerm Corp., Dallas, TX), which when placed on skin collects sebum, which turns the film transparent. When the film is placed on a black card the collected sebum appears as black spots (see Figure 19). The sebum output is usually assessed on the cheeks and forehead. The site is first cleansed for 15 seconds with a nonwoven cotton pad soaked in 0.1% aqueous solution of Triton-X100, followed by swabbing with hexane for 15 seconds. The Sebutape is applied to the test site for one hour, after which it is removed and placed on the plastic film attached to the black card. The card is placed under the stereomicroscope (OPMI-1fc, Zeiss, Germany) and illuminated by standardized fiber-optics light. The image is captured by a video camera (CCD-72, DAGE-MTI, Michigan, Indiana) and stored on the computer. The count and area of sebum spots are assessed by image analysis.

Figure 19: Sebum spots collected on the Sebutape in a one-hour period.

11.5.12 APPLICATION OF BIO-INSTRUMENTATION: SOME EXAMPLES

The three hydration measuring devices were compared in a multiple reading assay, in which eight sites were demarcated on the volar forearm and ten readings were obtained from each site using each of the devices sequentially. Each instrument measured a total of 80 readings. The normalized readings were plotted for Skicon, Novameter, and Corneometer, for site number and reading number (see Figure 20).

Figure 20: Plot of Hydration data from eight sites on the volar forearm using Skicon (A), Novameter (B), and Corneometer (C). Ten readings were collected from each of the eight sites. The data were normalized for each device so that hydration axis has the same scale (0 to 1).

The Standard Error of the Mean (SEM) was also calculated. Table 1 shows the data with Mean, Standard deviation (SD), and SEM.

The Standard Error of the Mean (SEM) is relatively low for all the devices, less than 10%. However, the Corneometer data show the least variance, SEM = 0.010 followed by Novameter, SEM = 0.018. The Skicon readings show relatively higher variance, SEM = 0.054.

The effect of lactic acid in changing the hydration of skin over a short period of time can be shown by measuring conductance by Skicon at various time-points post treatment. A single application of lactic acid 10% aqueous was applied to the cheek. Hydration was measured by Skicon before lactic acid application, and then after 30 min, 3 hours, 1 day, 2 days, 4 days, and 7 days. Figure 21 shows the effect of lactic acid over a period of seven days.

To demonstrate the hydration differences between "Dry" and "Normal" legs of human volunteers, Corneometer measurements were made on the legs of two dry-leg subjects and two normal-leg subjects. Ten readings were obtained from each subject, five from the left and five from the right. Mean hydration values showed statistically significant difference between dry vs. normal legs. A p-value of 0.00000000552 was obtained using a 2-Tail t-Test. Figure 22 shows the difference between dry vs. normal legs.

Figure 21: Single application of lactic acid 10% increases the hydration of skin significantly for a short period of time, i.e., 30 minutes and 3 hours.

TABLE 1

SKICON

SITE ↓ / Reading →	1	2	3	4	5	6	7	8	9	10		MEAN	SD	SEM
S1	60	62	48	66	48	52	56	41	47	71	>	55.10	9.539	0.055
S2	71	73	55	90	84	83	46	56	56	63	>	67.70	14.773	0.069
S3	45	52	53	49	44	54	51	63	54	33	>	49.80	7.927	0.050
S4	73	67	55	73	69	70	74	62	50	86	>	67.90	10.246	0.048
S5	59	79	72	81	74	71	72	54	54	65	>	68.10	9.712	0.045
S6	60	88	77	59	77	66	54	60	71	68	>	68.00	10.435	0.049
S7	46	59	41	67	57	67	53	41	56	54	>	54.10	9.303	0.054
S8	63	64	63	60	51	50	89	54	53	47	>	59.40	12.048	0.064

Max> 90 **Min> 33** **MEAN (All) > 61.26** **10.498** **0.054**

NOVAMETER

SITE ↓ / Reading →	1	2	3	4	5	6	7	8	9	10		MEAN	SD	SEM
S1	132	136	116	140	132	132	124	128	136	136	>	131.20	7.005	0.017
S2	130	136	116	136	128	136	132	128	136	134	>	131.20	6.268	0.015
S3	136	136	136	136	120	112	128	128	128	128	>	128.80	7.955	0.020
S4	128	132	148	140	122	120	142	136	138	134	>	134.00	8.794	0.021

S5	134	128	128	138	124	112	140	144	132	130	>	131.00	9.055	0.022
S6	134	140	140	136	142	120	142	132	132	134	>	135.20	6.613	0.015
S7	128	128	136	132	124	118	128	122	128	128	>	127.20	5.007	0.012
S8	130	124	128	140	124	112	136	124	132	132	>	128.20	7.800	0.019

Max> 148 Min> 112 MEAN (All) > 130.85 7.312 0.018

CORNEOMETER

SITE ↓ / **Reading →**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**		**MEAN**	**SD**	**SEM**
S1	48	47	47	46	45	46	45	45	45	46	>	46.00	1.054	0.007
S2	52	48	49	50	44	48	45	45	45	51	>	47.70	2.830	0.019
S3	49	47	45	48	46	47	47	48	46	47	>	47.00	1.155	0.008
S4	45	45	44	49	45	43	44	48	45	46	>	45.40	1.838	0.013
S5	46	45	46	48	47	48	47	49	46	47	>	46.90	1.197	0.008
S6	48	48	48	50	48	47	49	48	48	49	>	48.30	0.823	0.005
S7	45	43	43	44	43	42	40	44	42	44	>	43.00	1.414	0.010
S8	46	45	48	46	48	44	44	48	44	48	>	46.10	1.792	0.012

Max> 52 Min> 40 MEAN (All) > 46.30 1.513 0.010

Figure 22: Hydration measurements by Corneometer on dry vs. normal legs show statistically significant difference.

Experimental irritation was induced by applying sodium lauryl sulphate (SLS) 0.5% aqueous for 24 hours. The effects were evaluated at 3 hours, 1 day, 3 days, and 7 days post-SLS patch removal by measuring Redness (a*) and Brightness (L*) by Chromameter and TEWL by Dermalab as well as visually grading erythema. Figure 23 shows these effects over a period of seven days.

Figure 23: Evaluation of irritation produced by SLS 0.5% patch on volar forearm. Changes in Redness by Chromameter (A), Brightness by Chromameter (B), Visual erythema score (C) and TEWL (D) are shown over a period of seven days.

In another "Irritation by SLS" experiment, six SLS 0.5% patches were applied to the volar forearms, three on the left and three on the right, for 24 hours. Laser Doppler Imaging, side-lighted videomicroscopy, and UV videomicroscopy were done before SLS patch application and then at 3 hours, 1 day, 3 days, 6 days, and 16 days post-SLS patch removal.

Laser Doppler Images show strong erythema (high blood flow) on all the six sites at 3 hours and 1 day post-SLS patch removal. On day 3, erythema started to subside and on day 6, there was still some residual erythema. On day 16, erythema was completely gone. See the series of Laser Doppler Images in Figure 24.

Figure 24: Laser Doppler Images of the volar forearms, pre-SLS patch applications and then 3 hours, 1 day, 3 days, 6 days, and 16 days post-SLS patch removal.

Side-lighted videomicroscopy showed marked scaliness post-SLS patch on days 3 and 6. On day 16, scaliness begin to subside. Figure 25 shows the sequence of scaliness over a period of 16 days.

Figure 25: Side-lighted videomicroscope images of the volar forearms, pre-SLS patch applications and then 3 hours, 1 day, 3 days, 6 days, and 16 days post-SLS patch removal. Strong scaliness appears on days 3 and 6. Scaliness starts to reduce by day 16.

UV-videomicroscopy shows a trend similar to the side-lighted videomicroscopy, i.e., prominent scaliness post-SLS patch on days 3 and 6, then lower scaliness on day 16. Figure 26 shows the series of UV-videomicroscopy images.

Figure 26: Series of UV-videomicroscope images showing the evolution and resolution of scaliness post-SLS patch over a period of 16 days.

Silicon rubber replicas of skin show the distortion of glyphic pattern post-SLS patch on days 3 and 6, and then recovery by day 16. Figure 27 shows the series of replica images. Replica was not obtained at "3-Hr Post" time point because replicas don't form very well if the skin surface is somewhat moist.

Figure 27: Series of replica images showing the distortion and recovery of glyphic pattern post-SLS patch over a period of 16 days.

To show the changes in the degree of erythema and blood flow, oral niacin (250 mg) was used by a healthy volunteer and Laser Doppler Blood Flow Imaging of the cheek was performed at several time points. The blood flow values in Perfusion Units were calculated. Skin redness values were also evaluated by a colorimeter (Chromameter "a*") as well as skin surface temperature by an infrared thermometer. Figure 28 shows the series of Laser Doppler Images obtained at baseline (before niacin) and then at 20, 40, 60, and 80 minutes post-niacin.

Laser Doppler Images show increased blood flow after orally taking niacin 250 mg, with the maximum value recorded at 40 minutes; then the value was lower at 60 minutes and finally lowered to below the pre-niacin value at 80 minutes.

Figure 28: Laser Doppler Images show increased blood flow after orally taking niacin 250 mg. Facial blood flow peaked at 40 minutes, then reduced somewhat at 60 minutes and finally subsided at 80 minutes.

The increase and decrease of facial blood flow and erythema is shown graphically in Figure 29, as evaluated using the Laser Doppler Imager, Chromameter, and skin surface thermometer.

Figure 29: Measurement of skin blood flow and erythema by LDI, Chromameter, and skin surface thermometer (from left to right) over a period of 80 minutes post-niacin ingestion.

To demonstrate the erythema measuring capabilities of the Reflectance Spectrophotometer, Ultraviolet-B (UVB) light was used to generate erythema on the volar forearm. Two sites were exposed to UVB, one with minimal erythema dose (1-MED) and the other with twice the minimal erythema dose (2-MEDs). Using a Bifurbicated Fiber-Optic Reflectance Spectrophotometer, three measurements from each site were recorded. An adjacent normal skin site was also measured. A control spectrum was obtained from the upper inner arm. All spectra were referenced to a white tile. The ratio of the apparent absorbance of the control site (upper inner arm) to each of the three volar forearm sites are presented as the result. The differential spectra are shown in Figure 30. The Oxyhemoglobin peaks at 577 nm are considered as proportional to the intensities of erythema.

Figure 30: Reflectance Spectrophotometry showing the amplitudes of the oxyhemoglobin peaks at 577 nm after exposure of the volar forearm skin to 1-MED and 2-MEDs of UVB.

Decades of cumulative sun exposure of skin results in the gradual structural degradation and the appearance of an assortment of unwelcome signs of

photo-damage, viz. dyspigmentation, wrinkles, roughness, and dull, yellowish, leathery, and lax skin. Over later years of one's life the facial skin shows these signs of sun damage. Digital photography can demonstrate the difference between an older subject with photo-damaged skin compared to a young subject with relatively less photo-damaged skin. Figure 31 shows digital photos of a 62-year-old woman compared to an 18-year-old female.

The older photo-damaged face (B) shows more wrinkles, roughness, prominent naso-labial fold and dyspigmentation compared to the young face (A). The wrinkle and roughness features are enhanced in the parallel polarized photos while the cross-polarized photos show the dyspigmentation more prominently.

Digital fluorescence photos show enhanced pigmentation and in case of the young subject follicular casts and porphyrin fluorescence on and around the nose (Figure 32). The reddish fluorescence emission is from the porphyrin produced by p-Acnes.

Figure 31: Facial photographs of an 18-year-old female (A) compared to a 62-year-old female (B), in "Standard," "Parallel Polarized," and "Cross-Polarized" modes. Standard flash photo shows smoother skin, less prominent pigmented spots on the face of the young female compared to rough, wrinkly, and dyspigmented facial skin of the older female. The wrinkles and roughness are shown more prominently in the parallel polarized photos. The cross-polarized photos show the dyspigmentation more prominently.

Figure 32: Fluorescence photos with the excitation wavelength centered at 417 nm show enhanced dyspigmentation, more on the older face (B) compared to the younger face (A). The young face also shows fluorescing follicular cast (yellowish spots) on the nose and surrounding area, some of them showing the presence of porphyrin (reddish spots).

In another study, the dorsal forearms of young and old subjects were compared to their respective volar forearms. The dorsal forearms are generally more exposed to sun than the volar forearms. Therefore one would expect more photo-damage on the dorsal forearm.

The studies were carried out on the mid-volar and mid-dorsal forearms of each subject, with the assumption that the dorsal forearms would suffer a greater degree of photo-damage.

To show the relative photo-damage of volar vs. dorsal surface of the fore arms, videomicroscope images of the volar and dorsal forearms of a 62-year-old subject and an 18-year-old subject were obtained at 60× setting on the Hi-Scope probe. Figure 33 shows the differences in the images.

The cumulative photo-damage over decades of sun exposure is clearly evident in the older subject (A). Loss of glyphic pattern, scaliness, and dyspigmentation are clearly evident in the dorsal surface compared to volar surface. The younger subject (B), on the other hand, shows relatively slight change in the glyphic pattern and barely any difference in pigmentation.

Figure 33: Videomicroscopy at 60× reveals the differences in the relative photo-damage between the volar and dorsal forearms. The younger subject (B) shows slight difference in the glyphic pattern while the older subject (A) shows gross differences. The glyphic lines are practically obliterated, obscured by overlying thick scales, a common feature of photo-damaged skin. Hyper-pigmented patches are also typical of photo-damaged skin.

Fringe projection device, pseudocolored to show three-dimentional image, shows differences in photo-damaged skin of the dorsal forearm compared to the volar forearm. Figure 34 shows the 3-D images.

A mathematical construct of the primary image, after flattening the gross contour of the skin surface but leaving the microtopography intact, is presented. The color-coded image of the surface for heights show gross differences in the volar vs. dorsal forearm of older subject (A) while the younger subject (B) show slight

difference. The photo-damaged dorsal forearm of the older subject (A) shows gross distortion of the microtopography, loss of glyphic pattern, and surface details.

Figure 34: The color-coded 3-D images obtained with the fringe projection device show gross distortions in the microtopography of the dorsal forearm skin of the older subject (A) compared to the volar forearm. The difference between the volar and dorsal forearm of the younger subject is small.

The confocal scanning laser microscope (CSLM) images horizontal sections of skin at various depths showing the structures of tissue and cells. Figure 35 shows confocal images of normal skin (volar forearm) at various depths. In normal skin the confocal images of SC appears as a bright structure due to strong reflection. The wrinkle lines and surface structure along with some glyphic lines can be observed. The *stratum corneum* at a depth of 3.6 μm is shown in picture A. The cellular arrangement of *stratum granulosum* is shown in picture B at a depth of approximately 14.4 μm. Because of the curvature of the surface, some parts of SC are also shown at the edges. The picture C shows the *stratum spinosum* at a depth of 25.2 μm. Picture D shows the dermal-epidermal junction, with some capillary loops shown in the sub-epidermal layer, at a depth of 54.0 μm. The dermis is shown in picture E at a depth of 82.8 μm.

Figure 35: Horizontal sections obtained from the volar forearm by the Confocal Microscope at various depths of skin.

Confocal microscope can reveal the difference between the dark skin and light skin at the dermal-epidermal junction. The dermal-epidermal junction is a wavy surface forming conical projections. Dark-skinned individuals have thicker epidermis with more wavy dermal-epidermal junction compared to the light-skinned individuals. An example is presented in Figure 36.

The horizontal optical section of the skin obtained by a confocal microscope, at a depth of 80 microns, shows large bright circles in the dark-skinned individual compared to light-skinned individual. This is because melanin reflects 830-nm light strongly compared to other structures in skin. The dark-skinned individuals also have thicker epidermis with a wavier dermal-epidermal junction, and when a confocal microscope optically slices a horizontal section at the dermal-epidermal junction, larger circles of brighter spots are shown in the image.

Figure 36: Confocal microscope section at the dermal-epidermal junction of a dark-skinned individual (left) compared to a light-skinned individual (right). Melanin near the basal layer is observed as bright spots as the horizontal section slices the dermal-epidermal junction.

Cross-sectional images in the depth of skin can be obtained by Optical Coherence Tomography (OCT) device. This can be a complement to Confocal Microscopy, which images horizontal sections.

The cross-sectional image slice is 1 mm deep and 1 mm in lateral direction. Dermal and epidermal structures are clearly outlined. The epidermal thickness can be measured. OCT can easily be used to study the cosmetics and skin care products that can change the epidermal thickness. *Stratum corneum* (SC) is visualized as a bright line, and its thickness cannot be measured precisely unless it is grossly swollen. The geometry of isolated legions such as comedones and seborrheic keratosis can be visualized.

As it was shown by the Confocal Microscopy, the difference between dark and light skin can also be shown by the OCT at the dermal-epidermal junction. Figure 37 shows an example of an OCT image of dorsal forearm of a dark-skinned individual compared to image of a light-skinned individual.

The dark skin shows wavier dermal-epidermal junction compared to the light skin.

Dark skin Light skin

Figure 37: OCT images of dark and light skin. Dermal-epidermal junction is wavier in the dark skin.

In another example, OCT imaging showed the difference between sun-damaged skin to relatively less sun-damaged skin. Figure 38 show OCT images of the cheeks of a 60-year-old individual, with decades of cumulative sun damage, and a 20-year-old individual with relatively less sun damage. OCT image of the photo-damaged cheek of the older individual showed thinning of epidermis and flattening of the papillary junction compared to the OCT image of the young individual, which showed much thicker epidermis.

Figure 38: OCT image of the photo-damaged cheek (right) show thinning of epidermis and flattening of the papillary junction. The younger, relatively less photo-damaged skin (left) shows much thicker epidermis.

Ultrasound imaging is another method to capture cross-sectional images of the skin. The 20-megahertz ultrasound device has much lower resolution than OCT, but the images show full thickness of skin with some of the subcutaneous region as well. The epidermis appears as a line in the ultrasound image and its structure cannot be resolved. The thickness of the whole skin can be measured.

In one experiment, the reduction of cellulite on the thigh by a vibrating vacuum massaging device was shown by the ultrasound imaging. The ultrasound images were collected from the region of thigh showing cellulite, first before treatment and then after one month of daily massaging. Figure 39 shows the before-and-after images.

The image before massaging shows large pockets of cellulite fat protruding into the dermis at the base. After one month of massaging treatment the dermis is much thicker with very little fat protrusions.

Figure 39: The effect of massaging on cellulite as revealed by ultrasound. The ultrasound images were obtained from the region of thigh showing cellulite, before treatment (A) and then after one month of daily massaging (B).

The periorbital wrinkling of face, sometimes referred to as "crow's feet," have been studied extensively in the past, particularly with regard to anti-aging treatments. Fringe projection devices have been used extensively to show the reduction of the crow's feet.

To show the ability of a fringe projection device to assess the changes in crow's feet, a "lactic acid" model was used. Lactic acid 10% aqueous when applied to crow's-feet region produces a transient effect of partially effacing the wrinkles. This effect may last for a few hours.

The digital photos obtained before and after the lactic acid treatment show the reduction of crow's-feet wrinkles clearly (see Figure 40). The 3-D imaging by the fringe projection device also shows this difference (see Figure 41).

Using the fringe projection device, a "Crow's Feet Index" was calculated based on the cross-sectional areas of wrinkles at nine different locations. This crow's-feet index showed a marked reduction at 30 minutes after the lactic acid application. Figure 42 shows this difference graphically.

Figure 40: Digital photos of the crow's-feet wrinkle area, before lactic acid treatment (left) and 30 minutes post-lactic acid treatment (right).

Suction Device (Cutometer) is commonly used for measuring the elasticity parameters of skin. In one experiment, skin was exposed to ultraviolet light (UV) with a dose of 2-MEDs. Gross elasticity was measured by the Cutometer with a 2-mm probe at 1, 4, 7, and 14 days post-UV exposure. An adjacent Control (Ctrl) site was also measured.

Figure 41: Fringe projection images before (top) and after (bottom) lactic acid treatment. The images are color-coded for heights. The original images (A) were processed to remove the gross contours to show flat images but with the wrinkles intact (B). The geometric 3-D images are shown in (C).

Figure 42: The Crow's-Feet Index reduced dramatically at 30 minutes after the application of lactic acid.

There was an apparent loss of gross elasticity at the UV-exposed site on Day 1, which gradually recovered by Day 14. Figure 43 shows the effect graphically. Erythema generated by UV can cause some edema on the exposed site, which may influence the elastic properties.

Figure 43: The UV-exposed site showed apparent loss of elasticity at day 1, followed by recovery by day 14.

CONCLUSION

The methodologies and bio-instrumentation described in this chapter can be utilized along with the traditional clinical evaluation methods to test the efficacy of cosmetics and skin care products, to present a complete picture of improvements in skin attributes.

In the field of skin research we no longer depend solely on visual examination and touch; we now have instrumental methodologies based on physics, chemistry, biology, and engineering to study skin at the surface and in depth.

Effects of cosmetics and skin care products on the skin can be studied more objectively.

In this chapter a selected number of bio-instrumentations and methodologies are described. Indeed there are many more techniques available, some of them important for scientific research.

As a result of recent advancements in science, engineering, and computer technology, many new skin-measuring techniques are evolving, and one can expect more sophisticated instrumentations and methodologies in the near future.

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NANOMATERIALS CHARACTERIZATION

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ABSTRACT

Nanomaterials are materials that are produced with nanometer dimensions. These materials often exhibit widely different chemical and physical properties than their macro-sized counterparts. Nanomaterials are a rapidly growing field for many product categories, especially cosmetics and personal care. However, with the increased use in these fields, regulatory bodies around the globe have taken an escalated interested in their characterization and safety. To comply with EU and global nanomaterial regulatory controls currently being developed, researchers and manufacturers must have the ability to accurately characterize their products. This chapter provides an overview of analysis techniques available for the characterization of nanomaterials. It should serve as a starting point for those individuals interested in developing fundamental knowledge of nanomaterials based on their elemental, chemical, and physical properties.

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11.6.1 INTRODUCTION

In the cosmetics industry, as in many industries, the potential of nanotechnology to create major technological breakthroughs has clearly attracted considerable attention. It is quite an active innovation field as demonstrated by the number of

patents issued on nanotechnology by major cosmetic companies. Nanotechnology is enabling a fast-growing number of consumer products. This is evidenced by more than 1000 products claiming to contain nanomaterials (see Figure I.1a). Figure I.1b displays a breakdown of the nanomaterial application as a function of product category. The data in Figure I.1b show that the market is dominated by healthcare and fitness products, which on their own, represent more than 50% of nanomaterial-containing products placed on the market. In terms of cosmetics, nanomaterials are gaining traction in a variety of applications. For example, nano titanium dioxide is one of the most commonly used nanomaterials in cosmetics. Nano titanium dioxide is typically used in sunscreens exploiting its UV-inhibition properties. Carbon black is often used as an intense colorant, while nanoemulsions and nanosomes are being used to preserve the active ingredients in cosmetics such as anti-oxidants and vitamins.

The types of nanomaterials being used in this area generally can be categorized into two main groups: inorganic particles and organic-based liposoluble particles.

Figure 1.1. Number of consumer products claiming to contain nanomaterials as a function of a) time and b) subcategory.

a. Inorganic Particles

Inorganic particles are typically used in applications exploiting their enhanced UV-filtering or antibacterial properties. Cosmetic products, such as sunscreens, typically employ mineral-based materials like titanium dioxide or zinc oxide. For these materials, the UV filtering performance directly correlates directly with their diminished particle size. Titanium dioxide and zinc oxide are typically found in the size range far below 100 nm where their UV-inhibiting properties are found to be optimal. Nanoparticles, such as TiO_2 and ZnO, provide broad UV-protection and do not cause adverse cutaneous health effects.[2]

Silver nanoparticles are well known for their antibacterial properties[3,4] and are widely used throughout many industries.[5] They can be used as a disinfectant and anti-odor substance in textiles. They can also be found in cosmetic products such as deodorants, where claims for up to 24-hour antibacterial protection are often made.

b. Liposoluble Organic Soluble Nanomaterials and Their Delivery Systems

The category of liposoluble organic soluble nanomaterials includes specially designed metastable particle systems with properties to promote efficient and controlled release of active ingredients over time or triggered by different environmental conditions. This type of nanomaterial includes liposomes, nanoemulsions, nanocapsules, and solid lipid nanoparticles.[6]

Liposomes are concentric bi-layered vesicles with an external dimension below 100 nm. The internal aqueous part is entirely enclosed by a lipid bi-layer composed of phospholipids. These are generally regarded as safe products. Liposomes are used to promote targeted release of their contents, making them useful for cosmetic delivery applications.

Nanoemulsions consist of very fine oil-in-water dispersions of colloidal dimensions. They have droplet diameters smaller than 100 nm. Nanoemulsions are very fragile systems and are in a metastable state. The nanoemulsions are highly valued in skincare due to their positive sensory properties. Because of the extremely small drop size, they are compatible with parentera applications, and transfer through the skin with rapid penetration and hydrating power of the *stratum corneum*.

Nanocapsules are submicroscopic particles that are made of a polymeric capsule enclosing an aqueous or oily core. Encapsulation technologies were originally developed for drug-delivery applications but have been extended to cosmetic skincare applications. Different core/shell combinations are required based on the Active Pharmaceutical Agent (API) being administered and the location of where the drug is to be administered in the body. The same structure/property relationships exist in cosmetics applications. With regard to skincare, nanocapsules have been used to deliver UV-absorbing molecules to the surface of the skin, while also preventing percutaneous absorption.

Solid lipid nanoparticles are oily droplets of lipids that are solid at body temperature and stabilized by surfactants. They can protect the encapsulated ingredients from degradation and can be used for the controlled delivery of cosmetic agents over a prolonged period of time.

c. Nanomaterial Legislation in Cosmetics

Recognizing the increasing number of cosmetic and personal care products containing nanomaterials, European authorities have approved the amended recast of the EU Cosmetics Directive, introducing for the first time the mention of "nanomaterials" in an EU legislation. This includes the first ruled definition of a nanomaterial as "*an insoluble or bio-persistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.*[7] *Only cosmetic products for which a legal or natural person is designated within the community as the 'responsible person' shall be placed on the market"* (Art. 4, p. 4). The Responsible Person is responsible for ensuring that all the obligations related to product conformity have been carried out prior to introducing the product onto the European Market and more specifically in practice the responsible person should submit the following information to the European competent authorities:

- The presence of substances in the form of nanomaterials
- Identification including the chemical name (IUPAC) and other descriptors
- The reasonably foreseeable exposure conditions
- Labeling obligation: ingredients present in the form of nanomaterials shall be clearly indicated with "nano" in brackets

With the scrutiny of nanomaterials set to continue, we can expect changes to the EU and global regulatory environment as policymakers at all levels attempt to keep pace with understanding the chemical and physical characteristics of nanomaterials and how they impact both the environment and humans.

d. Nanomaterials Characterization

To demonstrate regulatory compliance and help produce and implement accurate implementation of EU and global definitions, utilization of the appropriate analytical instrumentation and methods is critical. Researchers and manufacturers must have the tools available to comprehensively understand their products as individual ingredients as well as when formulated into final cosmetics. This chapter provides an overview of the different analytical techniques available for the characterization of nanomaterials. It should noted here that we have focused on the characterization of raw materials rather than finished products. The full characterization of a finished product, like a cosmetic cream, will be much more complex.

Several regulatory and technical challenges exist for characterizing finished products. The first is to adequately define what a nanomaterial is in a multi-component system. For example, are aggregates of nanoparticles with length scales > 100 nm appropriately classified as nanomaterials even though the individual particles in the aggregates may be on the scale of 10 nm? The second is to determine if

toxicological and other properties are related to the size of aggregates or individual particles in the aggregates. More extensive testing is required to fully understand the relationships between particle size and properties in such systems. To that end, determining whether such a product *is* a nanomaterial, or indeed *contains* nanomaterials, will require continued development and refinement of the analytical methodologies currently available.

1. Particle Size, Distribution, and Shape

The ability to fully characterize particle size, and potentially shape, distributions is fundamental in the development, use, and regulation of nanoparticles and nanomaterials.

Particle size lies at the heart of the various international definitions of nanomaterials and particles. For example, the European Commission (EC) defines a nanomaterial, for regulatory purposes, as "a natural, incidental or manufactured material containing particles in an unbound state or as an aggregate or as an agglomerate and where for 50% of the particles in the number size distribution, one or more external dimensions is in the size range of 1 nm to 100 nm."[7] This form of definition itself raises many challenges for the analyst. A starting point is that it is necessary to be able to reproducibly and accurately measure the particle sizes of "raw materials"—including particulates, powders, clays, and fibrils. However, it is also a requirement that these particle size distributions can be characterized once the raw materials are incorporated into a final product form, where they may only comprise a fraction of the total product mass. It is certainly not sufficient to assume that the particle sizes in the product are equal to those in the starting materials—as processing can result in either fragmentation or agglomeration of the particulates (or both!). In many cases this is not yet readily achievable, and it has been recognized by a number of regulatory bodies that further method development is required before the measurements can be carried out on a routine basis.

Linsinger recently produced a very comprehensive summary of the different analytical techniques available for the characterization of nanoparticles, and their relative strengths and weaknesses.[8] It must be noted that no one technique will provide a full description of the particle size and shape distributions, and chemical composition. Below we outline several approaches for the determination of particle size, shape, and distribution.

1.1 Electron Microscopy Techniques: TEM and SEM

Microscopy techniques are probably the only way to characterize such particles directly. The other methods involve the measurement of some property (scattering power, surface area, etc.) of the nanomaterial and the application of some generally imperfect algorithm to translate the variations in the measured property into a size

distribution. The algorithms will inevitably involve simplifying assumptions, such as that the particles are all spherical, or of uniform density, and so a number-based distribution derived in this way will always be imperfect.

It is possible to visualize and explore shape and morphology directly with scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques.[9,10] Both techniques can look at features at or below the 1 nm scale. As their names suggest, SEM essentially produces an image of a surface, while for the TEM the electron beam has to pass through the sample—requiring ultra-thin specimens. Figure 1.1.c shows a TEM image of carbon nanotubes, illustrating clearly that visualization of a structure can often provide the most straightforward approach. The TEM image clearly demonstrates that the shape, internal structure, length, and width can easily be observed and subsequently measured. None of the techniques described below would provide an accurate description of the sizes of the tubes illustrated here.

Figure 1.1.c Transmission electron microscope image of carbon nanotubes. The scale bar in the lower left is 200 nm.

With most microscopy techniques, specimen preparation is critical to the outcome of the experiments. The manner in which a sample is prepared often is the most difficult part of the experiment and tends to be a skill set in its own right. Therefore, having the appropriate knowledge of different preparation techniques

and being able to perform these techniques proficiently is a key part of examining nanomaterials with electron microscopes.

In principle, the use of computer-based image analysis techniques can be coupled to electron microscopy to generate quantitative size and shape distributions. In practice, the methods are limited—particularly in the generation of statistically significant data. In general, these techniques will sample very small numbers of particles, and significant operator intervention is often required to use image analysis techniques to generate dimensional information. These factors, coupled with the relatively high cost of the instrumentation, also mean that electron microscopy is an expensive approach to materials characterization, if the equipment must be purchased and maintained by the research organization. Outsourcing is one way to help manage the costs to obtain high-end analytical support.

SEM or TEM coupled with energy-dispersive X-ray Spectroscopy (EDS) allows elemental information to be gained for individual particles. Variations in chemical composition may also be studied or mapped, even within individual particles. Again, it is important to note that almost all other techniques will analyze the ensemble properties rather than those of individual particles.

1.2 Probe-based techniques: STM and AFM

The capability to characterize localized properties such as size, morphology, orientation, and roughness in nanoscale materials has been greatly enhanced by the development of the scanning probe microscopies[11] [(scanning tunneling microscopy (STM) and atomic force microscopy (AFM)]. A great deal of information regarding these properties can be obtained from STM experiments; however, the use of STM necessitates an electronically conducting sample. This requirement limits the number of materials that can be studied by STM. AFM, on the other hand, can be used to study the morphological characteristics of both conductors and insulators. In fact, variants of standard AFM, including electrostatic force microscopy (EFM),[12] scanning surface potential microscopy (SSPM)[13] and conductive probe atomic force microscopy (CP-AFM)[14,15] have proven useful for the study of the nanoscale electrical properties of various conducting and insulating materials including metal-insulator composites,[16] semiconductors,[17] and nanomaterials.[18]

For both STM and AFM,[11] a sharp probe is rastered across the surface of a material using a piezo-electric tube scanner. Although the feedback mechanisms are different, the resulting images provide information on morphology, composition, and a variety of surface texture parameters. In either of these probe-based analysis techniques, there are several key experimental aspects that need to be carefully controlled, or at least understood, to properly characterize nanomaterials.[19] Both lateral and vertical calibration of the instruments needs to be performed with well-characterized reference materials so that accurate measurements can be made. A

variety of instrument-specific grids made from either metals or metal oxide substrates are commercially available from numerous probe suppliers. These grids are also available with VLSI certification and NIST traceability. One issue that confounds accurate size measurements by probe-based techniques is the influence of the tip shape and size. When measuring materials on the same order of the tip radius, severe tip-convolution effects can be encountered and this effect tends to be more prominent with AFM than STM. Numerous probe manufacturers have been developing high-aspect ratio probes with sharp tip radii for use in AFM measurements. A simplified drawing of the probe convolution problem is shown in Figure 1.2. The shape of a particle is more accurately defined with the sharper probe. However, it should be noted that with either case, the particle height remains the same.

Figure 1.2. Pictorial representation of how tip shape effects lateral size estimates for a nanoparticle. The path of travel for a normal probe and sharp probe are illustrated in the upper left and upper right, respectively. The lower portion shows stylized profile plots for the normal and sharp tips.

To circumvent the tip convolution issue, several research groups and software companies have developed tip deconvolution algorithms that enable the tip shape and size to be removed from scanning probe images in order to collect more accurate size measurements.[20, 21, 22] However, there is much debate in the literature

as to which algorithms are most appropriate. An alternative to these algorithms is to simply use the height measurement, rather than the lateral measurement to determine particle diameter. Estimates of particle diameter using the height measurement are fairly reliable, assuming the particles act as hard objects and are not pressed into the substrate, and that the particles themselves are not damaged under inappropriate imaging conditions.

A significant advantage of AFM over many other techniques is that a nanomaterial can be imaged in either air or fluid environments. This allows for development of an improved understanding of how the individual particles, fibers, or tubes behave in various media. Of course, as with all probe-based techniques, the analysis of these materials requires that they are adhered to a substrate when imaged in either air or fluid environments. The substrates in these experiments can be any number of materials, ranging from rigid glass or metals to biological materials such as skin or hair.

1.3 Dynamic Light Scattering

Dynamic light scattering (DLS) methods are commonly used on insoluble materials.[23] There are a number of different measurement technologies, but they all involve the measurement of the angular distribution of light scattered by the particles in solution (or in air). "Standard" DLS methods will measure down to around 40 nm, but cross-correlation methods can be used on particles down to a few nm in size. The algorithms used to convert scattering distributions to particle size generally assume that the particles are spherical, and so the methods should be used with caution on materials such as clays or nanotubes that differ markedly from the assumed geometry. Light-scattering methods are also rather poor in resolving components of multi-modal distributions.

1.4 High-resolution particle sedimentation

A method better suited to substances with multi-modal distributions is that of centrifugal liquid sedimentation or disc-centrifuge.[24] Essentially this technique fractionates a poly-dispersed distribution by time to sedimentation, producing improved resolution of different particle sizes. However, once again the conversion of the sedimentation time distributions into a true number-based particle size distribution is not straightforward, and there are also practical difficulties with instrumental dynamic range in acquiring an accurate distribution covering a very wide range of particle sizes. Nevertheless, disc-centrifuge methods can give an accurate picture of the multi-modal particulate materials down to a few nm in size. Figure 1.3 demonstrates the ability to separate a mixture of particles that exhibit average particle sizes of approximately 15 nm, 20 nm, 32 nm, and 100 nm. By comparison, the analysis of this same solution by dynamic light scattering would yield less resolution around the smaller particle sizes causing misinterpretation of the true particle size distributions.

Figure 1.3. Example of the resolution obtained for particle sizing of a multi-modal particle mixture using high resolution particle sedimentation.

11.6.2 SURFACE CHEMISTRY

In the traditional sense, the study of the surface chemistry of a material may involve the measurement of the degree of hydrophilicity or hydrophobicity, and interfacial forces. Due to experimental constraints, these properties are more easily performed on macroscale systems rather than ones that exhibit nanoscale dimensions, thus complicating the ability for researchers to understand these properties. However, measurements of the surface elemental and chemical composition can be used to gain insight into these properties. Here, the term "surface chemistry" relates to the analysis of elemental and chemical information at depths typically ranging from 2 to 10 nm. Obviously, as the dimensions of the nanomaterial become smaller, the definition of the surface becomes blurred, making these measurements more challenging.

a. Electron Spectroscopy

A key aspect of how nanomaterials interact with their host matrices is determined by the chemical and physical properties of their surfaces. In this regard, both Auger electron spectroscopy (AES) and X-ray photoelectron spectroscopy (XPS) are well suited for obtaining information such as surface elemental composition.[25] Although the beam penetration depth is fairly deep in both of these techniques, only the near surface (top 5–10 nm) of the material produces the electrons analyzed

in the experiment. The high surface sensitivity results from the short mean-free path that the generated electrons are able to travel before being ejected from the surface and out into vacuum. In either of these experimental approaches, it is necessary that the materials being analyzed are vacuum compatible. This condition is a requirement, as erroneous data can be obtained if the sample is changing when exposed to the ultra-high vacuum levels that typically reach 10^{-9} to 10^{-10} torr.

b. Auger electron spectroscopy (AES)

In the AES experiment the incident beam of focused electrons interacts with the material, removing an inner shell electron. An electron from the outer shell transitions down into the inner shell to fill the vacancy. During this transition, energy is released and another electron (an Auger electron) can be emitted.[26] Measurement of the kinetic energy of the Auger electrons allows for discrimination of the specific elements on the surface of the material being analyzed. Quantitation of the elemental composition down to about 0.1 atomic % is achievable.

High-resolution spectral analysis along with spectral imaging is possible since advanced optics allow for focusing of the electron beam to spot sizes as small as 50 nm.[27] This enables AES to be used to collect elemental composition information for single nanoparticles. Imaging is useful when elemental distribution of larger nanomaterials, or aggregates of nanomaterials, are needed.

Evaluation of the elemental composition as a function of depth is possible with AES. In this approach, an ion beam is rastered across the surface for a predetermined amount of time, after which the surface is reanalyzed in the same location. This process is repeated any number of times to create a plot of atomic concentration vs. sputter time (or depth if calibrated). Information from this type of measurement provides clues to the homogeneity of elemental composition. For instance, this can be used to better understand composition in materials that may exhibit core/shell structures or that have overlayers present on the surface of the underlying material of interest.

c. X-ray photo electron spectroscopy (XPS)

During the XPS analysis, the surface of a material is exposed to an X-ray beam, rather than an electron beam as is the case with AES. The X-ray beam penetrates several microns into the sample, but only a fraction of the electrons close to the surface escape into vacuum. The electrons originate from either core level or loosely bound valence levels. Similar to AES, XPS measures the kinetic energy of ejected electrons and can relate the kinetic energy to specific elements being present on the surface (see Section 8, Equation 2.1).[26]

Because of optics limitations, XPS lacks the ability to focus the incident radiation beam to practical sizes smaller than ~ 10 mm. Therefore, the analysis of single nanoparticles, nanotubes, or nanofibers is not achievable. With this drawback, semi-quantitative or quantitative information on composition can still be determined for nanomaterials, but it is done so by analyzing ensembles, or layers, of the specific nanomaterial.

In addition to elemental composition, information on oxidation state and bonding environments can be obtained from XPS.[28, 29] Through analysis of the individual high-resolution regional scans, the relative oxidation states for each element found on the surface can be determined. For instance, the carbon 1s region exhibits a single peak due to the photoelectron originating from the 1s orbital. The position of the photoelectron binding energy provides information on oxidation state. The presence of multiple peaks would indicate that carbon would be present in multiple oxidation states. Through peak deconvolution, the binding energy (derived from the measured kinetic energy) of each of these oxidation states can be determined and assigned to different classes of functional groups. For instance, not only can the total amount of carbon be quantified, but the relative amounts of carbon with various chemical functionalities can also be determined (e.g., relative amount of aliphatic, carbonyls, carbonates, fluorocarbons, etc).

Similar to AES, data in the form of elemental composition versus depth can also be obtained. Experiments are performed in a similar fashion to AES with the same types of ion beams. Although bonding state information can be obtained with XPS, care must be taken when interpreting bonding states after sputtering. It has been shown that ion bombardment can cause the reduction of the metal oxidation state upon sputtering. For example, Zhang and coworkers demonstrated that cerium in cerium oxide nanoparticles undergo reduction under defined sputtering conditions, which effectively misrepresents the true oxidation state of the material.[30]

Through peak deconvolution, the chemical states of the various elements found on the surface can be evaluated. Although these results are semi-quantitative or quantitative, they lack the chemical specificity to accurately build chemical structures of surface species. Gathering this level of information is only possible with surface mass spectrometry techniques.

d. Surface Mass Spectrometry

Analytical techniques such as secondary ion mass spectrometry (SIMS) are often employed to understand the chemistry of surfaces based on elemental and molecular information.[31] SIMS is performed by bombarding a surface with a focused primary ion beam. Numerous ion beam sources are available, including Ga^+, Cs^+, Bi_1^+, Bi_3^+, C_{60}^+, and other cluster sources, each of which affords advantages for

specific applications.[32, 33] After striking the surface, the energy from the primary beam is transferred to the material, resulting in the formation of charged species being removed from the surface. In this process the secondary ions with both positive and negative charges are individually analyzed. The surface sensitivity is also better than XPS and AES in that only the top 2–3 nm of the surface is typically analyzed. Thus the analysis of single monolayers is achievable. SIMS also has the advantage that it can detect hydrogen while AES and XPS cannot. Note however that, as with XPS, the limited lateral spatial resolution of SIMS means that in most cases it is only possible to obtain results based on an ensemble average.

1. Dynamic secondary ion mass spectrometry (DSIMS)

Dynamic SIMS is the technique of choice to quantitatively measure the amount of an atomic, or small molecular ion as a function of depth in a sample. DSIMS can readily measure relative changes in species versus depth, and some applications (like semiconductor doping) have been calibrated to obtain truly quantitative measurements. Due to the continuous flux of ions onto the sample surface, sample damage can occur. This typically happens with organic materials even if care is taken to reduce the ion flux. Sample damage can be particularly important when characterizing nanomaterials that have organic functionality of interest. A way to reduce the potential for sample damage is through the use of a softer surface SIMS technique called static SIMS, which effectively operates by reducing primary ion flux.

2. Static secondary ion mass spectrometry (SSIMS)

In static SIMS,[34] a primary ion beam bombards the surface of the material in pulsed mode, reducing the damaging effects of the beam flux. After bombardment, the positive and negative ions are expelled into the vacuum with the same kinetic energy, and then are subsequently mass analyzed. Time of flight (TOF) mass spectrometers can greatly increase the performance of the technique over quadrupole mass analyzers. In the TOF instrument, ion arrival time through the flight tube is measured and converted to mass through calibration. TOF–SIMS instruments can deliver high mass accuracy (up to 10 ppm), mass resolution up to 10,000, and detection limits into the ppb range.

11.6.3 SURFACE AREA AND POROSITY

Sorption analysis is commonly used for material characterization. For this testing, gases or vapors are exposed to solid materials at a variety of conditions and either the weight uptake or sample volume is evaluated. Analysis of these data provides information regarding the physical characteristics of the solid including: skeletal density (ρ_S), porosity, total pore volume (TOPV), pore size distribution, as well as activity and metal dispersion in the case of catalysts.

a. BET Surface Area

Although the underlying theory is known to have some weakness,[35] the BET technique remains one of the most popular methods for determining the surface area of powders and porous materials and is likely the most common sorption technique. Nitrogen gas is generally employed as the probe molecule and is exposed to a solid under investigation at liquid nitrogen conditions, i.e., at a temperature of 77 K. The surface area of the solid is evaluated from the measured monolayer capacity and knowledge of the cross-sectional area of the molecule being used as a probe. For the case of nitrogen, the cross-sectional area is taken as 16.2 $Å^2$/molecule.

For the case of spherical, nonporous nanoparticles, the BET surface area is related to the particle diameter (D), or radius (R), and the skeletal density (see Section 8, Equation 3.1).[36] Skeletal density is the mass of the solid divided by the volume of the solid excluding open and closed pores. For a nonporous solid, the skeletal density is the same as the geometric or envelope density. The BET surface area, therefore, can be calculated from knowledge of the particle density and diameter or, conversely, the average nanoparticle diameter can be evaluated from the measured BET surface area and knowledge of the material density. For a porous material, or one that has an unsmooth surface, the BET surface area is generally appreciably larger than its nonporous analog.

BET experiments are typically conducted to a relative pressure, P/P_0, of approximately 0.3 at 77 K, where P_0 is the saturation pressure.[37] One can think of the relative pressure in terms of relative humidity, i.e., the experiment is conducted to 30% of the saturation pressure of N_2 at 77 K (≈230 torr). At relative pressures above the point at which a N_2 monolayer has formed on the solid, capillary condensation occurs within the pore structure of the material such that the smaller pores are filled more easily and consecutively larger pores are filled as pressure is increased. When the saturation point is approached, i.e., P/P_0 is approximately 1.0, the internal pore structure of the material contains condensed (liquid) nitrogen. The total pore volume can be calculated by assuming that the density of liquid nitrogen (LIN) in the pores is the same as that of bulk LIN (see Section 8, Equation 3.2).

Figure 3.1 depicts a typical 77 K N_2 sorption isotherm with both adsorption and desorption branches of the isotherm measured. The hysteresis seen between the adsorption (lower) and desorption (upper) curves indicates the existence of mesoporosity (pores in the range of 20–500 Å) and provides information regarding the connectivity of the porous network. A detailed discussion of this phenomenon is provided by Gregg et al.[38] Also, illustrated in Figure 3.1 are the regimes of the isotherm, which are examined to evaluate the BET and the TOPV. Since the surface area is gauged by monolayer coverage of N_2 on the surface, it follows that the initial part of the isotherm is used for this analysis. In contrast, since the TOPV is evaluated by the maximum amount of condensed N_2, the latter part of the isotherm

is examined for this calculation. N_2 sorption is suitable to characterize materials with pores within the range of ~20 Å to below ~1500 Å (0.15 μm). For materials containing larger pores, mercury porosimetry is the preferred experimental technique and spans the pore range from ~35 Å to ~200 μm.

Figure 3.1. Typical N_2 adsorption/desorption isotherm measured at 77 K. Closed circles represent adsorption while open circles represent desorption.

b. Porosity

Since N_2 condenses in the pores of the solid, TOPV has units of cm^3 of void space per gram of solid. Skeletal density, on the other hand, represents the density of the solid portion of the material and therefore has units of grams of solid per cm^3 of solid. Skeletal density is typically evaluated by helium pycnometry experiments and represents the true solid density of a material when there is no closed porosity. A comparison of TOPV and ρ_S enables one to evaluate the porosity of a material (see Section 8, Equation 3.3).

Porosity reveals the total fraction of void space within a material but does not address the size of the voids or their distribution. Traditional pore size distribution analysis is performed by capillary condensation experiments such as N_2 sorption and the application of various forms of the Kelvin equation,[36] which relates the average pore size filled at a particular relative pressure. More sophisticated analysis methods are now available, such as density functional theory (DFT), but much needs to be known about the interaction energy between the gas and the solid surface to perform this analysis appropriately.

Figure 3.2 illustrates an N_2 isotherm with a well-defined pore structure. The sharp rise in the loading at low values of P/P_o indicates a high surface area (see Figure 3.1 for comparison), the abrupt loading transition within the P/P_o range of 0.3–0.4 reveals a unimodal pore size, and the absence of hysteresis suggests the existence of macropores since unrestricted monolayer-multilayer adsorption occurs at high P/P_o. The inset to Figure 3.2 shows the pore size distribution from a modified Kelvin analysis using a BJH thickness curve.[36]

Figure 3.2. Typical N_2 adsorption/desorption isotherm measured at 77 K. Inset is the pore size distribution evaluated from analysis of the isotherm.

c. Sample Preparation

Lastly, the consideration given to sample preparation (or activation) prior to sorption analysis is a key aspect of material characterization. To perform a meaningful analysis the material should be in a state that is representative of how it will be used and pretreated accordingly. Often materials are first tested in their pristine condition and then again after use in a process to examine whether it is still active or under what conditions the material can be regenerated to its initial level of performance.

Activation consists of heating a sample under conditions of dynamic vacuum or purging with an inert gas to remove adsorbed, volatile compounds from the surface. Isotherms and, consequently, BET and PSD results can be greatly influenced by sample preparation since the pore structure can be collapsed upon too stringent

activation or insufficiently cleaned (leaving the internal pore structure partially blocked) upon too mild a conditioning. High temperature activation, therefore, can either increase or decrease the porosity. Examples of this contrasting effect of temperature on the material structure are provided in Figures 3.3 and 3.4 below.

Figure 3.3. N_2 isotherms measured at 77 K. This is an example of a higher temperature sample activation leading to enhanced cleaning of a material and, consequently, a higher surface area and total pore volume.

Figure 3.4. N_2 isotherms measured at 77 K. This is an example of a higher temperature sample activation leading to a collapse of the pore structure of a material and a loss in the total pore volume.

11.6.4 PHYSICAL PROPERTIES

X-ray diffraction (XRD) is a nondestructive technique used to identify and quantify crystalline phases in a material.[39] Samples are typically analyzed as dry powders. However, crystalline phases in suspensions, gels, and creams can also be analyzed. XRD utilizes the elastic, coherent scattering of a monochromatic X-ray beam to produce a characteristic pattern of diffraction peaks from a periodic crystal structure. This pattern is dependent upon the arrangement and type of atoms in a crystalline material or phase. Differences in ordering and types of atoms between crystalline phases result in different observed XRD patterns. This provides a means to distinguish between species with different chemical compositions (e.g., TiO_2 and ZnO) and between polymorphs of the same chemical composition (e.g., rutile and anatase). The quantity of each crystalline phase in a sample can be determined from the linear dependence of relative peak intensities on the amount of each phase. The width of diffraction peaks can be significantly affected by the length scale of periodicity (crystallite size) in a crystalline phase and the presence of strains or imperfections in crystallites.[39] Consequently, properties that can be determined from XRD analysis include crystalline phase identification, quantitative crystalline phase analysis, total amorphous content, and crystallite size.

Modern XRD instruments are equipped with a variety of optics and detectors that allow the analyst to optimize the intensity and resolution of peaks in the diffraction analysis to extract the desired information from the data. Lower-resolution optics provide higher incident and diffracted beam intensities that can significantly reduce analysis time (10 min), but are not adequate for quantitative phase analysis and samples with multiple phases and significant overlap between large numbers of peaks. Higher-resolution optics can provide adequate peak separation for quantitative analysis, but result in significantly longer scan times (2–4 hrs) due to the significant reduction in beam intensity. Experiments can also be set up to scan only around peaks of interest and significantly reduce the total instrument time. This can be used to extract individual peak widths and calculate crystal sizes or to clearly distinguish between two closely spaced diffraction peaks from different phases. The recent development of area detectors further reduces the instrument time required for a single analysis from minutes or hours to seconds, although these are not as common in most analytical laboratories as point or strip detectors.

a. Phase Identification using XRD

Distinguishing between materials with the same chemical composition cannot be done via chemical analysis alone. This can be vitally important in determining the final properties of a mixture, e.g., the difference in ultraviolet light (UV) attenuation between rutile and anatase TiO_2 when used as a UV protectant.[40] However, XRD can be used to identify and quantify polymorphs like rutile and

anatase in TiO_2 powders due to the difference in the arrangement of Ti and O between the polymorphs. Anatase has a body-centered tetragonal crystal structure, whereas rutile has a primitive tetragonal crystal structure.[41] The two phases also have different unit cell dimensions, yielding distinctly different peak positions and intensities between the two polymorphs. The differences between crystal structures and the XRD patterns of rutile and anatase are immediately obvious from the simulated pattern shown in Figure 4.1. Even though the chemical compositions of the two phases are identical, the XRD patterns are distinct due to the different arrangements of atoms in each structure. For unknown samples, a list of diffraction peaks and their relative intensities can be generated from the XRD data. Search-match algorithms can then be used to search large databases of reference diffraction patterns to identify crystalline phases. Once crystalline phases are identified, their crystal structures can be used to determine the relative amounts of each phase along with other parameters.

Figure 4.1: Comparison of XRD patterns for rutile and anatase simulated from crystal structures. The inset shows the difference in structure between the two polymorphs.

b. Phase Composition using XRD

Calculation of the concentrations of crystalline phases in a sample is most accurately performed using the Rietveld Method.[42] The Rietveld Method involves the least-squares regression of structure, instrument, and sample parameters to minimize the difference between calculated intensities from the model and the observed XRD data. The starting model for the refinement includes an approximate crystal structure for each phase and a set of functions that describes the instrument,

sample, and background. Parameters in the model are varied as needed to produce a best fit and the final model is evaluated based on statistical figures of merit, chemical and physical considerations, and the residual between the model and data. For mixtures of crystalline phases, accurate peak profiles must be determined to ensure that peak convolution does not result in diffraction intensity from one phase affecting the refinement of parameters from a second phase.[42] Figure 4.2 shows a fit of calculated intensities to observed data from a sample mixture of Al_2O_3 (corundum), CeO_2, TiO_2 (rutile), and ZnO. The trace below the main plot shows the very small difference between the calculated and observed diffraction intensities after the final model refinement was performed. The calculated phase concentrations are given as a fraction of the crystalline portion of the sample and do not consider what is in the amorphous portion of a sample. However, absolute phase concentrations can be determined by adding a known amount of a highly crystalline reference material to a sample and normalizing the refined amount of the reference phase to the known amount present in the sample mixture.[42] This also allows the absolute amount of total amorphous material to be determined from the mass balance, although individual components of the amorphous portion cannot be distinguished by XRD.

Figure 4.2: Fit of calculated intensities from refined sample model to observed XRD data.

c. Crystallite size using XRD

The widths of diffraction peaks are affected by the size of the crystallites in a sample as well as crystal imperfections. Smaller crystallites and microstrain can lead to noticeable broadening of diffraction peaks, provided the optical configuration

of the instrument yields sufficient resolution. This is particularly significant when characterizing nanomaterials that typically involve length scales of the order where size broadening is observed in the XRD pattern. This is illustrated by the (121) and (220) peak profiles simulated for different crystallite sizes of rutile TiO_2 shown in Figure 4.3. Crystallite size can be calculated using several different methods with varying degrees of accuracy. The simplest and most common method is to use a single peak width to calculate crystallite size via the Scherrer equation. However, the accuracy of crystallite size calculated from the Scherrer equation[39] is reduced as crystallites depart from the assumptions of cubic crystallites without strain. A more accurate determination of crystallite size is obtained by line breadth analysis using multiple peaks in the diffraction pattern (Williamson-Hall method[43]) or by calculating size and strain from peak profile parameters refined using the Reitveld method.[44] It should be noted that the crystallite sizes extracted from XRD data are not generally the same as particle sizes observed in SEM, TEM, or light scattering techniques. Crystallites are domains with ordered, repeated structures that can be interrupted by grain boundaries. Several crystallites can grow together to form larger particles in powders or larger aggregates in suspension as shown in the inset of Figure 4.3.

Figure 4.3: Differences in simulated peak widths as a function of crystallite size, L_C, for rutile TiO_2. The inset illustrates the difference between crystallite size and particle size, L_P.

11.6.5 BULK METALS ANALYSIS

The two primary functions of elemental analysis in the cosmetics industry are to qualify raw materials and to quantify concentrations of trace elements that are toxic to the environment and personal health. The qualification of dry, raw materials, such as metal oxide powders or minerals, can be done using X-ray fluorescence

(XRF) spectroscopy.[45] The quantitation of trace and ultra-trace elements can be performed using flame atomic absorption spectroscopy (FAAS)[46] or inductively coupled plasma (ICP) techniques such as optical emission spectroscopy (OES)[47] or mass spectrometry (MS).[48] The analytical method is typically chosen based on the state of the sample, the concentration ranges of the analytes, the number of analytes, and the elemental species of interest.

a. X-ray Fluorescence—bulk and trace metals analysis

Raw material qualification is generally done to check for potential contaminants and to confirm the composition of materials. Minerals such as rutile and anatase (both TiO_2) are often mixed with zincite (ZnO) to produce a mixture with superior protection across the spectrum of UV light compared to the individual components.[40] The attenuation of UV over a range of wavelengths is dependent upon the relative concentrations of TiO_2 to ZnO. This ratio can be measured by comparing the relative amounts of Ti to Zn detected using XRF analysis of the raw material as a dry powder mixture. This analysis may also detect trace levels of other metal contaminants down to ~10 ppm and indicate the need for more sensitive methods for quantifying such contaminants. XRF is also useful for qualifying pure natural mineral raw materials due to differences in metal stoichiometry between different minerals and between similar minerals obtained from different geographical regions.

XRF involves the detection of photons that are emitted from an element during relaxation from an excited state following exposure to X-ray radiation.[49] These excited states occur when a low-energy electron is ejected by incident X-rays, resulting in a high-energy excited species. Relaxation to a lower energy state can occur via a higher-energy electron falling into the lower-energy hole. The difference in energy between the high-energy electron and the lower-energy hole is released as a photon. For a given element, multiple such transitions occur with well-known statistical frequency resulting in a spectrum of fluorescence energies (or wavelengths). The relative intensities of the observed fluorescence lines can be used to reliably quantify elements between Na and U at concentrations between 10 ppm to 100% in dry solids and powders. Figure 5.1 shows a typical set of XRF spectra collected from a mixture of metal oxides. XRF spectra can be analyzed semi-quantitatively using fundamental parameter models and single point calibration or quantitatively using a range of calibration standards. Sample preparation generally involves drying samples if residual moisture is present followed by grinding of the dry material. Alternatively, elastic samples can be prepared using a freezer mill to cool and embrittle the sample with liquid N_2 while grinding. Knowledge of the sample matrix, such as the presence of elements lighter than Na, can drastically improve the accuracy of semi-quantitative results.

Figure 5.1. XRF spectrum collected from a dry powder mixture of metal oxides. Individual plots are spectra collected using different crystal analyzers in the XRF instrument.

b. Trace and ultra-trace metals analysis using ICP-OES and ICP-MS

Trace and ultra-trace metal analyses are routinely performed using highly sensitive techniques to accurately measure the concentrations of heavy metals down to the parts per billion range. Concern about the potential health and environmental effects of certain metals present in cosmetics have motivated the development of guidelines to limit the concentrations of heavy metals based on exposure limits already in place in other industries. These guidelines recommend limits on metal concentrations of 20 ppm and below depending upon the metal species.[50]

Of particular concern are metals like lead and mercury, which exhibit toxicity to numerous organs and systems in the body.[50] Although their contributions to metal levels in the body from dermal exposure to lead and mercury compounds have not been as well studied as ingestion or inhalation, it is expected that compounds of both lead and mercury potentially present in cosmetic products would show similar toxicity. Chronic lead exposure has toxic effects on the cardiovascular, reproductive, gastrointestinal, and central nervous systems as well as kidneys, heart, and bones at all detectable blood levels and is particularly dangerous to children because it can significantly affect development of the nervous system.[51] Mercury compounds can significantly affect the kidneys, central nervous system, liver, and other systems after both acute and chronic exposure.[52] Recent cases involving

exposure to high levels of mercury in skin-lightening creams have illustrated the importance of metals testing to cosmetic products.[53]

At such low concentration levels, analytical methods such as FAAS, ICP-OES, and ICP-MS are required to provide accurate quantitation. All of these methods depend upon the ability to dissolve the sample into an aqueous solution while retaining the elements of interest throughout the sample preparation and analysis. Given the mixture of organics and metal oxides used in the cosmetics industry, it is not usually possible to simply dissolve samples in water. Strong acids or bases may be used to convert the sample to soluble species. The further addition of weak acids with appropriate counter ions can be used to further convert insoluble species. Heat and pressure can also be used to dissolve more difficult samples. Further care must be taken to ensure the stability of ions in solution. This can be done via the addition of other species, such as $AuCl_3$ to stabilize Hg^{2+} in an HNO_3 solution.[54]

Once samples are dissolved, accurate measurement of the dissolved elements can be done by one of the aforementioned techniques. FAAS can be a good choice if only a few elements need to be analyzed, but multi-element analyses are more efficiently performed using ICP techniques because all elements can be detected simultaneously without the limitations inherent to FAAS. Method detection limits (MDLs) are generally better and more uniform in ICP methods. However, MDL will differ based on ICP application. ICP-OES can give MDL down to 100 ppb and LOQ down to 1 ppm. For ultra-trace analysis, ICP-MS can give MDL down to 100 ppt and LOQ down to 1 ppb. Table 5.1 summarizes typical concentration ranges, MDL, and LOQ provided by each technique. Ultimately, MDL and LOQ are dependent upon the original sample matrix, purity of the reagents used during sample preparation, the cleanliness of the sample preparation vessels, environmental cleanliness, instrument contamination, and interferences between analytes. Contamination is generally minimized by maintaining scrupulously clean vessels and laboratory space and using high-purity reagents. Instruments are periodically cleaned and maintained to prevent carryover of trace amounts of metals between analyses and inhibit buildup of precipitates that can impede sample flow through the instrument.

Table 5.1. Elemental analysis techniques and typical concentration ranges of application, detection limits, and limits of quantitation for analytes in samples.

Technique	Range	MDL	LOQ
XRF	10 ppm–100 wt%	10 ppm	20 ppm
FAAS	1 ppm–100 ppm	100 ppb	1 ppm
ICP-OES	1 ppm–100 ppm	100 ppb	1 ppm
ICP-MS (quadrupole)	100 ppt–10 ppm	100 ppt	1 ppb
ICP-MS (magnetic sector)	100 ppt–10 ppm	100 ppt	1 ppb

Interferences between analytes can be avoided by choosing appropriate detection parameters. In ICP-OES, multiple emission wavelengths from the same element can be used to measure concentrations and look for significant differences in concentration between different wavelengths. In ICP-MS, interferences occur between isotopes and multi-component species that can form in the plasma. For quantitation of elements at concentrations below 1 ppm, ICP equipped with a quadrupole MS is typically used. However, overlap between polyatomic species with masses very close to those from the analyte isotope can lead to drastically inaccurate concentrations. These interferences can be identified using ICP coupled with a magnetic sector MS for separating isotopes from the interfering species. One such example is the interference of ^{63}Cu with $^{23}Na^{40}Ar$ for samples containing both Na and Cu. The difference in masses is only 0.0225 amu and the two species cannot be resolved using a quadrupole MS. This leads to measured Cu concentrations that can be many times more than are actually present. However, a magnetic sector device can be used to separate these masses when operated in medium or high-resolution mode. Figure 5.2 shows the single peak that is produced in low-resolution mode compared to the two peaks observed in medium- and high-resolution modes. High-resolution ICP-MS using the magnetic sector device also shows that very little interference is present near the ^{65}Cu peak and that this is the more appropriate peak to use for quantitation of Cu.

Figure 5.2: a) Single peak produced by interference between ^{63}Cu with $^{23}Na^{40}Ar$ and b) separation of individual masses using medium and high-resolution modes in ICP-MS with a magnetic sector device.

CONCLUSION

To comply with EU and global nanomaterial regulatory controls, researchers and manufacturers must have the ability to accurately characterize their products. There are a variety of tools available to comprehensively understand nanomaterials, each providing a portion of the fundamental information that is required for compliance. Having the necessary knowledge associated with what information each technique delivers is critical to accurately characterizing a material. This overview of analysis techniques should serve as a starting point for those individuals interested in developing an improved understanding of nanomaterials based on their elemental, chemical, and physical properties.

TERMS

TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
STM	Scanning tunneling microscopy
AFM	Atomic force microscopy
EFM	Electrostatic force microscopy
SSPM	Scanning surface potential microscopy
CP-AFM	Conductive probe atomic force microscopy
DLS	Dynamic light scattering
AES	Auger electron spectroscopy
XPS	X-ray photoelectron spectroscopy
DSIMS	Dynamic secondary ion mass spectrometry
TOF-SIMS	Time-of-flight secondary ion mass spectrometry
TOPV	Total pore volume
BET	Brunauer, Emmett, and Teller theory
DFT	Density functional theory
PSD	Pore size distribution
XRD	X-ray diffraction
ICP-OES	Inductively coupled plasma optical emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
FAAS	Flame atomic absorption spectroscopy
MDL	Method detection limit
LOQ	Limit of quantitation

EQUATIONS

Equation 2.1

$$E_k = h\nu - E_B - \varphi_{sp}$$

Where E_k is the kinetic energy of the emitted electron, hv is the energy of the photon beam, E_B is the binding energy of the electron, and ϕ_{sp} is the work function of the spectrometer.

Equation 3.1

$$\mathrm{BET}\left[\frac{\mathrm{m}^2}{\mathrm{g}}\right] = \frac{4\pi\,\mathrm{R}^2}{\frac{4}{3}\pi\,\mathrm{R}^3\rho_\mathrm{S}} = \frac{3}{\rho_\mathrm{S}\ \mathrm{R}} = \frac{6}{\rho_\mathrm{S}\ \mathrm{D}}$$

Where R is the pore radius, D is the pore diameter, and ρ_s is the skeletal density.

Equation 3.2

$$\mathrm{TOPV}\left[\frac{\mathrm{cm}^3}{\mathrm{g}}\right] = \mathrm{Max\ Loading}\left[\frac{\mathrm{cm}^3\mathrm{STP}}{\mathrm{g}}\right]\times$$

$$\left[\frac{1\ \mathrm{mole}}{22{,}414\ \mathrm{cm}^3\mathrm{STP}}\right]\times\mathrm{MW}\left[\frac{\mathrm{g}}{\mathrm{mole}}\right]\times\frac{1}{\rho_\mathrm{L}}\left[\frac{\mathrm{cm}^3}{\mathrm{g}}\right]$$

Where MW and ρ_L are the molecular weight and density of the probe molecule being used, which for N_2 adsorbed at 77 K have values of 28.01 g/mol and 0.807 g/cm^3, respectively.

Equation 3.3

$$\mathrm{Porosity} = \frac{\mathrm{cm}^3\mathrm{void}}{\mathrm{cm}^3\mathrm{solid} + \mathrm{cm}^3\mathrm{void}} = \frac{\mathrm{TOPV}}{\frac{1}{\rho_\mathrm{S}} + \mathrm{TOPV}}$$

Where TOPV is the total pore volume found by Equation 3.2, and ρ_s is the skeletal density.

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IN VITRO ASSAYS TO MEASURE EPIGENETIC MECHANISMS INVOLVED WITH CONTROLLING GENE EXPRESSION

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ABSTRACT:

This chapter will describe the analytical methods used to assess the epigenetic mechanisms involved in controlling gene expression. Although the information in this chapter is presented as a general overview of the methods, it nonetheless contains a great deal of technical detail with the intent that knowledge of these details can assist in both experimental design by the research and development scientists and also help marketing groups to understand how the data were generated along with the benefits or limitations of using these techniques. For readers who are not analytical chemists, or biochemical experts, the chapter is designed to educate and enhance the understanding of the role that epigenetics plays in normal and abnormal skin function. While this field has made great strides, there is still much to learn. Being able to at least be conversant in the current technologies opens the door to ingredient and product developers to successfully interact with their analytical counterparts, either internally or externally, to move forward towards their goal of producing novel products.

The term "epigenetic" can be defined as heritable changes in gene expression or phenotype that do not involve changes in the underlying DNA sequence of the organism. Heritable changes are those capable of being passed from one generation to the next; i.e., hereditary. To illustrate the power and importance of epigenetics one need look no further than our own bodies. Human cells contain the same approximate 30,000 genes regardless of cell type—and yet when keratinocytes divide, the two daughter cells remain keratinocytes. The gene expression pattern is transmitted from the mother cell to the progeny while the DNA sequence in the two keratinocyte daughter cells is unchanged and still identical to fibroblasts or any other cell type in the human body.

The reason for this maintained pattern of gene expression is thought to involve an overlying epigenetic control mechanism. The process by which this epigenetic control of gene expression occurs involves at least two basic pathways: DNA methylation and histone modification. Both DNA methylation and histone modification can control gene expression by restricting the access of transcription factors to gene promoters. These two types of modification can induce changes in the structure of the chromatin, such as chromatin condensation, in the region where the gene is located. This essentially leaves the gene promoter blocked from required transcription factors resulting in a silenced gene. Both mechanisms play an essential role in the epigenetic control of gene expression. An understanding of this phenomenon is of critical importance to the development of optimal cosmetic and personal care products. Not only is it advantageous to understand the basic epigenetic control process in normal healthy skin, but it is equally important to realize that epigenetic control mechanisms can adversely change in response to cosmetically relevant stressors such as aging, inflammation, UV exposure, or exposure to other adverse environmental stresses. By understanding the mechanisms of the adverse epigenetic changes it then becomes possible to design and test products with the intent of returning the skin to its normal healthy state. This chapter will discuss useful *in vitro* methods that can be used to examine two of the better-understood epigenetic mechanisms and help understand the impact of adverse stressors on normal skin function. The topic of epigenetics and its role in skin aging is also discussed elsewhere in this book in the section Anti-Aging Pathways.

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11.7.1 INTRODUCTION:

In 1951 Dr. G. Wyatt, at the time a young Ph.D. student, was working to improve current methods to separate DNA bases using paper chromatography. After finding a blend of isopropanol and HCl that gave a good separation, and well-defined "spots" for the four bases: adenine, guanine, cytosine, and thymine in test samples, Dr. Wyatt was surprised to find that upon running actual animal and plant DNA hydrolysate samples the assay produced an additional and unexpected fifth spot (Wyatt 1951). This fifth spot was quickly identified to be 5-methyl cytosine. While the presence of large amounts of 5-methylcytosine in human genomic DNA was interesting, its function remained largely unknown until 1975. In that year two papers were published predicting that programmed methylation and demethylation of cytosine bases in DNA would actually regulate gene expression (Holiday and Pugh 1975; Riggs 1975). The papers went on to suggest that the methylation of cytosine would not occur at random cytosine bases, but rather at specific DNA sequences containing key cytosine bases.

Over the decades, since the initial discovery of methylated DNA bases and their involvement with controlling gene transcription, it became apparent that modified DNA was not alone in its ability to control gene transcription through nontraditional transcription methods. Histones, the proteins that help package DNA into well-defined chromatin structures, were also discovered to be able to be covalently modified in ways that controlled gene expression. The potential of DNA methylation and histone modification controlling gene expression was an exciting prospect since it indicated that genetic changes could be inherited without altering the basic DNA sequence. This discovery also added an additional level of control above and beyond the basic mechanisms of the previously known cellular machinery for determining which genes were turned on or off and had the potential to explain many biochemical phenomena affecting the human body.

The basic mechanism of gene expression involves the binding of transcription factors to the promoter region of the gene. Transcription factors are essentially control proteins that when activated can bind to the promoter region of the gene and help to recruit the necessary enzymes that are involved in transcribing an RNA copy of the gene, which is subsequently translated to the target protein that the gene is responsible for making. When an RNA copy of the gene is being transcribed the gene is essentially "on." Conversely, when an RNA copy of the gene is not being made, the gene is referred to as "off" since without the transcribed RNA the final protein product of the gene cannot be made.

Transcription factors often work in concert with other regulatory proteins that can bind to repressor or enhancer regions also associated with the gene. Depending upon the combination of transcription factors and regulatory factors binding to

the regulatory regions of the gene, gene expression control can go beyond simply being "on" or "off" and intermediate levels of control can be achieved, which can further enhance or repress the basal level of gene expression. However, as intricate as this basic control mechanism for gene transcription is, DNA methylation and histone modification can further increase the control of gene transcription. This is done by altering the structure of the DNA in such a way as to control the access of these transcription and regulatory proteins to the regions to which they need to bind. Methylated DNA can bind to special proteins with methylation binding domains and, in essence, coat the DNA in proteins that prohibit access to the DNA by transcription factors. In addition, both DNA methylation and histone modification can lead to changes in the chromatin structure, such as chromatin condensation. Such changes can again prevent or allow access of transcription factor to their DNA binding regions. Since DNA methylation and histone modification-based mechanisms control gene transcription at a level above normal transcription factors, they have been grouped into a class of new control mechanisms termed Epigenetic.

Epigenetic control mechanisms are now known to be heritable changes, that is, the changes in DNA methylation or histone modification can be passed from mother cell to daughter cell and play an important role in normal cell function such as maintaining cell differentiation. However, sometimes epigenetic changes can be detrimental to a cell's performance. It is now thought that abnormal epigenetic control mechanisms may play a role in aging and cancer (Agrawal et al. 2010; Miller et al. 2011). Due to their ability to control global gene transcription, and their involvement in skin disease, the study of epigenetic control mechanisms has garnered a large amount of research interest by the skin care industry. Compounds that can influence epigenetic control mechanisms can be extremely beneficial in countering the effects of aging, preventing skin-based diseases, or even enhancing the normal function of the skin. This chapter describes some of the current methods used in skin care research when addressing epigenetic control mechanisms.

11.7.2 DNA MODIFICATION: DNA METHYLATION

It is now known that approximately 60% of the promoters within the human genome contain potential DNA methylation sites and that DNA methylation occurs primarily in cytosines that are followed immediately by a guanine residue (CpG) (Antequera and Bird 1993). These methylations are catalyzed by a family of DNA methyltransferases that consists of DNA methyltransferase (DNMT) 1, 3a, 3b, and 3l. This family of enzymes catalyzes the transfer of a methyl group from the methyl donor S-adenosylmethionine to cytosine resulting in the formation of 5-methylcytosine. Once donated to cytosine, this methyl group protrudes into the major groove of the DNA and can reduce the ability of transcription factors to both

recognize and bind to their specific DNA binding site (DeAngelis et al. 2008). The attached methyl group can also be bound by proteins with methylcytosine binding sites (MCBs). This binding can impact the level of gene transcription by at least two mechanisms: (1) proteins with MCBs can block the access of transcription factors to gene regulatory regions by directly binding to the DNA regulatory region of interest, or (2) they can also recruit histone-modifying enzymes to their location. Histone-modifying enzymes can modify histones at a number of locations via changes in the methylation, acetylation, phosphorylation, or ubiquitinylation status of key amino acids (these changes will be discussed in greater detail later in the chapter). This type of modification can have the net result of altering the local chromatin structure in a manner that again impacts gene expression. For the most part, these changes tend to silence genes; however, there are a small handful of studies showing that the expression of some genes can also be enhanced by these epigenetic changes.

Initial methods to examine DNA methylation used techniques based on either chromatography (Kuo et al. 1980), radiolabeling with tritiated S-adenosylmethionine (Duthie et al. 2000), or immunolabeling with anti-5-methylcytosine antibodies (Adouard et al. 1985). While these early methods were revealing, they were limited to simply determining the relative proportion of 5-methylcytosine within the genome and could not provide information on the methylation status of specific gene promoters or coding sequences. However, sometimes measuring the relative amount of global genomic methylation can be extremely informative. Such information is useful, for example when determining the impact of aging (Agrawal et al. 2010) or UV exposure (Mittal et al. 2003) on total genomic DNA methylation to determine if specific stressors can elicit general epigenetic changes before pursuing a more detailed study for specific changes. Such global measurements can be made with relative ease since the initially complicated and labor-intensive methods of chromatography and radiolabeling have now been replaced with rapid, high-throughput, sensitive ELISA-based methods (enzyme-linked immunosorbent assay). ELISA-based methods also have the advantages of low cost, relative to other methods for measuring DNA methylation. Thus, while ELISA-based methods may lack the ability to determine specific methylation sites, they have the benefit of being rapid and one of the least-expensive methods to measure changes in DNA methylation.

Since most DNA methylation ELISAs are competitive-based ELISAs designed to specifically measure 5’’-methyl-2’-deoxycytidine, the nucleoside version of the methylated DNA base, total genomic DNA must be isolated from the sample and broken down into individual nucleosides. DNA extraction from cells or tissues can be achieved using commercially available extraction kits. Once extracted, the DNA is re-suspended in water and denatured by incubating it for 5–10 minutes

at 95°C. Denaturation will convert the double-stranded DNA molecule into two single strands. Denaturation is followed by immediate cooling on ice, after which the single stranded DNA is enzymatically digested to nucleoside monophosphates with nuclease P1, followed by the removal of the phosphate groups using alkaline phosphatase. The remaining nucleoside solution can then be assayed using any commercially available ELISA to quantitatively measure the amount of 5'-methyl-2'-deoxycytidine. The amount of remaining nucleoside solution can then be normalized to the amount of original starting DNA in order to determine the proportion of DNA that is methylated.

While measuring global changes in DNA methylation can be easily accomplished using ELISAs, sometimes it is essential to not only measure global changes in DNA methylation, but to also determine where in the genome those changes are occurring. For example, while it may be interesting to note that UV exposure of keratinocytes results in an increase in global DNA methylation, the next question would be which genes are specifically being impacted by epigenetic mechanisms, such as the genes involved in cell proliferation, the genes involved in DNA repair, or genes involved with other critical cellular functions.

In general, there are two alternative methods for determining specific changes in methylation status within defined regions of the genome. These two methods are based on either microarray formats or sequencing formats. Microarray formats are designed to assess changes in global DNA methylation status across the genome. However, while they can identify if changes in DNA methylation are occurring within specific regions of DNA, they cannot definitively determine which individual cytosines are methylated if more than one potential methylation site occurs within the region. In contrast, sequencing-based DNA methylation analysis can determine which individual cytosines have been methylated within a given DNA region. However, due to the expense and complexity of sequencing based methods, these analytical approaches are normally limited to a handful of DNA sequence targets and are not well suited for global genomic measurements. Thus, these latter two methods provide a tradeoff between either obtaining global measurements of genomic DNA methylation (with limited detail on specific regions), or obtaining very specific methylation data on individual cytosines within a set of very well-defined DNA regions without genome-wide information.

With microarray based DNA methylation analysis methods, the initial step is the isolation of genomic DNA from the sample. This can be accomplished using most commercially available DNA extraction kits or established lab methods (Sambrook et al. 1989). Once isolated, the genomic DNA must be randomly fragmented into DNA strands of approximately 200–1000 base pairs (bp) in size using either sonication or restriction enzyme digestion (Weng et al. 2009). Once fragmented, a portion of the DNA sample is then used to enrich for methylated

DNA strands using affinity-based methods. With affinity-based methods, either an antibody that specifically recognizes 5-methylcytosine (Weber et al. 2005) or a protein containing an MCB (Rauch et al. 2006) is used to bind methylated DNA fragments. The antibody/protein DNA fragment complex is then precipitated, most commonly through the use of magnetic beads (Weng et al. 2009). Once separated from the antibody/protein used for precipitation, both the methylated DNA-enriched sample and the original DNA sample can be labeled with respectively colored fluorescent dyes and hybridized to a microarray containing complementary DNA probes specific for CpG-containing regions of the genome.

Upon being scanned and normalized, the two-color microarray provides an indication of the specific areas of the genome that contain methylated DNA. Current methylation arrays are considered tiling arrays. By this term we mean that the potentially thousands of bases that form the DNA sequence for any given gene (including both the promoter and coding portion of the gene) are divided into smaller portions and thereafter split into sequential features on the microarray chip. Thus, although each specific probe region on the array chip may only contain a DNA sequence corresponding to a 50–100 base portion of the gene of interest, the next probe set will cover the next adjacent DNA region. This process is then continued until the entire gene sequence is covered in the same manner that multiple individual tiles cover a single floor. With each probe for the region containing a portion of the whole DNA sequence for that region, this collection of multiple probes can provide complete coverage of methylated areas. Since most microarray chips now contain at least 250,000 probe sets, a good portion of the human DNA methylome can be covered with a single array experiment in a matter of a few days.

As indicated above, while DNA microarray-based methods for measuring DNA methylation can determine if methylated cytosines are present within a given region of DNA, they cannot definitively determine which cytosine within the region is methylated or if more than one cytosine is methylated. If a single probe region contains more than one cytosine methylation site then, depending upon the DNA fragmentation pattern, the array can potentially give the same signal if just one site within the probe region is methylated or if all of the sites within the probe are methylated, since all it takes is just one methylated cytosine to immunoprecipitate a DNA fragment. Generating smaller DNA fragments prior to enriching for methylated DNA may somewhat help to increase the ability of the microarrays to determine the level of methylation in regions with multiple methylation sites. However, to truly observe which specific cytosines are methylated within the genome requires true sequencing-based methods. Yet, some evidence suggests that within a given region of DNA, all of the potential cytosine methylation sites share the same methylation status (Eckhardt et al. 2006). Therefore it may be enough to know if a region contains some methylated cytosines, without having to determine

the details on all potential methylation sites within the region, since it can be inferred that they all share the same methylation status.

As you can see, microarray-based DNA methylation studies can provide a lot of data. With at least 250,000 data points the prospect of analysis can be quite daunting; however, the wealth of data obtained in these types of experiments is an extremely valuable asset. This is especially true since the field of epigenetic research is still relatively young and there are only a limited number of studies that have begun to address the epigenetic control mechanisms pertaining to skin cells. Since the main cell types of the skin (keratinocytes, fibroblasts, and melanocytes) have not been well characterized with respect to normal patterns of DNA methylation, let alone how stress may alter this pattern of DNA methylation, then experiments that examine global changes are the best option when specific targets are not known.

With respect to skin care research, DNA methylation microarray data can describe which regions of the genome are methylated and therefore potentially silenced. This type of data is especially useful when comparing different types of cellular stress since it will tell you how the cell is responding to the stress at an epigenetic level, allowing for the determination of whether the response is ultimately beneficial or harmful to the cell. It is these epigenetic responses to stress that represent an example of interesting targets in the product development and marketing aspect of cosmetic research. These epigenetic responses provide targets against which active ingredients can be screened to determine if the actives can either augment a beneficial epigenetic change or reduce a harmful response.

In contrast to microarray-based methods to measure genomic DNA methylation, sequencing-based methods can provide a very detailed analysis of precisely which cytosine bases have been methylated. The key to this precision is the reaction of both cytosine and 5-methylcytosine with the acid salt sodium bisulfite. When exposed to sodium bisulfite, cytosine is deaminated to form uracil (Hayatsu et al. 1970). Although 5-methylcytosine will also react with sodium bisulfite, the reaction rate is significantly slower than the reaction with cytosine. This reduction in reaction rate has allowed for the development of a method that will convert nonmethylated cytosines into uracil residues while methylated cytosines would remain as essentially cytosine residues (Frommer et al. 1992). Since sequencing reactions involve amplification of the target DNA via PCR, the uracil (which is normally found in RNA and base pairs with adenine) in the bisulfite-treated DNA sample is converted to a thymine residue (which is the DNA base that binds to adenine). This reaction results in the 5-methylcytosine remaining a cytosine. By comparing the DNA sequences of non-sodium bisulfite–treated regions of the genome to the same region *after* sodium bisulfite treatment, any remaining cytosine bases in the sodium bisulfite–treated DNA represent methylated cytosines, while

any cytosines in the original sequence that have been converted to thymines were nonmethylated cytosines (Zhang and Jeltsch 2010).

Sequencing-based methods can share some common approaches to DNA methylation analysis with microarray-based methods. In a manner similar to array-based methods, sequencing analysis can start with DNA samples that have been enriched for methylated DNA using the affinity/precipitation-based methods described above (Maunakea et al. 2010; Salpea et al. 2012). The enrichment for DNA methylation reduces the complexity and significantly reduces the cost of the subsequent sequencing data analysis. Rather than subject the entire genome to sodium bisulfite treatment, and then search for pockets of methylation, using the methylation- enriched DNA samples only those regions that contain DNA methylation are sequenced. Yet, despite using the methylated DNA enriched samples for analysis, there is still a considerable amount of sequencing that needs to be accomplished in order to obtain a detailed image of changes in the entire human DNA methylome.

As an alternative to the sequencing method described above, which uses DNA samples enriched for methylation to determine widespread DNA methylation, it is possible to use PCR to amplify small regions of interest to see if these discrete regions are methylated. All that is required in this method is to isolate genomic DNA from the sample, take a portion of that sample, and expose it to sodium bisulfite to differentiate between methylated and nonmethylated cytosines. Thereafter, PCR primers are designed that will amplify the region of interest in both the native DNA and bisulfite-converted DNA. The PCR products from both approaches would then be sequenced and compared to determine sites of DNA methylation within a discrete region of the genome.

a. DNA Methylation: Methyltransferases

DNA methylation is catalyzed by a family of DNA methyl transferases (DNMTs). DNMT1 appears to be the main isoform of the enzyme responsible for maintaining the methylation status of existing methylation patterns, while DNMTs 3a, 3b, and 3L are responsible for *de novo* patterns of DNA methylation (Yang et al. 2010). These enzymes catalyze the transfer of a methyl group from the methyl donor S-adenosyl methionine to cytosine, resulting in the formation of 5-methylcytosine.

Currently several companies produce commercial assays that are available to measure changes in DNMT activity in either purified enzyme preparations or in nuclear extracts from samples of treated cells or tissues. For DNMTs, there are two basic assay forms that are commercially available. Both forms of the assay require a source of the enzyme (either purified or nuclear extract), along with a DNA substrate containing CpG repeats. The first type of DNMT assay uses an ELISA-based format and ultimately measures the level of DNA methylation. The DNA substrate

is immobilized on a 96 well plate and incubated with the DNMT enzyme source in the presence of S-adenosyl methionine. After the incubation period the plate is washed and then incubated with either an anti-5-methylcytosine antibody or a tagged MCB protein. After washing the plate again, the antibody or MCB protein is detected using an appropriate detection molecule coupled to a signal-generating system that is generally of the colorimetric, fluorescent or luminescent type. The intensity of the signal generated will be proportional to the amount of 5-methylcytosine, which in turn will be proportional to the amount of DNMT activity in the sample.

The second type of DNMT activity assay is based on the accumulation of S-adenosylhomocysteine, which is formed from S-adenosyl methionine after it donates its methyl group (Dorgan et al. 2006). In this type of assay a DNMT enzyme source is combined with the DNA substrate and S-adenosyl methionine, but additional enzymes are added to the reaction mix to convert any S-adenosylhomocysteine to S-ribosylhomocysteine. The S-ribosylhomocysteine is used as a reactant—leading to the ultimate formation of a compound that can be measured spectrophotometrically or fluorometrically. This second type of assay has the advantage of not requiring wash steps and, since the entire reaction mix is combined at the same time, it allows for real-time monitoring of DNMT activity.

b. DNA Methylation: Pharmacological Agents

Commercially available assay kits offer the ability to both assess the level of DNMT activity in cells or tissues undergoing various treatments. They also provide the ability to screen potential materials for their ability to either inhibit, or stimulate, the activity of DNMTs by using purified forms of the enzyme family. Compounds that can impact DNMT function not only have a significant impact in potentially treating disease states that have a basis in altered DNA methylation patterns (i.e., cancer), but can also function as molecular tools for helping to understand the mechanisms behind the epigenetic controls involved with gene expression. As tools for skin care research, compounds that impact DNMT activity can play an important role as controls for DNA methylation-based studies. Table 1 provides a list of some well-established DNMT regulators.

Table 1. DNA Methylation-Related Agents

Agent	Effect on DNA Methylation	Mechanism of Action	Reference
5-Aza-2'-deoxycytidine (Decitabine)	Inhibitor	Inhibits DNMTs	Chuang et al. 2010
5−Fluoro−2'−deoxycytidine	Inhibitor	Inhibits DNMTs	Zheng et al. 2008

Agent	Effect on DNA Methylation	Mechanism of Action	Reference
Azacitadine	Inhibitor	Inhibits DNMTs	O'Dwyer and Maslak 2008
Zebularine	Inhibitor	Inhibits DNMTs	Yoo et al. 2004
(-)-epigallocatechin-3-gallate	Inhibitor	Inhibits DNMT1	Lyko and Brown 2005
MG98	Inhibitor	Reduces DNMT1 Expression	Lyko and Brown 2005
RG108	Inhibitor	Inhibits DNMTs	Lyko and Brown 2005
Procainamide	Inhibitor	Decreases DNMT1 affinity for DNA and S-adenosyl-methionine	Gravina et al. 2010
S110	Inhibitor	Inhibits DNMTs	Chuang et al. 2010

11.7.3 HISTONE MODIFICATIONS

Genomic DNA in eukaryotic cells is contained within the nucleus and heavily bound by regulatory proteins. The fundamental building block of this contained DNA is a unit called the nucleosome. Nucleosomes are composed of approximately 150 DNA base pairs wrapped around a core histone octamer. The histone octamer is composed of two of each of the following histone types: H2A, H2B, H3, and H4. Short sections of nonhistone-bound DNA (linker DNA) connect the regions of histone-bound DNA to form a nucleosome array, which has the appearance of a necklace composed of beads on a string.

Within the nucleosome, the core of the histone octamer forms the spool around which the genomic DNA is wrapped. While this core of the histone proteins is covered by DNA, portions of both the amino and carboxyl terminals of the histone proteins extend out from this core and have been termed the histone tails. These tails can be post-translationally modified at specific amino acids by several regulatory proteins. These modifications include methylation, acetylation, phosphorylation, or ubiquitinylation, which essentially involves the addition of a methyl

group, acetyl group, phosphate group, or a ubiquitin molecule, respectively, to the histone. Since these modifications occur outside of the histone core on the histone tails, these tail regions are free to interact with other regulatory proteins or even other nucleosome groups. These interactions have a dramatic effect on the overall structure of the nucleosome array and essentially result in nucleosomes that either become tightly packed together condensing the DNA and restricting gene transcription in the area, or exposing the nucleosome array, such that it becomes available to the transcription machinery and gene expression can occur.

Within the histone tails of each histone subtype there are numerous sites for post-translational modification (Tollervay and Lunyak 2012), which can lead to a foreboding number, at least from a researcher's point of view, of different modification combinations. However, some strides have been made to determine how histone modifications impact gene transcription. For example, the combination of acetylation of lysine 9 and dimethylation/trimethylation of lysine 4 on histone 3 in nucleosomes associated with the promoter regions of genes has been shown to result in a decondensed chromatin structure and the transcription of genes within the decondensed region. In contrast, the combination of trimethylation on lysines 9 and 27 on histone 3 within promoter regions has been associated with the inactivation of gene expression (Zhou et al. 2011). These basic modifications appear to be somewhat universal and may provide a starting point for examining epigenetic controls of specific genes in skin care research.

a. Post-Translational Histone Modification Assays

Assays for assessing post-translational histone modifications can vary greatly in their levels of complexity; however, most of the approaches have one thing in common and that is the use of antibodies that recognize post-translational modifications. Currently, there are commercially available antibodies that recognize general post-translational modifications (i.e., the dimethylation of any lysine residue), or very specific modifications (i.e., only the trimethylation of lysine 9 in histone 3), which provides researchers with a great deal of flexibility with respect to how to measure post-translational histone modifications.

One of the simplest approaches to studying post-translational histone modifications is to determine global changes using Western Blotting or ELISA-based techniques (Dai et al. 2005). In the Western Blotting approach, protein samples in the form of cell lysates or tissue homogenates are separated based on molecular weight using SDS-PAGE and then blotted onto a membrane solid support. The membrane is then probed using antibodies specific for the particular histone post-translational modification of interest and quantified using standard fluorescent/luminescent-based techniques.

In a manner similar to Western Blotting, ELISAs can also provide a measure of histone modification changes using a slightly different technique. Cell lysates or tissue homogenates would be added to well plates coated with a capture antibody. Ideally, the antibody is one that recognizes the post-translational modification of interest (if using a capture antibody for a very specific post-translational modification), or for the histone subunit of interest (if using a detection antibody that recognizes general changes). After incubating and rinsing the ELISA plate, a detection antibody would be added. This would be either an antibody that recognizes specific histone subunits, or an antibody that recognizes a type of histone modification, depending on what was used as the capture antibody. The detection antibody would then be detected using a secondary antibody or a streptavidin/avidin-based detection system and quantified using standard ELISA colorimetric- or fluorometric-based methods.

With both Western Blotting and the ELISA-based methods, the data generated by these types of assays will reflect global changes in the amount of specific types of post-translational modifications. While this data can be useful, for example if you are screening materials designed to impact a certain type of histone modification, it only reflects a net increase or decrease in the amount of the post-translational modification within the sample. It does not specify if histones associated with a specific gene are being impacted by the treatments being tested. To determine if histones associated with specific genes are being impacted by treatment with some ingredient, then the methods employed are very similar to those used to assay specific sites of DNA methylation: immunoprecipitation coupled with either microarray-based or sequencing-based analysis.

To determine which specific genes are associated with which specific types of histone modifications it is first necessary to ensure that the histones remain associated with their respective DNA regions during initial processing. This can be achieved by fixing cell samples with 1% formaldehyde, which is a reversible cross-linking agent that will cross-link the histone proteins to the DNA molecules they are associated with. After fixing the cells, the cells can then be recovered and broken open using a detergent-based lysis solution. Such a solution contains protease inhibitors to protect the histone protein from any proteases released by lysing the cells. Once the cells are lysed open, the genomic DNA and associated histones need to be broken into shorter DNA fragments by either shearing the DNA with sonication or digesting it with restriction enzyme-based methods. With the histones still associated to the genomic DNA, the fragmenting of the DNA will primarily occur in the linking DNA between the nucleosomes. This will not pose a problem, and ideally the DNA should be sheared/digested to fragments between 200 and 1000 base pairs in length such that the DNA region associated with the histone modification is small and therefore well defined.

After the fragmentation step, an aliquot of the DNA sample can be used for immunoprecipitation using an antibody that recognizes the post-translational histone modification of interest. The resulting immunoprecipitation will recover histone/DNA complexes that are enriched for specific histone modifications. After the immunoprecipitation step, it is necessary to reverse the DNA/protein cross-linking by heating the sample at 65°C for an extended period of time (i.e., six hours or overnight). The released DNA is then combined with proteinase K to digest any proteins that were associated with the DNA and then purified to remove the protein debris. This is accomplished using either standard DNA purification methods based on phenol-chloroform, or by means of commercially available kits designed to purify small DNA fragments.

Once the recovered DNA fragments are purified, they can be analyzed to determine what DNA sequences are associated with the specific histone modification that was targeted by the immunoprecipitation. This analysis can take two forms: either quantitative PCR-based experiments, or microarray-based experiments. Quantitative PCR can be used to detect and amplify specific DNA sequences. This is an excellent approach if a few, well-defined gene targets of interest are known. Custom primers specific for the DNA sequences can be easily designed and through the use of quantitative PCR methods, the relative abundance of each DNA sequence with the respective histone modification can be determined.

However, if a genomic-wide search is needed, then the best approach to determine which DNA sequences are associated with the specific histone modification is to again use a microarray-based approach. Several commercial companies have array chips available that are specifically designed to map immunoprecipitated DNA fragments to their respective position in the human genome.

b. Histone-Modifying Enzyme Assays

In contrast to the relatively discrete number of enzymes associated with DNA methylation, the family of histone-modifying enzymes is quite large. Generally speaking, this family is composed of histone acetyl transferases, histone deacetylases, histone methyltransferases, histone demethylases, protein kinases, and ubiquitination enzymes. Table 2 provides an overview of the family members associated with each of the different types of histone modification. *Since these enzymes are ultimately responsible for the histone modifications associated with the epigenetic control of gene expression, they represent valuable targets for studies seeking to determine if changes in their relative expression level or activity level has an impact on the expression of the gene of interest.*

Table 2.

Enzyme and Activity	Family Members	Target/Effect
Histone Acetyltransferase (Adds acetyl groups to histones)	GNAT superfamily (PCAF, GCN5), p300/CBP, MYST, MOZ, TAFII250, SRC-1, ACTR	Epsilon-amino group of lysine residue in histones/opens chromatin structure to promote gene transcription (Masumi 2011)
Histone Deacetylase (Removes acetyl groups from histones)	Class I HDAC (HDAC 1, 2, 3, 8, 11) Class II HDAC (HDAC 4, 5, 6, 7, 9a, 9b, 10, HDRP/MITR) Class III HDAC (SIRT1-7)	Epsilon-amino group of lysine residue in histones/closes chromatin structure to repress gene transcription (Karagiannis and Ververis 2012)
Histone Methyltransferases (Adds methyl groups to histones)	KMT1A, KMT1C (G9a), KMT3A (Setd2), PRMT1, SET7	Various lysine (up to 3 potential methylation sites) and arginine (up to 2 potential methylation sites) in histones/Various effects on transcription (Ng et al. 2009)
Histone Demethylases (Removes methyl groups from histones)	KDM1/LSD1, KDM2a, KDM3a, KDM4a, KDM4c, KDM6, KDM7a, PAD14, JMJD2, JMJD6, PHF8, EZH2	Various lysine (up to 3 potential methylation sites) and arginine (up to 2 potential methylation sites) in histones/Various effects on transcription (Ng et al. 2009)
Histone Kinases (Adds phosphate groups to histones)	ATR, ATM, DNA-PK, RSK2, MSK1/2, WSTF, NHK-1, Aurora B, AMPK, Protein Kinase C, Protein Kinase C Beta 1, Haspin, SNF1, IKK alpha, PKB/Akt, RSK2, PIMI, CHK1, PRK1, Zip Kinase, JAK2,	Various serine, threonine, and tyrosine residues/Various effects on transcription (Banerjee and Chakravarti 2011)

Enzyme and Activity	Family Members	Target/Effect
Ubiquitin Ligase (Adds ubiquitin to histones)	RNF8, Ring1a, Ring1b, Ring2, UbcH5c, BRCA1/BARD1, RNF20/40, RAD6A/B, UbcH6	Lysine 119 in histone H2a and Lysine 120 in histone H2b/Ubiquitination of H2a is associated with low transcription activity while ubiquitination of H2b is associated with high transcription activity (Ma et al. 2011)
Deubiquitinating Enzymes (Removes ubiquitin from histones)	USP16, USP21, 2A-DUB, USP3, USP22	Lysine 119 in histone H2a and Lysine 120 in histone H2b/Deubiquitination of H2a is associated with increased transcription activity while deubiquitination of H2b is associated with decreased transcription activity (Cao and Yan 2012)

While relative changes in the activity of the histone-modifying enzymes can be inferred by examining the changes in histone modifications using the methods described above, sometimes it is necessary to obtain a more direct assessment of enzyme activity. Currently, recombinant or purified forms of most of the histone-modifying enzymes are commercially available, as are suitable substrates. Alternatively cell lysates, or even better, nuclear extracts, can also been used as sources for histone-modifying enzymes. However, when lysates/extracts are used instead of a purified enzyme, it is often not possible to attribute any observed activity to a specific enzyme since cells contain many members of the same enzyme family with similar activities. On the other hand, if selective inhibitors are available, such as those that would specifically inhibit enzymes with a similar activity to that which you wish to measure—*but not impact the enzyme of interest*, then it is possible to obtain a reasonable measurement of a specific enzyme using lysates/extracts.

Histone acetyltransferases (HATs) catalyze the addition of an acetyl group to lysine residues in histones using acetyl-CoA as the acetyl source. While this acetylation can occur at lysine residues in all four of the histone subtypes, the acetylation reactions seem to occur only at select lysines that are within 20–30 amino acids of the amino terminal (Tollervey and Lunyak 2012). Thus, when studying the enzyme activity of HATs, it is possible to employ short peptide fragments consisting of just the 2030 residues of the histone amino terminal as a substrate. For

HATs activity assays, these short histone peptide fragments can be coated to ELISA plates (for example, using avidin-coated ELISA plates and biotinylated histone peptides). After coating the ELISA plate with the peptide and blocking the plate with a BSA solution, a mixture of HAT enzyme and acetyl-CoA can be added to the wells. The HAT enzyme will use acetyl-CoA to acetylate the peptide substrate. After removing the enzyme mixture the acetylated substrate can be detected using antibodies specific for acetylated histones. This antibody can in turn be detected using a secondary antibody coupled to a signal-generating compound and quantified using standard ELISA-based techniques.

Histone deacetylases (HDACs) catalyze the reverse reaction of HATs since they remove acetyl groups from histones. HDAC activity can be measured in a manner that is similar to the assay used for HATs, only substituting an acetylated histone peptide while retaining the same basic ELISA type approach. Alternatively, there are a few nonhistone HDAC enzyme substrates available. These commercially produced substrates still have an acetyl group on them, which is recognized and removed by HDACs; however, the remaining substrate is designed in such a way that removal of the acetyl group results in the generation of a colorimetric or fluorescent signal that is proportional to the level of HDAC activity. The added benefit of some of these commercially available nonhistone HDAC substrates is that they are cell permeable. This substrate can be added directly to cultured cells to allow the measurement of intracellular HDAC activity within the cellular environment.

Histone methyltransferases (HMTs) catalyze the transfer of methyl groups to both lysine and arginine residues in histones using S-adenosyl-L-methionine (SAM) as the methyl donor. While the majority of the methylation sites in histones are found within the 30 amino acids of the amino terminal of all four histone subtypes, histones H2a, H3, and H4 also have a few methylation sites near their carboxyl terminals. However, histone peptide fragments composed of just the 20–30 amino acids from the amino terminal are still excellent substrates for HMT activity assays.

HMT activity can be assessed using ELISA-based approaches as described for HATs and HDACs through the use of antibodies that recognize histone methylation. Alternatively, HMT activity has also been assessed through the use of radiolabeled methyl groups. HMT solutions can be combined with histone substrate peptides and SAM, which has been prepared to have a tritiated methyl group attached to it and allowed to incubate for a period of time. The radiolabeled methyl group will be attached to the histone substrate in proportion to the amount of HMT activity. At the end of the incubation period the reaction mixture is typically filtered in a manner that allows any remaining radiolabeled SAM to pass through the filter while the filter retains only the histone substrate peptide. The level of radioactivity in the filter can then be assessed and used as a measure of HMT activity (Greiner

et al. 2005). While this assay can be extremely sensitive for HMT activity, the use of radioactive material prevents its use in most laboratories. HMT activity can also be assessed by measuring the accumulation of S-adenosyl-homocysteine that is formed from SAM after it donates its methyl group. S-adenosyl-homocysteine can be converted through a series of enzymatic reactions to form end products that form compounds that generate either colorimetric or fluorescent signals. Again, the intensity of the signal would be proportional to the amount of S-adenosyl-homocysteine formed, which itself would be proportional to the amount of methyl groups donated due to HMT activity (Quinn and Simeonov 2011).

Histone demethylases (HDMs) will catalyze the removal of methyl groups from arginine and lysine residues in histones. Again, HDM activity can be easily assessed using ELISA-based methods as described above and making use of methylated histone substrates for the ELISA plate preparation. However, alternative methods to assess HDM activity are somewhat limited. One alternative method makes use of histone substrates that have been labeled with tritiated methyl groups (Tsukada and Nakayama 2010). When methyl groups are removed from lysines they are converted to formaldehyde. Since the initial methyl group was radiolabeled, the resulting released formaldehyde is also radiolabeled. By measuring either the accumulation of radiolabeled formaldehyde or the loss of radioactivity in the histone substrate the HDM activity can be determined. Again, this method involves the use of radioactivity, so it may not be available in most laboratory settings. However, a second option is available. Under certain conditions the activity of the HDM LSD1 can be coupled to enzyme systems that generate H_2O_2 (Forneris et al. 2005). Since there are readily available commercial probes that react with H_2O_2 to generate fluorescent signals, then by measuring changes in fluorescence the activity of demethylase activity of LSD1 can be determined.

Protein kinases, in general, add phosphate groups to target substrates. With respect to histones there are quite a few protein kinases that act upon the histones; however, there are relatively few histone phosphorylation sites. While the H2a, H2B, and H4 histone contain only a single phosphorylation site on their amino terminal residues (H2a also has a single site near its carboxyl terminal as well), H3 has two phosphorylation sites on its amino terminal. Despite the fact that there are fewer phosphorylation sites than methylation or acetylation sites, protein kinase activity on histone targets can still be readily measured using the same ELISA-based methods described above.

Ubiquitination refers to the process by which a group ubiquitin is attached to a protein while de-ubiquitination refers to the removal of an ubiquitin group. In contrast to most of the other histone modifications which predominately occur on the amino terminal tail of the histones, the most understood sites of histone ubiquitination occur only near the carboxyl terminal, and only on histones H2a and H2b.

Commonly used assays to measure histone ubiquitination still rely on Western Blotting-based techniques. In these cases cells, or tissues, are treated with agents, followed by lysing the cells and resolving the proteins via SDS-PAGE. Thereafter, blotting the proteins to a membrane and then probing with antibodies specific for histone ubiquitination is the final step in the process.

c. Pharmacological Agents That Impact Histone Modification

Many of the assays run on histone-modifying enzymes are used to screen potential compounds for their ability to either stimulate or inhibit these enzymes. Since these histone modifications can have a profound impact on gene expression, it follows that compounds that can influence the activity of these histone-modifying enzymes can be extremely powerful in terms of producing skin care benefits. Table 3 below gives an overview of currently well-established compounds that are known to impact these enzyme systems. These compounds can serve as either controls for enzyme-screening purposes or as excellent tools for skin care research applications.

Table 3. Histone-Modifying Agents

Agent	Target	Effect	Reference
Anacardic Acid	Histone Acetyltransferase (p300 and PCAF)	Inhibitor	Balasubramanyam et al. 2003
Butyrolactone 3	Histone Acetyltransferase (Gcn5)	Inhibitor	Biel et al. 2004
Circumin	Histone Acetyltransferase (p300)	Inhibitor	Marcu et al. 2006
CPTH2	Histone Acetyltransferase (Gcn5	Inhibitor	Chimenti et al. 2009
Delphinidin Chloride	Histone Acetyltransferase (p300)	Inhibitor	Seong et al. 2011
Garcinol	Histone Acetyltransferase (p300 and PCAF)	Inhibitor	Balasubramanyam et al. 2004
CTPB	Histone Acetyltransferase (p300)	Activator	Balasubramanyam et al. 2003

Agent	Target	Effect	Reference
AN-9	Histone Deacetylase	Inhibitor	Reid et al. 2004
APHA Compound 8	Histone Deacetylase 1	Inhibitor	Mai et al. 2008
Apicidin	Histone Deacetylase	Inhibitor	Ueda et al. 2007
BML-210	Histone Deacetylase	Inhibitor	Enzo Life Sciences Catalog
Butyrylhy-droxamic acid	Histone Deacetylase	Inhibitor	Fass et al. 2010
CAY10604	Histone Deacetylase 6	Inhibitor	Kozikowski et al. 2008
CBHA	Histone Deacetylase 1/3	Inhibitor	Richon et al. 1998
CI-994	Histone Deacetylase	Inhibitor	Moradei et al. 2007
Depudecin	Histone Deacetylase 1	Inhibitor	Kwon et al. 1998
HC Toxin	Histone Deacetylase	Inhibitor	McCaffrey et al. 1997
ITSA-1	Histone Deacetylase	Inhibitor	Koeller et al. 2003
M344	Histone Deacetylase	Inhibitor	Jung et al. 1999
MC1293	Histone Deacetylase 1	Inhibitor	Massa et al. 2001
MS-275	Histone Deacetylase 1/3	Inhibitor	Hu et al. 2003
1-Naphthohy-droxamic Acid	Histone Deacetylase 8	Inhibitor	KrennHrubec et al. 2007
Oxamflatin	Histone Deacetylase	Inhibitor	Kim et al. 1999
PCI 34051	Histone Deacetylase 8	Inhibitor	Balasubramanian et al. 2008
Pimelic Diphe-nylamide 106	Histone Deacetylase	Inhibitor	Chou et al. 2008
Pyroxamide	Histone Deacetylase	Inhibitor	Butler et al. 2001
PTACH	Histone Deacetylase	Inhibitor	Suzuki et al. 2005
SB 939	Histone Deacetylase	Inhibitor	Wang et al. 2011
Splitomicin	SIR2	Inhibitor	Bedalov et al. 2001
Suberoyl bis-hydroxamic acid	Histone Deacetylase	Inhibitor	Preston and McFarlane 1998

Agent	Target	Effect	Reference
Suberoylanilide Hydroxamic Acid	Histone Deacetylase	Inhibitor	Marks and Breslow 2007
Trichostatin A	Histone Deacetylase	Inhibitor	Wolffe 1996
Tubastatin A hydrochloride	Histone Deacetylase 6	Inhibitor	Sigma Chemical Catalog
Valproic Acid	Histone Deacetylase I	Inhibitor	Phiel et al. 2001
Butein	SIRT1	Activator	Howitz et al. 2003
Piceatannol	SIRT1	Activator	Howitz et al. 2003
Quercetin	SIRT1	Activator	Howitz et al. 2003
Resveratrol	SIRT1	Activator	Howitz et al. 2003
6-chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxamide	SIRT1>>SIRT2>SIRT3	Inhibitor	Napper et al. 2005
AGK2	SIRT2	Inhibitor	Outeiro et al. 2007
AK-7	SIRT2	Inhibitor	De Oliveira et al. 2012
Aristoforin	SIRTs	Inhibitor	Gey et al. 2007
Cambinol	SIRT1, SIRT2	Inhibitor	Tervo et al. 2006
CHIC-35	SIRT1	Inhibitor	Napper et al. 2005
EX-527	SIRT1	Inhibitor	Napper et al. 2005
Guttiferone G	SIRTs	Inhibitor	Gey et al. 2007
Hyperforin	SIRTs	Inhibitor	Gey et al. 2007
BML-266	SIRT2	Inhibitor	Kiviranta et al. 2006
Suramin	SIRT1, SIRT5	Inhibitor	Howitz et al. 2003
Sirtinol	SIRT1, SIRT2	Inhibitor	Grozinger et al. 2001
Tenovin-6	SIRT1, SIRT2, SIRT3	Inhibitor	Lain et al. 2008
AMI-1	Protein Arginine Methyltransferase	Inhibitor	Spannhoff et al. 2009
BIX 01294	G9a Histone Lysine Methyltransferase	Inhibitor	Kubicek et al. 2007
Chaetocin	Lysine Specific Methyltransferases	Inhibitor	Greiner et al. 2005

Agent	Target	Effect	Reference
3-Deazanepla-nocin A	Lysine Methyltransferase EZH2	Reduces EZH2 Expression	Simon et al. 2008
UNC0321	G9a Histone Lysine Methyltransferase	Inhibitor	Thomas et al. 2008
UNC0638	G9a Histone Lysine Methyltransferase	Inhibitor	Thomas et al. 2008
Daminozide	KDM2a, PHF8, and KDM7a Histone Demethylases	Inhibitor	Rose et al. 2012
GSKJ4	H3 Lysine 27 Demethylase	Inhibitor	Agger et al. 2011
JMJD3	H3 Lysine 27 Demethylase	Inhibitor	Agger et al. 2011
N-Oxalylglycine	JMJD2 Histone Demethylase	Inhibitor	Hamada et al. 2009
Tranylcypromine	BHC110/LSD1 Histone Demethylase	Inhibitor	Lee et al. 2006

CONCLUSION

Despite the decades since the initial observation of methylated DNA, and the realization of its potential to regulate gene expression, the understanding of the role of epigenetics in normal and abnormal skin function is by far incomplete. The basics for how DNA methylation and histone modification can impact general cell function are slowly being elucidated; however, there is currently very little information on how epigenetic-based gene expression control mechanisms can specifically impact the normal function of keratinocytes, fibroblasts, and melanocytes, which are the key cell types in skin care research and product development. In addition, not only is there relatively little information on epigenetic controls in the cell types under normal conditions, but also very little information on how epigenetic control mechanisms in these cell types change in response to relevant stressors, such as aging, UV exposure, inflammation, and adverse environmental exposure. Thus the field of epigenetics with respect to the skin research and skin care industry is very much open to a great deal of pioneering research, which can lead to the development of highly unique actives capable of working at an epigenetic level to regulate skin health, beauty, and well-being.

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SUSTAINABILITY & ECO-RESPONSIBILITY

SUSTAINABILITY AND ECO-RESPONSIBILITY: A GLOBAL APPROACH FOR THE COSMETIC AND PERSONAL CARE INDUSTRY

Editor's Overview

Alban Muller (President, Alban Muller Group)

In the cosmetic world, we have seen "the green wave," then the "organic tide," and now "the eco-responsibility tsunami." Do we have to consider that this wave will go by, allowing a new wave to come in? Like the ocean, waves come and go; some larger than others. At the time of this writing, we choose to consider that this eco-responsibility tsunami is probably more in line with various other converging evolutions and will rise and fall as previous and future waves. **Since we must surf this wave or lose our customers, let us consider some important current parameters:**

- **The first and most important parameter** we are all faced with is the rapid expansion of humankind, which, in the last century, has multiplied our numbers on this planet by a factor of four. This sobering statistic leads us to the perspective of a humanity numbering 9 billion people in the next few decades. As we anticipate this incredible increase, there will inevitably be an increasing collective pressure on the Earth's resources, thereby leading to concerns about their availability. *Thus, renewability becomes a key issue and concern in the whole industrial process*.
- **The second important parameter** is that consumers don't expect the same things from companies that supply their wants and needs any more. Not only do they want good value for their money, but they also expect that companies respect some values such as: the comfort of their employees, the offer of equal opportunities, and consumer safety along with an increasing respect for the global environment. For example, large U.S. distribution companies have recently embarked on programs to ban various chemicals suspected of being harmful to people or the environment.

When combined, these facets compel the formation of a new management approach that needs to show increased concern for the people and the planet, while not forgetting about profits. *The only way to maintain an appropriate balance of these parameters is to take a better look at our products and how we produce them*. By doing so, we can be innovative while finding new and economical ways to create new products with less impact on our environment.

As we explore these ideas in this part of *Harry's Cosmeticology*, 9th Ed., one important observation emerges:

What we see is that eco-conception can bring innovative concepts, themselves generating new approaches and new technologies.

The various articles in the section show two major points of view and some proactive pathways to achieving our goals.

- **The first pathway** consists of perfecting the existing industrial practices in order to reduce their global impact and produce better products (natural extracts and ingredients), which also results in superior performance when compared to their synthetic counterparts.
- **The second pathway** involves an exploration of the kinds of new technologies that could be introduced to allow us to benefit from natural molecules, even by way of emerging biofermentation technology.

It is obvious that all these efforts have the potential to create the new "greener economy," which in turn could be a source of technological progress.

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DEFINING SUSTAINABILITY AND HOW IT CHANGES THE INNOVATION PROCESS

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ABSTRACT

Innovation will be essential to addressing the increasingly critical sustainability challenges companies face, such as limited natural resources, energy constraints, and consumer demand. To address these emerging business drivers and meet the green demands of consumers, many companies are introducing sustainability practices into their businesses. These practices require companies to measure success not just monetarily, but also by the cost to the people who make the goods, the impact of goods and services on surrounding communities, and the price paid by the environment.

Once viewed as a fleeting consumer trend, sustainability is now seen as a new business paradigm. This chapter presents an overview of concepts that are essential to the continued growth and effective product generation for cosmetic and personal care products to compete effectively in this changing world. Innovation is essential to addressing these critical sustainability challenges that are reshaping industries and supply chains. Ingredients, packaging, and manufacturing equipment all play an important role in the environmental profile of a product. However, no company can source all of these materials in isolation. For a company to meet the eco-responsible standards demanded by consumers and required by regulations, it must be willing to innovate, improve transparency, and work with outside sources.

Open innovation (OI) is a well-recognized methodology that leverages technology assets both internal and external to the company to accelerate innovation. At its core, OI relies on transparency and collaboration. As such, there are many opportunities to exploit the synergies between OI and sustainability to improve environmental stewardship. Open innovation for sustainability (OIS) is driven by the reality of limited natural resources, public pressures, and high material costs to discover potential solutions for better waste and resource management. Companies successful in adopting OIS offer several best-practice techniques. Research also

indicates that OIS implementation leads to a more complete application of traditional OI. For example, effective OIS companies are more likely to collaborate with competitors and nongovernmental organizations; embrace transparency and sustainability throughout their organization; and share beneficial intellectual property to enable future sourcing and supply-chain development across the industry.

Businesses in the cosmetic and personal care industries recognize that OI can benefit both R&D and the bottom line. Thus, as the industry becomes increasingly aware and committed to sustaining planetary resources, it is critical to highlight how OIS can spur innovation while also advancing sustainability and eco-responsibility. While the focus of this book is cosmetic and personal care industries, this chapter purposely includes examples from beyond this focus to emphasize that OIS is most effective when cross-fertilization and technology transfer from many industries provide the stimulus for generating the next leap in innovation.

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12.1.1 SUSTAINABILITY—A CRITICAL BUSINESS ISSUE

Numerous industries and everyday citizens are beginning to awaken to the realization that the world cannot continue on the same path of disregarding the environment and its limitations. The reality of limited natural resources, energy

constraints, increasing pollution, tighter emerging environmental regulations, and green consumer demand have become new business drivers. These drivers not only threaten "business as usual," but have the potential to impact life on this planet. The imperative nature of these demands is reshaping industries and supply chains as companies seek to compete effectively in this new emerging world.

In the face of these emerging drivers, eco-responsible companies and governments have a heightened awareness of the planet's limitations regarding resources and have begun to adopt the principles of sustainability.

Sustainability as first defined by John Elkington (1998) is a focus on people, planet, and profit—a concept commonly referred to as the triple bottom line. Sustainability requires companies to measure their success not just by monetary increase, but also by the cost to the people that make the goods. Success is also measured by the impact on surrounding communities resulting from the creation of these goods and services, and the cost price paid by the environment. Once viewed as a fleeting consumer trend, sustainability is now seen as a new business paradigm (Nidumlo et al. 2009). *In fact, a recent MIT Sloan Management report (Haaneas et al. 2011) recently found that companies believe sustainability will eventually become core to any business.*

12.1.2 INNOVATION IS A CRITICAL BUT CHALLENGING COMPONENT OF ANY SUSTAINABILITY STRATEGY

Any organization attempting to produce goods and services that incorporate an awareness of planetary sustainability must inevitably innovate. In the first instance these innovations may be relatively "routine"—small adjustments for the company's current mode of operation to adopt technologies or practices well proven in other similar organizations. Ultimately, however, more challenging innovations are likely to be required—innovations that require larger departures from current practice and involve greater risks. This is especially true of innovations that enhance a business's sustainability, as they often require new or immature technologies that require varying degrees of development. For example, many waste-heat recovery, alternative-energy, and pollution-prevention technologies are not commercially viable, or the cost of these young technologies may impede their adoption.

It is often instinctive to automatically think of innovation for sustainability as primarily about technological innovation. It is certainly true that technical solutions are essential to mitigating many of a company's negative environmental impacts—for example, technologies to increase water efficiency, to increase the recyclability of packaging, etc. Nevertheless, other types of innovation can also play a critical role in enhancing an organization's sustainability practices—service innovation, business model innovation, financial innovation, etc.

A number of factors contribute to make innovation for sustainability particularly challenging:

- Sustainability cannot be solved in a vacuum. Companies must consider the entire lifespan of a product to truly address sustainability. This includes components from sourcing, manufacturing, shipping, disposal of waste, consumer usage, and eventual disposal by the consumer. The complex nature of sustainability requires a company to consider parameters both internal and external to the company.
- New technologies with the potential to improve sustainability are often at an early stage of development that can hinder adoption or move the cost of the technology beyond what a product can sell for.
- Governments are actively involved in generating regulations that require more sustainable practices. While this invention is often beneficial (e.g., by ensuring a level playing field between competitors, by creating financial incentives that improve the business case for taking action), it also introduces an additional set of risks. When will government regulate? What will be the exact requirements of the regulations? Will government change the regulations? Will variations in regulations across states, countries, and geographical areas impact corporate practices, especially for multinational endeavors?
- Greenwashing or overinflating a product or company's "green" attributes has become a consumer concern. Thus companies fear being accused of greenwashing, and this can often prevent sustainable innovation.

a. The Concept of Open Innovation (OI)

The novel aspect of the vast majority of innovations is typically the new combination of some preexisting ideas, capabilities, and skills or resources—rather than the creation of some fundamentally new knowledge. Open innovation is based on the idea that the greater the variety of these factors brought together, the more innovations are likely to be generated. A critical component of open innovation is that a firm must be actively encouraged to open its doors to source innovations from outside the company. For many companies, this differs from prior approaches to product development, as custom and practice has until now required corporate secrecy about their innovations/inventions and they have therefore chosen to highly protect intellectual property or trade secrets from prospective competitors.

OI follows the premise that it is likely that someone has addressed a similar problem in another industry and can provide possible solutions to an innovation need. This alternative approach tends to mean "path dependency": where firms have typically searched for new ideas in well-worn prior sources. An excellent example of this is that ingredient manufacturers for novel hair care materials frequently go to the carpet industry for their ideas since there is much common ground between these two types of products.

b. Open innovation and sustainability are synergistic

Many industries are beginning to realize that open innovation has the ability to enable sustainability goals and endeavors. Open innovation (OI) is particularly well situated for sustainability-focused innovation and is referred to as open innovation for sustainability (OIS). OI and sustainability inherently both require two important components: a high level of transparency, and collaboration.

c. Transparency

As sustainability is really focused on people, planet, and profit, a large element of success for a sustainable company is gaining the people's heart. Disingenuous attempts at sustainability and a track record of industry neglecting the environment in favor of the bottom line has created consumers that are skeptical when a company promotes its "green" attributes. Thus, credible green companies are now working to full disclosure, including disclosure of ingredients, packaging, and partners. These companies often invite outside groups to audit their sustainability practices and generate reports. This high level of transparency is critical to the success of any company pursuing green efforts.

In a similar manner, OI has the dual requirement of transparency. The act of seeking external partners and technologies naturally requires a greater degree of disclosure than a closed innovation approach. Many companies pursuing traditional innovation pathways work diligently to ensure that all innovation is kept secret and in-house. Companies active in OI often seek out intermediaries or post-innovation needs in public forums to help identify meritorious technologies. They additionally will externally leverage technologies that the company has developed with partners and other interested parties.

Companies unwilling to clearly define their innovation needs or negotiate openly will not gain the external partners they need to innovate and will not be successful at OI. These innovation activities necessitate that an OI company accept a greater degree of transparency into their development activities. Increased transparency is also being essentially forced on geographical areas by the regulatory actions of others. For example, in the cosmetic and personal care industry, European Union Regulations require documentation of toxicological properties that many small companies may not have. This results in an imperative for sharing of such data by larger companies with smaller ones so that full transparency is achieved.

d. Collaboration

The complexity of sustainability and the many parts that are required to truly follow a green path necessitate that a company must collaborate. No company can source all its ingredients, packaging, or manufacturing equipment eternally. Each of these components plays a significant role in the environmental profile of a product. For instance, a cosmetics company that uses all bio-based recycled packaging but uses

ingredients including phthalates, carcinogens, or ingredients tested on animals would struggle to define their endeavors as green. However, for this company to meet the green needs demanded by consumers and required by regulations, they must be willing to partner and work outside with different raw material providers to address these issues. Sustainability requires these types of partner collaborations due to the complexity of the problem. Moreover, collaboration is inherent to OI. To seek outside one's organization for external technology solutions cannot occur without some level of collaboration.

The dual needs of transparency and collaboration for both sustainability and OI mean that OI is an exceptionally well-situated innovation methodology to solve sustainability-focused needs. In fact, many sustainability companies have been found to follow OI approaches without a formal knowledge of OI or the intention to do so. These companies found that these approaches were just "right" for the technical challenge at hand. Furthermore, companies that have openly accepted an OIS approach and purposely use the methodology have seen greater gains related to their sustainability goals. OIS can be used to help to systematically increase a company's green profile.

12.1.3 INTEGRATION OF SUSTAINABILITY PRINCIPLES INTO INNOVATION PRACTICES IS EVOLUTIONARY

The process of incorporating sustainability principles into business practice is typically evolutionary. Figure 1.1 is a generalized model that illustrates the strategic drivers that push companies to become increasingly sustainable and the subsequent expansion of their innovation ecosystem by inclusion of a greater number of partners.

Figure 1.1: Sustainability drivers and the resultant expansion of the innovation ecosystem. Drivers are displayed as arrows and partner organizations are displayed within the ecosystem.

Companies often begin thinking about the sustainability of their products and practices, in the first instance, by relatively narrowly defined problems. Examples include government regulations (e.g., on emissions of air pollutants to the environment), consumer demand for a more sustainable product (e.g., recyclable packaging), and increasing cost of a resource (e.g., water) or a desire to decrease waste (e.g., raw materials). These early first steps are often accompanied by collaboration and inclusion within the innovation of partners such as their suppliers, government, and their consumers.

Once companies have started to make the transition to becoming more sustainable in some narrowly defined way, they are often exposed to new drivers to apply sustainability practices more broadly across their organization. Consumers, familiar with a few "green" products from a company, begin to expect that sustainability be fully embraced across the organization. These consumers may be unwilling to purchase products that do not meet the same levels of environmental stewardship. Thus consumer demand for a greener product may transition to a consumer requirement. This often also attracts the attention of a different workforce that is seeking to work with a company with principles. These employees once hired begin to change the company from the inside.

These increasing pressures internally from employees and externally from consumers push companies to quantify and publicly disclose their green efforts via approaches such as social auditing. Perhaps most importantly, accepting responsibility for environmental or social issues appears to change the way a company views itself. At this point society often becomes part of their innovation ecosystem. As Roger Cowe puts it:

Once a company has acknowledged it has to account for pollution . . . it is harder to deny wider social responsibilities. And once outsiders have been through the gates, it is impossible to stop them looking beyond one narrow aspect of business. Curiously, this odd little world of social auditing threatens to fuel a debate about the purpose and nature of 21st century capitalism which has escaped the politicians for decades.

Finally, companies can eventually reach a point at which the drivers they face are so strong or pervasive that they are pushed into completely embracing sustainability. One such driver at this stage is altruism. A company's values, and mission, can evolve to the point that improving its own sustainability, and that of the world around it, is seen as being a core part of their reason for existence.

Another critical driver at this stage can be *an emergent awareness of the physical scarcity of a resource*—that is, an understanding that the company's current pathway will ultimately lead to depletion of a critical resource to such an extent that the company's very survival will be threatened. One such example can be found with Nike. Nike realized that the very nature of how they compete as a company will be impacted by constrained resources in the future. To that end they developed an

Environmental Apparel Design Tool at a cost of $6 million to the company. The tool permitted Nike to assess the footprint, availability, and environmental characteristics of each technology incorporated into their products. Nike saw the value in this tool, but realized that its true value was diminished if it was limited to acceptance by one company as other companies may still be innovating in traditional, more resource-heavy methodologies. To that end, Nike shared this tool with industry.

Within the cosmetic and personal care community, destruction of natural resources (e.g., rare plants that are extracted for their essential oils) can often lead to a depletion of the critical resource, not to mention the devastating effect on the surrounding people, animals, and environment.

This understanding that corporate products and corporate survival are threatened by limited resources creates a superordinate goal, i.e., something that two parties normally in opposition to each other can agree to work together on as it is critical to their survival. This superordinate goal creates the greatest expansion of the innovation ecosystem as now companies begin to collaborate with their competitors on complex industry-wide innovation challenges and with nongovernmental organizations (NGOs) that industry has often had a combative relationship with.

a. Six key traits of sustainable companies

Through examining company behavior it has become clear that six traits are common among companies successfully using open innovation for achieving truly sustainable approaches:

1. **Systemic:** OIS companies are more likely to treat sustainability as a systems issue and understand that to be truly sustainable one must address the sustainability of the organization as a whole.
2. **Enterprise-level:** Within these organizations, sustainability is practiced at all levels, and all projects are evaluated for their environmental impact. This sets an expectation that sustainable solutions must be sought, whether from within the organization or from the outside.
3. **Committed:** These organizations are committed to sustainable practices for the long haul, understanding that future resource limitations will impact their industry and business model, and they are willing to take a "leadership" strategy. This contrasts with companies that practice sustainable innovation only in reaction to consumer demands (follower strategy) or industry regulation (compliance strategy).
4. **Collaborative:** OIS companies tend to have a greater number of partners in their innovation ecosystem than traditional OI companies. This is due to the fact that OIS often presents broader, more difficult, and more cutting-edge technology and sustainability needs that require expertise from multiple industries and partners.
5. **Invested:** These companies show a willingness to invest in building an

entire supply chain that can provide more benign offerings at all levels, and they will choose to follow their principles as opposed to focusing on short-term gain. They also are more likely to truly adopt a triple bottom-line approach that measures the success of their company equally on people, planet, and profit.

6. **Transparent:** OIS companies embrace transparency via internal and external reporting, as well as in sharing their innovation needs with outside entities, and believe this is a competitive advantage.

b. Few companies explicitly recognize and exploit open innovation as a tool to help them on this sustainability pathway

Collaboration and transparency are essential elements of both improving sustainability practices and successful innovation (whether for sustainability outcomes or some other desirable result). Companies attempting to improve sustainability practices inevitably must collaborate in order to solve problems. A bread manufacturer must engage partners all along the supply chain if it is to reduce the carbon footprint of a loaf of bread. There is a strong body of evidence proving that companies trying to innovate in any sphere will be more successful if they collaborate with partners for ideas, to support development and to take new products to market. Transparency is a critical tool for building trusting relationships in both sustainability and innovation. This overlap creates the potential for powerful synergies that can help accelerate both innovation and sustainability practices. Some examples of the way in which these synergies can be exploited are outlined below.

Exploiting synergies between innovation and sustainability

- In the process of become more sustainable, companies typically become more "open"—for example, they work with their suppliers, customers, and NGOs to understand sources of environmental or social impact along their supply chains. The relationships established in the process of opening up can then become an excellent community to work within an open innovation context. Companies can engage with this community to help generate new, innovative ideas and to collaborate during other parts of the innovation process. As another example, companies can work with suppliers on joint development of new technologies, or with NGOs to help test and validate whether new ideas will actually enhance sustainability.
- Adopting open innovation practices will demonstrate to a company's stakeholders that they are real about sustainability (since they're being actively engaged in the process)—which helps to build trust and overcome potential perception of greenwash.
- Adopting OIS helps companies recognize opportunities to work collaboratively with their competitors to address sustainability challenges

which have a direct business impact. For example, making IP related to sustainability practices freely available can help reduce pressure on finite resources (which will likely have some impact on the resources' price). It can also result in the industry as a whole being perceived by customers as a "sustainable" one—thus avoiding the possibility that one company with good sustainability practices is not tainted by a general perception of the industry as being unsustainable.

Few companies appear to actively link open innovation and sustainability initiatives. This appears to be due to a lack of awareness of the potential synergies, and to a relatively narrow understanding of the meaning of "open innovation" (e.g., assuming open innovation only means crowd sourcing).

c. Companies that practice open innovation for sustainability adopt a more complete model of open innovation

A number of common traits were identified during the course of our primary and secondary research into companies who are leveraging open innovation for sustainability outcomes. Additionally, it was found that the innovation ecosystem tends to expand as companies embrace OIS. This expansion of the innovation ecosystem alters the innovation process as shown in Figure 1.2.

Figure 1.2: The new product development pipeline for companies practicing Open Innovation for Sustainability.

The most significant and unexpected finding of this study was that these companies appear to be using a more complete model of open innovation than companies that have adopted OI but not for sustainability outcomes. Most companies adopting open innovation for general innovation purposes are relatively selective about just how open they are (few partner with direct competitors), and also about the direction in which ideas travel (most see OI as a tool for harvesting ideas, and not for sharing internal ideas out).

Companies practicing OIS, by contrast, appear to be more likely to develop partnerships with stakeholders sometimes seen as hostile—e.g., direct competitors or NGOs. For example, recently there has been collaboration between furniture manufacturers to identify alternatives to halogenated flame retardants.

It also appears that companies practicing OIS are more likely to release technologies or ideas out into the world, as opposed to just treating open innovation as another source of new product ideas. In one approach, a number of companies have either donated intellectual property or licensed IP for a modest fee. Websites now exist for sharing green IP for the common benefit. Two such examples of IP sharing are the World Business Council for Sustainable Development's Eco-Patent Commons and Nike's GreenXChange.

d. Practical lessons can be learned from companies that have recognized the synergies between sustainability and OI

The following practical lessons from companies who have sought to leverage the synergies between sustainability and open innovation can help to guide businesses interested in taking similar steps:

- **Understand that resource constraints will impact business.** In many situations limited resources are here and if they are not here now, they will be in the near future. These limitations will alter the cost of raw materials and fundamentally change the way a company does business.
- **Embrace openness in your innovation challenges and sustainability goals.** By partnering and working with numerous partners, organizations are more likely to develop robust products that address a greater number of sustainability challenges. This requires companies to take two important steps:
  –Strategically increase your innovation ecosystem.
  –Consider alternative points of view (e.g., NGOs and competitors).
- **Do not just take—you must also give.** There is a tendency for companies using OI to focus on bringing external ideas in, but not sharing out internal ideas. The critical challenges facing the planet necessitate the sharing of ideas related to sustainability. Many companies are now realizing that they can create new models that allow the sharing of these ideas. Additionally,

the sharing of sustainable technology helps to raise the ethical and green image of a company in the eyes of its consumers. Companies interested in sharing sustainable innovations should:

–Consider out-licensing of sustainable technology and IP donation of green innovations that will benefit the industry.

–Grow their supply chain with ideas built inside the company. This can help build a market related to these technologies that helps to mature these innovations.

–Spin-out green technologies that they will not develop internally so that these green innovations benefit the industry at large.

- **Prepare now for a triple bottom-line approach.** Many companies have already begun to follow the triple bottom line. However, a wider adoption of this approach is coming.
- **Do not focus innovation efforts solely on low-hanging fruit.** For many industries, the focus has been on smaller, easier innovations that require limited technology investment. However, the paradigm by which companies operate is changing. This will require new bold technologies that address environmental challenges.
- **Treat OI as a methodology to facilitate collaboration.** Collaboration is a key component to sustainability. OI is a methodology that can enable the collaboration needed to solve the grand challenges industry will face in the future.

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A BOTANIST'S VIEW OF SUSTAINABILITY: USE OF ABUSE IN THE PERSONAL CARE INDUSTRY?

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ABSTRACT

With increased public awareness of the finite nature of natural resources, there has been greater interest in living a sustainable lifestyle and reducing our footprint on the planet. In the personal care and supplements industries, the movement towards using more exotic and efficacious ingredients has led to concerns about their supply, particularly those that are wild harvested. As a result, sustainability has become not only a new supply goal but also a marketing mantra.

But what is sustainability from the biological perspective?

How do we assess the quantity of a raw material that can be harvested each year without destroying the population of the resource? The answer to these questions requires a biological and ecological evaluation of each raw material, whether it is harvested in the wild or cultivated. A basic model for establishing sustainability and harvest limits will be presented. Nature is a diverse but fragile source of important bioactive compounds, now and in the future, and must be protected as part of the process of commercialization and resource utilization.

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12.2.1 INTRODUCTION

From earliest recorded history, humans have used a broad range of plants for adornment as well as other aspects of personal care. It is now estimated that there are over 300,000 species of flowering plants on earth. Currently botanists are identifying and describing approximately 2,000 new species of flowering plants each year. It has been estimated that 70,000+ species remain to be discovered and described. Today, only a relative handful of the plants once used traditionally for decorating and perfuming the body, soothing the skin and protecting it from the elements are utilized in commerce. Great opportunity exists for the "rediscovery" and use of plant-based ingredients in the personal care industry.

As a researcher who has spent much of his career studying ethnobotany and the relationship between plants and people in a traditional setting, with people who still live as their ancestors did many hundreds of years ago, with few links to the global economy, this author has frequently observed indigenous people using plants externally, for example as cosmetics or washes. Table 12.2.1 provides examples of the categories of traditional plant uses that may offer guidance for those seeking to discover and develop active ingredients and new categories for personal care products.

Table 12.2.1: Traditional Uses of Plants: Their Application in Personal Care

- Anti-Inflammatory
- Anti-Irritants
- Antiseptic
- Body Decoration
- Cleansing and Conditioning Agents
- Fragrances
- Insect Repellants
- Moisturizers and Lotions
- Mouth and Teeth Cleaning
- Sunscreens and Skin Protectants
- Wound Healing

The process of identifying plant species that could provide raw materials of use for personal care and cosmetics involves a series of steps. These raw materials can be used as extracts, individual compounds, or their synthetic derivatives. First, one should have an excellent grasp of the literature on the species of interest or the type of activity (e.g., as anti-aging or sun protection) that is to be found in the plant world. This can help direct the search for species that can be screened for biological activity and also obviate the need for re-collection of species that have previously been investigated (unless of course there are new assays for testing).

The actual collection of plant samples can involve several strategies, including random surveys, phylogenetic surveys (Balick 1994), and ethnobotanically directed surveys (Cox and Balick 1994). The random survey has been likened to a shotgun approach, with large numbers of plants put through the available assays. Phylogeny, the study of the evolutionary relationships among organisms, can be employed if one is aware of a plant or compound with desired activity, and then searching its relatives in the plant kingdom to identify additional extracts or compounds that could potentially be of value.

The ethnobotanical approach involves learning the uses of plants by traditional peoples, evaluating their efficacy in an assay, and developing active extracts or molecules based on scientific evidence, as well as a documented history of use for a specific purpose. There are other approaches, but for our purposes these three ways of identifying plants for collection are a reasonable introduction to methods one should be familiar with to be effective in seeking out botanicals that can offer breakthroughs in new product performance and claims.

12.2.2 WHAT HAPPENS ONCE YOU FIND A SPECIES OF INTEREST?

Once a species of interest is identified, the next question for use in the personal care industry is whether the material is available in sufficient quantities to meet market demands, and if so, is scale-up possible within a reasonable time period if demand increases dramatically. Plant-based resources, unlike synthetically produced compounds, take time to grow. Some species can be produced in farms, while others, such as tree crops requiring years to mature or produce, must to be wild harvested. One must also take into account that from year to year the crop may vary somewhat in view of environmental factors.

The purpose of this chapter is to examine some of the biological and ethical issues involved in using plant-derived ingredients. In particular we examine those issues that have been identified through an examination of their use by traditional cultures, as well as providing a brief summary of the concept of ecological sustainability. **It is clear that this segment of the personal care industry is growing,** and the author would maintain that with this growth come increasing responsibilities related to the environment and peoples from whom the raw materials and ideas are obtained.

We describe below a six-element approach for the development of personal care products based on the ethnobotanical approach that should be seriously considered by all companies and individuals committed, or considering becoming committed to this Herculean task.

1) **Accurate identification of botanicals.** It is important that all botanical ingredients in a product, whether in an herbal medication or cosmetic cream, be properly identified. Every product should be prepared using "good botanical practices" including properly collecting, identifying, and permanently documenting the plant-based ingredients. Specifics on the concept of "good botanical practices" can be found in Balick (1999).
2) **Understanding why the plant is used in the product, and what part or form will give the best result to the consumer.** In order to maximize the properties of plant materials used in any formulation, it is essential that each plant ingredient have a specific purpose and utility. Each plant ingredient should be present in its most effective form, as opposed to being added as a device to promote marketing efforts! Educated consumers have raised the bar in the Internet age and both need and demand products that work.
3) **Truthful representation of the local uses of the plant in marketing efforts.** The current application of commonly eaten food plants in shampoos promoted as traditionally used is an example of ethnobotanically based misrepresentation. These products sometimes imply they are based on traditional cultural use when, in fact, they are not. It would be beneficial to have an industry-wide agreement limiting claims of product ingredients

having "centuries-old use" from around the world or by a specific culture, unless evidence can be shown through previously published or firsthand ethnobotanical studies that this is indeed the case.

4) **Making sure the environment is not degraded as a result of harvesting botanicals.** The "greening" of the personal care industry has led to an extensive demand for raw materials harvested from plants. Sometimes these plants are being harvested from wilderness areas without concern for the sustainability of the resources, ensuring that they thrive and can serve as a source of raw materials indefinitely. As a result of the growing demand for plant-based cosmetics, it is essential not to degrade the environment through overharvesting or unwise cultivation practices. The concept of a sustainable harvest will be discussed later in this chapter.
5) **Ensuring that local communities are not negatively impacted by the harvest of the plant.** As people harvest plant and other raw materials and ship them outside of their communities, a shortage of certain species that have traditionally been an important part of the local culture can arise. One example of this is the Brazil nut, now important in international commerce and primarily harvested from community lands. In creating markets for such products, industry needs to develop sources and production methods that will respect the people's rights to access these essential and important plants, which they use for food, medicine, sacred rituals, and other purposes.
6) **Under the spirit and intent of the United Nations–sponsored Convention on Biodiversity, compensation to groups and source countries where the materials and ideas were obtained.** Sometimes, the formula for developing, manufacturing, and selling "natural" products—whether they be herbal medicines or personal care lines—is to utilize ideas from traditional knowledge that have been previously published, and to manufacture these goods using raw materials at the lowest price. The fairest way of doing business in this era, as expressed by the UN Convention on Biodiversity (http://www.cbd.int/), is to compensate people and their communities for the knowledge leading to their use in commercial formulations. This will enable them to benefit from the production of these products, often receiving financial remuneration from sales of the final products, as well as being involved in their cultivation, production, and processing.

12.2.3 SUSTAINABLE PRODUCTION OF WILD-HARVESTED PRODUCTS[1]

Nowadays, the concept of sustainability is used in a rather cavalier fashion, with so many products—personal care and otherwise—being labeled as sustainably produced. In truth, we know very little about the sustainability of production for natural resources, including raw materials for the cosmetics industry, especially

involving products wild-harvested from tropical ecosystems. Yet, it is these exotic products that are often touted as "unique" in an industry searching for the next important active ingredient.

When the organic foods industry was in its infancy, someone observed that several-fold times more organic produce was being sold than was actually being produced; the same can be said for "sustainably produced personal care products" from the rain forest, for example.

The ecologist Charles Peters of the New York Botanical Garden has undertaken many detailed studies of tropical forest trees in efforts to determine the level of sustainable production or harvest of each species (Peters 1996). According to Peters, "a sustainable system for exploiting non-timber forest resources is one in which fruits, nuts, latexes, and other products can be harvested indefinitely from a limited area of forest with negligible impact on the structure and dynamics of the plant populations being exploited." A plant such as *Brosimum alicastrum* (*Moraceae* family), a tree found in Central and South America that is exploited for its protein-rich fruits, needs to produce over 1.5 million seeds to ensure that one tree will live long enough to reproduce. If most of the fruits produced by this species were to be harvested rather than left to grow in the forest, the population could become extinct over time. Similar portents of ecological disaster exist for many other botanical species.

Too little is known about the levels of sustainable harvest of many of the internationally important nontimber forest products (NTFPs), including the Brazil nut (*Bertholettia excelsa*). Some 200,000 people harvest the Brazil nut from the millions of hectares of Amazon forest where it grows, and they annually produce around 20,000 tons for the commercial trade. The harvest of this nut is one of the largest sources of cash income for many of the harvesters. In the early days of the Brazil nut "boom," no one considered what would happen 50 or 100 years in the future, following the harvest and sale of the majority of seeds produced by once-great populations of Brazil nut trees.

Quite simply, the mature, seed-producing trees that are the backbone of the population would die and not be replaced by younger trees, and the resource base on which these industries are built would disappear. This has happened in many areas where this plant is native, as Peres et al. (2003) confirmed in a study of populations of Brazil nuts that had been exploited for many years. Local people and their governments, along with commercial traders and conservationists, now acknowledge that overharvest of this wild plant will lead to its devastation, and today there is greater sensitivity to the importance of ecologically sustainable harvesting protocols being used in gathering this resource.

What, then, are the options for the continued use of NTFPs as a tool for economic development and conservation of biodiversity in the future? Dr. Peters suggests a series of steps for exploiting NTFPs in a sustainable fashion. First, the species to be exploited should be carefully selected, after such factors as the ease of harvesting and resilience of natural populations to disturbance are considered. A tree valued for its roots will be harder to harvest than one valued for its fruits, and the harvest of a species that produces fruits in massive quantities at one time of year will be easier to manage than the harvest of a species that produces fruits sporadically throughout the year.

Once the species has been decided upon, a forest inventory should be undertaken to learn where the resource is found in greatest abundance and the number of productive plants per hectare. Investigators then should estimate the quantity of the resource produced by the species (yield studies) per unit of time, by conducting yield studies of different-sized individuals in different habitats. The next step is to define sustainable harvest levels, and harvest no more than the annual growth of the stem or leaves. The sustainable harvest level of a fruit is more difficult to determine, as discussed above, because a certain amount of seed (to be determined) is required to maintain population structure over time.

When these four steps have been taken, the harvesting of the resource can begin, but the careful measurement should continue. The status of the botanical population should be monitored for signs that the forest is being overharvested. People should examine the status of adult trees periodically to determine whether the flowers are being pollinated, whether large numbers of fruits are being consumed by predators, and so on. If problems arise, the harvest should be adjusted to keep its level below the rate that would threaten sustainability. If such fruits become so popular based on their use in personal care and cosmetic products, then the purposeful and careful management of the resource becomes essential to the long-term viability of the products.

When necessary, people may intercede to replant areas that do not seem to be regenerating, clean out competitive species, or open up the forest canopies to allow more light to reach the young trees and thus speed their regrowth. The precise measurements that Peters recommends can be somewhat expensive and time-consuming, and only a few species have been studied from this perspective. However, plant populations may be threatened if harvests are determined by the demands of the marketplace rather than the needs of the ecosystem. As Dr. Peters notes, "nature does not offer a free lunch." In our enthusiasm to support conservation of the natural world by focusing on its usefulness to economies, we are perhaps inadvertently dooming elements of it to extinction. Peters's book, *Sustainable Harvest of Non-Timber Forest Products in Tropical Moist Forest: An Ecological Primer,* is available for downloading at http://pdf.usaid.gov/pdf_docs/PNABT501.pdf.

An alternate, less technical, but useful analogy for of explaining the concept of sustainability is to think of a wild plant resource as a type of bank account that you require in order to survive, in a situation where you have no other source of income. This account consists of principal and pays some level of interest each year. Your time frame for needing to derive benefit from this bank account is forever, so you need to be careful not to spend the principal, and in fact with some production systems (e.g., fruits) it is important to leave some of the interest that accrues so that the principal does not fall victim to inflation over time. Therefore, with materials such as canes and leaves, you can spend all of the interest (yield) in a given year, while with other materials, such as fruits, you can only spend some of the interest during that time.[2] In years of high interest this is a certain amount of money, but in years of low interest, this is a much smaller amount. Using this model, you will also need to subtract the inflation rate from the interest you are spending and add it back to the principal.

So it is with a natural population of plants that is producing a desired raw material. Use up the principal and pretty soon you have exhausted the resource. If you are harvesting fruits, then a percentage of the fruits must be left to regenerate new plants. If you are harvesting timber, then a percentage of the trees must remain to produce fruits that will develop into new trees, and so on. Determining the allowable harvest scenarios requires the process developed by Dr. Peters as described above.

Only when ecologically sound management plans based on scientific studies are developed for resource extraction will the use of those resources be able to contribute to the conservation of biological diversity as well as economic development and poverty alleviation of the population who are the owners or stewards of the particular botanical resource. It is only by following proper protocols that one can ensure ecological sustainability.

In addition to the ecological issues, there are a number of social issues that must be considered in the wild harvest or cultivation of plant species used commercially. A good example of this is the case history of marula (*Sclerocarya birrea*), a South African tree that is a source of food, timber, medicine, and oil used in the personal care industry. Details are discussed in Wynberg and Laird (2007). The authors describe issues including local laws and customs governing the use of this species, land and resource rights, and monitoring activities. They conclude that a combined approach to improving management that would involve both statutory laws and traditional customs would be most effective for this plant species. Each resource and context is different—all the more reason to look at the utilization and exploitation of a species individually, developing protocols that are economically and ecologically effective on a case-by-case basis.

There is great potential for use of some of the little-known plants found in remote corners of the earth in the personal care industry, but this potential can only be realized if a new and equitable framework for the discovery, harvest, and

trade of these raw materials is developed. We encourage product developers and marketers alike to use the material presented herein as an essential part of product development for the personal care and cosmetics industry. Look deeper than the products' performance lest there be a great commercial success but no raw material to allow for the growth of profits.

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1. This section was originally published in M.J. Balick and P.A. Cox. 1996. *Plants, People, and Culture: The Science of Ethnobotany*. W.H. Freeman, Scientific American Library, and updated for use in this paper.
2. Peters (2001) divides plant products into three categories: vegetative tissues (e.g., bark), reproductive propagules (e.g., fruit), and plant exudates (e.g., latex).

THE HERBORETUM NETWORK FOR PROMOTING LOCAL CULTURES AND BIODIVERSITY

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ABSTRACT

Biodiversity, living womb of the planet, is a pool for a variety of products and molecules of great interest to the cosmetic companies and a source of new plant ingredients. Preserving biodiversity is a life insurance for humankind and future generations, but also for cosmetic and pharmaceutical industries that explore and use the big natural reservoir of the planet.

To preserve and enhance biodiversity with a scientific, economic, educational, and cultural way, nature enthusiasts concerned with its preservation (scientists, botanists, lawyers, industrial players, etc.) came together in 2004 to create the Association of the Herboretum, and to make this "herbs' paradise" an open-air laboratory dedicated to plants for beauty, health, and well-being.

The Herboretum is a large field of thoughts, where we try to give a sense to the term "biodiversity" with a scientific approach that involves observing flora and fauna.

The Herboretum is a large field of preservation, including plants resources and wild plants that are protected by the use of environment-friendly farming practices.

The Herboretum is a large field of action, a learning center for all, where we try to transmit knowledge related to biodiversity and plant world.

To answer market needs and to provide practical solutions to companies that have realized the need to conserve natural resources to maintain their activity, the Herboretum has recently chosen to address these industry challenges by creating a unique interface between nature and phytocosmetics: the Herboretum Network, which aims at promoting biodiversity and local cultures worldwide. The Herboretum Network is a real tool for plant expertise and transmission of knowledge worldwide. It is also a good way for industrial players to promote a social and environmental approach that may lead to the winning of new market share.

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12.3.1 INTRODUCTION

In view of the challenges posed by our lifestyles and consumption styles, the health and beauty industry takes a very close interest in sustainable development and the preservation of natural resources that are used in production.

This is a relatively recent phenomenon. In contrast to the Industrial Revolution, when companies were solely concerned with their economic performance, over the course of the twentieth century corporate entities gradually integrated social criteria in response to civil society. Today environmental pressures increasingly feature in business-development strategies, and sustainable development is the fruit of this evolution.

In order to respond to this awakening of awareness and progression of mindsets in favor of environmental preservation, companies today try to find a sustainable-development model that suits them on a professional level while providing more incentives and integration. The economic argument, which was a barrier to changing opinions in favor of sustainable development, no longer stands up. The environment and the economy are no longer seen as in conflict. Sustainable development brings these two aspects together, and cosmetic and pharmaceutical companies are no longer worried about the cost associated with this change of model. Obviously the purchase of raw materials costs more; however, there are considerable savings in water and energy, and sustainable development adheres to environmental commitments. The argument finally seems to be coming down in favor of sustainable development.

Sustainable development starts with the search for raw materials. Simply buying these raw materials from those that sell them amounts to turning a blind eye to the extraction and production conditions. This can at times cause serious, even

disastrous, environmental problems if we are not careful. The infamous example of palm oil, which is used in biofuels, is one of the most shocking as it has led to the destruction of large parts of the Indonesian and Malaysian rain forest and therefore the loss of immense biodiversity richness.

To be concerned with biodiversity means taking an interest in the production of the raw materials: trusting certifications, imposing regulations on suppliers, and especially picking them up oneself in accordance with environmental and social considerations. This approach requires commitment and is a complex task that pharmaceutical and cosmetic companies are starting to take seriously.

Biodiversity, the planet's living network, is a veritable reservoir of highly interesting products and molecules for cosmetics companies as well as for the search for new plant ingredients. Protecting this biodiversity is life insurance for us and for future generations.

12.3.2 THE HERBORETUM, A TRUE OPEN-AIR PLANT LABORATORY DEDICATED TO PLANTS USED IN BEAUTY, HEALTH, AND WELL-BEING

Nature lovers passionate about its preservation (scientists, botanists, lawyers, industrialists, etc.) in 2004 formed the Herboretum Association, whose mission is to preserve and promote biodiversity, and develop the use of plants in a scientific, economic, educational, and cultural way.

The Herboretum literally means "grass garden," and in ancient Persian "garden" is *paradisia*. In this plant paradise all specimens are highly regarded, as each wild plant contains genetic capital with evolutionary potential. The Herboretum chose the cinquefoil as its emblem, the official plant of the *Rosaceae* family, which since antiquity has been known to have therapeutic properties.

The Herboretum is based in St. Ay, close to Orléans in the central region of France. It is at the heart of the Loire Valley, which is a UNESCO World Heritage Site. Its nine hectares are used for natural purposes and wild plants, as well as plants for cosmetic, medical, and well-being purposes.

a. An area of reflection—a scientific and natural approach

The Herboretum aims to give real meaning to the word "biodiversity"—a neologism whose meaning is often distorted from its original meaning and can be confusing for nonexperts.

Nature is the source of richness, but it is necessary to understand how and why. The Herboretum has chosen to adopt a scientific and natural approach—scientific in order to learn more about the world around us but also to analyze and extract the best of what nature can offer.

Nature is indeed very complicated, its balance complex, and humans have negatively influenced it by practices based on false interpretations that lack a global vision. As an example, people generally prefer a uniformly green lawn, but a lawn is made up of a maximum of just five types of plant, whereas a meadow is just as green while hosting as many as 200, which also provide more diverse shelter for the fauna. Another example: we do not like to see clover plants invading our pretty gardens and covering the bottom of our trees, even though they are a formidable fertilizer machine as they capture the nitrogen in the air and trap it in the soil. Clover is economic, ecological, efficient, and natural. Plants are essential to life; they produce oxygen, make up the basis of our diet, and have infinite resources.

–Observation of plants

With this scientific approach to nature, the Herboretum contributes to the building up of knowledge and cooperates with a "participative" and "predictive" science, thanks to the observation and follow-up of biodiversity principles and guidelines.

From description to knowledge, from knowledge to anticipation, observation becomes a means to predict the consequences on biodiversity of various perturbations, particularly global warming. Understanding the interactions between animal and vegetal species and the phenomena ruling their balance is born of observation. The Herboretum observes the frequency and abundance of these species, the presence of which may be visible thanks to efforts made to help and favor their settling down. All the data collected by a net of observers, among which stands the Herboretum, are then analyzed by scientists who regularly meet at the Herboretum to develop multidisciplinary exchanges for better understanding of biodiversity as a whole. Therefore the Herboretum is a meeting point of expertise on plants. Botanists, agricultural engineers, and professionals from the cosmetics industry come together to discuss the most precious things that nature provides to health and beauty and the conditions of their use.

–Observation of the fauna

In order to help the development of the small fauna in the gardens, five spaces have been organized or protected to favor it. These observation sites shelter indicators that allow us to follow the evolution of biodiversity, letting the Herboretum contribute, as a voluntary observatory, to a "citizen science."

1. The Bird Island

Between the two branches of the river, a small island (hard to reach, which preserves its quietness), has been organized as a Bird Island in order to provide birds with a space where they can easily eat, reproduce, and live peacefully. The planting of shrubs that will bear fruit all year long favors their stay and allows them to be prepared for tough winters. This project is led by the *Ligue de Protection des*

Oiseaux, which, recognizing the nature-valorization approach of the Herboretum, has granted the status of LPO Shelter to the Bird Island. More than 20 different species have been identified, among them the middle-spotted woodpecker, black woodpecker, green woodpecker, great spotted woodpecker, blue tit, coal tit, long-tailed tit, etc., and water birds such as heron, common kingfisher, and wagtail.

2. The Butterfly Meadow

A flowered meadow has been installed next to a nettle field in order to attract butterflies. Its flowers are shaped in such a way that butterflies may access the nectar and, to encourage their reproduction, the nettles, which are adapted for feeding their caterpillars, have been left standing. It has become necessary to take measures to safeguard butterflies, some species of which are becoming so scarce that they have disappeared in some places. Butterflies play a very important role in the pollination of flowered plants. The Herboretum registers the butterflies present on the domain and participates in the Garden Butterflies Observatory launched by the *Association Noé Conservation,* with the collaboration of the *Muséum National d'Histoire Naturelle*. The Observatory focuses on 28 of the most common butterfly species. Among the most beautiful butterflies observed at the Herboretum are the old-world swallowtail, the scarce swallowtail, the silver-washed fritillary, the comma, and the peacock butterfly.

3. The Beehives

Ten beehives have been installed to house bee colonies. These pollinating insects are indispensable for harvesting the many fruits of the orchard. A "bees' meadow" has been planted to provide them with a diversified source of food and may be a survival asset, as today bees are exposed to massive extinctions. This beehive symbolizes the close relationship between the world of flowers and the insects that find a place of balance and preservation in the midst of major cereal crops in the Beauce region.

4. The Bat Cave

Guided by their sonar, bats fly towards the insects they eat. These very useful species are becoming scarce and are included in the protected species. Eight species have been observed on the site, among which is a particularly rare and threatened one, the rhinolophe.

5. The Amphibian Riverside

Frogs—tree frogs (of a nice tender green), precious auxiliaries that are now protected—share the various habitats of the Mauves riverbanks, which are animated by the male songs during mating season. These amphibians are today sharply diminishing because of the degradation of the humid biotopes.

b. An area of protection, a long-term commitment to the protection of plant resources

At the Herboretum we aim to protect biodiversity by using environmentally friendly agricultural methods. This garden of preserved and shared biodiversity is landscaped with a big "garden of gardens," which expresses a diversity of natural or reconstituted ecosystems.

The Herboretum attracts over 1,000 different plant species, including 350 species that are characteristic of the region. Other species, on the verge of extinction, have the status of protected plants in the Loiret department.

The Herboretum primarily prioritizes indigenous species, demonstrating that, beyond environmental issues, day-to-day actions promoting diversity can be implemented in our regions. For example, Beauce hosts a wide diversity of plants particularly relevant to the cosmetic and pharmaceutical companies.

The Herboretum is composed of five ecologic gardens, five thematic gardens, and five faunistic observatories, which together constitute a big arena of biodiversity.

–Preservation of plants used in beauty, health, and well-being

In themed gardens, plant resources used in perfume, cosmetics, and pharmaceuticals are presented through different themes.

1. The Garden of Fragrances

The Garden of Fragrances offers a wide choice of fragranced plants and plants for perfumes, which exude subtle scents according to the seasons and during the day and night hours. Of these, we can appreciate the iris of Florence, the damask rose, honeysuckle, jasmine, fennel, etc.

2. The Garden of the Symphony

The fibers and colors square

This square is dedicated to plants to be woven, and to the tinctorial plants. Among the plants to weave we can see palmetto (*Chamaerops humilis*), hemp, flax, and ramie.

The tinctorial plants, which are the source of natural coloring matters used for centuries by various civilizations to dye vegetable fibers and food, are also displayed under the shape of a painter's palette. These include madder for red, pastel for blue, or gaude for yellow.

The Health Square

This square presents medicinal plants that are used in phytotherapy. This traditional medicine, which uses the active principles of some plants, nowadays raises a renewed public interest. Indeed, plants still have many more secrets to reveal to us; research still regularly isolates new molecules to fight diseases. Nearly 200

different medicinal plants are shown in this square: digestives (fennel, liquorice, wild thyme, sage, etc.); respiratory (mullein, thyme, hyssop, elecampane, etc.); vascular (lady's mantle, butcher's broom, purple loosestrife, etc.); and sedatives (California poppy, violet, goose grass, etc.).

The Beauty Square

The use of plants in the creation of beauty care products goes back to remotest antiquity. This long experience helps the phytocosmetic industry to make the good choice of plants as "safe values" and to use a vast number of medicinal and aromatic plants such as cornflower (soothing and softening), Mary's thistle (antioxidant et anti-redness), the root and leaf of great burdock (astringent and purifying), soapwort (toning and purifying), and sage and rosemary (antiseptics), etc.

The Better-Being Square

The plants of "better-being," usually used as food supplements, also bring benefits to humanity. Valerian or hops bring unwinding and relaxation, while curled dock or winter cherry gives energy and tone.

The Spells' Square

With a reputation of "kind fairies" or "dark witches," the so-called "magical" plants (bryony, hemlock, and columbine) have accompanied humans since remotest times. In the Middle Ages, these plants (and the famous mandrake) gave those who used them uncanny powers that could influence, rightly or wrongly, the course of events. Today, some of them are still at the base of our modern medicines (foxglove, belladonna).

3. The Flavors Garden

The Flavors Garden is a traditional orchard, but also displays aromatic vegetable and cereal plants dedicated to beauty, such as melon or lettuce.

4. The Garden of Regional Cosmetopoeia

This garden shows cosmetic plants that are grown in the Beauce region, including cornflower used for eye care, evening primrose to moisturize dry skin, carrot for its regenerating properties, and oat and meadowsweet for their softening properties.

5. The Garden of Temptations

The Garden of Temptations is organized around an orchard in which one finds about 40 apple tree varieties, 30 pear trees, various other fruit trees (medlar, quince, walnut, etc.), and a wide variety of red fruits. A rustic fence, composed of 14 native woody species, also shows what can be drawn from the local flora.

–Preservation of wild plants

In the Herboretum domain, five ecologic gardens have been preserved. They represent natural habitats that regroup wild plants perfectly adapted to their

environment (humid or dry soil, calcareous or acid soil, in the shade or on a sunny side, etc.). These plants constitute vegetal associations that are characteristic of a habitat in which some plants, named indicative, are well represented. These wild plants are also used by manufacturers of cosmetics and pharmaceuticals.

1. The Mint Meadow

This cool and sunny habitat shelters its cortege of plants adapted to this environment (fragranced mint, great nettle, burdock, etc.). Some plants have even developed strategies to survive in very humid habitats; among them is the bald cypress, which at earth level produces excrescences called cypress knees or pneumatophores, to tap the air that misses its roots. This meadow brings a procession of plants and animals that live in aquatic environments, such the heron, the kingfisher, and the wagtail of brooks.

2. The Orchid Ash Tree Garden

This forest habitat stands on a calcareous soil; it is structured by the presence of ash trees and shelters a shrubbery of viburnum and honeysuckle under which live in a herbaceous layer the plants specific to that environment and some scarce orchids (fly orchid, bee orchid).

3. The Cardamine Riverbank

Growing spontaneously along the banks of the river Mauves are species adapted to this habitat, which is liable to flooding: alder, carex, common comfrey, periwinkle, meadowsweet, coltsfoot, etc., and two species on the verge of extinction: narrow-leaved bittercress and adder's tongue.

4. The Burnet Meadow

This semi-humid meadow, also called mesohygrophile, is a carpet of gramineae among which numerous flowered plants come to grow, showing their flowers in season: cowslip, daisy, etc., then buttercup, great burnet (a small plant with purple flowers), etc.

5. The Cornflower and Poppy Field (harvest plants)

This cereal field has been recreated with its accompanying "harvest" plants, because they live at the rhythm of the harvests: cornflower, field poppy, corn cockle, etc. This kind of landscape has practically totally disappeared from our countryside because of the herbicides that have eliminated these plant species.

The bloom is spectacular: poppy, cornflower, corn cockle, chrysanthemum crops, and others give the image of landscapes that we had before the intensive use of pesticides. Butterflies are kings there; the old-world swallowtail, the scarce swallowtail, the silver-washed fritillary, the comma, and the peacock butterfly are regularly seen.

–An area of action, a place of learning for all

At the Herboretum we aim to pass on knowledge and information on biodiversity and plants; giving a taste of what is beautiful and providing information is one of the Herboretum's vocations. A place for eco-meetings, the Herboretum organizes numerous visits to educational workshops based on the theme of the five senses.

The Herboretum invites the visitor to an emotional encounter with nature that will promote a better understanding of its messages. The pleasure of beauty, source of amazement, goes together with the pleasure of knowledge, source of fulfillment. Beauty and knowledge lead to an awakening of the ecologic conscience and the rousing of the will to act. The ego-citizen becomes an eco-citizen, responsible and committed in favor of biodiversity: a true actor of sustainable development.

12.3.3 THE HERBORETUM ORGANIZES THEMED VISITS OF FOUR DIFFERENT KINDS: SCHOOL GROUPS, THE GENERAL PUBLIC, PROFESSIONALS, AND ORGANIZATIONS

a. School groups

Primary and secondary school groups are invited to the Herboretum in order to show them the richness of biodiversity from a sustainable-development perspective. Teaming with life, the Herboretum enables recreational learning and reflection thanks to an emotional contact with nature that appeals to all five senses. An educational program and diverse themed workshops are offered to children with the aim of raising their awareness.

As well as being educational, the Herboretum approach is interactive, active, and scientific, as it involves observation, handling, and reflection. The activities offered are integrated into the school programs. Where possible, a lesson in the classroom is given prior to the visit. The lesson is adapted according to the children's age level, thus facilitating the transfer of knowledge. The nature outing is adapted to children, who are very curious, have a need to be amazed, and are progressively discovering the world. By discovering biodiversity at the Herboretum, children discover the environment that is all around them and thus change their behavior, becoming more environmentally friendly.

b. The general public

The general public is also invited to the Herboretum. The activities involve guided tours, themed workshops, conferences, and various exhibitions. During the visits the guide presents biodiversity and explains how to protect it. The tour is interspersed with anecdotes in order to capture the visitors' attention while also using aspects of the land to its advantage. Activities are planned on a particular date with a predetermined theme.

c. Organizations

In order to encourage members of companies and organizations to develop more environmentally friendly behavior, the Herboretum organizes conferences and seminars on the protection of nature and regularly gives its analysis on the state of local biodiversity.

d. Professionals

Through the organization of its visits, seminars, and training days, the Herboretum is a real meeting place for plant expertise. Botanists, agricultural engineers, and professionals from the cosmetics industry come together to discuss the most precious things that nature provides to health and beauty and the conditions of their use. The Herboretum and its partners' mission is to change opinions and behavior, and motivate companies to take more responsibility.

12.3.4 THE HERBORETUM NETWORK, A UNIQUE INTERFACE BETWEEN THE PHYTOCOSMETIC INDUSTRY AND BIODIVERSITY

Cosmetic and pharmaceutical companies explore and use the planet's incredible resources as a natural reservoir. However, a significant proportion of the necessary natural substances are found in faraway countries. Following market upheaval and the increasing awareness of the need to protect natural resources necessary to continue their activity, companies must adapt and find sustainable solutions.

Increasingly knowledgeable and demanding consumers have understood the need to consume differently while taking care of themselves and nature at the same time. By demanding simple and natural products they insist on more transparency on products' composition and are concerned about certain substances present (aluminium, phthalates, silicone, etc.). They even seem to be more aware of the production methods used (Are they energy intensive? Water intensive?) as well as the raw materials used (Where do they come from? What is the carbon impact? Is the field fair? Is the sourcing sustainable?). They have become activist consumers.

However, at the same time consumers do not overlook the "pleasure" component, or the efficiency. In fact, the opposite view is taken. *Natural products must be able to offer the same efficiency as those with traditional synthetic ingredients, a criterion that helps to develop a positive attitude promoting innovation for natural formulas.*

In order to meet these needs and provide companies with concrete solutions, the Herboretum has chosen to highlight these industrial issues by creating a unique interface between nature and the phytocosmetic industry: the Herboretum Network promotes biodiversity and local produce on an international level.

The Herboretum Network was thus inaugurated on November 14, 2011 in French Polynesia and welcomed its first member: The "Groupement Interprofessionnel du Monoï de Tahiti" (GIMT) and its Motu Ovini botanical garden. It aims to bring together those who wish to be involved in the preservation of nature, especially the plant world, and who share the same values based on eco-responsibility.

We also point out that large companies are evaluated annually according to CSR (Corporate Social Responsibility) on their contribution to sustainable development. Consequently they deal with partners who adhere to eco-responsible values, a tool that weighs in favor of competitivity.

Bringing together knowledge and know-how, sharing skills and expertise, promoting local plant resources, and increasing the number of action- and awareness-raising centers on sustainable management and biodiversity—all of these commitments make up the heart of the Herboretum Charter, which unites the network partners who thus promote their image and brand through their social responsibility.

The Herboretum Network does not have borders, but is a network of gardens and shared values; a network of cultural diversity and valued traditions; a network of challenges to overcome in the face of the degradation of biodiversity; an intergenerational network promoting the natural heritage that will be inherited by future generations; a network of well-being and odes to the beauty of nature.

A veritable tool of expertise in the plant field and of knowledge transfer on a global level, the Herboretum Network fulfills the role of an interface and provides advice to companies that wish to contribute in the long term to the preservation of local plant resources and the carrying out of environmental projects. It is also a very good way of promoting a social and environmental strategy that could help to win large market shares.

CONCLUSION

The Herboretum and its network, as we have described above, presents a successful approach and model for the cosmetic and pharmaceutical industries worldwide to demonstrate that they respond to market needs, raising their profile while contributing to our planet's well-being as we move into the future.

THE ADVANTAGES AND POTENTIAL CONTRIBUTION OF LOCAL CULTURES FOR CARBON FOOTPRINT REDUCTION

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ABSTRACT

In the cosmetics industry, plants are very often seen as the source of active ingredients in the industry's finished products on the one hand, and as a marketing tool on the other. Indeed, thanks to biodiversity, plants provide us with a largely unexplored and practically inexhaustible reservoir of substances with cosmetological properties, from the active to the formulation ingredient. In a time when we are concerned with the protection of the planet, what better way is there than to use these plants, these veritable micro-factories that use solar energy, take in carbon dioxide, and release oxygen?

However, this plant-world must not be exploited regardless of the cost, but with awareness of eco-responsibility and sustainable development. As such, the producers of plant-sourced ingredients for the cosmetics industry must take certain essential principles into account in order to guarantee quality—as well provide for the safety and sustainability of the products produced from plants. This requires knowledge, awareness, and commitment to:

– An extensive botanical and physiological knowledge of plants;
– An in-depth awareness of the agricultural practices and initial processing;
– The ability to carry out agronomic research.

By following these precepts, *the cosmetics industry can make use of good-quality plants while also respecting biodiversity and being environmentally and human friendly*.

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12.4.1 INTRODUCTION

Every plant and every plant cell is actually a micro-factory that synthesizes thousands of different molecules. These substances represent a broad variety of chemical families, from carbohydrates, lipids, and proteins to more complex compounds such as flavonoids, terpenes, and alkaloids.

Not all these molecules are necessarily of interest to cosmetics manufacturers. In fact, sometimes a single molecule, and more often a small family of molecules or a combination of different molecules having a highly targeted function will offer the desired properties. In other words, only a very limited share of the molecules produced by any given plant is of true cosmetic value.

This being the case, and given today's ongoing focus on eco-responsibility and sustainability, manufacturers of plant-based ingredients for the cosmetics industry must gear their operations to three vital imperatives:

- Producing the plant biomass as close to processing facilities as possible;
- Using plants that synthesize the greatest possible quantity of beneficial molecules;
- Using plants that offer all quality and safety requirements.

To achieve this, the method for producing these plants for cosmetic use has to respect a number of principles that we will discuss below.

12.4.2 THE USE OF PLANTS IN COSMETICS

Greek mythology tells of a goddess, Panacea, who used plants to heal all ills. Her name became the modern word for "universal remedy." For a very long time, people have used plants to treat disease as well as to care for and beautify the skin. There were countless formulations for ointments, balms, and lotions that incorporated specific plants, reflecting empirical knowledge about their effects and properties. However, with the advent of chemical synthesis in the laboratory, traditional plant-based remedies were abandoned.

In recent decades, interest in medicinal plants has revived. Species are once again studied and sought after. This trend has been supported by a combination of factors:

- Cosmetics-sector abandonment of ingredients derived from animals in the wake of Bovine Spongiform Encephalopathy (BSE);
- Campaigns warning against excessive consumption of medications in so-called developed countries;
- A collective realization of the richness of nature and its biodiversity;
- Prestigious studies providing a scientific basis to confirm the plant properties described empirically by the ancients;
- Global awareness of sustainable development giving plants the importance they deserve in contrast to nonrenewable resources.

And indeed, what could be smarter than taking advantage of the extraordinary micro-factories available in the cells of living plants?

- Their primary energy source is sunlight, an inexhaustible resource.
- Their raw materials are:
  - Carbon dioxide, which is recyclable and, moreover, generated by human activity
  - Nitrogen, phosphorus, and potassium, recyclable through rational human intervention
  - Trace elements, which are inexhaustible provided there is no overexploitation
- Their infrastructure is arranged around various specialized organelles:
  - The power plant: during the day, energy is produced by chloroplasts, which are capable of converting solar energy into chemical energy with an efficiency unequaled by humans to date. At night, mitochondria go into action, by respiration using some of the molecules

synthesized by the chloroplasts to produce energy molecules such as ATP (adenosine triphosphate). It is noteworthy that plants are able to store solar energy.

- Biosynthesis units:
  - *Chloroplasts:* structures that synthesize mostly fatty acids, flavonoids, alkaloids, and terpenes
  - *Ribosomes:* the site of protein synthesis
  - *Dictyosomes:* the structures that synthesize polysaccharides
  - *Endoplasmic reticulum:* structure that manufactures more sophisticated molecules by combining simpler molecules. Above all, it serves as the transport and communication system between the cell's various organelles.
  - *Vacuoles:* storage cavities
  - *Nucleus:* the "hard drive" of the cell that contains the memory of all the processes performed by the micro-factory, to allow replication.

Figure 4.1 Biosynthesis units

However, the rush to develop new cosmetic applications for plants must be accompanied by the utmost scientific rigor and environmental responsibility. The actors in this development must never lose sight of the following aspects:

– Which plants should be picked in the wild, and which should be cultivated?

– Where, when, and how should plants be gathered?
– How can plants be processed into a form that preserves their properties and enables them to exert the desired effects?

We will discuss these points in the following sections.

12.4.3 PLANT ORIGIN

a. Name and identification

Plants can be collected from two sources: in the wild or from cultivated crops. Each source has its advantages, but above all, each one calls for certain essential precautions. Whether the plant is wild or cultivated, it must be identified using a number of requisite criteria to eliminate any risk of confusion:

1. Scientific name of the plant

Plant nomenclature uses a binary system consisting of a genus name and a species epithet. Latin is the universally adopted language of plant nomenclature. Valid names are defined by an international committee of botanists. Each Latin name is accompanied by the name of the botanist (in abbreviated form) who first described or defined the plant. For example, the scientific name of German chamomile is *Matricaria recutita* L., where the "L." stands for Carl Linnaeus, the 18th-century Swedish botanist credited with introducing this nomenclature.

2. Plant part used

The specific plant part actually used must be defined. Whole plants are rarely used, and the phytochemical composition can differ significantly between the various parts of a given plant. Such variations can result in different parts of a plant having different effects, or in a risk of toxicity from some parts of the plant that contain toxic substances. For example, the root of comfrey (*Symphytum officinale* L.) is widely used in cosmetics, although the plant's flower contains toxic pyrrolizidine alkaloids.

3. Botanical identification

Those who utilize plants must be able to recognize them through visual observation. Identification is based on the description of the macroscopic and microscopic characteristics of the plant part (e.g., shape of flowers and leaves for the macroscopic description and types of cells and cell wall characteristics for the microscopic description). The identification process also allows any unwanted foreign matter to be detected.

4. Phytochemical identification

This identification is achieved using various chromatography techniques: thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC). Chromatography ensures that plants have a uniform

chemical composition. Chemical identification is a useful counterpart to botanical identification: a nonconforming aspect may indicate another species, adulteration, or mixture with another plant.

How relevant the identification is depends on the representativeness of the sample. Indeed, within a given batch of plants, any contamination may be extremely localized. It is therefore advisable to follow a sampling plan specified for the purpose by the pharmacopeia.

5. Genomic identification

Today, it is imperative to authenticate the species, variety, and geographic origin of plants. Modern techniques centered on the plant genome are being developed and starting to find real-world applications.

It will soon be possible to identify the variety and species of plants using PCR-amplified (Polymerase Chain Reaction) and sequenced short fragments of DNA, especially mitochondrial DNA. The same method will also allow detection of the presence of other plants.

The same imprint can be used to track the plant all along the value chain and through to the commercial product; it can serve as the "signature" of the product. Genomic identification will ensure traceability, detect adulteration, strengthen the brand image, and help support claims of ethical production and preservation of biodiversity.

It will become fully operational once a reliable and extensive database becomes available.

b. Wild plants

Wild plants have been extensively used, although not always in accordance with biodiversity conservation principles. There is no doubt that some plants have become extinct or are endangered due to reckless exploitation by humans.

Rosewood (*Aniba rosaeodora* Ducke) is an eloquent example of this, one that triggered awareness of the dangers of irresponsible exploitation. In the early 19th century, French Guiana was one of the world's largest producers of rosewood essential oil. Production was carried out by traveling distillers who would cut down all the trees within an acceptable haulage radius of their processing facilities. Once they had depleted an area, they would move to an adjacent tract and repeat the process, disregarding the need to regenerate the species by planting new saplings. Without the introduction of protected status for the plant, accompanied by a replanting program, rosewood would have disappeared from Guiana's equatorial forest.

Today, the collective awareness of the need to preserve biodiversity led to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The text of the agreement was first adopted in 1973 in Washington, D.C. As of this writing, it has now been signed by 175 countries.

CITES classifies endangered plants in three appendices according to the severity of the threat of extinction. Appendices cover plants that it is prohibited to pick; those subject to restrictions as to the part picked; and authorizations covering cultivated plants alone. However, the existence of a treaty does not mean that simple common sense should not prevail when picking wild plants that do not feature on the CITES list.

Picking practices must ensure the long-term survival of plant populations in the wild, along with the related habitats. The target species' population density must be sufficient at the collection site.

Picking practices must be nondestructive for both the plant and the environment. For example, when collecting tree roots, the main roots must not be sliced or unearthed. When a species is used for its bark, the latter must be removed only on a single side of the tree, in longitudinal strips.

Those who gather the plants must possess sufficient knowledge of the target species. They must be able to distinguish that particular species from related species and/or species of similar morphological characteristics. They must also be instructed on all aspects of environmental protection and the conservation of plant species. They must understand the benefits for society of ensuring sustainable harvesting of wild plants.

c. Cultivated plants

Efforts to ensure regulatory compliance, safety, and quality coupled with concerns about eco-responsibility and the need to conserve biodiversity all make the use of cultivated plants a wise choice.

Cultivating plants within the framework of contracts between growers and manufacturers is a way to be sure of target species identification and to manage the risk of unwanted contamination. Moreover, cultivation permits varietal selection to breed plants that exhibit favorable agronomic characteristics (productivity, acclimation to the environment, resistance to disease) in addition to optimized concentrations of active ingredients.

With the current focus on reducing carbon footprints, an advantage not to be underestimated is that cultivated crops can be established as close as possible to the industrial processing site. The idea of transporting plants over thousands of kilometers is now as intolerable as it is unsustainable, given that only a very small portion of the plants will actually end up in a cosmetic product.

d. Good agricultural practices

Adhering to good agricultural practices is a form of quality assurance for plant materials destined to become raw materials for cosmetics manufacture. Good agricultural practices can improve the quality, innocuousness, and effectiveness of plant-based finished products. They also aim to encourage and support sustainable cultivation and harvesting of high-quality medicinal plants using methods that promote the conservation of plants and the environment in general. Certain fundamental principles must be followed at every step in the growing of the plant—from seed to seedling, from crop management to harvest, not to mention the role of personnel.

1. Seeds and seedlings

Seeds and seedlings must be of appropriate quality and, to the greatest possible extent, free of contamination and diseases. Quality at this stage promotes healthy growth of the plant.

2. Cultivation

Good agricultural management principles must be applied, including suitable crop rotation to meet the plants' environmental requirements. The emphasis should be on *conservation agriculture* techniques. These aim to conserve, enhance, and utilize natural resources more efficiently through the stewardship of available soil, water, and biological resources, combined with external inputs. This approach helps to preserve the environment and ensure strong, sustainable agricultural yield.

The choice of the crop location must consider the risk of contamination from ambient pollution of the soil, air, or water. If necessary, soils must be analyzed to determine toxic metals concentrations because some plants fix toxic metals selectively. Analyzing these risks makes it possible to limit the concentrations of toxic and heavy metals in the cultivated plants. In addition, the ecological impact of cultivation activities must be assessed and monitored because introducing crops of nonindigenous medicinal plant species can jeopardize the biological and ecological balance of the area.

Crop management practices will be guided by the plant's growth characteristics and by the part of the plant actually being used. In cases of absolute necessity, authorized agrochemicals may be used to protect medicinal plant crops. They must be applied at the minimum effective dose in accordance with regulations in effect. Applications must respect a specified minimum interval between treatment and harvest. Treatments must be recorded in a crop record.

Full compliance with all these conditions will ensure that the levels of pesticide residues in the plants will not exceed authorized limits.

3. Picking

To ensure the best possible quality of plant material, medicinal plants must be picked at the optimal time. That time depends on what part of the plant is being used. Moreover, the concentration of biologically active ingredients varies with the plant's stage of development. The timing of picking is determined by the quality and quantity of biologically active ingredients, rather than by the total volume of the plant part to be picked. The optimal period can be pinpointed by monitoring the concentration of actives over the course of the plant's life.

When collecting the plants, care must be taken to ensure that no foreign matter, weeds, or toxic plants are mixed in with the crop of medicinal plant material. Medicinal plants must be picked under optimal conditions. The material collected must be transported to a drying room without delay to prevent microbial fermentation and mold growth.

To limit the risks of deterioration of the plant, it is important to consider the distance between the plot and the drying and storage locations. The acceptable distance varies with the part of the plant collected. For example, seeds are usually collected dry, so the transport distances can be relatively long. Likewise for underground parts which, moreover, are often gathered in autumn or winter. In contrast, aerial parts picked in late spring or summer require much more limited transport distances—less than 30 minutes.

4. Personnel

Growers must have sufficient knowledge about each medicinal plant, namely, their botanical identification, cultivation characteristics and environmental requirements, and the techniques for picking and storage. They must be instructed on all aspects of environmental protection, conservation of medicinal plant species, and appropriate agricultural practices.

12.4.4 PLANT BREEDING

The growing of plants for medicinal purposes enables the use of certain varieties of a species that are most resilient to environmental, agricultural, and industrial pressures. This plant breeding is mainly carried out in accordance with traditional agricultural methods following two protocols:

a. Mass selection

This is a simple and cheap method that farmers have used intuitively throughout history. It consists of selecting seeds from the plants that respond best to the relative requirements and to replant them the following year. This method has enabled the domestication and improvement of plant species generally consumed by humans. However, very often the initial desired plant characteristics are not preserved over successive harvests.

b. Cross-breeding

This is the reason why more modern techniques are now used and are based on plants' genetic information, even though this is more expensive and takes longer. The aim is to create a new variety with the maximum number of desirable properties (rapid growth, increased yield, disease resistance, high concentration of active ingredients, etc.). This is achieved by cross-breeding plants that, generally, only have one of the desired criteria.

The techniques used will depend on the plants' atomic and genetic nature, and either sexual or vegetative reproduction will be used. Today research into molecular markers helps the selector to have a better understanding of the important genetic characteristics in order to maximize the efficiency of the selection programs. It enables rapid testing of the varieties and retention of those that have the desired characteristics. Selection aided by markers is indeed used to identify the agronomically desirable characteristics, such as yield or resistance to disease.

Plant breeding is an important tool for the future of plant use in cosmetics, an industry that must be exemplary in terms of sustainable development and protection of the environment. This selection must be guided by three major principles:

1. Level of yield and frequency of harvest, which remain a continuous area of research. The higher the production of biomass the lower the production cost and therefore the more competitive.
2. The plants' ability to be autonomous is equally fundamental. For example, the selection of varieties that are disease resistant enables the reduction or elimination of fungicides. This selection of resistant characteristics enables a reduction in material input; that is to say, products will only be used if necessary and at the lowest level possible, thereby reducing the risk of plant contamination.
3. Finally, the technological quality of plants must be at the heart of the selection programs in order to meet the processors' different needs. The concentration of active ingredient must be as high as possible in order to reduce the quantity of plants to dry, transport, and process, thereby significantly reducing the environmental impact. The carbon footprint of plant extracts used in cosmetics is significantly affected by the post-harvest processes such as drying, grinding, transportation, and the process of manufacture of the plant extract. Therefore a higher active ingredient concentration enables the use of less of the plant and reduces the carbon footprint.

The example of cosmetic extracts obtained from the flower of a selected variety of German chamomile (*Matricaria recutita* L.) shows that it is possible to obtain good-quality extracts equivalent to the concentration of active ingredient by using three times less plant mass. Also, the risk of undesirable contaminants is mathematically reduced by three.

Table 4.2

Composition	German chamomile Flower *Matricaria recutita*		Native extract (hydro-alcoholic extraction)		Standardised liquid extract	
	Usual variety	Selected variety	Usual variety	Selected variety	Usual variety	Selected variety
Carbohydrates (polysaccharides)	60%	60%	63%	56%	1.60%	1.60%
Proteins	20%	20%	5%	4%	<0.1%	<0.1%
Lipids	3%	3%	<0.1%	<0.1%	<0.01%	<0.01%
Active ingredients: flavonoids	1.00%	3.00%	5.00%	15.00%	0.10%	0.30%
Mineral products	10%	10%	17%	15%	<0.1%	<0.1%
Solvent: glycerine					98%	98%
Quantity of plant per 1kg of extract					100g	300g

Example of cosmetic extracts obtained from the flower of a selected variety of German chamomile (*Matricaria recutiw* L).

Furthermore, the carbon footprint of the obtained extract for the variety selected is 65% lower than for the extract produced with the usual variety. In fact, the best concentration of an active plant ingredient has a direct effect on the carbon footprint all along the production chain of the extract, from farming to waste reprocessing.

Figure 4.3 Carbon Footprint Example

12.4.5 FARMING METHOD

By whatever means the plants are farmed, the main concern is protecting the environment. In this context the ideal would be organic farming. Today, however, economic competition and at times environmental pressures mean that the organic process cannot always be used without sustainable risks. The unpredictable aspect of yield (and therefore price), the uncertainty of quality vis-à-vis the concentration of active ingredient, and the risk arising from uncontrollable factors leading to the loss of harvest make organic plants a luxury raw material. Only the most committed cosmetic brands that take on the high additional costs of organic ingredients and use it to their advantage, by using the significant added value in their marketing, can permit themselves to use organic plants.

Unfortunately the use of organic plants in cosmetics is very often overstated and they are used in cosmetic products simply to promote the product and not as a veritable active ingredient. The organic cosmetic label requires the use of a certain level of organic plant, which leads the less scrupulous companies to use the least expensive organic plant extracts which have little real effect.

Organic farming, in the domain of cosmetic and medicinal plants, therefore does not yet offer perfect supply security, which is necessary and indispensable to this industrial domain.

However, we can hope to envision it in the relatively near future, following the extensive research currently being carried out on crop management—with the goal of joining the two techniques of sustainable and organic farming.

a. Conventional farming (sustainable farming)

The majority of plants used in cosmetics come from conventional farming. Depending on the country, there is a very big difference in crop quality. Only a few countries, with strictly applied and monitored agricultural regulations, have a sufficiently high security level for risk-free use in cosmetics. For plants from a number of origins it will be necessary to carry out rigorous quality checks based on several criteria:

- Identification
- Falsification
- Contamination from other plants
- Contaminants:
  - Pesticides
  - Heavy metals
  - Mycotoxins
  - PAHs (Polycyclic Aromatic Hydrocarbons)
  - Others depending on origin

Plant processors increasingly demand sustainable farming from their partners, and such constraints mean that only some countries are able to meet such demands in practice. This method of farming has the aim of maximizing the producers' economic output while also controlling the input quantity (especially chemical substances such as fertilizers or other pest-control products) in order to limit their negative environmental impact as much as possible.

Today it is a sustainable production method that enables us to produce good-quality cosmetic plants that have a safe level of contaminants and are economically viable, if the Good Farming Practices are adhered to.

b. Organic farming

Organic farming is based on four major principles:

1. Organic farming must support and improve soil, plant, animal, human, and environmental health—which is one and indivisible.
2. Organic farming should be based on living ecological cycles and systems; it should work with them, emulate them, and help to sustain them.
3. Organic farming should build on relationships that ensure fairness with regard to the common environment and life opportunities.
4. Organic farming should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations as well as the environment.

The organic method of production is notably based on the avoidance of synthetic chemicals and GM products, the recycling of organic matter, and crop rotation. The aims are undeniably tempting, but currently remain largely utopian. They cannot always meet the pressures of a modern and innovative cosmetics industry. Furthermore, in the current conditions of use they do not necessarily guarantee the safe usage of plants vis-à-vis environmental pollutants. Monitoring on this level remains necessary.

12.4.6 INITIAL POST-HARVEST PROCESSING

The plants harvested are said to be in a "fresh" state and contain a variable percentage of water; ranging from 15% for a seed, to about 80% for an aerial part (leaf, flower), to over 90% for the fruit. In order to be preserved without being altered between harvest and extraction, the parts of the plant used must be frozen or dried. After being harvested the plant is very quickly subject to general natural biochemical or microbiological deterioration. The cold or the elimination of water from the plants stops these phenomena.

a. The fresh plant

Extraction from a fresh plant requires almost immediate use, as well as specific precautions in order to avoid deteriorated molecules, which are not initially present in the plant and which could be toxic. For example, this technique can be used in the case of extraction of essential oil where the hydro-distillation machine is used at the harvest site. Furthermore, the use of steam solves the enzyme and microbiological problems.

Additionally, cold temperatures, commonly at or below the freezing level, enables the prevention of enzyme reactions and microbiological fermentations without destroying the source of the phenomenon. The enzymes and the microorganisms are therefore preserved and are reactivated when defrosted, or during extraction, as long as a destruction phase is not carried out. This freezing technique can be expensive if the storage time is long and thus makes the carbon footprint bigger.

b. Dry plants

The drying technique is the most commonly used to preserve plants. However, this must also be perfectly handled. If it is carried out badly or in the wrong conditions it can be dangerous to quality and safety.

Drying allows the quick elimination of water in order to avoid enzyme reactions, which lead to deterioration and microbial growth. The percentage of water generally permitted is 12%. Below this level the quantity of free water is insufficient for the biochemical and microbiological mechanisms. The risk of the occurrence of undesirable, and even toxic, molecules can therefore be handled perfectly as long as the drying is carried out quickly and efficiently at an adequate temperature, which must be a compromise between speed and the heat sensitivity of the plants' active ingredients.

An interesting example of the above is the case of the erysimum, a plant from the *Brassicacea* family, which contains a glucosinolate-type molecule (sinigrin) that has numerous uses. This molecule is stored in the cell's vacuole where it remains stable. During the harvest the plant tissues may be lacerated, destroying the cellular compartmentalization and therefore putting the sinigrin in contact with a degrading enzyme (myrosinase). The sinigrin therefore breaks down into a series of molecules, for certain types into volatile and strong-smelling toxins, among others.

It must be noted that this process is part of the plant's natural defense strategy in the event of attack by parasites (insects) or microorganisms, as the degraded molecules play a repellant or antimicrobial role.

However, for the cosmetic use of erysimum it is vital to preserve the sinigrin integrity, which is molecularly very stable in the absence of myrosinase, in order to maintain its activity and harmlessness. From this deterioration problem point of view the erysimum drying temperature and conditions are optimal at 75°C, the necessary temperature to completely prevent myrosinase activity.

Figure 4.4 Sinigrin % after drying

This example shows the need to carry out the drying in controlled conditions with the appropriate tools. Even though natural sun drying is very environmentally advantageous it does not generally produce good-quality plants, as the oxidation phenomenon accelerates in direct light and climate variations, and external variants cannot be closely controlled. However, the use of drying equipment powered by solar, season and climate permitting, may be an option.

In addition, the artificial drying material must be adapted to the drying quantities and especially must not generate PAH (Polycyclic Aromatic Hydrocarbons) contaminants—highly toxic molecules produced by burning fossil fuels.

c. Storage

The quality of storage is vital for the preservation of the plant's quality. Even if the plant is perfectly dry at the start, it will deteriorate if the necessary hydroscopicity and temperature conditions are not met.

The increase in humidity in the plant can provoke certain plants to develop microorganisms, generally fungus, producing mycotoxins such as aflatoxins and ochratoxins. These are highly toxic molecules, as DL_{50} in animals is very often lower than µg/kg; this is the reason why it is important to have the correct storage conditions.

For certain plants that come from countries with hot, humid climates, notably rich in lipophilic components, systematic analysis is strongly recommended.

It is also important not to neglect attacks from harmful creatures such as rodents and insects. Trapping systems must be implemented in order to avoid deterioration and contamination of stored plants.

CONCLUSION

Today the cosmetic industry can have good-quality plants at its disposal, whether they are picked, or from sustainable or organic farming. However, this quality can only be achieved if every link of this chain is environmentally and biodiversity friendly, which is good for the planet and for human well-being.

Scientific research on plant physiology and genetic makeup, as well as on ecological cycles and systems, now enables us to acquire plants of an optimal quality. Such plants are safe to use, are economically viable, and also adhere to the **ideal key principles of organic farming:**

- Health,
- Ecology,
- Fairness, and
- Care.

COSMETIC INGREDIENTS FROM PLANT CELL CULTURES: A NEW ECO-SUSTAINABLE APPROACH

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ABSTRACT

Phenylpropanoids (PP) are plant secondary metabolites important for plant tissue protection from many different environmental stressors. PP are also reported to possess several highly significant biological activities that make them useful for cosmetic applications. While their antioxidant and anti-inflammatory activity is well known, many of these natural compounds are not available in sufficient amounts in plants, or are poorly standardized for industrial use.

To overcome these limitations, plant cell cultures have been developed from a number of plants such as *Echinacea angustifolia, Leontopodium alpinum* (edelweiss), and *Ajuga reptans*. Development of such cultures provides a means to obtain highly enriched and standardized phenylpropanoid extracts even from plants in danger of extinction.

Following optimization of culture conditions, plant cell cultures can provide a new, highly sustainable source of effective but rare biologically active substances for nutritional, cosmetic, and pharmaceutical applications. This process thus represents a potential turning point in the search for approaches to mitigating the loss of important botanicals by undesirable harvesting as well as reducing or even eliminating undesirable impact on the environment and indigenous peoples.

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12.5.1 INTRODUCTION

Plants and botanical extracts have always been a major source of food, cosmetic, and medicinal ingredients for humans, providing a wide range of biologically active substances. Indeed, not only the pharmaceutical industry, but also those involved in the nutritional and health sectors, use a multitude of substances and products of botanical origin, most of which cannot be obtained by chemical synthesis; and taxol is an important prime example [1]. Although some botanical extracts can be processed to reach highly purified substances, many extracts, even those used for traditional medicine, as a remedy for flu, and for wound healing such as *Echinacea purpurea*, still have a widely undefined and variable composition [2, 3].

In most cases, to provide the amount of plant tissue required to extract the active substances, standard agricultural and cultivation procedures have been adopted and these have generally offered the means to produce the quantities to be used. However, a number of limits intrinsic to this approach have become evident with the continual increase of regulatory demands and safety constraints. The main issues associated with these limits relate to the safety, availability, and standardization of the extracts and, in open field cultivation, all of these issues are deeply affected by still-uncontrolled environmental events. Safety of the extracted products depends upon the amount of pesticides, herbicides, heavy metals, and aflatoxins present on the collected plant tissue. Although many purification and quality-control levels reduce most of the risk, there are still detectable levels of these pollutants in many botanical extracts (4).

Standardization of complex botanical extracts is largely dependent on factors such as harvest location, timing and season of collection, mineral and microflora composition of soil, macro- and microclimate. Most of these are beyond human control. Finally, availability is the main limit for rare plant substances and

low-growth-rate plants since the amount of plant tissue to be collected may exceed sustainability levels and endanger biodiversity.

All of the above-stated limits apply also to the extraction of secondary metabolites. These compounds are essential for adaptation to continually changing environmental conditions, and protection of plant cells from external stressful challenges. However, these secondary metabolites are very frequently present in low amounts in the plant tissue. This is clearly the case of secondary products such as many therapeutically useful terpenoids and alkaloids, but also for phenylpropanoids (PP) and their glycosides—a large family of bioactive substances with several protective functions in plants [5], and medicinal applications as well [6].

One possible alternative to overcome the limiting availability of rare secondary metabolites is the use of plant cell cultures as a source for these natural products [7]. This possibility has been clearly shown to be feasible for the production of taxol, a terpenoid derivative [1], for pharmaceutical use in cancer treatment.

This chapter focuses on the use of plant cell cultures as a unique, comprehensive, and useful source for PP in cosmetic applications.

12.5.2 TRADITIONAL METHODS OF BOTANICAL SOURCING

Humans discovered very early on the benefits of herbal extracts not only as a source of food, but also for health and cosmetic purposes. In Egypt their use can be traced back almost to the earliest period of which burials have been found. Cleanliness, good fragrance, and personal appearance were highly regarded by the ancient Egyptians, Greeks, and Romans—and plants were the major sources for actives, pigments, and fragrances used by these civilizations. To meet all the different applications, a variety of collection, extraction, and storage procedures were set up in different cultures and ages.

For most plants, collection is still frequently made from the wild, although there is an increasing trend for the use of open-field or greenhouse-cultivated plant species, but these latter possibilities have been achieved only for a limited number of plants. The development of domesticated plant species is of great importance since the increased collection of herbal materials from wild plant populations, due to the expanding herbal product market, could drive overharvesting of plants and threaten biodiversity. Poorly managed collection practices could lead to the extinction of endangered plant species and the loss of natural resources.

Following plant collection, the product preparation requires extraction by use of selective solvents to provide separation of the biologically active portions of plant tissues. Most extraction procedures separate the solvent-soluble plant substances and discard all insoluble plant tissues that could make cosmetic formulation quite difficult. The extracts obtained were relatively complex mixtures of metabolites, and could range from liquid to dry powder form following solvent

removal. A variety of preparations known as decoctions, infusions, fluid extracts, tinctures, or powdered extracts have been popularly called galenics.

12.5.3 BASIC PARAMETERS INFLUENCING EXTRACT QUALITY

The basic parameters influencing the quality of a botanical extract are the plant parts used as starting material, the solvent used for extraction, the manufacturing process (extraction technology) used including the type of equipment employed, and the crude plant-to-solvent ratio. The use of properly identified plant material during the balsamic period in which the content of active substances is at the highest level, the use of appropriate solvent and extraction methods, and the adherence to defined manufacturing practices are all of critical importance to producing a good-quality extract. However, highly different extraction procedures have been developed by various cultures. Frequently, highly diverse extracts (in terms of substance content and functional bioactivity) are obtained from the same original plant material. This result points to the need for standardization of the herbal extracts and regulatory harmonization.

Despite the efforts of most manufacturers to guarantee the required quality levels of the plant extracts in terms of safety, availability, and reproducibility—via continual improvements in qualitative and quantitative analytical and extraction procedures—these standards cannot be entirely ensured due to the uncontrolled nature of the environment where plants grow and to a wide range of harvesting and handling processes.

Furthermore, because of the wide diversity of nations, areas, languages, practitioner training, and traditional use, a comprehensive and unified standardization has always been difficult, thus limiting information exchange and development. Although globalization is driving a strong standardization trend on traditional plant extracts for cosmetic applications, in many countries there is still a lack of commonly accepted and applicable information standards. This situation has become a critical issue.

As an alternative to these widespread limitations, a biotechnological approach utilizing cultures of plant cells or tissues could open new avenues in the production of botanical extracts. The technology of plant cell cultures has been endorsed by FAO (the UN Food and Agriculture Organization), which is an alternative method for the production of plant secondary metabolites. This methodology has merit for both pharmaceutical and nutritional applications [7]. Although it is a well-known technique in the scientific community, plant cell cultures have not yet been fully developed on an industrial scale, despite their numerous advantages over conventional methods. Indeed, in another more recent report [8], more than 90 cell cultures have been listed from different plant species as a potential source

of biologically active molecules for commercial use. However, production efficiency was the main limiting barrier for most of them to reach a full industrial development.

Not only are such plant cell cultures able to ensure a high degree of quality and safety, but they are also advantageous in terms of reproducibility in the composition of the finished product. Furthermore, besides being completely environmentally friendly, plant cell cultures also obviate the geographical, climatic, and seasonal variations that plague traditional plant harvesting, and finally, they guarantee a programmable and flexible production process.

Hence, when viewed from the perspective above, the major benefits that could come to herbal extracts from the use of plant cell culture technology are: safety, standardization, and availability.

12.5.4 ADVANTAGES OF PLANT CELL CULTURES: THE NEW ALTERNATIVE

Cultivation of plant tissue in synthetic media offers an alternative way of producing metabolites of interest to the traditional cultivation in fields or greenhouses, and several excellent general introductions to the technology are available [7, 8]. Traditionally, most of this work has been concerned with undifferentiated cells, but differentiated cells (such as in hairy root tissue) have also been cultivated [10, 11].

In plants, continuous growth is assured by the controlled proliferation of plant stem cells present in the primary meristem (apical and root) and lateral meristem tissues. Throughout their life span, these cells maintain totipotency, a very peculiar embryonic feature that allows them to originate all differentiated and specialized adult plant structures. Furthermore, many plant somatic cells present in differentiated adult tissues can reacquire more undifferentiated and embryonic features following specific biochemical and hormonal signals produced by plant tissue wounding [9]. This recovery of embryonic characteristics by wounded adult plant tissues allows for the extensive tissue repair and regrowth of large portions of plant organs.

The growth potential present in adult somatic plant cells is frequently utilized to asexually generate a whole new plant, a process known as somatic embryogenesis [10, 11]. The opportunity to control plant cell growth and differentiation has been one of the major driving forces for the development of plant culture technology. Plant biotechnology has made much progress by basically defining the essential nutrients required in the culture medium and identifying auxins and cytokinins as the major growth regulators controlling plant cells [12]. The plant cells can grow on a solid surface as friable lumps (called callus), or as small clusters of cells in a liquid medium called a suspension culture (Figure 12.5.1). These cells can be

maintained indefinitely provided they are subcultured regularly into fresh growth medium. Both callus and suspension-cultured cells generally lack the distinctive features of most differentiated plant cells and are biologically very similar to the undifferentiated cells found in meristem regions. (See Figure 12.5.1.)

Figure 12.5.1

From a single plant, a number of plant cell line strains can be properly selected based on the most interesting biochemical and morphological features of the cells. This is a long and time-consuming activity but can finally yield a stable cell line that maintains relatively constant metabolic traits. With time, however, as occurs for all living and replicating organisms, some of the cell features will change due to both genetic and epigenetic instability [13]. To avoid these undesired changes, cryopreservation of the most interesting strains is the major approach to properly storing the cell line for long-term maintenance, reproducibility, and industrial backup.

Plant cell cultures have emerged as an important alternative source to whole plants in many fields of research, both literally and figuratively! Unlike field-grown plants, plant cell lines are cultivated independently of climate, soil usage, season, day length, and weather conditions. Furthermore, there is no risk of contamination by mycotoxins, herbicides, or pesticides. Perhaps the most striking advantage of secondary metabolite production via plant cell lines versus whole plants is the simpler procedure for isolation and purification of compounds, especially when the metabolites are secreted into the culture medium [14].

Among various approaches for *in vitro* cultivation of plant cells, research efforts have been focused on suspension cell cultures, as they are more compliant with GMP procedures and they can be easily cultivated in large scale for many industrial and/or pharmaceutical purposes [15]. It has already been shown that plant cell cultures possess antimicrobial activities against a wide spectrum of bacteria and fungi. Furthermore, many pharmacologically active and commercially interesting secondary metabolites such as berberine, codeine, diosgenin, ginsenosides, morphine, scopolamine, taxol, vinblastine, and vincristine have been produced by plant cell cultures [16].

As previously mentioned, many secondary metabolites are essential for adaptation and protection of cells in continually changing and stressful environmental

conditions, and their cellular content is up-regulated as a response to a variety of external stimuli or elicitors. Both abiotic (UV, light intensity, temperature, and osmotic stress) and biotic elicitors (chemicals, bacteria, fungi, and insects or their derivatives) [17] are capable of stimulating the plant defense systems such as the induction of phytoalexin and phenylpropanoid biosynthesis [18]. Treatment with elicitors has gained special interest in many biotechnological fields to enhance the production of secondary metabolites. The use of biotic elicitors for the enhanced production of metabolites from plant cell cultures is already an essential tool to induce secondary metabolite production and has important outcomes for industry.

Upon elicitation of plant cells, one or more signal transduction pathways are activated by ligand receptor interactions that lead to the activation of a set of defense-related genes [19]. In cell culture systems, low elicitor concentrations and short incubation times are sufficient to induce cellular reactions because there are no cuticles or thick wooden walls that could hinder elicitor perception. Treatment of plant cells with elicitors such as chitosan, methyl jasmonate, and salicylic acid induce substantial modulations directed at establishing plant defense reactions and in many cases, result in enhanced production of secondary metabolites such as phenylpropanoids [20]. The diversity of biochemical pathways responding to elicitor treatment may reflect a switch from primary metabolism to primed secondary metabolism leading to the production of defense compounds.

12.5.5 SUSTAINABILITY OF THE BIOTECHNOLOGICAL APPROACH

The plant cell culture technology has also very important advantages over traditional methods in terms of environmental sustainability.

Once the plant cell line is established and optimized for a high content of bioactive metabolites, there is no further need to cultivate other plants in open fields or in greenhouses since cell growth is assured by the water and nutrients supplied to the sterile bioreactor during the process. This has deep and important implications on the use of natural resources as shown by a comparison of the resources, such as water, land surface, and solvents used by traditional and biotechnological methods for the production of 1 kg of echinacoside, in the case of *Echinacea angustifolia* (21, 22).

The production of 1 kg of echinacoside by traditional methods requires the cultivation of *Echinacea angustifolia* plants for three or four years so that the proper root size and the balsamic period of maturation is reached. On the average, roughly 1.3 metric tons of dry roots are collected per hectare (ha) of cultivated land (21). Each dry root contains on the average 1% of echinacoside, but in some cases can reach up to 2% of echinacoside. Taking into consideration this last overestimated case provides

a yield of 26 kg/ha in three years, which means 8.7 kg/ha per year. This provides the final figure of 1149 m^2 of soil used for the production of 1 kg of echinacoside. A similar calculation shows that, in the same period of time, the amount of water required for plant growth (22) for photosynthesis, biomass, plant evapotranspiration, evaporation, and soil percolation provides the amount of 1379 metric tons per kg of echinacoside. Finally, echinacoside also needs to be extracted from the roots, and this requires approximately 500 liters of organic solvents to be used.

When the biotechnological process based on plant cell cultures of *Echinacea angustifolia* is used to produce 1 kg of echinacoside, the surface required covers only 3 m^2 and the amount of water to be used is 1 metric ton, thus saving resources for primary uses and mainly for food production. Finally, the amount of solvents employed to extract the compound is 100 liters.

The difference involved in the use of natural resources is clear and very significant and is summarized in the following Table 1.

Table 1: Natural resources required to produce 1 kg of echinacoside by traditional and biotech methods

Resource required (unit)		**Traditional Cultivation Technology**	**Plant Cell Culture Biotechnology**
Surface	(m^2)	1149	3
Water	(metric tons)	1379	1
Solvent	(kg)	500	100

Source of data are from (21) and (22)

Other important advantages of the biotech process over the traditional approach in the extraction of active botanical substances are the total absence of pesticide use and the fact that no other plant is collected from the environment, which in the case of endangered botanical species, or very low-level substances, is an important element to preserve biodiversity and ecological balance. Finally, there is no need to use fertilizers, which are considered the major contaminants of drinking water.

12.5.6 PHENYLPROPANOIDS: STRUCTURE, METABOLISM, AND FUNCTIONS IN PLANTS

Far from being "secondary" compounds, as their belonging to secondary metabolism would suggest, phenylpropanoids play preeminent biological roles necessary not only for plant adaptation to their environment but also for basic plant functions. As frequently occurs in biological systems, compounds have many different structural and metabolic activities. An example of this is the role of glucose, which is both an essential source for metabolic energy as well as the necessary building block for

cellulose biosynthesis, a structural substance that in turn is definitely required for plant life, and whose evolutionary appearance allowed land colonization.

All PP derive from phenylalanine by enzymatic modifications removing nitrogen and adding catechol functions. Many PP absorb UV radiation, thus contributing to the protection of cells from UV-induced damages [23]. PP in some instances save the life of plants by protecting them from becoming food; thus acting as feeding repellents for insects and herbivores and acting as antimicrobial agents as well [24]. Besides these basic plant lifesaving roles, they improve and support plant life in many other ways. These include: assisting plant reproduction through the color and fragrance of flowers; helping seed dissemination through fruit color, fragrances, and taste; and allowing communication with other living organisms, such as the early signaling in plant-*Rhizobium meliloti* cross-talk, where the plants secrete PP to facilitate the symbiotic interaction between the bacterium *Rhizobium meliloti* to form nitrogen-fixing root nodules [25].

The details of the phenylpropanoid biosynthetic pathway and its complex regulation in plants have been extensively described elsewhere and are far beyond the scope of this chapter.

12.5.7 STANDARDIZATION, SAFETY, AND NEW POSSIBILITIES

All of the above-mentioned environmental benefits of plant cell culture technology are also associated with large improvements obtained in terms of product safety and quality.

The strict control of culture conditions, and the continuous selection of cell lines based on the most important features, considerably reduce the appearance of new variants and physiological aging phenomena. By this means the technology guarantees a reproducible profile of active metabolites, thereby overcoming the impossible-to-solve issue of variability linked to climatic and geographical conditions that occur in traditional agricultural methods. Furthermore, plant cell culture technology allows limits such as the natural biological cycle of the plant and the seasonality of the secondary metabolites to be bypassed, therefore guaranteeing full availability of the constituents at all times. Even the degradation of the active ingredients, which usually occurs during storage of the botanical material, is drastically reduced using this methodology, as the extraction procedure is performed immediately following the conclusion of the fermentation process.

Virtually any component or substance present in the meristem-like cultured plant cells can become an ingredient for cosmetic applications. The most interesting use of this biotechnology to date is the production of phenylpropanoids, a class of polyphenols that are highly concentrated in meristem cells. Echinacoside, obtained from *Echinacea angustifolia,* is an example, but also chicoric acid from *Echinacea purpurea*, teupolioisde from *Ajuga reptans*, or verbascoside from *Syringa vulgaris*

or *Buddleja davidii* are certainly compounds that can provide a large number of potential cosmetic applications and benefits, but are highly limited in view of their availability when harvested by conventional agricultural means. Examples of such cosmetic and personal care applications include anti-aging products for *Echinacea* extracts, lightening applications for verbascoside from *Buddleja*, and antioxidant use for *Syringa vulgaris*.

12.5.8 BIOACTIVE PROPERTIES OF PP FOR COSMETIC APPLICATIONS

PPs and their derivatives are of great interest, especially for cosmetic use as antioxidant, anti-aging, photoprotecting, and antimicrobial agents [6]. Much interest has been recently attracted to natural and synthetic PPs from the cosmetic and perfume industries [26]. Here we will focus mainly on a few bioactivity aspects of high interest for cosmetic applications.

Free radical scavenging, antioxidant, and metal chelating properties of PPs

There is a large amount of evidence that PPs and their glycosidated forms (PPG), like other plant polyphenols, are powerful antioxidants that act either by direct scavenging of reactive oxygen and nitrogen species, or by acting as chain-breaking peroxyl radical scavengers [27]. Polyphenols such as PP with two adjacent –OH groups, or other chelating structures, can also bind transition metals. These include iron, nickel, and copper, in molecularly chelated forms, which are poorly active in promoting free radical chain reactions [27, 28]. Several laboratories have measured the antioxidant activities of numerous PPs such as caffeic and chlorogenic acids as well as verbascoside and found them excellent inhibitors as oxidative radical scavengers in many different assays [29].

Anti-inflammatory and cytoprotective activity of PPs and their derivatives

Numerous publications have focused on the molecular mechanisms of biological activity of natural PPs. Several studies within the last few years have shown that verbascoside, a PP glycoside also known as acteoside, contains caffeic acid and hydroxytyrosol and is present in many plant species as well as plant cell cultures.

Molecular Structure of Verbascoside

This compound suppresses both the cytokine production of IL-8 and IP10 and its effect on the activation of NF-kB (30). Verbascoside is an active PP and has several anti-inflammatory properties. Verbascoside inhibits the activity of the COX-2 [31], iNOS, and nitric oxide production in a macrophage cell line [32]; suppresses the release of arachidonic acid and eicosanoid synthesis [33]; and activates Nrf2-mediated phase 2 enzymes, thus improving self-defensive mechanisms [34]. All these findings provided new insights into the molecular mechanisms involved in the anti-inflammatory activities of this PP. Several plant cell cultures have shown

the ability to produce verbascoside, among them *Syringa vulgaris* (lilac), *Buddleja davidii* (butterfly bush), and *Lippia citriodora* (lemon verbena).

Another extensively studied PP is chlorogenic acid, the ester of caffeic and quinic acids. It is one of the most abundant PPs in human diet and has been reported to decrease the incidence of chemical carcinogenesis in several animal models of cancer. Using the model of TPA- and UVB-induced neoplastic transformation of JB6P+ cells, Feng and co-authors [35] have shown that chlorogenic acid, as verbascoside, strongly inhibited NF-kB and AP-1, decreased the phosphorylation of p38 kinase as well as MAPK kinase 4, and activated Nrf2. The transcription factor Nrf2 plays an essential role in the antioxidant response element (ARE)-mediated expression of phase 2 detoxifying enzymes and stress-inducible genes.

CONCLUSION

The technology based on plant cell cultures, fine-tuned by IRB, offers a potentially unlimited availability of natural substances, even rare ones, that have already been utilized in limited amounts for cosmetic and nutritional applications. This increased availability, combined with a largely improved and reproducible safety profile, a standardized composition, and an extremely low environmental impact as compared with traditional extraction methods, results in the creation of a new possibility to utilize scarcely available, but highly active substances as cosmetic ingredients with truly significant benefits for both environmental and human wellness.

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ECO-RESPONSIBILITY APPLIED TO PLANT EXTRACTION

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12.6.1 SOURCING THE PLANT RAW MATERIAL: CULTIVATION IS KEY

We have seen in a previous chapter how important the various cultivation parameters were to guarantee a renewable safe availability for a reliable raw material. This is why we would recommend to always first select the plants coming from the "local biodiversity." The general approach we are describing will seem simple, but the devil is in the details . . .

Plants are living organisms and subject to all kinds of fluctuations—it is thus very important to carefully check each step of their transformation and perfect it with the view to ensuring the best possible extract. Quite obviously, the quality of a plant extract depends on the quality of the plant it is extracted from.

Cultivated plants should always be the first source to be considered, as the other approach (gathering in the wild) is not sustainable and can sometimes lead to dramatic mistakes.

Also, today, with the Nagoya protocol coming into place, collecting in the wild could have you facing biopiracy lawsuits.

Because the cosmetic industry may wish to take advantage of the actives of plants from various countries, all efforts should be made to promote the local cultivation of the "local biodiversity from elsewhere."

An interface now exists in the Herboretum Network (www.herboretum.org), an association promoting the exchange of information among various gardens protecting local biodiversity all around the world.

12.6.2 TRANSFORMING THE PLANT INTO A "DRUG" TO BECOME A COSMETIC EXTRACT RAW MATERIAL

Traceability starts at the seed level, then the field, the harvesting, the drying, and cutting steps until the powdering stage.

We also have seen that the selection of the part of the plant to be used as a raw material (the so-called "drug" (from the Dutch *droog*—"dried") for extraction was based on the necessity to get the best possible stable source for the active.

Indeed, plants are living organisms, accumulating actives in some specific organ (flower, stem, root, bark, rhizome) according to the vegetation cycle.

Sourcing the right vegetable material starts with the seed (are we sure we will have a homogeneous harvest of a "good plant" rich in this active?), but then goes on with the production stages (the agro-treatments) until the plant reaches maturity. At that stage, it may be important to check whether it contains the actives expected.

The harvest date can thus be scientifically decided, which leads to the following very important steps of pretreatment to keep the actives present as much as possible.

Once the harvest is done, one must actively proceed to the drying, because as soon as the plant is cut, it is subject to all kinds of enzymatic reactions that destroy the integrity of its contents.

The harvested plant should be brought as soon as possible to a drying unit (various systems of dry air ovens have been designed for that purpose).

After this first drying step, the plant must be cut into the part that will be the "drug" (leaves, stems, roots, flowers . . .).

It may be kept under this dried state in a warehouse, provided the temperature and humidity level are controlled.

Once we get to this "drug or purified agro-material," we can proceed to the next extraction steps.

Obviously, this "purified agro-material" needs to be analytically checked prior to its use in order to make sure we start from the raw material we wish to extract our actives from. It enables us to adjust the solvent/drug ratio. This ratio is important for the next steps, as more plant means more solvent, which means more evaporation and thus more energy consumption.

To improve extraction yield, it is a good idea to grind the plant, but it is a better idea to grind the plant just before the extraction process, because if grinding exposes more surface of the plant to the future extraction solvent system, it also exposes that same plant to the air and thus the oxidation and degradation processes.

Grinding just before extracting is thus key to improving the quality of the final extract.

12.6.3 EXTRACTION

We now need to review our options as far as extraction solvents and technologies are concerned.

a. The traditional extractions

They are very old and include the use of:

- Fire, in the first "perfumes" in Egypt for instance: plants (and resins) were burnt and their fragranced smokes and fumes were a gift to the divinities.
- Water, in all the teas, decoctions, and concoctions but also as a steam to evaporate and carry away the essential oils, which are then separated from the "distilled water" (or "floral water") by scooping the upper layer the essential oil forms by floating above the recondensed steam at the last stage of distillation

- Wine, which was first introduced by a Roman doctor of the 2nd century, Galen, who gave his name to this aspect of traditional pharmacy: the "galenic" part
- Alcohol was introduced around the 12th century by Arabs (who also invented the alambic)
- All through the Middle Ages, monks created all kinds of "long life elixirs," blending medicinal herbs and alcohol (Benedictine, etc.).
- Various vegetable oils or butters are used for macerations: this is how the Indian ayurvedic medicine is made.
- Beef fat was also used in the "enfleurage" technique to capture the smell of flowers, which was further extracted by solvents from the initial fat.
- Doctors used teas, decoctions, and concoctions; they also started using "tinctures" (alcoholic extracts).
- Since 1985, studies have shown it was possible to establish a comparison between the actives in teas traditionally used in medicine and the same actives in spray-dried extracts to recommend a scientifically estimated healing dose.

More recently, mainly for the cosmetic industry:

- Various industrial solvents such as propylene glycol, butylene glycol . . . of a pharmaceutical grade
- A propylene glycol obtained by the biofermentation of corn (propanediol)
- Glycerine cannot be considered as an effective solvent for a direct extraction through maceration: it is mainly a carrier, which means it will allow the dilution of an extract obtained by another extraction method. Glycerine comes from locally cultivated rapeseeds, which give an oil when transesterified and transformed.

Generally, we can see that the vegetable "drug" is prepared to optimize the action of the solvent. In many cases, this happens through cutting, grinding, powdering—in a word *exposing* the largest possible surface of the plant material to the action of the chosen solvent. Then, various techniques may be used to further improve the extraction of the actives by the solvent. These include heat, agitation, high pressure, and microwaves in order to make sure the plant material is fully exhausted.

b. The separation steps

We now have a liquid loaded with plant material and actives. We need to separate the solvent loaded with the actives we wanted from the plant material, which doesn't contain any actives anymore and cannot be used in a cosmetic product.

This will happen through various filtration systems, from the teabag to the nanofiltration membranes. In most cases filtration is performed on press filters, which may be sterilizing. It may be performed on centrifuge filters in a discontinuous operating mode. These "separation" systems may include pressure in order to force the actives out of the plant material.

c. The concentration steps

According to the final use of the extract, we have different approaches:

- We can leave the actives in the extraction solvent, which becomes the final carrier of the extract; in this case, the remainder of that extraction solvent will be kept in the plant waste, which makes recycling more difficult.
- We can decide to eliminate part or all of the extraction solvent (by distillation) in order to recycle it. This enables the option of putting the concentrated actives into a new carrier. In this case, the plant material may be recycled more easily; the solvents (water, alcohol) may be reused.
- If we don't wish to purify the extract too much, we can leave it in the extraction carrier. This gives most of the "first-generation cosmetic extracts."
- It may be decided at this stage to use some preservatives to make sure the extract will be germ-free before it is introduced into the final cosmetic product. The inconvenience is that consumers may have a negative perception of the preservative used, which will appear in the INCI listings.
- If we wish to get to a better purification stage, it is useful to evaporate the initial extraction solvents to get to the sole actives and go from there. This is the way followed to obtain eco-responsible extracts.

d. The eco-responsible steps around extraction

Separating actives from the plant material means that you generate a large volume of "exhausted" plant material as a waste. But this waste contains traces of the extraction solvents. It means that this reduces the biodegradability and disposability of this whole volume of waste. Because of the plant/extract ratio, these volumes may be large and create a "carbon footprint" problem for the factory. There aren't so many ways to go around this except choosing the solvents among those that may be easily biodegradable, which leaves us with water and alcohol (ethanol).

Fortunately, alcohol may be produced from renewable vegetable sources (sugar cane, sugar beet, wheat, corn). Of course, using alcohol implies working in an explosion-proof factory with good ventilation and explosion-proof equipment. In this case, we need to go through the basic maceration-separation (filtration) stages and then to a concentration stage to evaporate and then recycle the solvents. Generally, this concentration happens until one gets down to a thick liquid that then goes to

drying equipment. In this concentrated juice, one finds mainly water and actives, the alcohol being distilled into the concentrator and thus made available to be recycled.

e. After extraction and concentration: Drying

The drying process for concentrated extracts may be performed through different systems.

Traditionally, one may use closed stoves under vacuum to evaporate the solvents and bring the concentrated juice to a minimum level of solvents. The problem is that this process is very long (up to 48 hours), which may damage the actives. Furthermore, the "caramel-looking" "cake" isn't very easy to manipulate afterwards and cannot be ground into a powder very easily.

Classically, spray-dying equipment will allow a quick separation between the remaining solvents and the plant material to produce a powder. The major problem with this technique is that the concentrated plant must be blended with a carrier such as lactose or malto-dextrin, which favors the drying process by forming "flocks" that are "sprayed" into the warm air current, eliminating the solvents. Such carriers are diluting down the active while bringing sugars that could prove to be sticky into the future cosmetic emulsion.

Alternatively, one may use deep-freezing techniques, combined with vacuum. In this case, the energy consumption is high, combined with the use of refrigerating gases (which might be a risk to the environment (ozone layer).

Finally, another approach combines low-energy consumption and avoids the use of additives: it is zeodration, where the initial evaporation of water under vacuum at cold temperature (sublimation) is adsorbed onto zeolithes (specific clays), creating an exothermic reaction that will heat an oil that in turns heats the whole volume, which stays under vacuum to allow a full evaporation of the remaining solvents.

The main advantage of zeodration is to obtain a "native extract," all the actives without any additives, which enable their blending into any adapted carrier (glycerine, malto-dextrin, . . .) to give liquid (for cosmetics) or dried extracts (for phytotherapy).

f. Control steps

Of course, extracts are the source of activity in the cosmetic product and need to be identified and standardized.

The initial steps used TLC, then UV spectrophotometry and now routinely, H.P.L.C.

What matters is the "active molecules" contents: it is of course interesting to hear about the "drug-solvent" ratio, but what if the "drug" isn't of the right quality (the species wasn't rich in the requested active) or has been damaged by storage?

The focus should thus remain on the active that needs to be "standardized" to provide a constant ingredient to support the claims.

Extracts should be microbiologically clean. The new perceptions of consumers about preservatives may lead us to use alternative depollution techniques such as flash pasteurization to avoid any initial contamination in the extract.

12.6.4 AN ECO-RESPONSIBLE EXTRACT

Eco-responsibility is a new way to better manage a production with a long-term view. Until recently, the best manager was the one able to produce the best product at the lowest cost. Today, with long-term sustainability in mind, the best manager is the one able to keep the best possible balance among all the various parameters of a production, taking into account what happens before (raw materials), after (impacts of the final product), and during the process: How to improve the process to reduce the energy consumption? How to reduce the waste? How to recycle? How to handle effluents? Is my process generating toxic gases? What hazards are we facing?

It will be extracted from a perfectly traced raw material, ideally coming from a "local cultivation" close to the extraction plant in order to reduce the carbon footprint. The method selected to produce it should be using recyclable solvents to avoid wastage of solvents (as they "stick" to the plant material). Furthermore, the approach with a solvent-carrier method with the petrochemically derived propylene glycol or butylene glycol doesn't allow an easy disposal of the plant waste).

Using a solvent-maceration-filtration-concentration process allows leaving the plant waste with an acceptable level of water and alcohol, which are biodegradable.

The drying technique to eliminate the last drops of water needs to be low in energy. Perfecting each of these steps leads to a lower carbon footprint for the extract, well in line with the future legal requirements in Europe on carbon footprint labeling.

Using glycerine as a carrier avoids the preservatives, as glycerine will not allow germ proliferation. It is also a cell mediator for the actives and contributes to the global performance of the cosmetic product. The viscosity of the formulation of the final cosmetic will not be affected as it would with glycol-based extracts.

12.6.5 CERTIFICATION OR NOT?

Certification is generally a way for companies to advertise their efforts to use "better" grades of ingredients.

Organically grown plants are generally chosen for that purpose. Even though they don't bring any different "actives" as compared to the same plants cultivated in a more conventional way, they are considered as a more desirable source

because they are grown without any chemical fertilizers and haven't been treated by industrial pesticides. The lower impact on the environment to obtain them compensates for the higher cost of production related to labor and varying yields.

"Organic certifications" were initially designed to qualify "agricultural products." To extend that certification to ingredients to be used in cosmetics may sometimes happen to be quite complex. Organic certification of plant-derived materials may concern:

- **The essential oils**
  They are quite easily certified as "organic" because they are obtained by channeling water steam through the plant material, which evaporates the essential oil then to be collected as it floats on top of the water when the cooling process has taken place. As long as the production of the plant follows the rules of "organic farming," the essential oil obtained from this plant is "organic."
- **The floral waters**
  The easiest "organic extract" will be a "floral water" (by-product of the distillation of the plant to gain essential oils). Various approaches are being followed to create "organic floral waters": one stumbling stone is the preservation, as floral waters are made of "distilled water" and organic materials, both of which are prone to microbiological contaminations. Preservatives being mostly "chemical" (thus very typically "not organic"), the list of "useable" ones in "organic floral waters" is very short.
- **Vegetable oils**
  The lipidic fraction of some fruits or plants is traditionally obtained through pressing the plant material to get an oil. "Cold-pressed" oils will suffer less oxidation, even though the final qualities of such oils depend of the quality of the starting raw material and of its storage conditions. Again, being a "direct extraction process," oil pressing opens the door to "organic certifications."

  Of course, some of these "oil sources" could grow in debatable conditions: Is it wise to destroy the primary forest in equatorial countries to replace biodiversity by one main palm oil tree?
- **Plant extracts**
  Various parameters need to be taken into account: source of the "agro-material" (does it grow in a controlled "organic environment"—do we have to consider as "organic" the plants collected in some areas where farmers don't use pesticides nor fertilizers for budget reasons? Are we sure the other parameters have been checked (other contaminations—by heavy metals for instance)?

Then the extraction solvents—but do we need to establish a rule? The use of this or that solvent is prohibited; shouldn't we rather consider whether the level of traces of that solvent are beyond a level recognized as safe?

Finally, the carrier.

► **Other ingredients**
In cosmetics, other ingredients of a plant origin may enter into a formulation. They are the emulsifiers or detergents or the touch modifiers such as the silicone substitutes. Again, the rules around the use of such ingredients are very "technical," which creates a whole world of discussion for specialists, losing the general public a little more . . .

12.6.6 THE GMO (GENETICALLY MODIFIED ORGANISMS) PARAMETER

Of course, the plant ingredient should be coming from the conventional selection method: slow improvement by selecting the plants from the seeds. Fortunately, most of the "medicinal plants" used as a source for cosmetic ingredients are rustic and have been perfected by evolution only.

However, the problem may appear with vegetable oils from "industrial" corps such as wheat, sunflower, palm . . . Cosmetic chemists should be aware that traceability of the ingredients they want to use becomes each day more important.

12.6.7 ECO-RESPONSIBILITY APPLIED TO FORMULATIONS

In the field of cosmetics, consumers are increasingly informed and demanding, and have understood the need to consume differently to care for both themselves and nature. They have become consum'actors. They are seeking simple, natural products, and demand greater transparency regarding the composition of their cosmetics, especially concerning worries over certain substances (aluminum, parabens, phtalates, silicones, etc.).

They are even showing increasing concern regarding the methods of production used (are they energy-efficient? water-efficient?) and the raw materials employed (Where are they from? What is their carbon footprint? Are they produced by fair-trade sources? Is the sourcing sustainable?).

At the same time, consumers do not wish to compromise on the "pleasure" factor, or effectiveness; quite the opposite. Natural products must be able to provide identical performances to those of traditional synthetic components, which fosters a positive impetus in favor of innovation for natural formulas and a strong consumer demand.

A cosmetic product is not a perfumed pharmaceutical cream. It is more than that: it is a substance that needs to include a sensorial dimension: touch, fragrance, feeling . . . Modern cosmetology thus focused on the actives and on the "touch" and was able to design many "cosmetic ingredients," mainly based on sophisticated chemical science, transforming animal fat into detergents or emulsifiers, cross-linking moieties to create new "silicon" touches—in a word, perfecting the final products using all the resources of modern chemistry.

Consumers, however, have been exposed to many other sometimes contradicting messages, not always based on "good science" but biased by tabloids. Unfortunate scandals have tarnished many reputations: the BSE epidemy, the scares around "placenta being used in cosmetics coming from HIV infected women," the general anxieties around the pesticide-loaded food reducing masculine fertility, the promotion of "organically grown food," the articles on cattle raised eating hormones or antibiotics . . .

The initial trust in perpetual progress, which had brightened the future of the populations in 1850 with the "industrial revolution," turned into a suspicion that progress wasn't a sure thing after all.

All of this is now combining with new technologies that give consumers a better access to the information they feel relevant: it all started with little books giving explanations about INCI names; it was then perfected with the direct and speedy access through flash-codes to the fully commented INCI list, stating whether this or that ingredient was a "no-no" according to your own standards.

This of course has led to many changes in the formulation choices for ingredients. Natural cosmetics are no longer the combination of a "chemical base" blessed as natural by the addition of a 0.5% chamomile glycolic extract!

New efforts are now being made by various companies to provide the industry with new more natural and greener ingredients, obtained by "clean chemistry" methods.

a. Oily phase

1. Oils

The first relatively easy step has been to switch from mineral oils to vegetable oils. It wasn't that easy, because mineral oils and mineral oil esters have been perfected to show excellent features: no color, no smell, stability, no oxidation, and they are competitively priced. The manufacturing of vegetable oils on the contrary had to be improved to reach higher cosmetic standards. Ironically, the cosmetic industry ingredients comply with REACH while food-grade products don't. Nowadays, vegetable oils used in cosmetics show lower peroxide indexes, fewer color variations, and less smell than the first oils offered to the cosmetic market 20 years ago.

New "exotic oils" appeared: urucu from Brazil, kukui from Hawaii . . .

A replacement for whale fat was introduced as jojoba wax, initially growing in the desert of Arizona, today cultivated in the Neguev desert of in Argentina.

Then, in order to gain viscosity and get "richer touches," vegetable butters were introduced, together with vegetable waxes.

It was a progress of international trade as such ingredients came from Africa (shea butter, ilipé butter).

All these ingredients are natural alright, but are they sustainable in the sense that they have to travel long distances to be used by the various cosmetic industries, which impacts their carbon footprint.

Such considerations have led the Cosmetic Valley, in France, to launch studies with the view to better exhaust a vegetable biomass to gain various derivatives: it is the Granolin project, which will reintroduce flaxseed cultivation into the central region of France. Flaxseed may be pressed to produce an edible-grade oil, rich in linoleic and linolenic acids, but which may show interesting skin care properties. At the same time, the fibers may be used as an insulation in concrete walls or panels (muffling sounds and protecting against temperature variations).

2. Vegetable oil and vegetable oil esters

Another interesting plant has been studied with a philosophy of the "global use of the biomass": milk-thistle, which grows around Chartres. The seeds give silymarin, a liver-protecting medicine. After silymarin extraction, the "cake" of their seeds is further processed to get the milk-thistle oil, which is rich in omega-6.

Further transesterification (an Ecocert-acceptable process) gives a mono-ester that brings an interesting touch to the whole emulsion or shampoo and could be a good substitution for more currently used silicones.

3. Antioxidants

If we look into vegetable oils, we need to look into antioxidant systems. Fortunately, plants are solar machines and have all developed specific antioxidant systems. One of them is to be gained from rosemary and performs very well in all the oily phases. Such approaches show that there is a good future for promoting "local" sources of biodiversity riches.

b. Water phase

With the "labeling issues" in mind, one may envision introducing various ingredients into this water phase to increase the "natural" or "organic" qualification rate.

Floral waters but also plant infusions may be replacing the classical “deionized water” or the “thermal or source water” (because transporting water may prove to be less sustainable). Plant extracts of course are easy to use in such water phases.

The most important part is the preservative system: in the same way plants have developed their own antioxidant systems, they also have created their own preservative systems.

A very interesting natural (but on all positive lists of preservatives) preservative is salicylic acid (extracted from a natural source and gained through “clean-chemistry” accepted methods), which shows astonishing results on aqueous systems and excellent performance in emulsions.

THE INDUSTRIAL FRAME: CONCRETE, GREEN SOLUTIONS FOR PRODUCTION AND WASTE MANAGEMENT

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ABSTRACT

Can anyone not feel concerned about environment protection today? Endangered plant and animal species? Global warming? Melting glaciers? These recurring subjects are increasingly covered by the media and are a matter of general acknowledgment. One cannot continue to live in our world without focusing on these issues, which are crucial for the future of our children and, in the long term, for the survival of the human race. In order to protect nature, know it passionately, and use it reasonably, we professionals must reflect daily on the best way to adapt our industrial means of production to meet the demands of environment protection. Our efforts in terms of ecology are made at each step of the production chain, and there are numerous solutions for organizing industrial production in an eco-friendly way.

Industrial players today can commit to protecting the environment on various levels of their activity—by establishing an eco-design approach for ingredients and products, by developing nonpolluting products and processes, by sourcing renewable raw materials (and no unmonitored harvesting), by respecting ISO, HACCP (Hazard Analysis Critical Control Point) norms for food products, and by using GMPs (Good Manufacturing Practices) for all products. They can abide by the European Cosmetic Directives and REACH (to measure the impact of substances on the environment), using environment-friendly materials such as recyclable boxes, biodegradable inks, corn-starch packaging, etc. They can also reduce and recycle waste, thus saving and recycling resources such as water.

Thus we present here two examples of concrete and eco-responsible solutions adapted to the industrial frame: zeodration, an environment-friendly top-quality technique, as an alternative to conventional drying processes for plant extracts, and water and biodiversity gardens, an original innovation to restore wetlands in industrial areas.

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12.7.1 AN EXAMPLE OF AN ALTERNATIVE, ECO-FRIENDLY PROCESS FOR PLANT EXTRACTION: ZEODRATION, A UNIQUE ECO-RESPONSIBLE SOLUTION TO DRY PLANT EXTRACTS

Dehydrating a product is quite easy nowadays: there are several techniques and all have their own advantages and disadvantages. Two main techniques are commonly used:

- **Under vacuum**, such as lyophilization (deep-freeze drying), which uses very low temperatures (with ice sublimation) during a long period of time, and is very demanding in energy
- **Spray drying**, taking place at a very high temperature during a short period of time and generally needing a carrier (most often malto-dextrin)

By permanently focusing on improving extracting processes, the Alban Muller Group uses innovative and economical energy tools, such as the zeodration drying technique. Zeodration seems to be the perfect, eco-responsible alternative drying technique as it produces little discharge, which is mainly water. Therefore the process is thoroughly clean and eco-friendly. It has also a very low energy consumption as it uses the heat produced by the exothermic reaction. Furthermore, due to the total absence of refrigerating fluids, the continuity of the process is ensured, whatever future environmental regulations may request. Zeodration is a high-quality alternative, environmentally friendly and implying fewer constraints.

a. The principle

The product is placed in a drying unit and put in vacuum. The depression creates a decrease in temperature down to about –20°C, which provokes the evaporation of the water contained in the product. Steam is adsorbed on zeolites, a phenomenon

that naturally generates an exothermic reaction. The energy produced is collected by exchangers and used to heat the product during the drying cycle. Hence zeodration implies a very low energy consumption. The reactors containing zeolites are regenerated once they are saturated with water.

In nature, zeolites are kinds of crystallized clays that form from volcanic ashes. Their water adsorption capacity can reach 30% of their mass and their regeneration temperature is 250°C, their residual water being 1 to 3%. The process uses synthetic, very pure-grade zeolites with pores of 4 ångströms in diameter, the same size as water molecules. Pore calibration works like a selective trap, allowing water molecules only to penetrate and excluding larger molecules such as aromas, pigments, or other volatile solvents.

The conditions of temperature implemented (≤40°C) guarantee that zeodration is a dehydration technology perfectly suitable for all plant extracts—liquid or semi-liquid—for cosmetic, pharmaceutical, or food applications. It allows for fully respecting the structure of the actives, especially those that are heat-sensitive, while preserving the organoleptic properties and the best possible solubility of the extracts. At the end of the drying cycle, "native" extracts (without carrier) are obtained. After being crushed and sieved, they can be processed for a wide range of uses.

In some cases, adding an adjuvant may be necessary to stabilize the product and to standardize the batches. If maltodextrin is used, the extracts are similar to those obtained by spray drying, aside from the fact that the powder is thinner and the color often lighter. Varied adjuvants can be used to get an easily compressible powder to make tablets, to improve the free-flowing ability of an extract, or to avoid adding carbohydrates, for instance.

b. Ecological advantages

Zeodration produces little discharge, which is mainly water. Therefore the process is a thoroughly clean and eco-friendly process. It also has a very low energy consumption, as it uses the heat produced by the exothermic reaction. Furthermore, due to the total absence of refrigerating fluids, the continuity of the process is ensured regardless of future environmental regulations.

As we are concerned about the environment from the beginning of a product's life, we can find solutions such as the zeodration technique to optimize a manufacturing process, but also to preserve the reservoir of biodiversity for its raw materials and water.

12.7.2 WATER AND BIODIVERSITY GARDENS AN ORIGINAL INNOVATION: RESTORING WETLANDS IN INDUSTRIAL AREAS

Since its creation in 1978, the Group's policy has been based on its initial and founding pledge: respect for humanity and the planet. The company is permanently aware of how it draws on the reservoir of biodiversity for its raw materials and water and is determined to help preserve this ecological capital, the source of its economic growth.

a. The project's origins

Businesses are intricately linked to what biodiversity provides and how ecosystems function. Through their activities, they weigh heavily on the causes of erosion in biodiversity. In the face of irrevocable scientific observation, the issue has become crucial: by 2050 a quarter of the world's species will have disappeared if nothing is done.

Wetlands are the most threatened areas (50% have disappeared in France, according to the biodiversity survey). Conscious of this threat and considering that the situation is neither unavoidable nor irreversible and inaction unacceptable, the Alban Muller Group decided to contribute to the restoration of wetlands in industrial areas. The Group purchased 4,500 m² of land adjacent to its production plant to create water and biodiversity gardens.

Every day, 100,000 liters of water are used by the industrial site for plant extraction and cosmetic production. How can we give this precious resource back to nature? After the effluent has been treated in the large waste areas next to the plant, the water is returned to the recovered ponds and wetlands.

The project began in 2007 and lasted a year: the basins and water channels were dug. The water used in the manufacturing processes is treated upstream, then stored in huge vats before being sent to the recovered gardens.

Once the effluent has been treated, the water is returned to nature. These wetland gardens have helped restore the biodiversity environment and contributed to the development of a wide range of animals.

b. Resources implemented

These wetlands were recovered with, in particular, the introduction of specific plants (reeds, ranunculuses, wild irises, lilies, bamboos, etc.) and the creation of habitats to draw animals to the area. Three beehives were built to encourage pollination, along with an uncultivated area specifically designed for bees on the remaining industrial wasteland to ensure a regular pollen supply.

Surrounding the gardens, wooded hedges of bushes and shrubs form green corridors that offer shelter and cover for small animals while fertilizing the soils. These hedges also serve as windbreaks that protect the wetland environment.

c. The return of animal biodiversity

By re-creating the wetlands, our water gardens have witnessed a spontaneous return of flora and small animals to the recovered habitats. The new riverbanks have encouraged the return of amphibians (frogs, tree frogs, newts, etc.), the most endangered species because of their disappearing biotope. Insects, birds, and small mammals have rediscovered habitats where they can reproduce, protect themselves, feed, and shelter.

d. A sensory environment conducive to awareness

The aesthetic principle behind our water gardens is that of a landscape composed of ponds and channels surrounded by plants, the whole generating balance and harmony. Organized around a theme of the five senses through a specific selection of plants, the gardens offer a sensory experience and an introduction to biodiversity. Our employees thoroughly enjoy these gardens, which play a role in improving the working environment and their well-being. Mindful of the garden's evolution, they help track the plant and animal development.

One of our key objectives is raising the awareness of our different client-partners and suppliers to environmental issues. Members of international firms, institutions, and delegations have visited our gardens, drawing inspiration from what we have achieved.

MANUFACTURE OF COSMETICS

MANUFACTURE OF COSMETICS SECTION OVERVIEW

As you may have noticed, this part of the book has been placed near its end. We have discussed many things in the world of cosmetics and personal care such as ingredients, anti-aging theories, and substrates including, but not limited to, skin and hair. We have delved deeply into formulating, marketing, intellectual property aspects, and global regulatory issues, to name but a few of the important areas one needs to understand as state of the art in this, the 9th Edition of *Harry's Cosmeticology*.

As we near completion of this work, we have one more major topic (besides packaging) that needs to be covered.

It is my opinion, that no matter how novel your ingredient, or its delivery system, or its formulation, sensory impact, fragrance, packaging etc., once your prototype product gets out of the lab, it must be scaled up successfully or all the work that has led to a product about to become a Brand will be for naught.

Scaling up is usually a two-step process; first to pilot scale and then to full-scale production. It is my experience that these transitions are typically fraught with angst. Some of it arises from the unfortunate fact that the ability of both laboratory developers to understand and work with individuals who are charged with making their products on a larger scale are often lacking the fine edge of successful communication. This observation, by the way, is a two-way street—for the large-scale people must also understand the laboratory people.

Even the distinction between "chemist" and "chemical engineer" creates a schism in the context described above.

To be complete in our vision to truly survey cosmetics and personal care, we must inevitably provide an understandable foundation of what the issues and approaches are when "scaling up."

To this end, we have engaged Bruce Victor and his team at Estée Lauder to provide the necessary training. Bruce is uniquely qualified for this daunting task, since he and his associates wrote the 8th Edition of *Harry's* on this subject!

Since so much has changed since the 8th Edition, you will find an enormous amount of new technology to learn about and understand. It is also my opinion that without knowing something about this area, and being able to use the information to successfully interact in the trek from concept to lab to pilot plant to full-scale production, your greatest product concept is doomed to failure.

It is with pleasure that I present the cosmetics manufacturing section by Bruce and his team. I have also taken the liberty of including two other chapters in this Part. These deal with novel emulsification and extraction technology and stand on their own, distinct from the chemical engineering, equipment, and approaches to manufacturing the many different forms of cosmetic and personal care products elsewhere in the book.

Enjoy . . .

And Learn . . .

Meyer R. Rosen

Editor-in-Chief

PART 13.1

COSMETIC MANUFACTURING PROCESSES

Editor: Bruce Victor

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ABSTRACT

Delivering the intended benefits to the consumer is the key goal for the formulating chemist. Success will help the company to grow the business. This objective is best achieved when the process used in production is controlled and reproducible. A formulator who is knowledgeable of scale-up techniques and the use of both pilot and production equipment has an advantage when the product moves out of the laboratory and into production. Armed with this knowledge, the formulator can best simulate the plant process and plan bench work effectively to ease the scale-up process and help ensure a smooth transition.

This chapter provides a brief understanding of the more important unit operations—mixing, heat transfer, and mass transfer. This is followed by a discussion of equipment, procedures, and principles commonly used in the cosmetics industry to manufacture and fill both wet and dry products. Also included is a brief discussion on drug (active) delivery systems and their special formulation/process requirements and interactions. This leads to the concept of formulations based upon the process instead of a process designed to handle the formulation. The sections on scale-up of both wet and dry products are particularly important since product quality and stability may be adversely affected as the process progresses from bench to production. For this reason it is recommended that the formulator plan the use of bench equipment and procedures carefully with pilot and full-scale limitations in mind.

This background, combined with time spent in both the pilot and manufacturing plants, will better equip the formulator to carry out successful new product introductions.

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13.1 INTRODUCTION

This chapter begins by providing a brief understanding of the more important unit operations, namely mixing, heat transfer, and mass transfer. This is followed by a treatment of equipment, procedures, and principles commonly used in the cosmetics industry to manufacture and fill both wet and dry products, including some newer approaches to the emulsion system concepts. Knowledge of this background, combined with time spent in both the pilot and manufacturing plants, will equip the effective formulator to carry out successful new product introductions.

The sections on scale-up of both wet and dry products are particularly important since cosmetic and personal care product quality and stability may be adversely affected as the process progresses from bench to production. For this reason it is recommended that the formulator plan the use of bench equipment and procedures carefully with pilot and full-scale limitations in mind. We highly recommend formulators to generate a purposeful coordination and communication with pilot plant and full-scale manufacturing experts in order to achieve a mutual understanding that each approach, from bench to full-scale, has its own inevitable strengths and weaknesses and it is only through excellent communication that successful production will be achieved.

Formulating cosmetic and personal care products is an ancient art. Originally, these products contained ground minerals in an oil or grease. They were initially used by men to exaggerate their features during battle, to conduct tribal ceremonies, and to differentiate different tribes or clans. The use of cosmetics by women began in ancient Egypt. Color products accentuated facial features; plant and animal essences provided a scent to the hair and body (minimizing body odor); and greases and oils were used to treat the skin. Skin care did not really change over the years and typically involved the application of natural oils or glycerin and rosewater preparations.

A Brief Chronological Survey

The first widely used cosmetic dates back to AD 200 when the Greek physician, Galen, published a formula that contained only rose water, beeswax, and olive oil. This formula remained essentially unchanged until the late 1800s, when borax was added to the basic formula to form a simple cold cream preparation. The principal cleansing agent at the time was a lye-based soap made by mixing potash or lye with animal or vegetable-based fats. The art of soap-making became more refined throughout the second millennium.

The era of modern cosmetics emerged in the 1940s with the widespread use of synthetic surface-active agents. These materials, commonly called surfactants, modified the surface tension of the oil and water phases and enabled the formulator

to mix them together to form a composition that was stable for at least the commercial shelf life of the product. These preparations were called emulsions and the surface-active materials used to form them were called emulsifiers. Personal care products continued to evolve throughout the latter part of the 20th century. Raw material manufacturers improved aesthetic sensations through the use of new, more refined natural oils and synthetic emollients, thereby allowing product forms to become more diverse. Traditionally skin care products were moisturizers. They were used to treat dry skin by plasticizing and softening the hard, rough, tight, scaly manifestations of damaged skin.

In the late 1960s and early 1970s functional skin treatment products emerged. Product performance expanded beyond the amelioration of superficial dryness, and benefits evolved to a higher therapeutic level. The boundary between cosmetics and dermatological drug products began to blur. Problems such as aging, uneven skin pigmentation, slack skin, cellulite, sensitive skin, oily skin, and dryness were identified and agents were sourced, or developed, to address these conditions—most as cosmetics, some as dermatological (drug) products. These skin disorders were often associated with conditions such as sunburn, acne, or the need for topical analgesia, etc. The personal care market started to borrow from the latest advances in medical research. Processing these new formulations has become even more important to maintain the required aesthetics and stability required by the consumer.

In the 40 years since the late 1970s, formal chemical engineering concepts began to be introduced into the manufacture of cosmetics and personal care products. There appeared a schism between formulators (chemists) and process engineers (usually chemical engineers). Their scholastic training overlapped, but communication within an organization was often far from perfect. Yet, while these disciplines can be very diverse, the successful development of commercial cosmetics requires a smooth transition from bench to pilot to full-scale production.

Within the recent past, OTC (over-the-counter drugs) protocols have added multiple layers of regulations and paperwork to be handled from both R&D and Production. Anti-dandruff shampoos, antiperspirants, and sunscreen products are much more common. Also included in these changing times is the emphasis on "organic" and "natural"—two words that indicate potential issues related to raw materials (consistency), formulation (control of aesthetics), and process (control of the uncontrolled).

In this chapter, we focus primarily on the chemical engineering principles without which pilot and full-scale manufacture would be nonexistent. In the spirit of *Harry's* long history of explaining concepts in simple terms, we have taken the approach of examining the areas most important to gaining a true understanding of what it takes, at the full-scale production end of the process, to generate mass-scale

production of products creatively generated in the laboratory—both through the creative thinking of "ingredient-seekers" to formulators to (and from) marketers. We seek to acknowledge all of the people who contribute to making new products and brands successful.

We begin with Unit Operations, the heart of understanding the basics of full-scale production.

13.1.2 UNIT OPERATIONS

When studying scale-up operations, wherever they originate, and no matter how complex, one considers a limited number of physical phenomena. These are separated into discrete yet interdependent events called "unit operations." The manufacture of cosmetics can be described in terms of four operations:

- Fluid Flow / Mixing
- Heat Transfer
- Mass Transfer and
- Filtration

All phases of cosmetic manufacture involve aspects of each.

Fluid Flow attempts to define operations involving movement of fluids and solids exhibiting fluid-like behavior. Mixing and agitation, pumping, and metering of liquids are typical operations studied in fluid flow.

Heat Transfer involves the movement of thermal energy in a gradient from areas of higher temperature to areas of lower temperature. Heating and cooling of cosmetic bulk by conduction, convection, and radiation are studied.

Mass Transfer operations define phenomena that involve movement of components of a mixture in a concentration gradient from areas where the concentration of a component is high to areas where it is lower. Cosmetic processes can make use of mass transfer by absorption (e.g., addition of binder to a cosmetic powder) and diffusion (e.g., migration of an active ingredient). The formation of an emulsion itself can be described in terms of mass transfer.

Filtration is not usually a unit operation of major importance in cosmetics manufacture except in the production of spirituous preparations (astringents/toners, colognes, aftershaves, and perfumes). It is possible to regard filtering as unmixing, and certainly the flow characteristics of the filtered product are again of prime importance. Filtration is usually included in the process to ensure clarity. The use of sub-micron filters for the sterilization of water is discussed elsewhere in this chapter.

Each of these four unit operations involves mathematical treatments that are, perhaps, inappropriate for this book and not pertinent to our practical use of the operations. Their theoretical treatment can be found in any good chemical

engineering textbook, such as *Unit Operations of Chemical Engineering* by McCabe and Smith.

Mixing and heat transfer are the most critical operations and will be discussed in some detail [1].

a. Mixing

The subject of bulk cosmetics manufacture revolves around satisfactory mixing. There are several types of mixing employed as indicated in Table 13.1 below.

Table 13.1: Scope of mixing operations within the cosmetics industry

Type of mixing	Examples
Gas/Liquid (a) Cohesive (b) Segregative	(i) Dispersion (aeration and gasification) (i) Deaeration or degassing
2. Liquid/Liquid (A) Miscible (a) Cohesive (b) Distributive (B) Immiscible (a) Cohesive (b) Distributive (c) Segregative	(i) Chemical reactions (formation of salts from acid and base) (ii) Blending (spirituous preparations, clear lip gloss products) (iii) Pumping (low-viscosity system) (i) Blending (flow controlled) (ii) pH control or soap formation from fatty acid and base (iii) Pumping (high-viscosity system) (i) Emulsion formation (dispersion–addition rate is not critical) (i) Emulsion formation (dispersion–addition rate is critical) (i) Coalescing / Settling (phase separation)
3. Solid/Liquid and Liquid/Solid (a) Cohesive (b) Distributive (c) Segregative	(i) Dissolution (of water-soluble dyes, preservatives, powder surfactants, etc.) (ii) Suspensions and dispersions (mascara, pigments in castor oil and in other liquids) (iii) Hot pour products (lipsticks, etc.) (i) Controlled addition of liquid binders or actives to powders (i) Filtration, sedimentation, decantation
4. Solid/Solid (a) Segregative (b) Cohesive	(i) Free flowing powders discharged from hoppers, etc. (i) Face powders, eye shadows, and all dry mixing

Types of mixing:

Cohesive mixing - natural or forced combining of particles during blending
Segregative mixing - natural or forced separation of particles during blending
Distributive mixing - controlled or uniform diffusion of particles during blending

Table 13.1 represents a convenient way of classifying the mixing processes most commonly found within the cosmetics industry. Almost every cosmetic manufacturing process includes at least one mixing operation and often more than one type is involved. For example, the manufacture of a pigmented emulsion-based foundation cream may include:

(i) Preliminary dry blending of pigments and excipient (type 4b- i).
(ii) Dissolution of oil-soluble and water-soluble materials separately in their appropriate phase (type 3a- i and type 2Aa- ii).
(iii) Dispersion or suspension of pigments in the oil or water phase (type 3a- ii).
(iv) Mixing of the two phases to form an emulsion, possibly with the formation in situ of soap as part of the emulsifier (types 2Ba and 2Bb).
(v) Adjustment of pH (type 2Ab- ii).
(vi) Deaeration of the bulk (type 1b- i).
(vii) Shade matching (type 3a- i or -ii and iii).
(viii) Pumping into a storage vessel (type 2Ab- iii).

Not only are all these operations different from each other, but at each stage the characteristics of the bulk are quite different and require a different set of processing characteristics to achieve an optimal economic process. Not surprisingly, the optimum is rarely achieved throughout the process.

The subject of **pumping** is not clearly separated from that of **mixing** since the pumping process implies the forced flow of product. Any flow will naturally introduce an element of mixing if the product is not already homogeneous. Further, since flow is a common element of both processes, the same product characteristics (e.g., rheological behavior) must be taken into account in each process. Different types of pumps provide different quantities of shear to the product and thus provide different degrees of mixing. The “standard” in the industry is the positive displacement pump, which imparts little shear and thus little mixing to the product. The centrifugal pump works similarly to a propeller mixer in a frame. This pump can impart a great deal of shear and produce vigorous mixing. It is typically used with lower-viscosity materials.

1. Quality of Mixing

Mixing can only occur by relative movement between the particles of the constituent components. Three basic mechanisms for achieving this relative movement are bulk flow, convective mixing, and diffusive mixing.

- **Bulk flow** (which includes shear mixing, cutting, folding, and tumbling) occurs in pastes and solids when relatively large volumes of mixture are first separated and then redistributed to another part of the mixing vessel.
- **Convective mixing** involves the establishment of circulation patterns within the mixture (e.g., propeller mixing).
- **Diffusive mixing** occurs by particle collisions (e.g., thermal diffusion and concentration diffusion). In miscible liquids of sufficiently low viscosity, the thermal energy of the constituent molecules may be enough to achieve a good mixing by thermal diffusion without additional energy.

It is incorrect to assume, however, that the relative movement between mixture particles brought about by these mechanisms always results in an improved mixture quality or homogeneity. Many mixing problems arise from the tendency of mixture particles to segregate or aggregate during attempts to mix them, particularly with powders.

Segregation is defined as the preference of the particles of one component to be located nonrandomly in one or more sites in a mixture. The size of the nonuniformities in an imperfect mixture is sometimes referred to as the "scale of segregation" and the difference in composition between neighboring volumes is the "intensity of segregation." Segregation is fortunately not a major problem in cosmetics manufacture although it does manifest itself occasionally (e.g., the flotation of pigments during lipstick processing).

Aggregation is defined as the preference for the particles of one component to join with another component or components and then travel through the batch as a group. The aggregation can be physical (mixing of different-viscosity materials), physical bonding (powder agglomerations), or Van der Waals attractive forces leading to agglomeration of an emulsified droplet forming a loosely bound cluster of many individual droplets that may be shear sensitive and therefore produce smaller clusters at higher shear rates and pseudoplastic rheological behavior (i.e., viscosity decreases with increasing shear rate). To best control the distribution of some raw materials, aggregation may be required; cohesive powders may be included in the formula or the process may be used to develop an aggregation that is easily controlled. Surfactant/emulsifier selection and concentration can control the degree of agglomeration and final product stability/behavior.

Some products can be made without heating but these systems preclude the use of higher melting-point materials that can add richness to the aesthetics of the

final product. Further, if the rate of mixing is high, there is a chance that air can be entrapped in the emulsion. This phenomenon causes an undesirable decrease in the specific gravity of the product and an artificial increase in product viscosity. Any variability in processing can lead to a range of undesirable rheological and textural properties. This issue can occur even if the formulation is not modified.

The term **"product by process"** is well known in the patent art and describes the above-stated phenomenon. If two or more formulators prepare the same product, the resulting compositions may vary considerably. This variation can occur even though each person utilized the same lots of raw ingredients. This occurs because it may be very difficult to exactly reproduce all of the processing parameters used to make an emulsion, for example. If any of the processing variables are modified unexpectedly, particle size variations may occur, or the crystalline properties of the emulsion can be compromised. Table 13.2 is a chart containing the results from an experiment to determine the effect of processing on the final properties of a 5% petrolatum-containing cream. All preparations contained the same lots of ingredients. The data demonstrate that the viscosity and specific gravity can vary dramatically depending upon the processing parameters employed to make the batch.

Table 13.2: Petrolatum Cream (5%) - Standard Emulsion Test Results

Sample 1: Optimum manufacturing procedure				
Sample 2: Overheated phases				
Sample 3: Forced cooling				
Sample 4: Ambient cooling				
Sample 5: Paddle mixing				
Sample 6: Rapid homogenization				
Sample 7: Under heated phases				
Sample	*Specific Gravity*	*Initial Viscosity (cP)*	*Viscosity (cP) 24 Hr @ 25°C*	*Viscosity (cP) 24 Hr. @ 50°C*
1	0.912	99,970	140,580	26,560
2	0.937	85,910	93,720	29,690
3	0.952	93,720	109,340	29,700
4	0.941	51,550	96,840	131,200
5	0.959	62,480	85,910	78,100
6	0.803	112,460	124,960	20,620
7	0.931	51,550	96,840	20,600
Note: Viscosity measurements were taken with a Brookfield LVT model viscometer				

There is foreseeable uncertainty at the "bench" level in the laboratory that a typical 500-gram to 2000-gram laboratory preparation will translate well to a manufacturing environment. This concern is often well founded. To deal with the vagaries of scale-up, the product may be subjected to a wide range of processing variations in order to optimize the conditions of manufacture. Products made at each level of scale-up are typically subjected to accelerated stability testing in order to ensure the integrity of the product for its anticipated shelf life. A significant alteration to the past "tried-and-true" systems or the development of an entirely new system is often laced with unknown issues that can severely jeopardize the introduction (timing) of a new product.

2. Mixing Rheology

Apart from the "dry" powder processing discussed later, the processes listed in Table 13.1 involve mostly liquids present in sufficiently large quantities as to impose fluid characteristics on the mixture. Although there are similarities between the flow of powders and the flow of liquids, it is easier to set up and sustain flow patterns in liquids. This makes liquid mixing processes easier to perform with a much larger variety of equipment from which to choose.

However, even for liquids the science of mixing has not yet been sufficiently developed to enable the optimal mixer to be designed for a given process from purely theoretical calculations. Much of the knowledge we have is empirical and has been accumulated from trial-and-error practical experience. Application of Computational Fluid Dynamics (CFD, a method of mathematically modeling moving systems) promises to enhance the theoretical understanding of how forces are transmitted through a fluid by a given mixer design. The use of **tomography** in specially designed laboratory and pilot systems (glass or clear plastic vessels) have provided actual measurements that can be used to verify CFD results and designs. Most of these are specialized, controlled systems using plastic beads or liquid latex spheres moving through a water or a glycol system. The liquid makes the movement easy to see and measure using lasers. Several agitation blades have been developed, modified, and improved since the 1990s using this technology. CFD now allows the models to be developed using computers alone. 3D printers can then be used to create the test pieces to confirm the designs.

By definition, fluid mixing occurs when an applied force (e.g., a moving mixer blade) creates a velocity gradient, also known as the rate of shear, in the fluid. The "layers" or "particles" that make up the fluid are moving at different velocities relative to each other while the mixing blade is exerting the force. On the other hand, agitation occurs when the fluid particles and blade are moving at the same velocity, much like droplets of cream swirling around in a cup of coffee. In the coffee and cream example, mixing does not occur until blade speed is increased sufficiently

to create a velocity gradient that breaks up the cream droplets into a fine dispersion that we know as "café-au-lait."

Figure 13.1: Shear rate and agitation profiles [2]

Not only is there a great variation in the physical form and properties of substances that the cosmetics industry needs to mix, but there is also a divergence of purpose. As indicated by Table 13.1, it is convenient to consider two cases separately; the first, in which the liquid components are all mutually soluble (miscible liquids), and the second, in which some or all of them can coexist as separate phases (immiscible liquids, at best partly soluble in each other).

Some mixing operations involve simple blending of miscible ingredients, for example the blending of color solutions into miscible liquids and the blending of oils, alcohol, and water in perfumes and colognes. Mixing of miscible liquids represents the simplest mixing operation in cosmetics manufacture and is achieved by developing bulk flow throughout the vessel. On the other hand, the formation of an emulsion from two immiscible phases, the suspending of a gelling agent, and the distribution of pigment agglomerates in a viscous liquid all require hydraulic shearing to break up the constituents of the mixture into finer particles during the mixing process. For this reason, such mixing is referred to as **high-shear mixing** in order to distinguish it from **simple blending**.

On the industrial scale, mixing occurs as the result of forced bulk flow within the mixing vessel. Two types of flow can be distinguished—laminar and turbulent.

Laminar flow occurs when the fluid particles move along streamlines parallel to the direction of flow. The only mode of mass transfer is by molecular diffusion between adjacent layers of fluid (Brownian motion). Heat transfer is accomplished by the same type of mechanism—conduction from layer to layer.

In **turbulent flow**, the fluid elements move not only in parallel paths but also on erratic and random paths, thus producing eddies which transfer mass from one layer to another. For this reason, turbulent mixing is rapid compared with other mixing mechanisms. Heat transfer is also influenced by forced convection—moving heat from higher- to lower-temperature areas along eddies created by turbulent mixing.

When a liquid at rest is slowly stirred the flow is laminar, but as the liquid's velocity increases flow will eventually become turbulent. A valuable aid in describing the critical point at which laminar flow becomes turbulent is due to Osborne Reynolds who, in 1883, first characterized turbulence. The dimensionless number that bears his name, N_{Re}, can be calculated for an impeller mixing in a tank as follows:

$$N_{Re} = \frac{C\,D^2 N \rho}{\eta}$$

Where N_{re} = Reynolds Number In Dimensionless Units

D = Diameter Of The Impeller In Centimeters

N = Impeller Speed In Revolutions Per Minute

ρ = Density Of Liquid In Grams Per Cubic Centimeter

η = Viscosity Of Liquid In Centipoise

C = Conversion Constant Of 1.67×10^{-4}

Equation 13.1 Reynolds Number

Experience has shown that the onset of turbulence occurs at Reynolds Numbers above 2,000. For fully developed turbulence, Reynolds Numbers greater than 10,000 are required and these higher values are found in many cosmetic mixing processes. As shown in Equation 13.1, it becomes more difficult to achieve turbulence as the viscosity (η) increases. Between 1000 and 10,000 centipoise, the viscosity range of many cosmetic products, turbulent flow can be achieved without the need for an excessive amount of power. For highly viscous creams and pastes, mixing raises certain problems since the flow pattern in the mixer is invariably laminar. Under these circumstances, distributive mixing (cutting and folding) is more applicable than turbulent mixing. Turbulence not only provides rapid mixing but also influences dispersion (mass transfer) and heating/cooling (heat transfer).

Figure 13.2: Velocity gradient of flow through a pipe

As can be seen by examining flow through a pipe Figure 13.2, there is a velocity gradient between the layers of moving liquid. A similar gradient can be found

with the movement of all materials. When Newtonian (or ideal) liquids flow, the relationship between the force causing the movement (F) and the velocity gradient (ν) between the layers of moving liquid is as follows:

Figure 13.3: Newtonian flow [3]

F/A is commonly referred to as the "shear stress" and the velocity gradient (ν) as the "rate of shear." Assuming an ideal fluid, as velocity of flow increases, so does shear stress. This is the force that breaks up the weak bonds holding together pigment aggregates or other immiscible phases into droplets. Shear forces are also produced when liquids flow under laminar conditions, but under these circumstances the energy used to generate flow is dissipated largely as heat. During turbulent flow, the energy is dissipated in disorder; eddies are produced whose size and intensity depend upon the viscosity of the liquid and upon the force F. For a liquid of a given viscosity, the droplet size of an emulsion or the fragmented size of dispersed pigment agglomerates depends primarily on the energy input from the agitator, the velocity gradient, and the nature of the forces holding together the disintegrating entities. Unfortunately, the simple model shown in Equation 13.2 has limited application in cosmetics manufacture.

$$F = \eta \times A \times \nu$$

Where F = Force in gram-centimeters per second squared

η = Coefficient of Viscosity of Liquid in gram per centimeter-second squared

A = Cross-sectional Area of the liquid in square centimeters

ν = Velocity Gradient in centimeters per second

Equation 13.2 Force relationship to viscosity

The majority of products exhibit non-ideal (non-Newtonian) behavior, which can often be more appropriately described by the Equation 13.3.

$$F = (\eta_{app})^n \times A \times \nu$$

Where: F = Force in gram-centimeters per second squared

η_{app} = Apparent Viscosity of Liquid in gram per centimeter-second squared
n = exponent
A = Cross-sectional Area of the liquid in square centimeters
ν = Velocity Gradient in centimeters per second

Equation 13.3 Force relationship to apparent viscosity

In the case where the exponent, n, has a value between 0 and 1, the type of behavior is "pseudoplastic." The basic difference between materials exhibiting this property and ideal or "Newtonian" fluids is illustrated in Figure 13.4. As can be seen, pseudo plasticity is manifested by a decrease in viscosity with increasing shear rate at constant temperature. Many cosmetic liquids exhibit this behavior, especially emulsions and suspensions of particles 1 μm or less in size. Pseudoplastic viscosity loss is usually reversible; since apparent viscosity decreases as the shear rate increases and then increases along the same path as the shear rate is decreased; thus, when left unstirred long enough, the fluid will recover some or most of its original viscosity. The magnitude of the pseudoplastic effect is variable with the identity of the fluid, although a fall in viscosity of 25% when the rate of shear is doubled is not unusual.

Figure 13.4 Rate of shear (ν) plotted against viscosity (η) or apparent viscosity (η_{app})

Three other types of rheological behavior are also worth noting, although they are less frequently encountered in cosmetics processing. A truly "plastic" fluid exhibits viscosity versus shear rate curves similar to those of pseudoplastic materials, but a certain force must be applied before any shear (or flow) takes place. "Dilatant" materials show the opposite effect, viscosity increasing with shear rate, Figure 13.4 c. The term "thixotropic" is often used erroneously to describe pseudoplastic behavior. Thixotropic liquids exhibit a decrease of viscosity with time at constant shear rate, not with increasing shear rate as Figure 13.4 b shows for a pseudoplastic material. However, like the pseudoplastic material, the thixotropic liquid usually recovers most of its original viscosity after the mixing force is removed. An excellent overview of the various rheological behaviors and both their mathematical characterization by empirical constants as well as their useful

graphical characterization can be found in Part 1 of the *Rheology Modifiers Handbook* by David B. Braun and Meyer R. Rosen, 1999, William Andrew Publishing/ Elsevier Publishing. The authors, well familiar with the discomfiture of mathematics in the chemist's realm have provided graphical representations of the various equations and pointed out the value of what they term "Practical Rheology," where the empirical constants in the many equations can be usefully related to performance parameters of cosmetic and personal care products.

In the mixing of fluids, all three mixing mechanisms—bulk flow, turbulent diffusion, and molecular diffusion—are usually present. As viscosity increases, however, and turbulence becomes more difficult to establish, the parts played by turbulent and molecular diffusion become less important. Mixing equipment can be divided into two categories, depending on whether or not turbulent conditions prevail, as follows:

Table 13.3: Types of mixing equipment

Laminar Shear / Distributive Mixers	**Turbulent Mixers**
Helical Screw / Ribbon Blenders	Turbine-Agitated Vessels
Two-Blade Mixers	Pipes
Kneaders	Jet Mixers
Extrusion Devices	Sparged Systems
Colanders	High-Speed Shear Mixers
Static Mixers: Low N_{Re}	Static Mixers: High N_{Re}
Contra-Rotational and Planetary Side-Wiping Blades at Low Speed	Contra-Rotational and Planetary Side-Wiping Blades at High Speed

Control of mixing parameters and of the rheological characteristics of the bulk allows the manufacture of products with optimum homogeneity and stability. Heat and mass transfer are functions of the mixing process. The same mixing parameters that achieve ingredient homogeneity also influence heat and mass transfer. Good heat-transfer and good mass-transfer are both consequences of good mixing.

3. Heat Transfer

The topic of heat transfer in cosmetic operations does not require mathematical treatment as much as methodologies for its use and control in processing and scale-up. Heat can be used to one's advantage in a formulation. One must be aware of the fact that what can be done on the laboratory bench may not be achievable in production. The process team must be aware of the capabilities of available production-sized kettles and of the amount of heating and cooling capacity available to them.

The manufacture of most cosmetic creams and lotions involves the formation of an emulsion in which an oil and/or wax phase is combined with a water phase. This

combination is made most often at higher temperatures. The even distribution of temperature is of major importance in the formation of a good emulsion. Control of the rates of heating and cooling is required in order to scale properly from bench to production and if the formulation is to be reproducible from batch to batch.

The relationship of heat and mass transfer to mixing is similar and can be treated as analogous phenomena. The degree of heat transfer depends upon a relative temperature difference (temperature gradient), which provides the driving force toward equilibrium. Equilibrium means that the temperature difference between transfer medium and product is zero. In reality, the maintenance of a gradient is required to meet the desired endpoint in heating or cooling the product, namely the "set point temperature." At this temperature we want to stop the heating or cooling process, and this is done by making the gradient zero.

The mathematical relationship between heat transferred (Q) and temperature gradient (ΔT_{LM}) is expressed below.

$$Q = U_o A_o \Delta T_{LM}$$

Where: Q = Heat transfer in watts

U_o = Overall heat transfer coefficient in watts per square meter °C

A_o = Heat transfer area in square meters

ΔT_{LM} = Log Mean Temperature gradient between the product and the heat transfer fluid (difference) in °C

Equation 13.4 Heat transfer

ΔT_{LM} takes into account the multiple heat transfer losses in the process system. The amount of heat transferred is directly proportional to the size of the temperature gradient, the contact area between heat transfer surfaces, and the magnitude of the heat transfer coefficient.

U_o is composed of a number of factors and is expressed as follows:

Where: U_o = Overall heat transfer coefficient in watts per square meter °C

H_c = Individual heat transfer coefficient between the process fluid (the product) and surface—based on the area and Δt (temperature gradient) between the surface and the fluid—in watts per square meter °C

H_{dC} = Individual heat transfer coefficient between the process fluid and surface— when that surface is fouled—in watts per square meter °C

X_w = Thickness of vessel wall in meters

K_w = Thermal conductivity of vessel wall in watts per meter °C

H_{dH} = Individual heat transfer coefficient between the heat transfer fluid (steam or cold water) and surface—when that surface is fouled by scale—in watts per square meter °C

H_H = Individual heat transfer coefficient between the heat transfer fluid (steam or cold water) and surface—based on the area and Δt (temperature gradient) between the surface and the fluid—in watts per square meter °C

Equation 13.5 Heat transfer coefficient

$$\frac{1}{U_O} = \frac{1}{H_C} + \frac{1}{H_{dC}} + \frac{X_W}{K_W} + \frac{1}{H_{dH}} + \frac{1}{H_H}$$

As the individual heat transfer coefficients increase, the overall heat transfer coefficient (U_o) increases. Fouling of either surface decreases the U_o. The fouling that occurs on the heat transfer fluid side of the vessel is usually caused by boiler steam scale or scale from untreated cooling water. This can be corrected by regular cleaning of the vessel jacket, and by use of water additives to minimize scaling.

The fouling that occurs on the process fluid side of the vessel is caused by buildup of the product due to its own viscosity and lack of turbulence (mixing) which makes this "boundary layer" thicker, preventing good heat transfer. The factors H_{dH} and H_{dC} are referred to as "film coefficients." There are thin layers of fluid that are in immediate contact with the surfaces of the vessel, both on the process side and the heat transfer fluid side. These thin layers represent the "film," which is essentially at rest. The film coefficient is a measure of the thermal conductivity through this resting fluid.

The importance of mixing as a unit operation is clearly demonstrated by its effect on the product film coefficient, H_{dC}. Higher mixing decreases the thickness of this film layer and permits more rapid heat transfer between the surface of the vessel and the bulk product. During heating, the film can be lower in viscosity than the product, which will improve heat transmission. It is during cooling that the product is subject to a significant decrease in heat transmission due to the increase in viscosity at the cool surface of the vessel. The mixing speed may need to be

increased in order to produce the turbulence necessary to make the film thinner so that the bulk can be cooled at a satisfactory rate. The increased shear may be detrimental to the product, which may force a tradeoff between cooling rate and shear rate. A side-wiping agitator can remove this film and replace the cold bulk with warm fresh bulk to be cooled. The removal of the cold layer is of prime importance in providing the proper shear in mixing as well as the specified cooling rate. The relationship between mixing and heat transfer is important information needed for scale-up.

The mass transfer coefficient has a mathematical form similar to the heat transfer coefficient. Its units are in kilogram moles per square meter second in the MKS system. Qualitatively, it is a measure of how much mass is transferred per unit of available surface per second. The heat transfer coefficient is a measure of how much heat is transferred per unit of available surface per second. The driving force for heat transfer is the temperature gradient, ΔT. The driving force for mass transfer is ΔC, the concentration gradient. Mixing influences both in the same way. The formulation team must consider both when developing and scaling a process.

4. Types of Reactors and Their Use in Cosmetics

The most frequently used "reactor" in the cosmetics industry is the CSTR, or continuous stirred tank reactor. This is typically a jacketed stainless-steel vessel with agitation provided by propeller blade, anchor blade, side-wipe, homogenizer, or any combination of these. This type of reactor is used in batch processing. Jacketing the vessel allows a heat transfer medium (cooling water, glycol, steam, hot water, etc.) to circulate. Heat transfer is accomplished by conduction from the inner surface of the vessel, which is heated or cooled by the medium, to the batch. Mixing the batch in the vessel increases the efficiency of heat transfer by forced convection. In batch processing, all raw materials are added to the vessel in a specified order, and the reaction mixture should be homogeneous after each operation. Ideally, each incremental sample of the batch, from any location, should be the same as all others. Proper mixing will ensure homogeneity of the batch (mass transfer) as well as efficient heating or cooling.

In **semi-continuous or continuous processes**, it is more common to use a "plug flow reactor." Also called a pipeline reactor, it resembles just that. Unlike the CSTR, the contents of the pipe are not homogeneous throughout the length. Each incremental cross-section of the pipe is different in concentration (contents), and in temperature, from all others. Heat transfer in this type of process is attained by use of a jacket around the pipe similar to the CSTR. Increments of the pipe are heated or cooled as required by the process. As each increment, or plug, moves along the pipe it is subjected to a different set of concentration and temperature conditions. Each increment is homogeneous within itself, but is different from all others along the pipe.

Batch reactors are suitable for use in small-scale operations using expensive raw materials. This is more typical of the cosmetics industry where quality control is performed on a discrete batch-to-batch basis. When deviations occur, they occur in a particular batch and can be easily isolated. Continuous processes lend themselves to large-scale operations. When deviations occur, they can be harder to isolate and correct due to the overall variations along the reactor. Both systems require instrumentation to record and control process parameters. The batch-process parameters to measure and control are heat/cool rate and times, mixing speeds and times, and degree of vacuum (if a vacuum kettle is used).

Plug-flow reactor parameters to measure are rates of addition (to measure and control concentrations at any point in the pipe), temperature at each critical point in the reactor, mixing speeds (if dynamic pipeline mixing is used), pH, viscosity, conductivity (for complex emulsion systems), pressure/vacuum, and overall rate/product formation. The plug-flow reactor makes up for its advantages by requiring precise measurement and control devices, as well as alarms to alert operators to process variations. The choice of the particular reactor type depends on quantity of product to be made, complexity of formulation, sensitivity to reaction parameters of heat and mass transfer, fluid flow, and regulatory issues (e.g., over-the-counter, or OTC, drugs versus non-drug product).

5. Emulsion Processing Equipment—Heat Transfer

The most common heat transfer method used in the cosmetic industry is jacketing of a vessel/pipe. The jacket may be used for either heating or cooling with steam, water, or other heat transfer medium (specially designed oils and glycols). Most liquid products can be made using this type of cooling regardless of viscosity. Because of the necessity to mix the batch uniformly, excess mixing may occur during long cooling cycles, which can cause viscosity and stability variations.

It is important in scale-up to keep the overall heating and cooling rates the same through the changes in batch size. In heating, manufacturing will typically use steam. It is not recommended that the steam pressure on the kettle be higher than 15 psig (pounds per square inch gauge, or 1 bar). Saturated steam at a pressure of 15 psig creates a jacket and kettle wall temperature of 250°F (121°C). Steam pressures greater than 15 psig produce higher temperatures. The higher temperatures could cause degradation of ingredients on the walls of the kettle (particularly oxidation at the liquid-air interface). Other than the issue of burning material—cooling is typically a more critical process than heating. This is particularly true of emulsion products or products containing waxes. If this type of product is cooled in the laboratory in 20 minutes but requires a cooling time of 120 minutes in manufacturing, the resultant batches will not be identical. The microstructure of the emulsion or waxes could be totally different, affecting viscosity, feel, absorption, appearance and/or stability.

To overcome this problem, colder cooling media will be used in manufacturing than in the laboratory. The problem caused by this solution is that the product will be exposed to a much colder temperature at the wall of the kettle. This can result in premature solidification of waxes. To determine whether this is a concern or not, batches should be made in the laboratory or pilot plant using both fast and slow cooling rates.

Tube-and-shell or plate-and-frame heat exchangers can be used with some low-viscosity products, which are sensitive to shear. The larger ratio of surface area of cooling to product volume inherent in the design of this equipment allows heat transfer to be achieved quickly and efficiently. Only testing on the pilot or production scale will determine whether the product can acceptably be processed by this method. There are no simple laboratory systems available. Heating or cooling media, supply hopper, product transfer pump, and the heat exchanger must all be designed to work together for the product.

Products that are viscous can be pumped through a scraped-wall heat exchanger, Figure 13.5. This piece of equipment has a surface area to volume ratio that is less than the last two pieces discussed, but can provide cooling at rates four times or more than those in a continuous stirred tank reactor (CSTR). The disadvantage is that it can add considerable work to the product at lower temperatures. If the product requires a faster cool than can be achieved in a CSTR, and it can withstand shear at lower temperatures, the scraped-wall heat exchanger may be the process equipment of choice. Other heat exchanger designs are available; some are more compact or of sanitary design, which aids in the cleaning and sanitization process.

Figure 13.5: Scraped-wall heat exchanger

13.1.3.1 WET SYSTEMS—SINGLE PHASE (MISCIBLE) SYSTEMS

The science of mixing is far from complete. Designers of mixing equipment are not yet able to produce, from simple design principles, the optimum piece of equipment for a specific job even if they are given the necessary parameters and fundamental characteristics of the process and formula. One of the reasons for this is that the complete mathematical description of the flow pattern of fluid within each mixing vessel is extremely complex and difficult to achieve. Progress, however, is being made using the mathematical tools of dimensional analysis and modeling [4, 5, 6, and 7]. As an illustration of the practical usefulness of the data that can emerge from this analytical approach, a brief description of the relationship between some of the relevant parameters follows.

a. Flow Patterns: Fluids with Low or Medium Viscosity (< 5,000 centipoise)

Flow patterns in agitated vessels can be resolved into three principal types: tangential flow, radial flow, and axial flow.

In ***tangential flow***, the liquid moves parallel to the direction of the impeller. Movement of liquid into the surroundings is small and there is little movement perpendicular to the blades except in eddies near the tips. Tangential flow may be observed in paddle mixers operating at low speeds or in liquids of sufficient viscosity to prevent centrifugal flow from being developed, Figure 13.6a.

During ***radial flow***, the liquid is discharged outwards from the impeller by centrifugal force. If the moving liquid strikes the wall of the vessel, it splits into two sections, circulating back towards the impeller where it is re-entrained. The splitting of the flow at the wall induces further turbulence and mixing. There is usually some element of radial flow in all stirred vessels, but flat-blade turbines and disks with radial paddle blades or teeth are specifically designed to produce flow patterns that are primarily radial, Figure 13.6b.

Axial flow, as the name implies, takes place parallel to the axis of rotation. Usually the impeller or propeller blades are pitched so that liquid is discharged axially—the direction of flow may be from top to bottom of the vessel or vice versa, Figure 13.6c. Generally speaking the most difficult flow pattern to maintain is one of axial flow.

By far the most common form of mixing in liquids of low or medium viscosity (< 5,000 centipoise) is achieved by forced convection in stirred vessels. The motion of the liquid produced in the vessel must be sufficiently intense to sustain turbulence. Since it is unlikely that turbulence can be generated uniformly throughout the whole content of the vessel on the production scale, the liquid must be circulated continuously around the vessel so that all of it passes through those regions where turbulence develops. Thus, the number of important basic mixing parameters to be considered is two: the extent of turbulence, and the circulation rate of the contents.

(a) (b) (c)

(a) tangential, around the circumference of the vessel

(b) radial, centrifugal off the peripheral tips of the blade

(c) axial, in the direction of the impeller shaft (baffles in vessel)

Figure 13.6: Flow patterns with different agitators

Viscous liquids showing shear-thinning characteristics present considerable problems to the cosmetics processor. The fluid close to the rotating impeller of a mixer is sheared at a high rate and so becomes relatively mobile. But as this is pumped away from the impeller, regions of less intense flow and hence of much higher viscosity are encountered. Turbulence is rapidly damped out, decreasing the

turnover in the vessel and slowing down the mixing process. Scraper blades are often included on an anchor sweep, or contra-sweep to ensure good agitation along the vessel walls as the batch viscosity increases during cooling.

b. Impellers for Liquids of Low and Medium Viscosity

Paddle mixers produce mainly tangential flow and are usually mounted centrally because of their large diameter compared with that of the tank. For viscous liquids, the paddle is often of an anchor design equipped with scraper blades that remove product from the walls of the vessel.

Marine type ***Propeller mixers***, Figure 13.7a, are restricted to use with low-viscosity fluids, since their pumping capacity becomes localized at viscosities above 5000 centipoise. They have pitched blades whose angle varies along the length from center to tip. Flow patterns developed by propeller mixers have a high axial component and the rate of circulation around the vessel is high. Though the ideal impeller diameter to tank diameter ratio (D/T) is 0.33 ± 0.05, propeller mixers are usually of relatively small diameter, typically three-bladed, and are used at speeds between 150 to 2500 RPM. Such stirrers are used extensively in the cosmetics industry for simple blending operations. Alternate propeller mixer designs are available. These are commonly called **axial flow impellers**.

Figure 13.7: Designs of turbine impeller

Many portable mixers are of the propeller type. If the mixer is mounted centrally in the mixing tank, Figure 13.8 a, the surface becomes depressed and a vortex is formed. This is because of the natural movement of liquid to be drawn from above the impeller and towards its center. Generally, vortices are to be avoided because of the low order of turbulence and likely air-entrapment. Thus, propeller mixers should be mounted eccentrically, that is, offset from the center of the vessel at an angle other than perpendicular to the batch surface, Figure 13.8 b. Offset propeller mounting improves mixing efficiency by eliminating vortices, increasing turbulence, and improving batch turnover in the vessel. If the mixer must be mounted centrally, an alternate method of minimizing the formation of a vortex is through the addition of baffles, Figure 13.8 c. The baffles are normally equally spaced (e.g., four at 90° separations) and fixed to the vessel walls for efficiency. To improve ease of cleaning of the vessel, the baffles may not be fixed, but they then will not be sized the same to maintain the flow pattern required.

Figure 13.8: Portable mixer positioning

A *turbine prop* is a common impeller type used in cosmetics processing since it can cope with a wide range of viscosity and density. For liquids of low-viscosity, the flat-blade, axial-flow impeller is sometimes used, Figure 13.7 b. Slightly more viscous fluids are more efficiently mixed using the axial-flow flat-blade turbine shown in Figure 13.7 c. For very viscous materials, multiple radial-flow turbine blades, curved backwards in the direction opposite to the rotation, may be used, Figure 13.7 d. These require a lower starting torque and seem to give better energy transfer from impeller to liquid. Turbines produce a large flow of liquid with minimal shear and horsepower. They are normally mounted perpendicular to the top of the vessel with three or four baffles attached to the vertical vessel wall to reduce tangential and radial flow. Baffles help to induce and control the flow in both axial and radial directions. Used without baffles, the axial component generated by turbines remains secondary to the radial flow component, and the turbine does not efficiently turn over the entire batch. Typically, impellers of this kind are used at rotational speeds of 100 to 2000 revolutions per minute (RPM) as compared to the low speeds of 15 to 50 RPM for paddles.

c. Power Consumption

Power consumption is of great relevance to the economics of the mixing process. The choice of the wrong equipment can lead to the consumption of vastly greater quantities of power than are necessary to achieve the desired end result. On the other hand, sufficient power must be available and applied to the fluid to ensure that the endpoint of the mixing process can be achieved in a sensible time [5, 6, and 7].

As can be seen in Equation 13.6, a change in impeller diameter has a more significant effect on the power required than a change in impeller speed. This is emphasized in the design of most drop-in homogenizers through their small head design powered by a large drive motor.

$$P \propto N^3 D^5$$

Where: P = Power in watts

N = Impeller Speed in revolutions per minute

D = Impeller Diameter in meters

Equation 13.6 Power relationship to impeller speed and diameter

d. Pumping Capacity and Velocity Head

Perhaps the most powerful concept to arise from the analytical approach to mixing concerns the way that power provided by each type of impeller is actually transmitted to the fluid. This relationship can be expressed generally as

$$P \propto Q\,H$$

Where: P = Power in watts

Q = Pumping Capacity in liters per minute

H = Velocity Head in meters

Equation 13.7 Power relationship to pumping capacity and velocity head

Q is the pumping capacity of the impeller (the volume of fluid displaced directly by the impeller in liters per minute) and H is the velocity head—this is related to the shear rate experienced by fluid leaving the impeller. A large slow-moving impeller might produce, for example, a large pumping capacity and a low-velocity head, while a small impeller operating at high speed might produce a lower volume of fluid pumping but at a much higher-velocity head. Most cosmetics production processes require high pumping capacity, while others require high shear rate. It is useful to know the parameters that affect both these functions and how they interrelate.

For simple blending operations (the manufacture of shampoos or colognes, for example) pumping capacity of the impeller is often of the greatest significance. Under conditions of laminar flow, the number of complete changes of the bulk (batch turnover) required to bring about homogeneity is approximately three. For a turbine operating in turbulent conditions, this is reduced to about 1.5. However, given that only a fixed amount of power is available from the motor, it is not likely that a relatively small turbine will have sufficient pumping capacity to push a fairly viscous product around by even this amount. Not surprisingly, the factor that determines whether power is used as pumping capacity or velocity head is the ratio of impeller to tank diameter (D/T).

Examining the relationship between power consumption and D/T ratio for equal process results in a given vessel completes the picture, Figure 13.9. The use of a D/T ratio larger than 0.6 lowers the power required to achieve the same end result. At the same time, this implies the use of lower impeller speed, and this inevitably means that the torque required to drive the mixer increases dramatically [8]. The amount of torque that a given mixer is able to generate depends largely on its construction. It becomes a question of economics whether to invest in a more substantial (and expensive) mixer in order to reduce the power consumption needed to achieve a given mixture quality.

Figure 13.9 Power and torque relationship for equal process results [9]

e. Mixing Time

Another important measurable parameter is mixing time, t_m. This is the time taken to achieve the desired degree of homogeneity in the mixture. There are many methods by which this characteristic may be measured, but perhaps the most obvious is the time taken for a soluble dye to become uniformly dispersed throughout the mixing vessel (e.g., in the manufacture of a colored shampoo). The relationship between mixing time, t_m, and the degree of uniformity can clearly be shown if some index of mixing level can be established.

A simple example of this would be the ratio of color intensity between the top and bottom of the vessel contents at intervals after dye is added to the top (so that uniformity is achieved as the mixing index, M, approaches unity). This comparatively simple experiment should give rise to a curve similar to that shown in Figure 13.10. Since the approach of M to unity is asymptotic, t_m is difficult to measure accurately unless a colorimeter or other optical measuring device is available.

Once t_m has been established, however, more useful insight into the parameters controlling mixing rates may be gleaned from relationships such as that illustrated in Figure 13.11 [5, 6, 7, and 10]. In Figure 13.11a (which relates to a viscous liquid in which turbulence is not established), t_m, the mixing time, has been replaced by the product of rotational speed N and t_m, that is, by the number of revolutions of the impeller. M, the mixing index, has been replaced by log (1-M) to present the plot in a linear fashion.

Figure 13.10 Mixing index, M, plotted against time to give mixing time, t_m

Figure 13.11: Mixing index, M, plotted against number of revolutions, Nt_m
(a) Newtonian fluids (b) non-Newtonian fluids

It is interesting to note that for Newtonian fluids (ideal response between stress and strain) precisely the same plot is produced whatever the viscosity of the medium and speed of the impeller. In other words, only the number of impeller revolutions determines the change in mixing index. This is not true of non-Newtonian fluids—plots A, B, and C in Figure 13.11 b, which represents liquids showing

increasing divergence from Newtonian behavior. This illustrates the difficulties of mixing non-Newtonian media in which flow is damped out rapidly by regions of high viscosity away from the vicinity of the impeller blade.

f. Influence of Vessel Shape

The ratio of tank dimensions can be an important factor in determining the efficiency of any mixing process. It is sensible to perform a simple blending, such as alcohol-water, in tall cylindrical vessels of small cross-section using a propeller or turbine mixer with two or three sets of blades located two to three blade diameters apart on the same shaft. This stirred tank setup allows the capability to make variable batch volumes with high axial flow. For low-viscosity lotions, a hemispherical kettle often replaces the dished bottom conical tank to maximize the batch turnover achieved with an offset-mounted propeller mixer. Normally the ratio of kettle diameter to kettle height is close to 1:1. In the production of an emulsion of medium viscosity, it is desirable to keep the height of the vessel between 1 and 1.5 times its diameter.

g. Flow Patterns: Fluids of High Viscosity

As the viscosity of the mixture increases, it becomes increasingly difficult—and eventually mechanically impossible—to produce turbulent flow within the mixing vessel. At viscosities of 100,000 centipoise or above, flow is inevitably laminar, power consumption high, and the rate of mixing exceedingly low. In such systems, the input power should be used to create disorder (mixing) but is used to create heat. The rate of temperature rise is dependent upon the energy input, the thermal conductivity of the mixture, and the efficiency of the cooling surfaces; but the range 1° to 3°C per minute would include many cosmetic mixing processes of this kind.

Generally it is difficult or impossible during industrial mixing of highly viscous materials to dissipate heat faster than it is generated. This is particularly true if the mixing vessel is of large capacity (in which the ratio of volume to heat-exchange surface is high) and if appreciable films of chilled liquid are allowed to build up on the walls of the vessel, so insulating the contents from further cooling. The increase in temperature associated with such processes has both advantages and disadvantages. On the one hand a rise in temperature might cause a decrease in viscosity, making mixing more efficient, and might also help in the melting or dissolution of some of the components of the mixture. Taken too far, on the other hand, the decrease in shear stress caused by the fall in viscosity can decrease the efficiency of stress-dependent processes. This can be seen when breaking up and dispersing pigment agglomerates. The increase in temperature may damage the product by causing the thermal degradation of heat-sensitive components such as

actives, preservatives, and perfumes. The relatively high-energy input required while mixing viscous materials also influences the mechanical construction of the mixing machinery and the method by which mixing is achieved.

h. Impeller Types and Mixers for High-Viscosity Fluids

Propellers and turbines, as already mentioned, work best under turbulent conditions at relatively high rotational speeds. In viscous products (given that such speeds are attainable at all), flow is confined to the regions very close to the impeller. Large stagnant regions in the vessel exist where no mixing can occur without the employment of some secondary mechanism. To eliminate these stagnant regions large impellers such as paddles, gates, anchors, and leaf impellers may be used. These sweep a much greater proportion of the vessel and produce more extensive flow. Usually such impellers are designed to have close clearances with walls, giving a degree of wall scraping. This helps to eliminate buildup of unmixed materials at the walls, provides a region of high shear for dispersing aggregates and lumps, and may improve the wall heat transfer to and from the bulk.

Such impellers provide extensive flow but only of the tangential and radial variety. Axial flow, and therefore top-to-bottom mixing, is almost totally absent. This is a limitation of the design. Several suppliers have developed agitation techniques that include special baffles (often of variable pitch) that induce flow in both the downward and upward directions. For this reason, recycling the batch either externally through a pump or with time using the pitch of the sweep blade becomes an important consideration.

An alternative approach to the problem created by lack of flow in viscous media is the use of impellers that progressively sweep the whole contents of the vessel while the mixture remains stationary. Examples of this include the mixer in which a helical screw sweeps the wall of a conical mixing chamber, Figure 13.12. Equipment that exhibits a greater degree of distributive mixing may be utilized for more viscous products such as mascaras and toothpastes. Such mixers are designed to produce bulk flow and laminar shear by spatial redistribution of elements of the mixture. Perhaps the most commonly encountered mixers of this type are of the single or double action planetary type or the two-blade “dough” mixer. Their essential feature involves the cutting and folding of a volume of the mixture and the physical replacement of it into another part of the mixture where it is cut and folded again.

Figure 13.12: High solids blender [11]
Figure Courtesy of Charles Ross and Son Company, Hauppauge, New York

The static mixer is essentially an in-line mixing device in which mixtures flowing through a pipe are cut and folded by a series of helical elements in a circular tube, Figure 13.13. These elements (which do not move—hence the name "static") turn the flowing mixture through an angle of 180°. Since alternate elements have opposite pitch and are displaced 90° to each other, this causes the bulk flow to reverse direction at each junction. The leading edge of each element becomes a

cutting device, splitting and refolding the mixture in and on itself. This type of static mixer works well with thick materials. Other static, or motionless, mixer designs utilize the divergence and convergence of flows to induce mixing with some control as to the shear developed. This type of static mixer works well with thin materials. It can be seen that with both high and low viscosities, excellent mixing can be achieved in a relatively short length of pipe and with relatively few static elements. Each element splits the product stream in two and combines the two new streams with parts of the previously split streams. Other styles of static mixers are also available that offer specialty features such as adjustable gap settings and lower pressure drops.

Figure 13.13: Static mixer elements

Finally, mention must be made of extruders, in which a helical screw forces the bulk mixture to flow down a tube. Here, the pressure generated can be enormous, as in soap plodding, and such energy can cause materials with high viscosity to undergo laminar flow and change structure. The actual flow pattern produced is complex, being a combination of pressure and drag flow within the tube [12].

13.1.3.2 WET SYSTEMS - MULTIPHASE SYSTEMS

a. The Emulsification Process

Two major immiscible phases (referred to as “oil” and “water”) together with the emulsifier are brought together to form an emulsion. If the chemistry is favorable, very little energy is needed to produce a stable product. To minimize the level of emulsifier, it may be possible to use energy to force the emulsion to a stable endpoint.

The level of energy required will be formula dependent. Under turbulent conditions, one phase (usually the discontinuous or internal phase) is broken up into

droplets (predominantly by the action of shear stress imparted by turbulent eddies). The droplets are distributed throughout the other phase (the continuous or external phase).

While the droplets remain larger than the majority of the flowing particles, they will continue to break up into ever-smaller droplets. Eventually a point is reached in this process when the available power creating the turbulence cannot provide the shear stress necessary to reduce the droplet size any further. At this stage an emulsion exists containing droplets of a certain mean diameter ranging from d_{min} to d_{max}. Provided that it is correctly chosen, the emulsifier prevents the rapid coalescence of these droplets, and a stable emulsion may be formed.

In order to obtain products of maximum stability that can be made consistently from batch to batch, it is generally desirable to keep the droplet size distribution as narrow as possible. In a CSTR, droplet size is smallest near the impeller in the region of greatest turbulence whereas the maximum droplet size is to be found in any quiescent region of the tank. Thus it can be seen that d_{min} is fixed by the power available to generate turbulence, and d_{max} depends on the efficiency of the mixing in the tank to produce a good circulation rate, to bring all the contents through the region of maximum turbulence. This will minimize the distribution range between d_{min} and d_{max}.

Superimposed on the effect of circulation patterns in the vessel is an additional factor affecting the particle size range of the droplets. For a given vessel and impeller the effect of increasing the mixer speed should be to reduce the particle size range to a minimum, after which a further increase in speed could give rise to instability and coalescence. In practice, coalescence does not take place if a sufficient quantity of emulsifier is present; nevertheless it is important to attain the correct impeller speed to reduce the particle size range to a minimum.

b. Orientation of Phases

In any emulsion the orientation of the phases (that is, whether the oil or the water phase is continuous) is determined principally by the choice of emulsifier and the volume ratio of oil to water. Usually there is a range of volume ratios over which either phase may be dispersed, depending upon the method of manufacture.

Initially, only one phase is present in the mixing vessel containing the impeller. The second phase will form the dispersed or discontinuous phase upon its addition. If the second phase is combined with the choice of emulsifier, which eventually leads to a volume ratio at which the system is more stable with the second phase being continuous, then the emulsion will spontaneously invert and the continuous and discontinuous phases will switch. When an inversion takes place, it is very often accompanied by an abrupt change in droplet size. Where this droplet size

change is a decrease, the inversion leads to a more stable emulsion and gives rise to a valuable method of manufacture. This inversion process is often an effective method of producing a uniform, fine droplet size and may improve stability of the emulsion. Chemical formula, processing temperature, and energy must all combine at the correct levels for an inversion process to be reproducible in both the Laboratory and Production. Available energy level in Production is usually the limiting factor.

c. Addition of Surfactant

In a batch manufacturing process for emulsions, there are four possible methods of adding the emulsifier:

- dissolving or dispersing an emulsifying agent in water
- dissolving or dispersing an emulsifying agent in oil
- dissolving or dispersing an emulsifying agent in both the water and oil
- adding the water and oil phases alternately to an emulsifying agent

Normally, the emulsion process starts with a water phase to which the oil is added. An oil-in-water emulsion is initially produced, but an inversion to a water-in-oil emulsion may take place if sufficient oil is added. If the emulsifier is added to the oil phase, this mixture may be added directly to water to form an oil-in-water emulsion; if the water is added to the surfactant/oil mixture, a water-in-oil emulsion is formed initially.

Some emulsions are stabilized by “soaps,” which are formed at the interface between the two phases. In this case, the fatty acid is dissolved in the oil and the alkaline component is dissolved in the water. The two phases can be brought together in any order. The neutralization reaction, which forms the emulsifier, takes place as the phases are combined.

A less-used method is one in which both the water and oil phases are added alternately to the emulsifying agent. Usually, the small improvement in product quality obtained by the use of this method does not warrant the complication it causes in the manufacturing procedure.

d. Emulsion Temperature

The primary reason for operating above room temperature during the manufacture of an emulsion is to ensure that both phases are in the liquid state. In particular, the oil phase may contain fats and waxes that are solid at room temperature. The water phase is customarily heated slightly above the temperature chosen for the oil phase so as not to cause any sudden solidification upon blending. There is, however, an interesting variation: emulsification between a hot oil phase and a cold (usually

room temperature) water phase. An illustration of this procedure in which mixing and homogenization of the phases take place simultaneously is shown in Figure 13.14. The advantage of such a method is the saving of time and energy by not having to heat the aqueous phase and by reducing the time and energy required to cool the product to room temperature.

A secondary reason for heating a phase is to minimize microbiological concerns associated with a raw material. Preservatives are normally added to a formula to control any issues produced by the consumer. Minimizing growth can be performed in the process by using high-energy homogenizers (an inefficient use of the equipment) or by phase temperature (holding the phase at an elevated temperature for an extended period of time). A challenge when looking to move from a hot water/hot oil process to a cold water/hot oil process is whether or not the microbiology team supports the process change. It does not help to make batches quickly if they fail microbiological testing.

Figure 13.14: Hot/cold processing emulsion system

e. Emulsion Processing Equipment - Mixing

It is evident from the discussion so far that there are two important elements of emulsion processing: shear for emulsification and particle size reduction and flow for circulation of vessel contents through the region of maximum shear. Flow is also important in the heating and cooling of the emulsion. Most emulsion processing vessels are equipped with a jacket through which steam or hot water or cold/chilled water can be circulated for temperature control. To be effective, the mixing mechanism must include adequate flow to and from the vessel walls, as discussed earlier in this chapter.

For these reasons, most emulsion batch processing vessels contain a high-shear turbine or rotor-stator homogenizer (bottom, side, or top entry). High flow in one design, Figure 13.15, is provided by a paddle-style anchor agitator with scraper feet, which meshes with two fixed baffles (mating blades). The agitator can be run intermittently in either direction to provide a complete mix. In another design, Figure 13.16, concentric central shafts carry blades that turn in opposite directions and sweep the area in-between (counter-sweep). A frame holding the outer blades carries spring-loaded or product-pressure scraper blades to prevent buildup of product on the vessel wall, while mixing in the higher-viscosity region is thus enhanced. Mixing can be further enhanced by recirculation of the batch through the homogenizer and depositing it on the top of the batch to enhance plug flow. The recirculation piping may be an integral part of the vessel design (as shown in Figure 13.15) or an added feature in the production facility utilizing an external pump and piping (permanent line or hoses).

The need to combine all of these mixing capabilities into one vessel has led to the design shown in Figure 13.15. The following capabilities are available with no additional piping or equipment needed:

- Universal in application. It is capable of handling the entire viscosity range from light lotions to heavy ointments.
- Efficient presentation of the batch to the homogenizer for all batches. Efficient mixing ensures that all parts of the batch will see the same quantity of energy.
- Vacuum capable for maximum production efficiencies (high mixing speeds without aeration) and specific gravity control capabilities. Product integrity is not compromised during processing since aeration during mixing is not a factor.
- Clean-in-place and sanitize-in-place capable. The system is designed to be sanitary and maintain appropriate controls to meet the requirements for food, drug, and cosmetics manufacture.

Alternate designs are available from many suppliers in an effort to meet the above requirements (e.g., Figure 13.17). It is important for personnel purchasing equipment to understand the flexibility that is available in light of the processing requirements of new and upcoming products.

Figure 13.15: Batch emulsion processing vessel—SYMEX® Style - homogenizer in the down position [13];
Figure Courtesy of Schröder and Boos, Misch- & Anlagentechnik GmbH & Co. KG, Bremerhaven, Germany.

Figure 13.16: Batch Emulsion processing vessel—Lee® Tri-Mix Turbo-Shear® Style [14]
Figure Courtesy of Lee® Industries, Philipsburg, Pennsylvania.

Figure 13.17: Batch emulsion processing vessel—AGI™ Mixer Triple Shaft, homogenizer in the up position [15]
Figure Courtesy of Chemineer®-Greerco®, North Andover, Massachusetts.

f. High-Shear Mixers and Dispersion Equipment

The mixing equipment that has so far been covered in this chapter is designed primarily to produce bulk flow patterns of sufficient intensity to allow mixing. In the majority of cases, the pattern of shear and turbulence developed within the mixture varies according to the viscosity of the bulk, the method of producing flow, and the volume within the mixture under consideration. For certain applications, however, it is desirable to generate a very intense degree of shear stress in the mixture, and for this purpose specialized equipment is available. The uses to which such machines are put in cosmetics processing include the breaking up of pigment agglomerates and their dispersion in liquids, the rapid fracture and dispersion of gelling agents (e.g., bentone clays, polymeric cellulose derivatives, and alginate-type polymers), and the size reduction of internal phase droplets in emulsion products.

g. Batch Homogenizers

All three types of batch homogenizers (top mounted, bottom mounted in the kettle with or without recirculation and in-line) operate under the same principles. Liquid is drawn into the inlet by the suction created by the rotating blades, or teeth, of the rotor (centrifugal force). The liquid is then forced through the stator configuration, subjecting the fluid to high mechanical shear at the rotor/stator interface. After the fluid exits the stator it is subjected to high hydraulic shear as it returns to the vessel or pipeline. The design of the rotor and the stator dictates the magnitude of shear stress developed by the homogenizer. *High-flow/low-shear homogenizers* normally employ a flat or pitched design with an open stator design, Figure 13.18 a. The stator will have slotted, round, or other discharge port configurations and discharges radially or axially.

Figure 13.18: Rotor/Stator designs

Major applications of the high-flow design are for traditional creams and lotions and other low- to medium-viscosity products where frequent particle

interaction with low shear rates is required. High-shear/low-flow homogenizers are characterized typically by an intermeshing toothed rotor/stator design, Figure 13.18 b. The rotor and stator frequently have multiple rows of teeth, which operate at a gap range of 0.005”–0.015” (0.127–0.381 mm). The close tolerance increases the shear stress but has the effect of reducing the batch turnover rate. Major applications for this design are makeup pigment dispersion, mascara production, and low-emulsifier cream and lotion formulations that depend on mechanical energy for emulsification. Some vessels may allow interchangeability between high-flow/low-shear and high-shear/low-flow homogenizing heads. These units provide maximum flexibility for the production of a wide range of finished products ranging from thin lotions to thick ointments.

Some new designs provide additional flow and shear control through interchangeable slot configurations (a technique also used in laboratory homogenizers) and a rotor/stator design where both components can move in both directions. In theory, a wide range of flow rates and shear rates can be produced in one vessel using this new rotor/stator design.

A disadvantage of the top-entering homogenizer is the tendency of the mixer to cause aeration due to vortex formation—particularly when the homogenizer head is not completely covered. For this reason, high-shear devices are often incorporated into the bottom of the processing vessels. Additional advantages to the bottom-mounted design include the following: low-volume processing with minimal aeration, consistent agitation throughout the mass, virtually no minimum batch size, and fewer seal problems and less wear due to shorter shaft length. If the homogenizer is the primary mixer in the vessel, the outer tips of the rotor may wear rapidly, leading to increased clearance and decreased efficiency. Since no adjustment is possible, worn rotors must be replaced at considerable cost. Some designs also allow the homogenizer to be used as the discharge pump simplifying the total operation.

Generally, high-shear rotor-stator mixers may be used either for batch processing in a mixing tank (as a drop-in unit) or as in-line devices when encased in a suitable chamber. Used as batch mixers, they are capable of generating considerable turbulence because of the great velocity with which fluid is pumped out of the mixing head. In the high-viscosity region, pumping capacity decreases significantly. In this situation, use of an in-line device can be more effective. A positive displacement pump should be used to minimize viscosity effects during homogenization. The in-line homogenizer routinely has a significantly lower batch turnover rate than a drop-in homogenizer due to the restrictions of the recirculation arm, especially as batch sizes increase. This limitation may be overcome since it is highly effective as a finishing step for products during discharge. It should be remembered that mixing time is required to ensure that every part of the mixture has passed through the shear head at least once.

h. Continuous High-Pressure Homogenizers and Mixers

High-pressure homogenizers require a continuous process. This may be part of a recirculation line for a one-kettle operation or it may involve multiple kettles with a single pass or multiple passes through the device. The problems with most high-pressure systems are the high initial and operating costs and the low production-processing rate. The advantages lie in the small particle sizes that can be created during processing of both emulsions and solids dispersed in liquids.

Perhaps the highest shear stress is generated by the valve homogenizer, which is still extensively used in the production of emulsions with very fine internal phase droplets. A valve homogenizer, Figure 13.19, consists of a high-pressure pump, which forces product through a small orifice at pressures of up to 30,000 psi (2000 bar). Rapid expansion of the product after traveling through the orifice produces very fine emulsion and dispersion particles that are smaller than those produced by most other equipment.

The roughly premixed product at I is forced through the valve seating at A and leaves via O. T is a tapered shaft whose position can be adjusted with screw head C. S is a powerful spring against which the product is pushed through the narrow valve orifice.

Figure 13.19: Valve homogenizer

An alternative to the valve homogenizer is the ultrasonic homogenizer. When high-intensity ultrasonic energy is applied to liquids, a phenomenon known as "cavitation" occurs. Cavitation is complex and not easily understood. As ultrasonic waves are propagated through the fluid, areas of compression and rarefaction are formed, and cavitation is produced in these rarefied areas. When the ultrasonic wave passes on, these cavities collapse and change to an area of compression. It has been demonstrated that the pressure in these cavities, just before their collapse, can be as high as several thousand bars. Most of the effects of ultrasonic homogenization in liquids are attributed to the powerful shock waves produced immediately following the collapse of such cavities. Units are available for both batch (like a drop-in homogenizer) and continuous processing. Most production systems will use the continuous or flow-through cell technology. One design of an ultrasonic homogenizer is illustrated in Figure 13.20. The energy developed through the cavitation process will wear specific components. These will need regular replacement to maintain process efficiency.

The roughly premixed product enters at I. It passes through orifice A and is subjected to intense ultrasonic energy by vibrating blade C. The treated product leaves via O. The meter, M, and "tuning" devices at B combine to allow the operator to maximize the settings for each product

Figure 13.20: Ultrasonic Homogenizer

A third alternative to the valve homogenizer is a design that forces fluid, at high pressure, through an orifice into an expansion and impingement chamber, Figure 13.21. These systems can usually achieve higher pressures than the other

designs described, from 15,000 psi (1000 bar) up to 40,000 psi (2757 bar) and higher. These systems are often used in the processing of liposomes and special dispersions. Costs of these dispersions are high, and production flow rates are low.

Figure 13.21: Multi-stream high-pressure homogenizer

i. Processing of Water in Silicone Emulsions

Advances in silicone technology have offered an alternative set of synthetic chemicals to the hydrocarbon compounds that have traditionally been used in the cosmetics industry, both as emulsifiers and as oil phase ingredients. In the case of emulsifiers, many specialty products can be derived by selective block copolymerization with hydrophilic materials along the siloxane backbone of the chain. The result of this block copolymerization is a molecule with a strongly hydrophobic Si-O-Si backbone with a 130° bond angle that allows a series of hydrophilic sites to rotate freely about that backbone with minimal steric restriction.

In working with silicone emulsifiers, described in great detail elsewhere in this book, the key processing step in forming a stable emulsion is allowing enough time for the randomly oriented hydrophilic sites to uncoil and become oriented to the water phase with the less polar and highly flexible Si-O-Si backbone following the curvature of a single water droplet. Failure to allow time for this orientation and coating action to progress to completion usually leads to a less stable emulsion that loses viscosity over time and can lead to syneresis (separation of the phases) as the water droplets coalesce. Thus, normal processing of water-in-silicone (W/S) emulsions involves introducing the water phase into the silicone phase very slowly with low-shear agitation to produce a “crude” emulsion that, after a mixing period, is “finished” by a high-shear mixing step. Failure to follow the above process can yield an emulsion that meets initial viscosity specifications and looks fine but deteriorates in two to four weeks into a low-viscosity product that may even bleed (syneresis).

Low-shear turbulent mixing can be accomplished with a turbine, sweep, or propeller blade turning with a “tip-speed” below 900 feet per minute (4.57 meters per second). Gentle mixing is essential in order to allow the silicone emulsifier

molecule to uncoil from a random orientation. The implication is that excessive hydraulic shear can interfere with proper orientation of these large molecules. The slow water transfer under low-shear mixing results in a "crude" emulsion of large water droplets suspended in the "open" structure of the oil phase.

"Slow" water transfer is a relative term that is very dependent on the chemicals involved. Thus slow transfer can mean transferring a water phase, which is normally between 35% and 65% of the batch, into the oil phase over a period of 40 to 220 minutes. Another way to help the silicone emulsifier uncoil is to "seed" the oil phase with 5% to 10% of the water phase. The seeding step involves the slow transfer of a small percentage of the water phase into the oil, followed by a mixing period before the slow water transfer is resumed. The primary difficulty with this method is that two transfer pumps may be required to control the rates at the two extreme transfer rates.

In either case, a gentle mixing period follows the water transfer, with the batch sometimes showing a 1,000 to 5,000 centipoise build in viscosity, indicating that it is ready to be finished.

The finishing step involves converting the large water droplets of the "crude" emulsion into fine droplets that make up the internal phase of the W/S product. High-shear mixing is normally needed in this step. It should be applied as a single pass through a high-shear device with the shear applied via an orifice plate, colloid mill, or in-line rotor-stator homogenizer. A drop-in homogenizer can provide sufficient shear, but batch uniformity may produce acceptable test results of the batch with long-term stability issues due to the variation in final droplet size distribution.

Production of water-in-silicone emulsion systems requires as much attention to the "crude" emulsion step as it does to the finishing step. The water transfer time cannot be cut short. Cold (room temperature) emulsion formation eliminates cooling time providing a faster batch cycle time, provided the production equipment can match the shear energy applied in the lab. These are the two keys to successful W/S processing.

j. Liposome Production

Liposome formulation in the cosmetic industry normally utilizes phospholipids and produces either single- or multi-lamellar vesicles. High-pressure homogenization is the main method for liposome processing. There are two processes commonly employed for manufacture. Both methods are used to achieve a uniform-sized single- or multi-lamellar vesicle. The first utilizes a piston/valve homogenizer at an 8,000–10,000 psi (551–689 bar) operating pressure. Production requires four to five passes to achieve a uniform size distribution of the vesicles. Additionally, a temperature reduction step is required between passes to avoid degradation of

active materials and destruction of the vesicles. This method produces vesicles in the range of 300–500 nanometers in diameter. The second method uses two product streams which impinge at 20,000–30,000 psi (1333–2000 bar). For specialized applications, pressures up to 60,000 psi (4000 bar) are available. This method also requires 4 to 5 passes, with temperature reduction between passes. It will result in much smaller vesicle sizes, typically 50–200 nanometers.

Non-phospholipid liposomes can also be produced. High pressures are not usually required for their formation. They are formula-specific, temperature sensitive, and may require specific agitation for their formation. Special controls may be required during their production. Typical vesicle sizes are in the 100–500 nanometer range, with process pressures of 200–1000 psig (13–67 bar).

13.1.3.3 WET SYSTEMS—LIQUID–SOLID SYSTEMS

The production of a cosmetic product often involves the incorporation of a powdered solid material into a liquid. The objective may be to dissolve the powder completely (as with true solutions like salts or preservatives in water), to produce a colloidal dispersion of water-swelling particles (as with bentones and other gelling agents), or simply to disperse insoluble materials, such as pigments. To do this efficiently with a wide range of powder particle sizes, surface characteristics, and liquids of varying viscosities, a variety of mixing equipment is available.

Perhaps the easiest of these processes to carry out is the dissolution of a fairly large, smooth-faced solid, such as salt. The initial incorporation of each crystal into the liquid involves the complete replacement of the air-solid surface with a liquid-solid surface. This may be considered to be a three-stage process of adhesion, immersion, and spreading, Figure 13.22. Immersion is complete when the liquid has displaced all the air and the surface of the crystal has been completely wetted by the liquid. A low surface tension results in a low contact angle (between the liquid and solid), which aids this process. This is a result of the production of a positive spreading coefficient wherein not only does the surfactant decrease the surface tension of the liquid but it also reduces the interfacial energy at the liquid-solid interface.

Not all powders used in cosmetics have favorable size and surface characteristics. The majority are of extremely small particle size and highly agglomerated. The agglomerates will have a complex structure with an uneven surface and will be perforated by cavities of irregular shape. The complete wetting of such structures, involving the penetration of liquid into all the crevices and cavities together with the expulsion of air, is much more difficult. It should be noted that penetration into cavities requires a low contact angle but a high surface tension. This is in conflict with conditions for easy wetting.

Even more complex is the immersion of powders, which swell in liquid to form dispersions of colloidal size, since the particles on the outside of each agglomerated mass tend to swell and adhere to each other, retarding penetration of the liquid to the still-dry core.

Figure 13.22: Immersion of a solid in a liquid

The forces holding these agglomerates together are precisely the same as those described later in this chapter in the section on powder mixing. The obvious difference, of course, is that these agglomerates are situated in a fluid medium, the physicochemical characteristics of which may enter into the reckoning of bond strength, ease of separation, and likelihood of re-agglomeration. Consequently, the theoretical treatment of particle-particle interaction in liquid media is even more complex than that for dry solids.

In cosmetic processes, the de-agglomeration of solid particles in liquid media can be brought about by a variety of machines. In lipstick processing, for example, pigments are pre-dispersed into castor oil or any other suitable liquid by preparing a coarse mixture that is then processed in a triple roll mill, colloid mill, ball mill, or sand mill. These machines are used specifically because they can deal effectively with high solids content and high viscosities.

For less viscous formulas (e.g., the dispersion of pigments into the aqueous phase of an emulsion), a high-shear device of the rotor-stator type may be used. In this case, ensuring that the whole contents of the vessel are entrained into the shearing head by secondary stirring can shorten processing time. However, the pigments may be externally dispersed, as in the lipsticks mentioned above, before addition to the aqueous phase of the emulsion. Depending on the amount and type of color being used, a rotor-stator type of shearing device may increase the batch processing time.

In view of the fact that a ball mill utilizes only one pass to develop the color (and this may be done outside the manufacturing kettle), it is especially efficient

for this method. A wetting agent should be used when dispersing pigments in this manner to aid in de-agglomeration and to minimize re-agglomeration after processing. As with all de-agglomeration, shear stress is critical for the particle disintegration of the agglomerate.

For soluble powders, the enormous increase in the solid-liquid interface brought about by immersion and de-agglomeration ensures that the actual processor dissolution can proceed at the maximum possible rate. For insoluble powders, however, there remains the problem of maintaining a good stable dispersion. De-agglomeration is usually a reversible phenomenon and it can usually be assumed that the opposite process, flocculation (particle accumulation), can take place simultaneously.

In the case of dry powder–powder dispersions, stabilization can be achieved by the introduction of particles of larger size to which disintegrated agglomerates can adhere. In some cases this can be applied to solid-liquid systems, for example, by preextending pigments onto talc before adding them to a liquid foundation base. In many instances all the solid particles might be too small a size for such a process. Under these circumstances, rules similar to those used in emulsion technology can be applied. Thus, some or all of the following means can slow the rate of flocculation:

- The use of surface-active agents (sometimes as polymer coating of the powdered solid) to inhibit flocculation by steric hindrance.
- The manipulation of electrostatic charges on the surfaces of the powder particles.
- The manipulation of the viscosity of the dispersion.

Surface-active agents play a part at two stages in the process of manufacturing a stable dispersion. It has already been seen that the lowering of the solid-liquid contact angle speeds up the wetting process. In practice, the best results are often achieved not with a surfactant, which measurably lowers the surface tension of the liquid, but with what is sometimes described as a surface activator, which reduces the interfacial tension between solid and liquid. These surface activators (which are best described as "dispersing agents") can, if correctly chosen, cause an immediate improvement in the quality of the dispersion, which is manifested by an increase in the color intensity.

The rules for choosing a dispersant are similar to those used for surfactants in emulsions; part of the molecule must have affinity for the liquid medium and part for the solid. A significant difference exists, however, between emulsions in which the two phases have very different chemical affinities) and dispersions of solids in which a hydrophilic solid is dispersed in water. Powders can be processed to coat their surfaces with a chemical (usually a polymer) of suitable characteristics

to allow easy wetting and dispersion. Nowhere is this more clearly demonstrated than in the case of water-wettable (hydrophilic) and oil-wettable (hydrophobic) grades of titanium dioxide. In this instance, the same grade of titanium dioxide can be coated with different resins to modify the surface in such a way as to make it wettable by either water or oil. Formulation problems resulting from flocculation are normally resolved before scale-up. Thus, the addition of shear-thinning gums to nail lacquers and of colloidal thickeners to the aqueous phase of emulsions helps to slow down flocculation without materially influencing the basic flocculation process itself. However, reheating of liquid pigmented foundation products sometimes results in unexpected changes of hue, which are often erroneously ascribed to phase-inversion. The truth is that the rate of flocculation of an intrinsically unstable dispersion has been increased because of a drop in viscosity caused by the heating process.

a. Suspension of Solids

If a particulate solid is dispersed in a liquid in which it does not dissolve, if the suspension so formed is allowed to stand undisturbed in a vessel, and if the densities of the two components are dissimilar, some degree of flocculation will eventually take place. Where the particles are present in sufficiently low concentration to have a negligible effect on the viscosity of the suspension, the establishment in the liquid of flow patterns of sufficient turbulence can achieve re-suspension.

The suspension of solids in agitated tanks is frequently encountered in cosmetics processing as an aid to dissolution or as a means of obtaining a good dispersion of particles prior to a change of viscosity of the liquid medium by gelling or cooling. Although the theory concerning flow patterns in agitated tanks has already been discussed, it is necessary to reiterate that it is axial flow that is of prime importance in the movement of solid particles away from the top or bottom of a tank. At the lower viscosities a correctly sized propeller mixer at the correct angle for mixing is the best method for achieving the axial flow. See Figure 13.8 b.

Three conditions can be recognized during the production of a suspension, namely complete suspension, homogeneous suspension, and the formation of bottom or corner fillets [16].

Complete suspension [17, 18, 19, 20, and 21] exists when all particles are in motion and no particle remains stationary on the bottom or surface for more than a short period. Under these conditions, the whole surface of each particle is presented to the fluid, thereby ensuring the maximum area for dissolution or chemical reaction.

Homogeneous suspension exists when the particle concentration and (for a range of sizes) the size distribution are the same throughout the tank. The homogeneous suspension is always more difficult to achieve and to measure than the complete suspension. Nevertheless, homogeneous suspension is very desirable for certain types of cosmetics applications, and particularly so for continuous processing. In practice, for such processes the requirement is only that the particle-size distribution and concentration in the discharge of the homogenizer head and the vessel are the same.

Sometimes heavier particles are allowed to collect in corners or on the bottom of the vessel in relatively stagnant regions to form fillets. This may have the practical advantage of very large savings in power consumption compared with the energy that may be needed to achieve complete suspension (provided, of course, that this saving offsets the loss of the active/solids in the fillets).

In general, it may be said that a propeller at a 45° angle or turbines offer the best advantage for rapid suspension at low power consumption. If, on the other hand, radial-flow agitators need to be used, these should be of relatively large shaft-length to impeller-diameter ratio, be placed close to the bottom of the tank, and have turbine blades extending to the shaft to prevent problems with central stagnant regions.

b. Milling Equipment

There are many products in the cosmetics industry that require de-agglomeration and particle-size reduction. Lipsticks, concealers, foundations, and mascaras are examples. They have high solids content and are composed of pigments, fillers, and other powders dispersed in a liquid base. It is often necessary to reduce the gritty feel that these powders impart on the skin as well as to maximize the color development of the pigmented phases. By taking the portion of the formula containing the oversized and agglomerated solids, one can mill it to wet out and develop the ingredients. A pigmented phase should be milled until the shade cannot be developed further and the grit cannot be detected by touch. The method of milling will determine the required viscosity of the slurry to be processed. Colloid mills, ball mills, and three-roll mills are used extensively to de-agglomerate, and to produce the required feel and shade development. The colloid mill requires the lowest viscosity slurry of the three mill types mentioned and the three-roll mill requires the highest viscosity slurry.

Figure 13.23: Particle-size comparison for milling equipment (some processing may require multiple passes)

c. Colloid Mills

Colloid mills are used as in-line devices. They work by hydraulic shear. The energy they produce is imparted to the product in a thin film. Colloid mills can be used to de-agglomerate pigments or to disperse solids in liquid phases through recirculation. They can be used to build final viscosity in an emulsion system, as a continuous device.

The colloid mill consists of a rotor, a rapidly rotating conical member, which may be smooth, toothed, or grooved, and a similarly machined stator into which the rotor fits. Product is pumped through the mill inlet, and the fluid mixture is forced through the small clearance [ranging from 0.004” (0.1mm) to 0.040” (1.0mm) gap size] between the rotating rotor and stator. The gap size can theoretically be set to 0.001” (0.025mm); however, 0.004” is typically used as a minimum to allow for tolerances due to wear in the rotor and stator. Figure 13.24 illustrates a typical design.

Figure 13.24: Colloid mill

There are three important process variables that affect product output: retention time, tip speed, and gap size. Each of these is a known quantity or can be measured, and each can be adjusted to give the desired result.

Retention time is the amount of time the product is exposed to the milling energy. The longer the product is in the mill, the smaller the particle size will be. Retention time is dependent on the flow rate, rotor speed, backpressure, and the gap size. The flow rate is easily measurable and should be controlled by a positive displacement pump. When rotor speed, backpressure, and gap size are kept constant, flow rate is the only variable controlling retention time. Average tip speed, the linear velocity averaged across the surface of the rotor, is the shaft speed multiplied by the average rotor circumference. Increasing the tip speed imparts more energy to reduce the particle size. Likewise, lowering the tip speed imparts less energy, which lowers particle-size reduction. By varying the gap size, the product is forced through a different opening and a different void volume in the mill. Equation 13.8

Colloid mills are effective tools for reducing the size of particulates and dispersing agglomerates. They are usually scalable from laboratory to production sizes if the style, retention time, tip speed, backpressure, and gap size are all kept constant.

d. Ball Mills

Ball mills are typically used as a side batching or in-process device. Unlike the colloid mill, they are not normally used on the finished product, just the pigmented sub-phase. Ball mills are used extensively in the de-agglomeration of solids (up to 60% concentration for some pigments) into a liquid medium and development of the pigment shades.

The rapid movement of grinding elements (media), which take the form of pebbles, balls, or finer sand-like particles, 1 millimeter or less in diameter, results in the breakdown of agglomerates. The mill style may be horizontal or vertical in which a rotating agitator (rotor) causes the grinding media to rapidly collide. The powder particles to be milled are subjected to both impact and high shear. Heat energy is produced during the milling process, so the milling chamber should be jacketed for controlled cooling. This will protect the product from reaching an unsuitable temperature. Figures 13.25 and 13.26 illustrate the design of vertical and horizontal ball mills. The annular gap or screen is used to retain the media in the mill and allow the milled product out. The milled product or phase may have to be filtered to ensure that any broken or worn media is removed.

Figure 13.25: Vertical ball mill

Figure 13.26: Horizontal ball mill

Before a sub-phase can be ball-milled, the powders and pigments first must be pre-dispersed; the quality of this pre-dispersion may affect the quality of the finished grind. It is typical to design the process to have a ratio between in-feed particle diameter to media diameter of 10 and 20 to 1. For example, if the in-feed particles are between 50 and 100 microns in diameter, the milling media should be 1.0 millimeters in diameter [22]. If the media diameter to pre-dispersion phase diameter is not matched, problems may occur. If the particles in the phase are too

large, they will enter the ball mill and plug the screen or gap. Although no oversized material will exit the mill, no material will be processed. If the media is too large, particles of the phase will pass between the media and not be efficiently milled. If the size of the media is too small, the power required will be higher and the particle-size distribution exiting the mill will be wider than expected. Some particles will be exposed to high energy as they are processed through the mill, while some will see less as they travel a different route through the mill.

Choosing the proper media size can help control the finished distribution. There are five important process parameters that affect the milled product: retention time, rotor tip speed, media type, media size, and media loading. Each of these is a known quantity or can be measured, and each can be adjusted to give the desired result.

Retention time is the average amount of time each particle is in the milling chamber. Generally, the longer a particle is exposed to the milling energy, the smaller the particle size will be. Retention time is a function of the void volume in the mill chamber and the volumetric flow rate of the slurry. The equations for retention time and related void volume are:

$t = V_v / Q$ and $V_v = V_c - V_m$

Where: t = retention time in minutes

V_v = Void (Available) Volume of the Mill in liters

V_c = Volume of the Empty Mill Chamber, with the Rotor in-place, in liters

V_m = Volume of the Media in the Mill in liters

Q = Volumetric Flow Rate of the Product in liters per minute

Equation 13.8 Mill retention time

The void volume is a function of the media loading (quantity and size used for the process). As the media loading (V_m) increases, the void volume (V_v) decreases, decreasing the retention time for constant flow. The void volume parameter (V_v) is easily controlled before processing. The flow rate is easily measurable and can be controlled by varying the speed of the product supply pump. Slower flow rates will produce higher retention times, generate an increase in the number of collisions, and yield a smaller particle size. The rotor speed controls the movement of the media, which controls the collision energy imparted to the slurry. Greater shaft speeds generate more collisions, which helps to reduce the particle size.

Choosing the proper media is crucial in getting the desired end product. Size and composition are the important factors. The media diameter must be greater than the annular gap size; otherwise media will be found in the product and

normally is at least three to one media diameter to gap. Common media types used are sand, stainless steel, glass, zirconium oxide, and zirconium silicate. The hardness (or density) of the media will determine adequate grinding versus wear on the chamber. Harder (or denser) media will reduce particles better than less dense ones. However, the interior of the mill may become pitted and wear faster. Media which is too brittle may fracture and be found in the product. The breakage pattern of the media (e.g., pulverization, crumbling, or slivers) should ensure that no long slivers or damaged media are passed through the screen and are found in the finished product. Ball mills are widely used in the cosmetics industry today. They are effective tools for grinding and reducing agglomerates. They are usually scalable from laboratory to production sizes if the equipment style, retention time, rotor tip speed, media type, media size, and loading are kept constant.

e. Three-Roll Mills

Three-roll (roller) mills are typically used as side batching or in-process devices. Like the ball mill, they are not normally used on the finished product, just on the pigmented sub-phase. Unlike the ball mill, particles are ground down to reduce their agglomerate size. Roller mills are used extensively in the dispersion of high solids content (and high-viscosity) pigments into liquid media and for full development of the pigment. Typical solids content for a roller mill grind is 50% or higher.

The roller mill consists of three rolls each operating at different speeds and a takeoff knife or doctor blade. The mill reduces particle size by using shear (adjacent rolls operating at different speeds) and mechanical pressure. The first roll (the roll nearest the operator) operates at the slowest speed, the middle roll is faster, and the third roll is the fastest. Material is transferred from roll 1 to roll 2 to roll 3 where it is scraped from roll 3 with a knife-edge blade. In order for the material to move from one roll to the next efficiently, it is necessary for the material being ground to have some tack. Without tack, material transfers very slowly, if at all; but if the material is too tacky, it may pass through the mill too quickly.

The percent solids content of the slurry should be adjusted based upon how well it performs on the roller mill. This needs to be developed in the laboratory. The slurry should be observed at several different points in the process, both in the laboratory and during manufacture.

Production size three-roll mills are manufactured with a slight crown (outward curve) to each roll. This is done so that as pressure is applied the rolls will flatten. The material coming off the knife blade is referred to as the tongue. If not enough roll pressure is applied, the tongue will be longer in the middle and shorter on the ends as seen in Figure 13.27 a. If too much pressure is used, the tongue will be

longer at the ends of the roll and shorter in the middle, as seen in Figure 13.27 b. With the proper pressure, the tongue will be even all the way across the knife blade/tongue onto the takeoff plate as seen in Figure 13.27 c.

Figure 13.27: Three-Roll mill pressure control

Three-roll mill procedures are time consuming; however, the mechanical energy imparted to the product is very effective. Due to general safety and cleaning difficulties, three-roll mills are not preferred for production. Due to the design of the feed system, particularly during the cleaning operation, items can be drawn into the gaps, called "nips," which pose a safety hazard to the operator. As the rolls are worn, minute pitting occurs. These pits can hold fine pigments and lead to contamination problems with subsequent batches. This enhances cleaning difficulties. Fortunately, based upon the availability of raw materials, it is usually de-agglomeration not particle-size reduction that is required, so alternate systems can be considered.

13.1.4 FILLING

Most cosmetics are filled from stored bulk in machines specifically designed to handle the packaging units of a particular product. Great care must be taken in choosing and setting up these machines. The main problems are due to the limitations of the machines themselves rather than the product being filled. There are at least two areas, however, where a special understanding of production filling requirements and constraints, and formula characteristics are essential for achieving efficient production. These are the molding processes (lipsticks, wax-based sticks, and alcohol-stearate gels—discussed under warm and hot fills below), and compression processes (compressed eye shadow, blushers, and face powders—discussed under filling pressed powder, later in the chapter).

Different products require different types of equipment to be filled effectively, which result in low cost per unit, high filling rates, low maintenance of the systems, and controlled product characteristics. When selecting the proper equipment, two key questions must be answered:

- What are the physical characteristics of the product to be filled—viscosity, flowability, temperature, shear sensitivity, potential for aeration, etc.
- What type of container is being filled—bottle, jar, tube, godet, etc.

The answers to these questions along with (to a lesser degree) the speed at which the product needs to be filled, will determine the type of filler that will be needed. Products are to be handled and transferred from their storage container to the appropriate filling equipment in a clean environment. Cosmetic GMPs are to be practiced and maintained. All equipment must be properly cleaned and sanitized prior to use.

a. Filling Parameters:

There are number of parameters associated with filling that directly impact the aesthetics and quality of the filled cosmetic products. These parameters are:

Type of filling equipment

Temperature of the product at fill

Mixing & shearing of the product just prior to and at fill

Filling speed—velocity through the nozzle

Nozzle size—inlet and outlet diameter, sometimes length

Viscosity & flowability of the product

Specific Gravity

Cooling rate (if filled at an elevated temperature)

Container type and fill opening

b. Filling Machines:

The three types of equipment used for the filling of cosmetics can categorized as follows:

(1) **Positive Displacement Piston filler**—This type of filler is used in many applications across the cosmetic industry. It is capable of handling products with varied viscosity, fluid consistency, and pressure application. It is regularly used to fill products that are of medium to high viscosity. The filler is very accurate achieving consistent fill and is suitable for attaining and maintaining weight claims.

This filler uses reciprocating motion causing fluid to move from one location to another within the unit by way of the rotary valve. The rotary valves ensure that the product moves in one direction while the entrapped fixed portion of the product moves from pump suction to the discharge or dispensing nozzle. The rate of a product's flow inside the piston is proportional to speed of dispensing.

Products such as hot-pour foundations, creams, lotions, viscose lip products (gels and glosses), and mascaras are generally filled using this technology. An example is shown in Figure 13.29.

(2) **Gear Pump filler**—Gear pumps are versatile, operate consistently in either flow direction, and give a constant and even discharge regardless of pressure fluctuations due to supply level changes. It consists of two gears that generate suction on one side and drive the product out to the dispensing nozzle. This filler is often designed to recirculate the product and is normally used to handle products easily deaerated. It can help to control product variability when a weight claim is hard to achieve, particularly if solids can easily settle out (for thin products that contain pearls and pigments to keep the powders suspended during dispensing). A less accurate weight claim is associated with products that need recirculation during fill. The fill is often based upon a timing cycle—product circulates, then fills, then circulates. This filler is used for products that are less viscous, and flow easily such as lotions, and liquid make up. These fillers should not use a gravity hopper since the flow rate will change as the product level changes during the fill.

(3) **Vacuum/Pressure filler**—In vacuum/pressure equipment, the product is either pushed via a pump or gravity system, or pulled via a vacuum system to the container. When the fill reaches the set height, any additional product (overfill) is directed away from the package through a return port incorporated in the filling nozzle, Figure 13.28. The vacuum is used to draw the product into the container to a set height. In these systems, product continually flows through the nozzle, bypassing the nozzle outlet when it is

not filling. Both the excess fill and the bypassed product are returned to the filling hopper. The product is then available to be reused during the filling run. A level sensing system may use a slight air pressure flow that is delivered through a control line attached to the tip of the filling nozzle. A control sensor notes the change in backpressure once product has filled the package and reached the sensing tip of the control line. The sensor then stops the delivery of product to the filling nozzle.

(4) Both types of Vacuum / Pressure / Fill-to-Level filling systems fill to a set level, regardless of the container size, shape, or capacity (internal dimensions of the package). They allow a great deal of volumetric variation while delivering an aesthetic look to the fill. This may be of critical importance for some package designs. The production of plastic packages is much better controlled, dimensionally, than for glass packages. The variation on a glass package volume can be as high as 10% and still be considered acceptable.

A **tube filler** is another form of positive displacement piston filler that is generally used for filling both low- and high-viscosity products such as creams, lotions, gels, conditioners, cleansers, lip balms, and toothpastes. The tube filling machines are equipped with hot-air or hot-element sealing jaws and are configurable to seal the plastic or laminated metal tubes by using automated heating and crimping (press) after dispensing is completed. Metal tubes are crimped using multiple sets of jaws to fold over the tube ends in various patterns.

c. Filling – Low-Viscosity Products (Lotions, Toners, Liquid Makeups)

Vacuum / Pressure / Fill-to-Level equipment can take advantage of the flowability of low-viscosity products to produce a very efficient fill. See Figure 13.28. Low-viscosity products can aerate, foam, and striate during the handling and filling operations and directly affect the esthetics of the fill by producing under fills, wet packages, poor seals, low production yields, etc. Shear-sensitive products raise special concerns when setting up and operating the filling system. Poor setup will result in high recycle and bypass rates, which will continually add shear to the product, and increase the probability of aeration and the potential for contamination. Foam in the filling hopper will probably be produced. By choosing an improper pump type (centrifugal versus positive displacement) and size or misadjusting the vacuum level, significant shear on the product can result. Nozzle sizing is often determined by the constraints of the package. Small nozzle size, along with high production rates, may not work well together. The rates may have to be slowed when small nozzles are used to control the flow near the top of the bottle and limit the shear on the product. These factors can all limit the production rates, and are all key production concerns when any new formula is produced. For efficient production and improved control, low-viscosity products depend on check valves fitted into the nozzles.

Figure 13.28: Liquid fill-to-level system

d. Filling – High-Viscosity Products (Creams, Mascaras, Masks)

Products in this category will not flow readily without the assistance of an outside force. A Positive Displacement (PD) filler system is required. A PD system includes a controlled pump that works in tandem with a piston or related device, which can overcome friction and flow problems to move finished product through the filler, Figure 13.29. With very viscous products, a pressure hopper and/or ram pump is also needed to supply nonaerated mass to the filling chamber (aerated mass will produce inconsistent fills). The ram pump system contains a plate that lowers into the container as the product is filled. The pump pressurizes the supply container and minimizes aeration as the product is filled. Pressure is often required to maintain product flow to the filling nozzle. A crowder may also be designed into the hopper for this purpose. It is designed to push the mass toward the nozzle slowly while maintaining an air-free environment.

A piston, or positive displacement pump, is then used to dispense the product in a controlled manner. By design, these fillers dispense a reproducible quantity of product for each rotation of the chamber (piston cylinder or pump head). To dispense different quantities, the piston stroke length or the number of revolutions of the pump can be adjusted. High-viscosity products will typically "break off" cleanly from the nozzle tip. Special nozzles are rarely required except to control the curl, or wave, that can be developed upon completion of the fill (ice cream curl).

Figure 13.29: Positive displacement filler

Mascaras and masks are generally filled at room temperature. Agitation inside the filling hopper or holding drum is not recommended or required during the filling process (high potential for aeration).

The filling/dispensing speed should be carefully adjusted based upon the flow characteristics of the product. Too fast a rate may create air at the discharge point. A void can be created inside the package, which may affect the fill weight, product aesthetics, and possibly the long-term stability of the product. The nozzle size is determined by the vial or component size, desired fill-weight and shear sensitivity of the mascara—typically as large a diameter as can fit into the package while still allowing air to escape during the fill. For mascara development, it may become very important to know early if the vial's wiper will be received placed in the package. This will force a smaller nozzle to be used, which may shear the product unacceptably.

A product's specific gravity (SG) is critical in determining the minimum/maximum fill weight within a container. Even though the fill weight may be satisfied, aesthetic requirements may not be met (particularly important when the package is clear). For example; a 10-gram fill within a vial may be an issue for a shaded product line—if the product has an SG of 1.0 for a red product and an SG of 0.975 for a blue product, the red shade may fill acceptably while the blue one may be overfilled and have an under-label weight of 9.99 grams.

Troubleshooting for aesthetic and texture: Parameters to look for and address during the filling process are aeration, voids, fill weight, and micro.

Beyond the use of special nozzles, high-viscosity products may require special handling procedures to fill the package without voids. Bottom-up fill capabilities are often required. This also allows a minimum of residual product to remain on the nozzle tip, possibly affecting the fill of the next package. In bottom-up filling, the nozzle will dive down to the bottom of the package and rise as the level of product increases in the package. The timing between the rate of the nozzle rise and the

product flow rate must be mated for consistent fill. Sometimes the rates may be stepped instead of continuously controlled to ensure that product flows into all the lower corners of the package. Some difficult-to-fill packages may even require the finished package to be spun to induce the product to move into all of the corners of the package. The packaging of viscous products in clear containers without visible voids is difficult to achieve consistently even with the current state-of-the-art controls and should be avoided. The use of opaque bands on the package to hide potential areas of voids may make the filling operation appear more successful.

Some products will be packaged with a disc placed between the top of the jar and the cap. The purpose of the disc is usually to minimize the air contact with the product in the filled package. This may be due to the activity of the raw materials in the formula, and to the microbiological sensitivity of the product. Other functions of the disc are to minimize a separation of the product at the surface, or to prevent moisture from accumulating after a warm (above room temperature) fill. The determination of proper and acceptable disc contact are troublesome specifications for the line operator and mechanic. One hundred percent coverage is preferred. However, any overfill of the package would mean the disc no longer fits and that product will be squeezed out of the package into the threads. Significant underfill may mean no disc contact at all. Typical contact is between 50% and 80%. The design of the package and the filling system used will determine the actual range. It is typical to produce a bottom-up fill with the ice cream peak for improved contact. The peak is pressed down when the disc is placed, ensuring contact.

Products of low viscosity typically flow easily; they do not require the use of a PD system. PD systems are not as production efficient (they are relatively slow) when compared with the alternate systems described, which can provide higher production rates with fewer problems. However, most low-viscosity products can be filled on the same equipment as used for high-viscosity products (which is often done in the laboratory or pilot plant). Since low-viscosity products typically need check valves fitted into the nozzles, this may slow the filling rate further.

e. Filling—Traditional Lotion Products

Positive displacement pump technology is used for filling lotion products. The product is pulled from the holding hopper by a piston into an entrapped area and pushed to the discharge area to be dispensed. This filler is particularly suitable to use for emulsions because it performs controlled shear to the product during dispensing.

Filling the emulsions does not normally require heating. Most creams are filled at room temperature. However, there are creams that do require a minimum amount of heat (up to 40°C maximum.) in order to flow and allow circulation in

the filling system for the ease of dispensing and to meet aesthetic requirement. Hot fill of emulsions will be discussed in a different section.

Creams and lotions that required cold fill (room temperature) normally do not need mixing inside the filling hopper. However, if heating/warming is involved then sweep mixing inside the holding tank is required to ensure product uniformity. It should be noted that the holding tank must be covered at all times to prevent volatile loss (typically water). Most emulsions are sensitive to mixing; therefore the agitation speed must be carefully adjusted to control shearing and prevent separation of the product.

Adjusting and control of the dispensing speed is particularly important as faster speed can increase the shear to the product at the filling nozzle. It may also create air bubbles and leave dispensing mark on the surface of the filled component and therefore affect the aesthetic.

Troubleshooting for aesthetic and texture: examine aeration (SG) and voids. If the product is low in specific gravity, adjust hopper pressure, hopper minimum and maximum loading (head pressure), minimize the distance between product supply container/tank and hopper, check/adjust the nozzle dispensing rate/speed, and adjust and check loose fittings and hoses. Voids may be corrected by checking the nozzle for nicks and bends. Check the product specifications (aged product may form air pockets during storage, adjust dispensing speed (less swirl in the package), and adjust line speed.

f. Filling – Shear-Sensitive Products

The package will have a direct effect on the minimal shear that is added to the product during filling. Typically, a large-diameter filling nozzle will produce less shear on the product during filling than a smaller size. A tube usually has a large opening by design. Therefore, the mass is rarely sheared during filling. Most bottles have a limiting neck size, but plastic is usually larger than glass for the same size cap. Jars may allow the most flexibility but can be a major problem if small promotional pieces are to be handled.

For shear-sensitive products, the pump used to supply the filler as well as whether or not to allow recirculation must be examined. Specially designed low-shear pumps, along with careful setup by the operator can minimize recirculation and control the quantity of shear that may adversely affect the finished product. The limited available adjustments on the equipment may be critical and force some shearing during filling. It may not be possible to maintain all of the “perfect” settings on the systems available to production. The low-shear pumps have special requirements, which can limit their use for normal processing, for example special seal concerns, limited flow rates, and difficulty in cleaning.

g. Filling Shampoos, Conditioners, Cleansers—Products That Aerate

A gear pump filler equipped with diving nozzle is typically used to dispense this type of product. Fill is done at room temperature; these products do not require heating during the filling process. If warming is required for flow, aeration will take place.

The filling speed is critical in filling these types of products. Dispensing speed must be carefully adjusted as it may create unnecessary air or bubbles at the point of dispensing.

Troubleshooting for aesthetic and texture: imperfections to look for and parameters to address during the filling process include aeration and voids. Microbial testing is also important, as most "No Tears" formulas are neutral pH. Every effort must be made to verify air-free bulk is getting to the filling nozzle. To keep from creating voids inside the filled components the appropriate nozzle is required. Controlling dispensing speed and line speed is also important for avoiding air pockets in the tube. These products may be thin enough to allow the air during filling to rise and not affect the products' stability or characteristics, but unless the package is opaque, the fill aesthetics will be poor once the air rises to the surface and the package then looks underfilled.

h. Packaging Lines

Fill-to-level packaging systems lend themselves very well to rotary configurations. A great deal of filling operation is performed within a short period of time. The time can be increased, with a minimal loss of productivity, by increasing the size of the filling circle instead of expanding the entire filling line. With a rotary table, a small increase in table diameter translates to a large increase in available time for the filling process. Therefore, the flow rate can be reduced. Rotary fillers are also used to minimize floor space required for large production runs. Different filling actions can be performed in a limited space, around the rotary table, e.g., bottle placement, filling, cap placement, and bottle transfer to the remaining portions of the packaging line.

For large production packages, greater than 16 ounce (473 milliliter), slower lines are often needed to minimize the shear stresses on the finished mass during extended filling times. For these large-size packages, almost any type of filler can be used. Unlike the related smaller packages, the majority of the production time will now be used during the filling cycle, not bottle handling, capping, and labeling. This slow speed line is often set up in a straight line along a wall to maximize the space in the room for other, higher speed, production lines, Figure 13.30. These orphan lines are usually operated with less automation and utilize production personnel as schedules permit.

Figure 13.30: Straight Line version of a Rotary Filler

i. Warm and Hot Fills—Creams and Dispersions

Many cream products develop their unique pickup and application aesthetics because the products set up in the package, typically a jar. This requires the fill to be hot (typically 60°C to 80°C). A product that must be filled at an elevated temperature offers a different set of obstacles to overcome. The most important thing to remember is that after bulk manufacture and storage, the product must be heated before filling. This may amount to remanufacturing the product. Most products that require a warm, not hot, fill do not reprocess well and must be filled just after batching. This enforces a Just-In-Time (JIT) operation with its associated production concerns, such as microbiological testing not being completed before filling. Possible concerns include the requirement for warm holding tanks with special agitation, the availability of packaging components, the availability of the production line, and special handling of the finished package.

Moisture is a warm and hot fill problem that is always present and is based upon the formula, package, and filling area. If the product is anhydrous, the only concern is high humidity in the filling area. Moisture from the air in the package's headspace may then condense on the surface of the filled package as the product

slowly cools. Droplets of water can easily become locations for significant microbiological growth since it will not combine with the product. Fortunately, this high humidity condition is rarely a concern. If the product contains water, however, moisture can easily collect on the surface during cooling as the water leaves the product at the air interface.

When filling into a tube, this is of minimal concern. The tube is squeezed in use. Any minimal moisture in the headspace will be blended with the product and sheared as it exits the orifice. When filling into a jar, a special cooling operation may be required to minimize moisture on the surface. This cooling operation is time, temperature, and airflow dependent. Its purpose is to cool the filled mass and evaporate any moisture on the surface at the same time. Evaporation is necessary from both an aesthetic and a microbiological standpoint. A disc may be added to the filling process to help keep the available headspace to a minimum and cut down on the time required after filling to seal the package. However, if the amount of disc contact is insufficient, a pool of water may still collect and be the first thing the consumer sees upon opening. A typical cooling tunnel will allow the package to cool for at least twenty minutes with 10°C to 25°C air flowing around and over it. Dust and dirt, as well as microbiological, contamination are concerns during the entire cooling time. A standard protocol should be followed for all fills from the laboratory bench through the pilot plant and into production for consistency and uniformity.

j. Warm and Hot Fills—Godet Products

When a product is not filled at or around the optimum temperature, its hardness or texture may be adversely affected. This is readily noted with the change in payoff or application when filling godets (small pans). Adverse effects can also occur when the product is not remelted to at least the minimum melting temperature. The best way to determine both of these temperatures (reheat minimum and pouring maximum), which may or may not be the same, is to run a heating and cooling curve on a Differential Scanning Calorimeter (DSC). By reading the peaks on the heating curve, one can determine the minimum melting temperature of the highest melting wax. The peaks on the cooling curve indicate at which temperature the waxes will start to crystallize out of solution. Because of solubility factors, waxes will usually crystallize out of solution at a lower temperature than is needed to completely melt them. The optimum filling temperature is usually somewhere above the crystallization temperature and usually, but not always, below the melting point of the highest melting wax.

If a DSC were not available, a rule of thumb would be to remelt the product to a temperature at least as high as the melt point of the highest melting wax in

the product. The optimum filling temperature would then become a trial-and-error procedure on the filling line. A good starting point could be determined by pouring a number of pieces at different temperatures in the laboratory.

A key problem for production is in trying to match the aesthetics on the filling line of the "approved" sample, which was carefully poured into the container in the laboratory. A filled product on the manufacturing line may never match the surface appearance of a laboratory-poured sample. This is a particular problem when filling godets. Once the formula is ready for manufacture, a test fill should be performed on the actual filling line or a similar piece of equipment that can be scaled to the actual filling line. This is done to determine true operating parameters and to develop standards that the manufacturing facility can achieve within the expected production rate.

The filling temperature of the product should be the first parameter to be set. The temperature of the product exiting the nozzle is equal or greater in importance than the temperature in the filling kettle. The transfer from the reheat vessel to the filler is done manually or via a transfer pump—follow all local safety considerations (as an example, some facilities do not allow manual transfer above 45°C).

For all hot-pour kettles, the filler must start by purging the product through pistons, transfer hoses, and nozzles. To ensure product uniformity in the filling hopper, both bulk and filled pieces are to be checked within certain time intervals. If the filler is stopped for an extended time, then it must be purged prior to resuming filling into components. When using heated nozzles, make sure the product is not being overheated just prior to filling, particularly if the line is idle. If heat is continuously applied, the section will overheat during the idle times. Unheated nozzles require the mass in the filling kettle to be kept a few degrees warmer than the desired filling temperature in order to provide the correct initial filling temperature. Once the temperature at the nozzle has reached the proper temperature, the kettle temperature may need to be reduced.

Once the correct filling temperature is established, there are other filling parameters that may affect surface appearance and can be set by trial and error. Some of these variables include the nozzle outlet's size and shape. Smaller-size nozzle generally leaves a pouring mark on the surface of the compact. Large-size nozzle may minimize any hot-pour aesthetic filling issues (but may drip onto the transfer belt or the next pan). Tapered nozzle with suck-back capability can facilitate a better fill and minimize most aesthetic issues. Some less complicated nozzle types that may be experimented with are a flared head (outward like a funnel or inward, making a smaller diameter at the tip), a showerhead type (large opening with multiple holes), or a straight-through nozzle. The change in nozzle design can be used to minimize dripping and change the pour dot on the surface of the godet. A

moving belt may also shift the pour mark to a location that is aesthetically acceptable. The nozzle's internal diameter and flow rate determine how forcefully the product enters the container. The distance from the filling nozzle to the container affects the spreading of the product into the container.

The different types of container used (steel, aluminum, tin, or plastic) all dissipate heat at different rates, which affects the spreading and cooling rate of the product and often the surface finish. The filling belt speed determines how evenly the product fills the container. It is critical to maintain the temperature, as it can impact the aesthetic and texture of the final product as it flows to the edges of the package. Preheating empty containers just prior to dispensing helps to evenly flow the bulk product to the edges. The purpose of post-heating onto the filled pieces is to even out and smooth the surface of any pour mark, bubbles or pinholes. This applies to all hot-pours. Preheating works particularly well for metal pans. However, if too much heat is applied, the texture of the product may be altered, pearlescent pigments may migrate to the surface, or pigments may settle to the bottom.

The type of conveyor used on the filling line will affect the filling operation—for example, a metal belt will hold heat, while a rubber or fabric belt will not. However, metal belts may be needed for pre- and post-heating. The use of a cooling tunnel for quick cooling of the filled pieces will also affect the finished product's aesthetics. Too quick a set may develop an improper surface appearance or crystalline structure. As stated earlier, a good deal of trial and error is needed to establish filling parameters for a hot-pour product.

Most anhydrous products require constant mixing inside the pre-melt tank and filling hopper. Mixing is required in order to prevent particle settlement or flotation and to avoid burning mass on the vessel's walls, especially when the temperature is elevated. (Heating at most filling lines is done electrically since the plumbing services for steam and condensate are not usually available.) Mixing speed is adjusted based on the texture and quantity inside the vessel to achieve optimum agitation. It must be noted that mixing speed is to be monitored so that no air is introduced to the pre-melt batch inside the kettle before the transfer or filling operations.

Specific gravity (SG) plays a significant role when it comes to filling. Products that are aerated fall outside the specified range and the air bubble or voids can be evident in the filled pieces. If the bulk is found to be aerated, then vacuum must be applied to remove the air from the product.

Two methods can be used to cool the filled products—(1) force-cool; (2) air-cool. Depending upon the nature of the products and test trials performed, it can be determined what type of cooling is needed to achieve the desired aesthetics. Force-cooling is normally done by passing the filled component through a cooling tunnel that has adjustable temperature controller. If air-cooling is desired, longer

conveyor belts may be required to allow the mass to cool to a functional temperature to allow handling of the component/package. To maintain aesthetics from formula development into production, the same system must be used to cool the filled pieces. Allowing the pieces to air-cool on a counter for a few hours is rarely reproducible by production. Examine short-term storage in both a refrigerator and a freezer to help develop conditions that can be used by both facilities.

The following aesthetic defects and subsequent corrective actions may improve the end results.

- Pinholes: Adjust mixing speed, filling speed, temperature, angle of heat gun for pre/post-heat, check mass quantity in the vessels, apply vacuum.
- Voids: Adjust preheat, mass temperature, nozzle position and size, determine/adjust appropriate use of diving nozzle, filling speed, adjust angle of heat gun for post- or preheat.
- Discoloration or pearl floatation: Adjust filling temperature, mixing speed, adjust angle of heat gun for post- or preheat.
- Sweating: Adjust line speed, adjust filling temperature.
- Cracking: Adjust filling temperature, temperature in cooling tunnel, adjust post-heat, adjust belt vibration, and handling.
- Pour-marks: Adjust angle of heat gun, adjust nozzle position, filling (dispensing) speed, nozzle size.
- Indentation (dimple): Adjust line speed, adjust cooling temperature, and adjust post-heat.

k. Warm and Hot Fills—Lipsticks, Lip Balms, Suppositories

When filling into a mold (e.g., lipsticks, lip balms, suppositories), key areas of concern are the following: aesthetics, aeration, cooling rate, shrinkage, and finishing steps. The DSC is often used to minimize these issues similar to the godet fills mentioned earlier.

Molds come in two basic designs—book and individual. Book molds contain several sections (leaves) that are held together to allow for filling multiple cavities. These are typically between two and five pieces. The use of the mold allows for complete or partial use of the cavities by the operator. In general, there is a large amount of metal around each cavity to provide good/quick heat transfer. Book molds are usually aluminum, coated/treated aluminum, or stainless steel. Individual molds are built into a filling system. These molds—metal (aluminum, brass, or stainless steel), plastic (used for individual bullets and then discarded or included as part of the package), or a silicone/rubber—are filled and cooled as part of an automatic system's operation. Less metal is involved, so a cooling medium is required to control the product temperature.

All molds may require preheating or warming prior to the fill to aid in flow to the tip/edges. Most book-style molds are filled with an overflow condition. The overflow is removed prior to removal of the bullets. This ensures a complete fill after cooling, which often produces sinkholes at the top (fill end) of the stick. Sinkholes are a result of a contraction of the solidifying product that begins at the cooled surface. The cooling of the mold may take place through a chilled bath (liquid), on a chill-table (metal contact), chill-tunnel (circulating air), or in a refrigerator or freezer (circulating/stagnant air). Variations of these techniques can be duplicated from the laboratory through production. Air-cooling in the laboratory, however, cannot be duplicated in a production facility. Most godets contain very little mass, so production can simply use a longer belt to allow for a longer cooling time, when compared to the laboratory. Cooling time is more important for bullet-shape products that may partially set quickly, but finish developing their crystalline structure after an extended time, which may range from several minutes to 24 hours. This makes immediate handling of the just-molded stick a problem for many production designs. Automated systems may have the ability to perform a second fill to fill-in the sinkhole. New aesthetics should then be examined when compared to the laboratory fill.

"Old-time" lipsticks contained high levels of waxes with castor oil. These sticks would shrink a consistent amount during cooling. This shrinkage allowed for easy removal from the molds and consistent insertion into the components. Newer sticks develop a gel structure, which may not shrink as much. This means it is more difficult to remove from the mold, and components may need to change to ensure functionality. Be aware of the potential issues during development—extra cooling or lower-temperature cooling may minimize the difficulty, if the production facility has the equipment available.

Silicone/rubber molds were developed to minimize this contraction issue associated with many newer formulas. After the filling and cooling cycles, the mold stretches away from the bullet allowing for easy removal and insertion into the component. Decorations are also possible, as the bullet does not slide out of the mold. Cooling rate is however an issue to be examined. Silicone and rubber are natural insulators, so the temperature removal is less efficient than for metal molds.

Lipsticks are normally filled in a mold with the finished tip down. Lip balms are normally filled directly into the case, top up. Other hot-fill products can be produced using variations of these two methods. Sinkholes are sometimes believed to be good for a stick product, assuming they do not interfere with the lift mechanism. They are said to offer two outer surfaces for increased strength. The additional strength is formula related and should be reviewed for every shade of every product produced (rarely done). To minimize aeration of the molded sticks, the mass may have a vacuum applied to it just before filling. This assumes the mass

to be liquid and not contain key volatiles that may be removed under vacuum conditions. The quantity of vacuum necessary will be formula and temperature dependent. Improved filling methods can also minimize aeration during fill. These methods include the use of diving nozzles, slanted molds for improved flow into the cavities, and slow flow rates to minimize swirling of the mass as it leaves the nozzle. Most aesthetic defects and subsequent corrective actions for lipsticks are the same as those for godets discussed earlier.

Since the apparent volume of product is different in a stick from a godet, if a softer texture is desired, then a lower fill temperature is used. On the other hand, to achieve a more rigid (harder) stick, higher-temperature fill is often used. Small temperature adjustments are often required to address the normal variations in raw materials over the life of the product. Acceptable ranges should always be "suggested" during the initial transfer from R&D to production. If a significant change is required, R&D should investigate the cause even if the aesthetics are acceptable using the "new" filling temperatures.

Finishing of the sticks is product specific. Most lip balms have a waxy texture that is acceptable to the consumer. Also, the finished package is rarely elevated completely before use. Therefore, a minimum of treatment is required. Lipsticks are not as simple. The consumer will often raise the bullet to near its full height before use; the entire appearance must be acceptable since it is continually in view. A "flaming" operation is usually performed to improve the finished appearance of the sticks. Although a gas flame may be used, alternate heat sources are also used: heat guns (hot-air heating) and lamps (induction and convection heating). The heat is used to melt any mold marks or other imperfections in the lipstick and to produce a smooth, shiny surface appearance. Different production techniques are available ranging from fully automated systems (that include the pouring, molding, insertion into cases, and "flaming") to 100% manual systems. The choice will be dependent upon the formula, the finished package, and the production requirements. Some of the testing and assessment criteria that are done to ensure quality of the lipstick bullet, are to evaluate the hardness, strength, texture, payoff, stick finish/shine, discoloration, bullet alignment/insertion, and pinholes.

l. Antiperspirants and Deodorants

Antiperspirants (A/Ps) and deodorants are processed much like other cream and lotion emulsions using similar equipment and processing methods. High-shear mixing (homogenizer or colloid mill) is used to ensure complete dispersion of the solids, typically the actives, in the formulas. One must remember to follow government regulatory guidelines for A/Ps, which are regulated as "over-the-counter" (OTC) drug items in the United States. Most deodorant sticks contain very low levels of solids, but the filling issues are similar.

Stick formulations offer a filling challenge. Like lipsticks and other hot pours, the filling operation can be more critical than the processing of raw materials into the final formula. For sticks to set properly:

- The mass must be hot enough so that it flows into and around the component elevator.
- The mass must eliminate "white spotting" caused by the batch crystallizing on the case walls.
- Pour marks, typically along the case wall created by hot spots where liquid is ejected from the filler nozzle, must be eliminated. Post–surface treatment is awkward at best.
- The mass must set up quickly without shrinkage. A quick set is needed to prevent the suspended solids from settling and to effect a good surface appearance. Shrinkage produces a poor appearance to the consumer and can affect the package mechanism (the product may shrink away from the mechanism so the package cannot function).

Thus, the formulation must be held just above its drop point with sufficient agitation so that the active ingredients, usually alumina complexes, are kept uniformly suspended while the product is filled. The drop point can be accurately determined using a DSC, or by measuring the temperature at which a solid sample liquefies and "drops" out of a test apparatus. Jacketed hoppers with mixers that are capable of holding mass within ±1°C, heat-traced lines and filler assemblies, preheating of the packaging component(s), and the use of cooling tunnels are normal for stick manufacture. In large-scale operations the mass is often recirculated from a jacketed mixing vessel through a customized filler manifold on a specialized filling line. A number of these filling operations, and the equipment associated with them, have been patented. Because of the complexity, it is advisable to utilize "make-and-fill" or semi-continuous processing for A/P and deodorant sticks. If the filling is separated from the compounding step, it is imperative that measures be taken to ensure that uniform product be delivered to the filler. The bulk can easily striate as it sets up in storage. The solid bulk must be sliced vertically for proper remelting, or the entire contents of each storage container completely melted and mixed to uniformity before it is brought to filling temperature.

13.1.5 SCALE-UP

The cosmetics industry has been considered collateral to the fashion industry. The driving force for the product development efforts of both industries is the marketplace. The cosmetic chemist is faced with the challenge of formulating products with active ingredients, rheology, and aesthetic characteristics dictated by the marketing group. Laboratory-scale equipment is used to develop and achieve the

desired product characteristics. The chemist's beaker is the place where the scaling process begins, which will culminate in production's vessels. It is common to develop a process that fits current manufacturing equipment because the time to launch a new product is almost always short and the lead-time to purchase and install new equipment is almost always long.

It is not unusual for a product that was successfully developed in the laboratory to exhibit quite different characteristics when it is transferred to production. As the product is transferred from a small, laboratory-scale apparatus to large-scale production equipment, a difference in conditions is experienced. Even if a pilot plant is available for an intermediate scale, there is no guarantee that problems will not be encountered during manufacture of full-scale production batches. Scale-up, which is of fundamental importance to the efficient manufacture of cosmetics, is an extremely complex subject because the variables that determine the distribution of forces within a processing vessel vary considerably as its size and volume change.[8]

Process scale-up is typically an engineering function, and this chapter is written to provide information to the formulator that would not ordinarily be available. In general, the formulator will develop a product without sufficient awareness of the problems that can arise during scale-up (other than raw material variations from the vendors). The pilot plant is then an essential and valuable tool in choosing the equipment and processes needed for full-scale production. This is an area where a fundamental understanding of unit operations and a willingness to experiment proves most fruitful.

The items most often considered by the process team during scale-up from the laboratory bench to production are:

- Mixing
  Impeller dimensions and types (e.g., 1.5 pitch marine style, 316 stainless steel)

  Impeller tip speed (e.g., RPM and diameter)

  Impeller diameter to tank diameter (D/T ratio)

  Turnover rate (number of batch turnovers per unit time)

  Flow profile (based upon the impeller and vessel configuration and batch loading)
- Heat Transfer
  Heat transfer medium

  Heating and cooling rates

  Maximum and minimum temperatures (batch and vessel walls)
- Mass Transfer
  Phase transfer rates

The task of the process team is to understand which characteristics are controlling the process, which ones must be kept constant, and which may be allowed to vary.

One method of scale-up is to develop the product on the bench using plant (full-scale) limitations. In this method, mixing times, heating/cooling rates, and phase addition times will be held constant from the laboratory to the pilot plant, and into full-scale production. The disadvantage of this method is that it will stifle creativity at the bench due to current manufacturing limitations. The existing plant becomes the model, and even the pilot plant will be designed to mimic its environment and limitations. If a cosmetic company is to prosper, it must continue to expand its technology base with new and innovative products and up-to-date production equipment in which to make them. Basing all scale-up on the plant will not lead to the degree of innovation necessary to achieve vigorous corporate growth. A more progressive approach to the scaling process makes newer technology accessible to the chemist through the pilot plant and the processing team. The formulator now has the resources of a process team to not only guide a new product from bench to production, but to suggest and institute new technology to improve the entire process. In this approach, the formulating chemist and process team will drive the development effort forward so that the manufacturing plant will purchase and install new technology to manufacture new products.

a. Agitation

There are many different types of mixers used in the cosmetic industry. The marine propeller works well at viscosities below approximately 5000 centipoise. Above this viscosity paddle-type mixing is usually required. For improved reliability, three rules to apply during scale-up are:

- The same design propeller should be used in the laboratory, pilot plant, and in manufacturing.
- The mixer tip speeds (linear velocity) should be maintained within the same magnitude.
- The batch turnover rate should remain constant.

The batch turnover rate is calculated using the pumping data supplied by the propeller manufacturer. The liters per revolution (or gallons per revolution) data supplied are usually based upon water as the medium being mixed. As stated earlier, the mixing time required to ensure that every part of the mixture has passed through the mixing impeller at least once, is for three times the theoretical flow rate.

The scale-up team needs to be aware that propeller mixers used in the laboratory are drastically oversized. Increasing the diameter or speed of the mixing

blade will increase the horsepower requirement throughout scale-up. Increasing the blade diameter has a much greater effect on horsepower than increasing speed. To maintain scalability from the laboratory through production, a standard D/T and tip speed should be used. This will cause the most difficulty for the formulating chemist since the movement in a 1-kg beaker will appear to be minimal. It is the job of the Process Team to understand this limitation and adjust to a scalable impeller when the product enters the Pilot area.

b. High-Shear Mixing

High-shear mixing typically refers to the use of a homogenizer. There are many homogenizers on the market. As with propeller mixers, their characteristics need to be determined and understood before use. Unfortunately, the data available through the manufacturer is often minimal and may not relate to the formula under development. Data developed by the manufacturer most often were gathered using water as the working fluid (or the data may be based upon a single formula from another industry, typically pharmaceuticals). Actual data must be developed for each process and refined for each formula. To make scale-up easier, the shear developed over time must be quantified. The change in viscosity over time and/or particle-size reduction over time data are required.

The same types of problems exist with homogenizers as with propeller mixers: homogenizer speed, peripheral speed, homogenizer diameter to tank diameter (D/T ratio), turnover rate (flow per unit volume), homogenizer diameter, and flow profile. As with propeller mixers, the equipment used in the laboratory is usually oversized. In-line homogenizers allow for additional control and can make the scale-up process easier, but may require very long process times. Drop-in homogenizers are more difficult to scale-up since additional agitation is required to help ensure all particles or dispersions are passed through the homogenizer.

When scaling products that require rotor/stator homogenizers from the laboratory to production, several major criteria should be addressed. The first consideration is to keep the design of the homogenizer constant whenever possible. Second a constant shear rate should be achieved by maintaining the peripheral (tip) speed of the rotor constant for all batch sizes. Tip speed is defined as the linear velocity of the rotor tip at a given speed, and is calculated as follows:

$$T = (\pi \times D)\ (R)$$

Where T = Rotor Tip Speed in meters per minute

D = Diameter of the Rotor in meters

R = Rotor speed in revolutions per minute

Equation 13.10 Tip Speed

The third consideration should be the flow rate through the homogenizer. By maintaining the batch turnover rate constant for all batch sizes, the shear rate per unit time will be maintained. This ensures that the same shear is imparted to the product over an equal time period (this is to be controlled in the Pilot area as well as throughout production). Additional concerns are homogenizer diameter to tank diameter (D/T ratio), turnover rate (flow per unit volume), and the flow profile.

c. Heat Transfer

When a product has been satisfactorily produced on the bench, the set of parameters used in its manufacture must be duplicated in the production environment. Heat transfer on the bench is controlled most often by a steam table or hot plate for heating, and a water/ice bath for cooling. Agitation is provided in order to ensure the reaction mixture is homogeneous in both temperature and composition. The formulating chemist is very concerned with the batch process temperatures because, in order to form a proper emulsion, the waxy components must be completely melted. Other raw materials have maximum and minimum temperature parameters (to maintain stability and efficacy), which also must be respected. During mixing of the oil and water phases, one must maintain a particular temperature and provide enough agitation to allow the proper formation of the emulsion. The combination of temperature and agitation should ensure the desired results.

What is often not given proper consideration is the heat transfer rate. How fast or slow the product is heated or cooled is often critical to bringing the process successfully from the bench to production. The process team is faced with the problem of duplicating the bench process (small batches, short heat/cool cycles) in production (large batches, slow heat/cool cycles). A one-kilogram batch can be heated from 25°C to 85°C in 20 minutes. This is a three degree-per-minute average rate. This rate is easy to attain in the lab, but is difficult or impossible to attain in production on a 2,000-kg continuous stirred-tank reactor (CSTR). If this average rate must be attained in order to make satisfactory product, the process team will have a difficult time. In order to increase the heating rate, process development and production must raise the steam pressure to attain a higher kettle jacket temperature. This can lead to situations where components of the batch can be burned onto the kettle wall. Active ingredients and preservatives may be overheated, voiding their usefulness in the product. Carbomer and gum solutions may produce flakes on the kettle side, which under side-wiping conditions may result in specks and/or graininess in the final product.

Once a batch is at a high temperature, the time at which it remains there may be critical due to volatile and heat-sensitive raw materials. Fortunately, the heating rate is seldom a scaling problem because its rate is rarely the critical processing factor.

The cooling rate is often more critical than heating rate because of the need to allow waxes and gels to set their structure in the desired manner. If the product is a complex emulsion with an inversion temperature, attaining the proper rate of cooling through this point is absolutely critical. Because the energy-input rate due to mixing must be constant, lengthening the cooling time in production may result in a ruined emulsion due to overmixing (the cooled waxes are now worked more than those in the laboratory, so a different structure develops).

The chemist normally will not use a heat transfer medium other than tap water for cooling. This can range from 10°C to 18°C or more depending on location and season. Ice may be added occasionally, if the product must be quick-cooled. The process team must also attain the same cooling rate upon scale-up. If too fast a cooling rate is demanded, the process team may respond by providing a jacket temperature lower than normally used by the chemist in the laboratory. This may result in freezing or crystallization on the kettle surface. If the product contains many waxes, crystallization may occur prematurely and the final product may be grainy, lumpy, and not meet specifications.

The reason for the variation in time-versus-temperature data when a product is scaled from a beaker in the laboratory to a CSTR in production is the ratio of kettle volume to jacket surface area available for heat transfer. In any CSTR scale-up system, as one goes from a small volume to a large one the volume of material to be heated/cooled increases as a cubic function, whereas the surface area increases as a square function. This exponential difference must be overcome in order to provide the proper heat transfer rate. As a typical illustration of this processing dilemma, a one-kilogram cream or lotion batch may be cooled with tap water at 12°C from 78°C to 30°C in approximately 16 minutes. This is a 3°C per minute average cooling rate. For the same product in a 100-kg kettle, using the same temperature tap water at optimum flow rate, the average cooling rate slips to approximately 1.5°C per minute. If we scale to a 500-kg kettle, this rate is even less favorable. Typical rates can be as low as 0.15°C per minute or less, depending on jacket quality, design, etc. What happens during this additional cooling time as we scale from small to large? Mixing is prolonged, and may not duplicate the product cooled on the bench by the chemist at a rate of 3°C per minute. For this reason, *the chemist must strive to keep laboratory conditions similar to what is attainable in production*. And, where the Chemist cannot maintain the conditions, the Pilot Plant **MUST** maintain them.

Not all products react adversely with bench-to-production variations. There are many products robust enough to withstand all but the most extreme variations. But as the science of cosmetics progresses, emulsion systems for creams and lotions, soap systems for shampoos/conditioners, and gels for body washes are becoming very sophisticated and more sensitive to process variations. Thus, heat

transfer and heat/cooling transfer rate should always be considered carefully during formulation and scale-up.

d. Mass Transfer

In scaling up or down, phase addition times should remain constant. It is important that the rate of dilution remain constant as the batch size changes from the laboratory bench to production. This is particularly true for emulsion products during the emulsification steps. Maintaining this requires a conscious effort at the laboratory level. Since production often cannot transfer at rates that the chemist uses at the bench (seconds), without safety being a concern, laboratory processing must be based upon actual production capabilities. Even a slow laboratory transfer may be too fast for production once the rate is scaled. One hundred grams in thirty seconds for a one-kilogram batch is a slow dripping addition. For a 2000-kg production batch this becomes 200kilograms in 30 seconds (with comparable agitation in the vessel). Since the mixing rate is slower, particularly for viscous systems, the dilution and related transfer rate should also be slower. Most emulsions will have a transfer phase of 10–30% of the batch. In the pilot plant and in production, this phase should transfer in 10 to 30 minutes (silicones are a noted exception). *Laboratory operations must allow for similar addition rates*. Since the transfer phase is usually hot, partial additions over the expected time period are better than a slow addition at an uncontrolled temperature.

13.1.6 DRY SYSTEMS

Table 13.1 distinguishes between two types of solid-solid mixing operations: those concerned with segregating powders, and those with nonsegregating or cohesive powders. The essential difference between these two categories relates to the properties of the powders themselves and, in particular, to the freedom with which individual particles have to move independently of their neighbors. Free-flowing powders exhibit many process advantages (such as easy storage, easy flow from hoppers, smooth flow into packages), but have the disadvantage that they tend to segregate unless all the constituent particles are of very similar shape, density, and size. Cohesive powder, on the other hand, lacks mobility and individual particles are bonded together and move as clumps or aggregates. Although segregation is not a problem (except, as can be seen, at very small scales of scrutiny), cohesive powders are difficult to store and do not easily flow from hoppers (see Bulk Powder Storage).

In a powder mass, there are forces at work that tend to make the particles bond to each other and these are balanced by the gravitational mass of the particles, which causes them to fall apart again. Although the bonding forces, for a given powder, are largely independent of particle size, their gravitational mass is not. Since the gravitational forces are based upon the mass of the particles, particles will stick together only when they are small enough for the gravitational forces acting on them to be much smaller than the bonding forces. Powders composed primarily of such particles exhibit cohesive characteristics and those consisting of larger particles tend to be free flowing. To a first approximation, the division between the two types of powder is approximately 50 μm; below this particle size, powders are generally cohesive.

A - Titanium Dioxide	B - Magnesium Carbonate	C - Mica
D - Zinc Stearate	E - Micas coated with Titanium Dioxide	F - Talc
G - Organic Pigments	H - Inorganic Pigments	

Figure 13.33: Range of particle size of some powders used in cosmetics

Figure 13.31 shows the particle size range of commercial grades of some powders commonly used in cosmetics production; by inference, it will be noted that they are all predominantly cohesive in nature.

The nature of the bonding forces between powder particles is of fundamental importance to many industries and is well understood.[23-26] The characteristics of these forces, which are essential to understand, are as follows:

- The forces operate over very short distances. Particles must be brought into very close contact to obtain maximum agglomerate strength (as in pressing).
- The forces are greatly enhanced by the presence of any liquid—particularly if it is easily capable of wetting and spreading over the particle surfaces (like most powder binders).

- The forces that create the agglomerates are very much weaker than those holding the particles themselves together. That is, it is much easier to break up agglomerates than it is to break up a primary particle (most milling is just de-agglomeration, not true particle-size reduction).
- The probability of a small particle bonding to a larger one is much greater than that of a particle bonding to another of the same size.
- Particle shape has as important a relationship to the bonding force as particle size since available surface may determine if particles can get close enough to bond.

a. Blending Equipment

Powder eye-shadows, face powders, and powder blushers are commonly composed of the following types of material:

Talcs	Pigments	Liquid binder systems
Micas	Pearl agents	Preservatives

The order in which these ingredients are processed and the mixing that carries it out are specific to the type and color of product produced.

The completed process is specific to, and depends largely upon, the types of equipment that are used. A satisfactory powder product, when examined under high magnification, is seen to consist of small agglomerates or single particles of the pigments adhering to and covering the surface of the larger talc or mica particles. Improperly processed powders contain larger agglomerates of pigments existing as discrete entities and separate from any talc or mica particles and unevenly coated talc or mica particles. When rubbed, for example between finger and skin surface, such improperly processed powders change hue as these agglomerates are broken and the smaller pigment groups are released and follow their natural tendency to coat the larger particles and the skin. This process is often referred to as the "extension" of pigments onto talc or mica. This "extension" is often intentionally performed separately as an intermediate step in the process. This allows the overall process to flow smoothly while allowing extra processing and testing of the "extension" before use.

The processing of bulk pigmented powder products is dominated by the need to achieve adequate "extension" on an industrial scale. Of all the devices that have from time to time been tried, none has proven more popular than the hammer mill, Figure 13.32.

Since the pre-mix is an additional operation and adds to the processing time and cost, the mixer that is selected must be as efficient as possible. In the past, the most widely used mixer was the "ribbon blender," which comprises a horizontal drum containing a rotating axial shaft that carries ribbon-like blades. In such a device, the pre-mix can take between 20 and 60 minutes. Other mixers are now the standard (V-Mixer and Double Cone), which utilize higher energy input and are quicker to achieve the same level of randomness in the finished mix. Table 13.6 summarizes the properties of some of the more conventional powder mixers. Since it is relatively easy to achieve good mixture quality (at a large scale of scrutiny) in cohesive powders, any mixing device will eventually produce a satisfactory, even distribution of components. This assumes that no dead spots, where mixing does not take place, are present. The dead spots can be "controlled," typically through manual scraping by the operator during processing, if required.

It is typical to add the binder as a liquid after the preliminary mixing stage. Solid waxes may be included in the binder if they are added at a sufficiently elevated temperature to ensure complete liquefaction. The binder may be poured or pressurized through a suitable orifice in the mixer. It is preferred to spray the binder into the mixer cavity as an aerosol through a venturi or similar orifice/nozzle device. This procedure helps to distribute the liquid more evenly and avoids the formation of wet, lumpy areas in the powder body. Detrimental heat can develop during the manufacture of these high solids formulations. Many times liquid binder sequences (oils, fragrances, preservatives, actives, etc.) are added that must be well mixed to avoid spotting. If they are heat-sensitive or volatile, allowing the blending or milling operation to overheat these components may damage the integrity of the batch. (Most spray nozzles are designed using water. Surface tension of oil binder tends to produce a much poorer quality spray than a water blend. Care must be taken when choosing nozzles, as each binder may behave differently, but production may have limited nozzle capabilities.)

The separation of large agglomerates of powder and binder, which takes place in the milling step that normally follows the binder addition, assures the completion of the wetting process provided the binder is correctly chosen. Should the binder still appear to be unevenly distributed after the passage of the powder through the mill, the product can often be rescued by passing it through as fine a mesh sieve as possible. (Additional milling has its own concerns that are discussed in later sections.) The addition of the binder through high-pressure nozzles improves the fineness of the spray. However, some blending vessels cannot handle the higher pressures, usually 100 to 3000 psig (6 to 200 bar). In general, the finer the liquid addition, the more control of the finished batch is available. An alternate design to the high-pressure spray concept uses cutters to improve the distribution of the liquid and may not require the additional milling step. The cutters provide the additional energy to disperse the binder as smaller droplets in a uniform fashion.

Table 13.6: Conventional Powder Mixers

Type of Mixer	Batch / Continuous	Main Mixing Mechanism*	Speed of Mixing	Process Concerns	Ease of Cleaning	Energy Consumption	Quality of Extension
Horizontal drum	B or C	Diffusive	Poor	Quality of mix	Good	Low	Poor
Löedige/Littleford -type (Plough-shear) (with cutters)	B	Convective	Good	Dead spots at ends	Fair	Medium	Fair/Good
Ribbon Blender	B	Convective	Poor	Dead spots at ends	Fair	Low	Poor
Nauta Mixer (with cutters)	B	Convective	Good	Slow top to bottom blending	Fair	Low	Fair
V-Mixer (with cutters)	B	Diffusive	Fair	Batch size to volume of equipment	Good	Medium	Fair
Airmix	B	Convective	Good	Noisy	Poor	Low	Excellent
Double Cone or CBM** (with cutters)	B	Convective and Diffusive	Excellent	Batch loading	Good	High	Fair
Bowl Granulator (with cutters)	B	Convective and Diffusive	Excellent	Batch loading and scale-up	Good	High	Excellent
Extruder	C	Diffusive	Poor	Formulate to the equipment	Fair	High	Fair
Fluidized Bed	B	Convective and Diffusive	Good	Formulate to the equipment	Fair	High	Poor

B (Batch)—Discrete processing of ingredients

C (Continuous)—Continuous processing of ingredients

* All Mixing Mechanisms include Bulk Flow

Cohesive Mixing: natural or forced combining of particles during blending

Distributive Mixing: controlled or uniform diffusion of particles during blending

** CBM—Containerized Batch Mixing

Batch loading will determine the effectiveness of the mix in any particular vessel. Overloading the processing capacity of a vessel will lower the quality of the mix and possibly increase the temperature during processing. Undersizing the capacity will limit production and affect the quality of the liquid binder distribution. *All scale-up should ensure proper and consistent batch loading throughout—bench, pilot, and production*. For CBM and related higher-energy systems, the loading is between 5 and 8 kilograms per cubic foot (28.3 liters) of vessel contents. Acceptable products can be produced within these ranges. For bowl granulation, the loading is less than 20% of capacity and cannot be varied from vessel size to vessel size.

The use of the liquid binder to assist in the development of color is a technique to be used only under special circumstances. The liquid binder will assist the high-shear equipment to disperse the pigments onto the chosen substrate. However, the pigments will be much more temperature sensitive to color shifting after the liquid has been added. Therefore, each lot of the chosen colors will extend out differently. Extending the pigments and then properly distributing the binder as separate steps can minimize this effect. A special case needs to be considered when dealing with organic pigments. These will darken significantly with heat. Some processes are designed to include this effect. However, a slight hydrogen sulfide odor may be noted when this takes place. Also, the color is not a more intense version of the original shade, but a less vibrant, dirtier, deeper version. To match the shades produced in the laboratory, all development and production must use the same method of extending the pigments along with the same temperature controls.

Pearl agents, especially the titanium-coated micas, present a special problem. Many of these brittle materials, which depend on their size to achieve the desired effect, are prone to disintegration in the hammer mill. For this reason, they usually have to be mixed into the bulk after its passage through the mill, necessitating an additional mixing operation. The pearls are normally added after the binder has been uniformly distributed throughout the batch. Again, this is to minimize the work energy applied to the pearls. Some pearl coatings can handle the additional oils normally associated with the binder. In those cases, the pearls may be added before the binder. The lower energy of the "cutters," Table 13.6, compared to the hammer mill, can be used to disperse the binder with "minimal" effect on the

pearls. The pearl may be added into the bulk in the same device used to perform the preliminary coarse mixing, providing the device has been cleaned.

It may sometimes be necessary to pass the bulk finally through a sieve or coarse screen to break up agglomerates of pearl and to ensure its even distribution. Some formulations use pearls that are processed through the hammer mill to develop a particular feel. Less expensive materials should always be evaluated, if this is required for a successful product, rather than purchase specialty-sized particles designed to impart appearance effects that are then resized to meet physical and not visual aesthetics. A minimum of energy should be applied to pearls to maximize their benefit to the product while minimizing the cost of the product.

Treated pigments and pearls may allow additional flexibility to the formulation since less processing should be required to attain particular aesthetics. As an example, a lower binder level may be used in a high-pearl formula (making for easier processing with less milling) because the treated/coated particles accept the wet binder easier with fewer spots and streaks.

Figure 13.32: Hammer Mill

b. Shearing Equipment

The hammer mill was designed as a comminution, particle-size reduction machine. The standard design consists of a fast-rotating shaft fitted with freely swinging hammers mounted in a cage that is equipped with a breaker plate against which the feed is disintegrated, chiefly by impact from the hammers. The very high speed at which the hammers move (60–80 m s^{-1}) increases the chance of a hammer making

contact with each particle and with particles making contact with particles. The dwell-time of particles within the chamber is controlled by the placement of different-size screens over the exit. Alternate designs exist that have different hammer designs or no swinging hammers with the rotor being a single unit. The alternate designs offer some cleaning benefits because the disassembly is easier and simpler.

Hammer mills are very efficient in the comminuting of brittle particles in the range of 1500–50 micron, but below this size their efficiency (the probability of direct impact) falls off rapidly. This is fortunate, since it means that small-particle-size cosmetic talcs and micas (not pearls or pearl extensions) can be passed through without being substantially altered. At the same time, however, the very high rotational speed of the hammers and the airflow within the chamber ensure that there are enough weak secondary impacts (particle-wall and particle-particle) to break the much weaker pigment agglomerates—which may be up to 500 micron in diameter. The primary particle size for most pigments is 0.1 micron in diameter. The mill is primarily a de-agglomeration device. The disintegrated agglomerate fractions then stabilize by becoming coated onto larger talc or mica particles and should not be further changed by subsequent passes through the mill equipped with the same screen. The tip-speed of the mill should be controlled to ensure that its energy level is higher than any other energy level developed during subsequent processing. For example, if a batch of powder is initially milled through a coarse screen for color development and then milled through a fine screen for improved binder dispersion (at the same tip speed), the shade of the product will change. This is because the pre-milled colors will see a higher level of energy once the binder is added and the batch is milled. The addition of binders may aid in the development of color; however, the quantity of energy and type of equipment used will determine the possible particle-size distribution that will be produced.

Nevertheless the hammer mill, in its role as an extender of pigments onto talc or other substrates, has certain disadvantages. For example, most of the extremely high energy it makes available is wasted and is largely dissipated in heating the powder. From the viewpoint of energy consumption, a hammer mill is very inefficient. The feed-rate and therefore the processing time for all but the smallest batch sizes of powder is very slow. The screen size is adjustable, but one does not set the screen at the size particle that is needed. The finest screen is normally 0.010-inch (0.254 mm) wide slits approximately 0.5 inches (12.7 mm) long in a herringbone pattern.

An attempt to speed up the process by the substitution of exit screens of larger diameter opening reduces the residence time in the mill. This often results in inadequate extension, necessitating a second, third, or fourth pass through the mill. On the other hand, increasing the residence time of powder within the grinding chamber by decreasing this opening size can cause the screen to become blocked with compacted and bridged powder, resulting in overheating and damage to both

the machine and product. The design of the hammer mill allows a high volume of air to pass through it during the milling operation. This airflow can assist in minimizing a temperature rise during processing, but it cannot eliminate it. The airflow will also cause a fraction, usually less than 2% of what is being milled to be lost as fines (small particles that are easily carried through the air-handling system). If the process is not properly adjusted, the losses can be as high as 30%. If this high level of milling is required for the process, an alternate high-shear device should be examined, like the jet-mill, turbine mill, or classifier mill.

Perhaps the biggest disadvantage of all is that the hammer mill is a noisy, continuous processing device being used for batch processing. Since it is a continuous process device, it must be fed with a powder mixture that has already been effectively mixed, otherwise the color of the product exiting the mill will change as each section of unmixed bulk passes through. This preliminary mixing must be efficient, particularly if there is minimal mixing during the subsequent processing. It is not necessary for any extension to be achieved at this stage. The formulator will normally include an excess of the substrate material in this sequence to ensure a complete extension of the pigments. Subsequent blending will then be used to produce a uniform finished product.

The hammer mill has a design flaw that must be remembered. Some powder can drop vertically through the unit without being affected by the rotating hammers. It is typical, when milling pigments, to pass the batch through the hammer mill at least two times to increase the probability that all particles are hit and extended at least once. Alternate milling designs may have the same design problem or exit from a side or top panel to minimize this concern.

c. Alternatives to the Hammer Mill

The drawbacks to the hammer mill, as used for powder extension, have led to a search for other machines that can fulfill this function more satisfactorily. The ideal equipment would probably have the following properties:

- It would be capable of breaking up weak particles in the size range 0.5–50 μm without damaging talc or mica particles of similar diameter.
- It would be a low-energy device, consuming little power itself without heating the powder mixture excessively.
- It would be both a batch- and continuous-processing device capable of mixing and extending in one operation.
- It would be rapid: processing times of less than 10 minutes for 100 kilograms.
- It would not cause aeration of the powder (since this causes problems in later processing).

- Its efficiency would not vary with the cohesiveness of the powder—it would not be affected by poor flow characteristics of the raw materials.
- It would be quiet, clean in operation, and easy to clean.

Other comminution devices have been shown to produce extension. Pin mills (inefficient when compared to a hammer mill) and fluid energy or jet mills (too efficient at particle-size reduction when compared to a hammer mill) are two examples. No alternative seems to work as efficiently as the hammer mill at spreading the pigment onto the talc or mica.

In recent years, however, the development of high-speed powder mixers, which are also capable of producing some degree of extension, has brought the industry closer to the ideal. Two types in particular are worth mentioning. The first of these is best described as the horizontal vortex mixer. As an alternate high-speed mixer, it is often referred to as a plough-shear device, because of the unusual shape of the mixing paddles, which rotate on an axial shaft in a cylindrical-like horizontal mixing chamber (similar to a ribbon blender). These paddles cause the powder from all parts of the chamber to be thrown about in such a way that it all passes rapidly through a zone occupied by a series of rapidly revolving blades off a separate shaft referred to as a "chopper." The chopper is largely responsible for the powder extension and may be switched on or off independently of the main axial drive.

The second type is a high-speed modified bowl granulator. It has a propeller- or anchor-shaped blade of "good aerodynamic" design that rotates very rapidly in the dished base of the mixing bowl. This design mixer has a separate "chopper" shaft to increase the available energy to extend the pigments. Mixing and dispersion occur at the point of collision of the powder particles (in the upper part of the mixing bowl) by particle-particle and chopper-particle collisions. The flow pattern in modified bowl granulation vessels must be properly scaled from the laboratory through the pilot plant and into production. Specific batch sizes, with less than 20% powder loading, are required to maintain the proper flow characteristics. The tip speeds are usually matched for all of the process sizes being utilized. This will help to control the time factors in scale-up. Often, special designs are required to ensure scale-up including upgraded motor controls and blade speed operating ranges.

Both types of mixer have been used as partial or complete replacements for the traditional blender-hammer mill combination. Variations of these mixers have also been used in the solids-liquids processing area.

d. Batch Color Correction

It is not unusual for the bulk powder product, even though it has been correctly processed, to require color correction in order to obtain a satisfactory match to the

standard. Since any addition of pigment or talc may need to be extended, a passage through the mill is necessary. A common procedure is as follows: After the preliminary process has been completed, a small quantity of the bulk (usually 2–5 kilograms), which is assumed to be representative of the whole, is passed through the mill with a coarse screen (≥ 0.125”). (This is to simulate the final milling step in a typical powder process.) This is examined in the laboratory, and if necessary, a pigment addition specified. This correction is added to the small quantity of the bulk product, mixed in roughly by hand, and the small quantity is re-milled through a fine screen (between 0.010” and 0.035”), twice. The screen size matches the one used during the original process. The twice-milled sample is returned to the remainder of the batch and remixed in the original mixer. A further sample is then removed and the process is repeated until a match is obtained. This method works well as long as the formula can handle the additional pigment without affecting the “feel” or other specifications (testing for international markets or for drug products requires an alternate adjusting method). The finished formula rarely matches the 100% formula originally developed by the Chemist.

There are a number of minor variations to this procedure, which are adapted to suit individual companies; the most important of these is the use of pigments previously extended on talc or mica and stored as such. This has the merit of speeding up the correction process by only requiring the extension to be blended into the batch without the fine screen milling to eliminate particle specks or streaks.

Full formula extenders are used throughout the industry to ensure that adjusted batches continue to match the 100% formula. However, this requires the production area to produce at least five additional batches, depending upon the pigments used in the formula—typically white, red, yellow, black, and colorless. The colorless extender is to “base out” the batch if the shade is too intense. This method is particularly useful if the extenders can also be blended together to make small batches of some shades, which provides some additional flexibility to production. The equipment necessary to make a full batch may be more elaborate than the equipment necessary to blend extenders (typically no high-shear device is required to produce the finished blend).

Another adjustment protocol uses adjusting base(s). This is a full formula extender without the pigment (or pearls). The pigment (and pearl) is added and usually milled into this base, keeping the ratios of the formula constant. This now-full formula extender is added to the batch for adjustment. Now, only one additional batch is required for production to make and store. The use of an adjusting base will be determined by the formula. If the formula without pigment is too wet, the adjusting base will not store well (see Bulk Powder Storage). Separate adjusting bases may also be needed for pearl and nonpearl shades. The first adjusting method described is the simplest, but it may produce the most batch-to-batch variations in final formulas.

When pearl agents are part of the formulation, unless an unpearlized standard is provided, the pearl must be added in the correct proportion to the laboratory sample before color can be assessed. Pearl is normally added to the bulk in the last stage of the manufacturing procedure. Adjusting the shade of a product is often very difficult due to the lighting effects of the pearl. It is important to train the operator on physical and visual examination of the product. The use of light boxes, as a controlled lighting source, is also important. However, it is critical that a standard method of evaluation be maintained from the development stage through the Quality Assurance evaluation of finished goods.

e. Powder Grinds for Creams and Lotions Batches—Dry Mix

If milling of the pigments in a liquid media is not available due to the formula parameters, a dry mix may be needed by Production to shade a cream or lotion batch. The logistics of this type of process requires multiple awkward controls without gaining improved shade control of the finished mass. The procedure is outlined as follows:

1. A batch of the cream or lotion is made without the pigment. This batch must be acceptable from all stability parameters, as it will be used to adjust the shade of the pigment blend.
2. A pigment batch is made. This includes mixing and milling of the pigments and filler powders until a drawdown shows no color specks or streaks.
3. A quantity of the pigment-free bulk is blended with the pigment grind. The same mixing as in production should be scaled for this operation (usually about one liter, similar to the formulation laboratory).
4. The finished laboratory batch is compared to the standard.
5. The appropriate shade adjustment is made on the pigment blend, including milling.
6. Steps 3 through 5 are repeated until the pigment batch produces a finished batch that matches standard.

The powder grind is then used in the actual production of the finished product. Since the powder was pre-matched, no shade adjustment should be required. If these operations—pigment processing and finished cream or lotion production—are performed at different locations, a great deal of finger pointing may ensue if a batch is made without matching the standard. Sometimes a specially colored, full-size production batch will need to be made and blended with the batch that does not match standard to minimize color variations. The use of a dry-mix process is to be discouraged during all phases of the development and production of the finished product.

f. Loose Powders

Loose powder production uses similar equipment and follows a similar process to that of pressed powders. The blending operation is relatively simple with little

high-shear energy needed—except for some preservatives, a little color, and for the addition of oil or fragrance. The requirements for chemical uniformity are lower (from a specifications standpoint) for a loose powder than for a pressed powder. The loose powder is usually examined as a gross or large-quantity powder. The pressed powder (cake or tablet) is usually examined based upon the actives or the pickup from a brush or sponge.

Particle-size distribution and the associated handling concerns are usually more critical with a loose powder than with a pressed powder. Inhalation concerns will often require the formula and process to be very specific and therefore limit the Chemist on flexibility of formula. Oils and waxes may be added to the formula to intentionally agglomerate the finer particles due to limited availability of raw materials with the preferred (safer) particle-size distribution. This addition may be performed as a side phase and added to the main batch as a previously processed ingredient.

The packages for loose powders are larger than those of pressed powders. Therefore, larger batch sizes are normally produced for loose powders. Loose powders may contain a small pigment phase that requires milling, but most do not. Even high levels of fragrance usually do not require a great deal of milling since if the mass is too wet, it will agglomerate and no longer be loose. Without the need for higher energy during processing, less efficient mixing systems can be used by production. The batching times for these large, loose powder batches may be longer than for a similar formula pressed powder. The oldest, slowest machines are usually saved by production for loose powder work. Most loose powders are fragranced, while most pressed powders are not. Removing fragrance from equipment can be very challenging and difficult. Large batch sizes and simplified quality testing ensure that a small quantity of residual fragrance has a minimal effect on the next batch to be processed.

g. Filling Loose Powders

Loose powder filling machine or auger fillers are used to fill non–free-flowing powder into containers. This type of equipment is relatively low maintenance and gives high-accuracy filling. The machine works both on auto or manual configuration. It consists of a hopper equipped with sweep blade connecting to an auger. As the blades turn, the powder moves inside the hopper and the auger displaces the product through the funnel into the container held under the dispensing nozzle. The electronic sensor provides the precise filling cycle through an enclosed pulley unit, brake, and clutch that is fixed on auger shaft. Once the sensor sends signals, the powder filler machine will fill-in the automated quantity of powder product into the container through the auger. The two designs either start and stop through a timed cycle or based upon a particular number of revolutions of the auger screw. Cosmetics loose powder containers are usually plastic jars.

An alternate filling system pulls a vacuum on the filling container. The level of vacuum is controlled to "pull" the powder into the container to meet the proper weight. The powder must be free-flowing for this type of filler. If the vacuum seal is not perfect, the packages will get dusty from overfill.

Filling is dependent upon the flow characteristics of the powder. Rotary volumetric fillers are used to fill powders at high production rates. These systems are designed to enable the powder to freely flow into moving chambers that control the weight and/or volume of powder to be filled. The chambers are designed to completely release the pre-set quantity of product into the package (usually boxes or bags) just prior to sealing the package. Automatic dishwashing detergents, powdered bath products, and cereals are packaged this way. Due to variations in bulk density that may be enhanced through shipping, these packages are completely opaque and are typically labeled "Note that settling may occur during shipping and storage." The volumetric filling system design can tightly control fill variation of the package assuming the variation of powder to be minimal.

h. Filling Pressed Powders

There are several different types of press powder machines available. Most machines are equipped with powder feed system that use a linear-vibratory feed or free-flowing feed to supply the pans with loose powder to be pressed. Hand distribution of powders into the cavities with pans is a key variable to be continually examined by the Chemist and Quality Assurance. Although hand "raking" of powder can be very efficient, slight variations in loading into a pan can give very different aesthetics. The Chemist will often weigh powder for individual pans being pressed. The production variations need to be understood when providing samples for evaluation. All the presses can produce acceptable tablets within the pressure range of 100 to 1000 psig (6 to 70 bar). It is important to remember that the pressure across a single pan is not the same pressure across several pans unless the total surface area remains constant. Different pressing systems are as follows:

- **Top-down press:** This is an automated powder press machine that is normally used for large-quantity runs. It is used for pressing face powder, eye shadow, or blush into pans. It is designed in a manner that the single or multiple punch press driven by hydraulic or electric piston from top moves down to stationary module where pan is located. Pressing cloth is normally fed from a roll.
- **Bottom-up press:** The mold moves up and is driven by piston into single or multiple stationary punches. This style of pressing helps the powder to deaerate better than the top-down method. Deaeration depends on the ribbon cloth and the gradual pressure applied until the target pressure is

achieved. Pressing cloth is normally placed manually in single sheets. This press machine is used for face powder or eye shadow.

- **Manual press:** This is a single-press machine that is used for small runs. It is also used for blush and eye shadow that are pressed into unusually shaped pans. This single-pan application operates with manual pump via hydraulic piston to press the powder pan. To regulate the amount of pressure applied on the powder, a pressure gauge installed on the press reads the pressure of the top punch when pressing on the mold pan area. The pumping should stop when the desired pressure is achieved.

The design of the manual press is such that usually the punch is driven downward to the stationary mold with loose powder in the pan. However, in a laboratory unit, the bottom plate moves up with the loose powder in a pan and the punch is stationary.

All of the different systems used to produce pressed powders have certain components in common. The powder must be presented to the filler. The appropriate cavity is filled and the cavity is placed under pressure. The pressed unit is collected, tested, and placed in a package for shipping.

In presenting the powder to the filler, it must be uniformly presented to each pan. This may require that a "fluffing" be performed just prior to filling. This fluffing may be as simple as rolling the drums of powder before use to evenly aerate the mass. It may be as formal as a container with a mixing blade designed to aerate the powder as it travels to the filler. It may be an air conveyor system that aerates the powder as it is moved. Aeration is not a preferred action, since the air must be pressed out of the powder in the next step. However, the change in density after storage may be necessary to achieve uniform production at the press over multiple shifts.

Variations in tablet press quality may be due to flexing of the structure, cycle rate, internal pressure measurement, pan variability, and fill variability. Formulation will affect very few of these variables. However, it is important for the formulator to understand the variability of the press during formulation. If the pressing pressure range for an acceptable tablet is too small, due to the acceptance of other characteristics (payoff, coverage/sheerness, packing density/durometer, size and shape of pan, type of applicator, etc.) good production runs will not be the norm. A small variation in the pressing pressure should not produce a significant change in the aesthetics. The formula may not need to be adjusted, but an acceptable pressure range may be required.

Pharmaceutical tablet and soap pressing are separate specialty presses. Pharmaceutical tablet presses are designed for near continuous operation and can be used to generate over 10,000 psig (667 bar). They can produce over 10,000 tablets

a minute using dual-station operation, depending upon the tablet size. Since these are normally used in the pharmaceutical industry only, the tablet is not pressed into a pan. Similar controls are needed to those of the pressed eye shadow, but the flow characteristics are much more critical. The small volumes must be filled easily and consistently to provide the correct level of drug/active to each tablet. Improper settings will very quickly force thousands of dollars worth of materials to be rejected.

Sometimes, the powders will be partially agglomerated to better control their flow through the production system. Pressing times are still an issue, so production machines are often rotary with the rotating table being very large to provide sufficient pressing/dwell time for each tablet. Soap pressing may be performed at very low pressures, which are both formula and package dependent. The slurry of bar soap is injected (sometimes using an extruder) into a mold, minimizing uncontrolled air while the bar shape is formed. The soap bars are then dried and packaged. Molds are typically brass or sometimes stainless steel. There are periodic uses in cosmetics, like bath and toilet bowl additives, that have smaller production requirements. The key part for both of these presses is the control of the process from raw materials through the exit of the press. Since production is continuous, any delay in testing or uncontrolled operation can ruin huge quantities of finished goods.

Parameters used to control pressed powder products:

- Target pressure: This element plays a balancing act in keeping the powders together in a tablet form in order to pass and meet the general QA tests standards, such as drop test, payoff, and texture.
- Press/dwell time: The press time is critical to form a tablet and deaerate the powder to be pressed, and it normally ranges between half a second to two seconds in order to complete the pressing cycle. The dwell time is the hold at the maximum pressure to ensure the pressed tablet does not bounce back after compression. An extended dwell time can sometimes produce a different product feel. This does not usually affect the product, but it is an operation that may be a variable for the press. Any increase in dwell time is a decrease in production capacity.
- **Mixer/augur speed:** The augur positioned inside the holding tank helps to move the powder towards the dispensing nozzle. The longer the rotation time, the more consistent powder is dispensed, but the more the powder may have pre-compacted producing flow issues.
- **Type of pressing ribbon:** The type of ribbon used is driven by aesthetic and press deaeration. The pattern and weave of the ribbon cloth is selected based on several reasons such as type of powder, aesthetic appearance, and desired final quality. The weave of the cloth (threads per inch [or millimeter] and the size [diameter] of the threads) is used to produce a consistent

texture across the finished pan. The tighter-weave ribbon provides better aesthetic with finer finish but at the same time traps air inside the pressed tablet, which may cause a spongy look and breakage. The more moderate weave or less coarse ribbon cloth helps the powder to deaerate during press. It also provides better payoff and texture; however, the aesthetic may not be as pleasing since it leaves a larger print pattern on the pressed tablet. If the mass contains too much air for the cloth to vent, the pressed piece may immediately crumble once the pressure is removed or it may bow as the air bubbles in the powder are released from being under pressure. If the pressing cloth is too fine for the air to release, a second press may be necessary. In normal operation, the pressing cloth is changed and the press is operated a second time. Some automated machines can perform these two presses at different pressures to best control the density of the powder throughout the pan—start low, finish high. This double operation will cut production capacity by approximately 40%.

- **Press number:** Some products require multiple pressing to achieve the desired characteristics. Usually the first press is done at a lower pressure to dissipate the air and the second press applied at a higher force or target pressure to achieve the final formed tablet. (Some tests have been done by the equipment vendors to validate the finished pressure from multiple presses. The results indicate no change in pressure value, but possibly less aeration in the finished press.)
- The design of the mold and die will determine the net pressure available for each pan, and therefore the capacity limitations. Different pan materials hold and dispel static charge differently. Most pans are steel or aluminum (if water is involved in the formula or the end use). Some pans are plastic. The handling characteristics are very different. Pan shape and depth will affect the flow of the powder prior to and during the pressing operation. Sharp corners make for easy breakage. Round pans handle high pressures more evenly across the entire surface.

i. Powder Scale-Up—Batch

Scale-up of powders is dependent upon many processing parameters. These include the following, which must be measured and controlled from the laboratory bench, through the pilot plant, and throughout production.

- the batch loading by volume and/or by weight [to be maintained]—which affects the flow patterns in the compounding vessel
- the shear being imparted on the powders and powder dispersions (typically measured as tip speed) [to be maintained]—which affect the color

and color development of the raw materials

- the process time [to be scaled or maintained]—which affects the overall production capacity
- the batching temperatures [to be maintained]—improper processing can produce temperature elevations of over 40°C from the normal batching temperature
- the batching procedure [to be scaled or maintained]—due to the use of high-shear, detailed procedures will maintain the quantity of shear consistently through all production
- the sequencing of the phases [to be maintained]—changes in sequence will affect the color or intensity and consumer characteristics ("feel") of the finished product
- the raw materials [to be maintained or at least controlled]—examples: (1) variations in dye strengths, in different lots of pigments, will change the finished shades and require color adjustments; (2) changes in particle-size distributions will change the look of larger-particle pearls; (3) changes in the source may shift the color range from red with yellow to red with blue

Batch loading, the quantity of powder to be produced in a given vessel configuration, is a particular problem for all new formulas. If a new shade is being made of an existing product, a history through production has already been developed. A new product may be less dense, more cohesive, more brittle, contain a higher pigment load, a higher binder level, a higher pearl level, or be more temperature sensitive and harder to press than a formula already in production. All of these variables can affect the batch loading. If the loading is too high, the process will usually create a higher intermediate temperature during processing. The spraying characteristics (spray pattern and particle-size distribution) of the binder will also be affected if a constant addition time is to be maintained; the flow rate will be changing as the process scales-up. If the batch is being milled *in situ*, using a bowl granulator for instance, the loading will significantly affect the color development. Unfortunately, multiple laboratory, pilot, and production batches may be required to determine and fine-tune the proper loading.

Process time has some additional flexibility with powders that is not usually available in liquid systems. Since heating and cooling are not usually taking place during the mixing or shearing operation, the actual time necessary for a step to take place is less critical. However, the steps should be performed in a very consistent manner with times that can be duplicated. If the scale-up time from the laboratory to the pilot plant for one product is one to ten, it should be maintained for all similar products that use the same equipment system. If it is not, some process variable is not being controlled and could become a major problem for production.

The development of accurate and detailed procedures is key to proper color development and shade matching of products. The protocols used to evaluate finished bulk, finished/packaged products, and, where necessary, intermediate sequences must be as controlled as is the equipment being used. Evaluation of esthetics can be difficult to translate to an intermediate process test. Standard measurements of rotational speed, temperature, and time may be all that is available. These may be sufficient. In addition, motor current draw, on a high- or low-shear drive, may be used to indicate that a spray addition was uneven or that a batching scale is out of calibration. Both of these problems would make for batches that would be a challenge to shade match as well as an increased opportunity to produce rejected product. New pieces of equipment used to measure powder flow characteristics have recently been introduced to the pharmaceutical and cosmetics markets. These instruments can provide some numerical indicators on flow and shear during development and production of powder phases and batches.

Production may schedule work as a series of batches. An example for loose powders may be based upon fragrance intensities (light level or scent to heavy). They may schedule a series of pressed powders batches based upon color intensities (white to yellow to red to black/dark). These scheduling groups can help Production to minimize the effects of a poorly cleaned system or an elaborate cleaning procedure. Some of the newer equipment designs allow Clean-In-Place (CIP) operation. (CIP is an automated system for cleaning equipment following an exact protocol the same way every time. It should only be used when a system is designed and tested to ensure that proper controls make the system clean every time. New products should always be "validated.") Most of the older equipment, notably the ribbon blender, cannot be CIPed due to the design limitations of its sealing systems, which may leak and may not be very water-friendly. Cleaning of a ribbon blender can be a slow, tedious process and is rarely 100% effective.

Production schedules can minimize the effects of this problem. To meet other regulatory requirements, equipment (batching vessels and sometimes mills, weighing areas, and compounding areas) is often designated for organic pigments only or inorganic pigments only.

Powder systems, with their inherently low water level (activity) are rarely a microbiological problem. However, the use of preservatives is included in most formulas due to the package design and the methods of use by the consumer. Even if the equipment has not been completely cleaned before the next batch, it must be sanitized. This may produce "clean" dirt, but the finished product will not be a site for potential microbial growth while the consumer uses the product. The newer systems that allow CIP usually also allow SIP (Sanitization-In-Place). The

preferred method is to use steam. The residual heat will dry the vessel. Chemicals may also be used for sanitization, but these could leave a residual and affect the next product.

13.1.7.1 WET CONTINUOUS PROCESS

The objective of building a continuous process, as compared to the batch processes discussed earlier, for an emulsion, liquid/liquid, or liquid/solid cosmetic product is generally the need for high-volume production. It may also be desired for just-in-time manufacture or to make a special product where intense energy is required to form a viscous product such as a hair-styling gel. Examples of the most common cosmetic products made via this method include hydro-alcoholics, creams, lotions, shampoos, hair conditioners, hair dye developers, and hair-styling gels. Color cosmetics, such as liquid or cream makeup, may also be possible provided color correction is not required.

Formula composition is maintained using stoichiometric calculations usually by means of a series of piston pumps, connected by a common shaft with a variable-speed motor/drive. Piston pumps are generally used to allow for higher pressures with accurate delivery. Each phase in the formula requires a separate pump to control its composition in the total formula. Each pump is calibrated for weight delivery by adjusting its stroke length at a constant motor/drive speed. This will lock that ingredient or phase into the proper formulation. Production rate is then increased or decreased by speeding up or slowing down the drive motor. Pumps employed may be of several designs depending upon the application. Sanitary pulsation dampeners may be employed downstream to smooth out flow, but oftentimes are not necessary. Other components of the process may include some or all of the following items:

- a static mixer for pre-mixing some or all of the formulation constituents
- a dynamic mixer to provide additional mixing energy to set the emulsion or dispersion
- a scraped-wall, plate-and-frame, or tubular heat exchanger to cool the product if required

The final components in the system prior to storage or filling are usually pH or conductivity and viscosity in-line measuring. This equipment is used to monitor quality. They will usually control adjusted pump delivery to maintain the finished product specifications or sound an alarm to shut the system down. The complete process may be freestanding and require considerable space, or mounted on portable skids and rolled up to a production filling line for just-in-time manufacture depending on the complexity of the design, size, and application.

Figure 13.33: Simple Continuous Process Liquids System

a. Emulsion Products Requiring Cooling

Experience has shown that for lotions, creams, etc., it is usually possible to form a crude emulsion by metering the oil and water phases and gum phase first into a static mixer, Figure 13.33. The crude emulsion is then finished either in a dynamic in-line mixer and/or a scraped-wall heat exchanger where other temperature sensitive colors, fragrances, and actives are injected just before the heat exchanger inlet. This minimizes their exposure to heat to just a few seconds during the rapid cooling process within the heat exchanger. Injected ingredients should be added into the centerline of the pipe at a velocity equal to the mass velocity of the formula passing that point. This will minimize concentration pockets in the stream. The advantage of the scraped-wall heat exchanger is that it can be used as a final mixing element by controlling its internal shaft speed (mutator) during very rapid cooling to set product rheology prior to storage and/or filling. Cooling capacity and rate is very high in these units, being four to five times more efficient and substantially more rapid than cooling in a CSTR. Some products, however, cannot tolerate the turbulence and/or cooling rate produced in these units. Plate-and-frame or tubular heat exchangers may be employed where cooling rate is less critical and can be slower. Product viscosities are usually low, so films do not build up in the heat exchanger and limit temperature control. These can also be used when mixing energy during cooling is detrimental to product rheology.

There are two things to keep in mind when one needs to make an existing batch-made product on a continuous flow process.

(1) The continuous-made emulsion will have a smaller mean average particle size and a much narrower particle-size distribution compared to the batch-made product. This may change product rheology and affect such things as rub-in break and feel on the skin. This occurs because every particle of the continuously made product is exposed to much more uniform mixing energy than is possible in batch-made product. Experience has shown, however, that through careful tuning of the continuous process, the rheological properties can be brought close enough to those made by batch process so that the consumer cannot detect a significant difference.

(2) One should be aware that continuous-made product, particularly lotions, may have a much lower initial viscosity, perhaps as low as 20% of that compared to initial batch-made product. This is due once again to the intense mixing energy imparted in either the in-line mixer and/or the scraped-wall heat exchanger. However, the rate of viscosity build may be much faster achieving the same value as the initial batch viscosity within the first two or three hours. This may require that the product be stored for several hours prior to filling to prevent filling issues like a premature shutoff of the filling nozzles. This would produce uneven fills due to rapid viscosity changes during filling. Normally, the product equilibrium viscosity will reach the same value as batch-made product within a few days, if total and finishing work energy in the heat exchanger (mutator shaft speed and velocity of product) have been respected.

b. Emulsion Hair Conditioners

Emulsion hair conditioners may require much more sensitive temperature control during the process prior to filling or storage than that of most other products. A typical arrangement for this type of product would require the following in sequence:

- heated oil phase
- heated water phase
- metering pumps for the above
- static mixer
- plate-and-frame heat exchanger—a simple design that is easy to maintain with excess area to control the exit temperature of the crude emulsion to within ± 0.5°C of a predetermined intermediate temperature (temperature is chosen based upon DSC curves and set point of oil-phase ingredients)
- dynamic mixer (typically rotor/stator)—used to set the product rheology at that temperature

- another plate-and-frame heat exchanger—to cool the product prior to storage or filling
- backpressure valve—to control the residence time in the dynamic mixer
- in-line pH and viscosity instrumentation and mix systems—used to monitor product quality, adjust phases, or note alarm conditions and shut the system down

It should be noted that the use of a scraped-wall heat exchanger would not work in this application due to destruction of the product viscosity at lower temperatures. Experimental work is required to determine the correct temperature of feed to the dynamic mixer combined with the correct rotor/stator configuration, speed, backpressure, and product flow rate, to obtain the desired results prior to scale-up.

c. Hair Gels

Hair gels are generally processed cold. They consist of a water phase containing polymers and styling agents, and a solvent phase containing neutralizer, colorants, and other desired ingredients. These phases are generally metered together at room temperature into a static mixer to form a crude gel followed by a dynamic mixer to complete the gel structure. The finished gel then passes through in-line pH and viscosity instrumentation prior to filling or storage. Instrumentation can be provided to adjust the neutralizer phase pump based on pH feedback and/or viscosity. Experimental work is required to develop process parameters such as the number of static mixing elements required, flow rate, dynamic mixing speed, backpressure required for different production rates, and phase tolerances required, ensuring control of the formulation.

d. Scale-Up of Continuous Systems

A pilot system producing between 0.5 and 5 kilograms per minute of product containing all of the main components envisioned for the full-scale production system is recommended to work out the process. This size system lends itself well to the use of small commercial equipment. It can also demonstrate product characteristics and stability, determine operating parameters, and develop data for control of the process and the sizing of full-scale production equipment. In addition, it makes pilot runs of 30 minutes to two or more hours reasonable to handle to examine formula and/or process changes.

The pilot system should be designed with maximum flexibility built into each component of the process. This will allow collection of scale-up data over the entire operating range. The system would include such things as variable-speed drives on phase pumps, dynamic mixers, and scraped-wall heat exchangers.

Sufficient instrumentation should also be provided to ensure accurate recording of temperature, pressures, and speeds of dynamic equipment during experimental runs so that material and energy balances can be calculated for each component. The following scale-up rules to keep in mind:

- pipelines should be sized for equal velocities and pressure drop
- static mixers should be the same style and contain the same number of elements
- dynamic in-line mixers should have the same rotor/stator design, number of stages, variable-speed drives, and rotor tip speeds where possible
- scraped-wall, plate-and-frame, or tubular heat exchangers should be connected for overall countercurrent heat transfer for maximum efficiency and sized to match developmental parameters—equal velocity throughput, residence time, mutator shaft tip speed where applicable, and overall heat transfer coefficient

The data, when supplied to the engineering department and the equipment manufacturer, will aid substantially in getting the proper full-scale equipment specified and in place. The production equipment typically will not have the same flexibility as the pilot system. Changes to the production system can usually be justified when the next product is developed. By controlling the flexibility/components of the production system, fewer errors occur during the life of the product, producing a more consistent product for the consumer.

e. Production Design Considerations

If heated or cooled phases are required in the process it is necessary to trace, insulate, and control the temperature of pipelines from the bottom of the heated phase tanks to and including the metering pumps and the return lines to the top of the phase tanks for weight calibration purposes. Tandem phase tanks may be required for water and oil phases, etc., since materials must be melted, dissolved, or chilled. One phase tank is thus "online" while the other is being prepared. The alternative to this is to size the vessels for a shift or more of production. In some cases it may also be necessary to control the temperature of phase lines on the pump discharge leading to the point where the initial emulsion or dispersion is formed in order to prevent "salting out" or solidification in lines. The need for this must be determined in the pilot plant.

If the product requires "aging" to ensure conformity to specifications, bulk storage of mass may be required. If so, capacity should be based upon the longest age time and testing that may be required (the storage tanks may not be upgradeable while the processing system can be).

Weight/mass calibration of each phase must be established at constant drive motor speed set at the actual flow conditions and line pressure that will occur during production. If the calibration is not performed at constant drive motor speed, the formula will not be correct once production starts. This requires installation of an adjustable valve in each recycle/calibration line to pressurize the pumps and recycle lines to ensure accurate calibration. Each recycle line should also have a check valve at the kettle return discharge point to keep the line full for weight accuracy. Suitable controls and instrumentation are required to allow flow to process or recycle for each phase independently, and to divert all phases to recycle or process simultaneously. These requirements are necessary to prime all process lines for calibration and production startup. An adjustable backpressure valve should be installed at the process end to keep pipelines full, control residence time, and optimize performance in the mixing and heat exchange equipment.

Once the stroke positions are set properly for each phase pump, calibration is complete. Startup is accomplished by first adjusting the drive motor to the selected speed and allowing the system to run in recycle mode under production line pressure conditions. Each phase process line is then primed to its injection point. Next, if heated phases and product cooling are required, the process portion of the system is made ready for product manufacture by passing hot water through the in-line mixers and heat exchangers (see Figure 39.35). This is necessary to bring the pipeline, mixers, and heat exchange equipment up to equilibrium conditions so that once the process is initiated a minimum of rework is created.

Production then begins by switching all pumps to process mode and simultaneously cutting off the hot water flow. Product exiting the heat exchangers is diverted to drain or reworked for a few seconds before allowing it to pass through the Quality Control in-line measuring equipment and into the storage or filling systems. This allows all the product temperature controls to reach equilibrium.

The system is shut down in reverse order as it was started up. Simultaneously the pumps are diverted into their recycle position; hot water is flushed through the pipeline, mixers, and heat exchangers and routed to the drain. This flushes the system in preparation for cleaning and sanitization for the next run. Supply and recycle lines from and to the heated phases and ingredient tanks are blown back to the tanks with air pressure, and are cleaned and sanitized as required.

13.1.7.2 DRY CONTINUOUS PROCESSING

Continuous production of powder products requires flexibility in the formula and large volumes for filling. Typical examples are powder water softeners, automatic dishwasher detergents, and bath products. In general, these products all share a few characteristics—they do not foam a great deal, they do not contain a large quantity

of oils or other liquids, the powders are the active ingredients, and the raw materials all flow well, Figure 13.34.

The powder formula must be flexible to allow for a larger variation than with creams and lotions due to the different processing controls used in a continuous powder process. Typically, the raw materials are stored in bulk. Bulk storage expands the range of temperature and humidity the powders are subjected to compared with laboratory storage conditions. The ingredients for the formula are metered automatically or sub-phased as large batches to be used continuously. Therefore, only gross adjustments (color or fragrance level) can be made under controlled circumstances. Exact shades can rarely be reproduced. Even with modern process controls, the mixing systems will have normal variations. Without continuous in-line testing, finished product samples are grouped together for a general analysis. If the variations that resulted were unacceptable, recalls of finished goods would need to be made. This is a time-consuming and costly operation.

Figure 13.34: Simple Continuous Powder System

a. Bulk Powder Storage

The two factors that have an important effect on stored cosmetic powders are moisture and pressure. It is not always appreciated that a small increase in relative humidity can give rise to sufficient moisture in the stored powder to change the main mechanism of particle-particle bonding, which will increase the bond strength of agglomerates by a factor of 2 or more. Such an increase in cohesiveness can make the handling and flow problems already inherent in cosmetic powders perceptibly worse and can change the processing characteristics of (for instance) an eye shadow to the point where all the pressing machine settings may have to be altered to compensate.

In the same way, powder bulk that has been stored in vertical containers exhibits increasingly difficult flow characteristics as the container gradually empties. The lower layers, having been compressed by the weight of powder above them, become increasingly cohesive as the bottom is approached. If oils or moisture has been added to the powder, they may migrate towards the bottom of the container and produce a significant product change between the top and the bottom. For this reason, it is sometimes better to store powder blends in a large number of small, well-sealed containers than in loosely covered large bins. Each container should be mixed before removing any of its contents.

If the variability of the powders being stored is known, or at least controlled, the flow characteristics within a container can be adjusted through the design of the container. The most efficient design would have the first particle that was dropped in be the first particle that comes out (FIFO). Most simple silo designs do not function that well. Powders will settle towards the edges as fines while only a center core of material leaves the silo. This is termed funnel flow. Modifications to an existing silo are always cumbersome at best. Therefore, vibrators, or sometimes air jets are added to enhance the flow of material. Often, the operators use sledgehammers on the body of the silo or the outlet cone.

Design improvements have led towards an almost FIFO design called mass flow. Several variations have been designed, but they all require specific knowledge of the powders being stored, such as particle-size distribution, particle shape, density, angle of repose, etc. The angles of the sidewalls are designed to ensure that the particles cannot sit and accumulate. A change in the powder being handled may require a new silo, Figure 13.35.

Figure 13.35: Hopper Flow

Mass flow versus funnel flow, as pictured above, shows the general physical difference of the two hopper/silo designs. The volumes and the outlet diameters are the same for the two hoppers shown in Figure 13.35. The funnel flow design will allow flow through a center core with finer particles gravitating towards the walls of the vessel. This will produce a change in particle-size distribution as the level in the hopper changes. It will also enable easier "bridging" of the outlet of agglomerated material. The mass flow hopper, by design, allows the lowest material to leave first while the top material leaves last. There is virtually no mixing or separation of the horizontal layers.

Often, the hopper supplier will design the slope of the sidewall to be sharper than a critical friction value for the particular material. As noted through the illustrations, the mass flow hopper is taller and narrower than the funnel flow design. If the hoppers are being installed to meet a particular volume requirement and there is a height limitation, a mass flow hopper may not be a true option. Alternate designs can be used to improve the flow characteristics of the silo; these designs modify the flow with internal changes, typically baffle plates. These alternate designs do not allow for complete cleaning without special attachments and will still have some particle-size distribution changes during use.

Industrial bulk containers (IBC), in an assortment of sizes, shapes, and materials of construction, are commonly used to store large volumes of powder. They can range in design from a large bag (sometimes with elaborate linings) to a skid-mounted silo. Since they hold less material than a "normal" silo, segregation due to particle size is less. These containers are normally filled at the supplier and emptied at the cosmetics manufacturing facility. Therefore, there is no concern that the first

material received by the manufacturing facility will be the last out during use, as is the case in a poorly designed silo. It is assumed that the manufacturing facility will control the IBCs and use them FIFO. The key concern for the cosmetics and drug industries is contamination. Special bags have been designed to minimize sifting, which is the loss of powder at the seams of the bag. Sometimes, special one-piece liners are inserted into the bag to minimize sifting. It is assumed that if powder can come out, moisture and microbiological contamination can enter.

The cost of liners can be a problem. Single-use liners can be very expensive, but can be maintained microbiologically clean until use. Multiple-use liners may control sifting, but require cleaning and sanitization between uses. This may leave unwanted residuals in the bags, which can become incorporated into the next powder. The older, skid-mounted style containers are stainless steel and were originally designed to handle liquids (275 gallons in the same skid space as four 55-gallon drums). These containers do not work well with powders and often have cleaning and sanitization problems for liquid use. Newer designs can empty powders completely but the containers must be cleaned, sanitized, and dried before reuse. Microbiological concerns continue to exist for these containers.

Once the bulk container has been filled with powder, a transfer system is used to move the powders through the process. The typical conveyor systems fall into three main categories. The first design is pneumatic. An air blower is used to fluidize and then move the powders through special tubing. If the line sizes are not well matched to the flow rates, some segregation of particles may take place. If the powder must be kept dry, low-moisture air must be used to move the powder. This technique can be used to move large quantities of materials over very long distances with minimal product-to-product contact. Some particle-size breakdown takes place, which is often dependent upon the length of the run and the bends during the run. The second design is mechanical with product-to-product contact. A screw conveyor is a classic example of this technique. Another is a chain within a tube or housing. The screw is used to move the powder at a very controlled rate. By design, the distance between supply and deposit cannot be large. Since the movement of the screw or chain physically pushes or pulls the powder, degradation and compaction of the powder often take place. The third design is mechanical without product-to-product contact. Examples are bucket and belt conveyors. These conveyors move the powder in discrete increments with little detriment. Unfortunately, the atmospheric conditions must be controlled, particularly if moisture is a concern. This operation is usually open to the air, and microbial growth is always a finished-product concern.

All conveying systems have an inherent limitation when moving powders. Dust explosions can take place when dust levels reach a critical point within the conveyor operation or dust collector system. The explosions are actually deflagration (explosion with a propagation speed ranging between 0.1 and 100 meters per second, which generates pressure waves that are much faster than the flame front) not detonation (sudden burning without propagation). As with all burning conditions, oxygen and the combustible material must coexist along with an ignition source. The ignition source is often a static discharge or a worn or damaged component in the system. The combustible material is usually an organic dust—sulfur, starch, wood, flour, sugar, carbon, rubber, and some plastics are examples. When the dust reaches sufficient concentration, and its powder reaches minimum ignition energy because an ignition source is available, an explosion will take place.

The environment must be controlled to minimize the explosion potential. Hybrid explosions, due to a combination of materials in an isolated space, present an even greater danger. Each material may require extra controls when contained with other materials. The danger is when several materials are near their combustion points and join in the explosion. An example could be a low level of cornstarch being blended with iron oxide pigments. The system may be designed to safely handle the pigments, but the cornstarch will accelerate any explosion while the pigments continue to burn and limit how the system can be handled. Special design changes may be required and are dependent upon the raw materials being examined, the process, and the environment. Some possible control methods include the following:

- change the particle-size distribution from the supplier to minimize finer particles
- change the conveying system to minimize particle degradation
- the addition of a liquid spray to the dust atmosphere to agglomerate the fines, cool the powder to lower the ignition energy, minimize static build-up, and lower the oxygen level in the atmosphere
- increase the level of fines in the controlled environment to lower the oxygen level in the atmosphere to a point where an explosion cannot occur

Additional methods should be examined to minimize risks that may not be apparent during the development of a product, but that may be critical during production. Some of the raw material concerns to be aware of include the following: Explosibility Index (Kst), Minimum Ignition Energy (MIE), Minimum Ignition Temperature (MIT), Limiting Oxygen Concentration (LOC), Minimum Explosible Concentration (MEC), Volume Resistivity (Ω), Charge Decay Time, Electrostatic

Chargeability, and Self-heating Onset Temperature. The raw material supplier may be able to address these issues if specifically requested. Since non-bulk storage and dust collection are less of a concern, the information may not be automatically made available for small lots used for laboratory testing. These tests can be performed by a few outside laboratories where a one-meter sphere is used to force an explosion and measure the values. Tests run between $3000 and $5000 per ingredient or formula being tested (2012 values in the U.S.).

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COLD-PROCESS EMULSIFICATION PRODUCING SUB-MICRON DISPERSIONS: FORMULATION AND AESTHETIC ENHANCEMENT OF COSMETIC AND OTC PRODUCTS

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ABSTRACT:

In view of the trend to reduce costs and increase convenience of emulsion preparation, especially in these days of consumer safety concerns, energy saving focus and environmental consciousness, new technologies are needed to address global concerns. One potential solution utilizes an alternate approach to formulating conventional emulsion-like products. This approach enables cosmetic scientists to prepare personal care and OTC drug products more rapidly and more reproducibly. Unlike conventional emulsions, with typical particle sizes exceeding 3 microns this process generates sub-micron, oil-in-water micelles, which are 1/10th to 1/20th the particle size of conventional emulsions.

Oils and other hydrophobic materials used in these systems have different physical and chemical properties which are distinct from conventional emulsions where the properties of both the oil and water are modified by the nature and quantity of the surface active agent(s) employed. Hydrophobic materials take on very different, desirable tactile properties when the dispersed phase particles are reduced significantly in size. The resulting sub-micron systems can be easily mixed into a rheologically modified matrix and are able to generate either conventional or highly unique tactile experiences. On a laboratory scale, the products are prepared in a single beaker so there is no need for separate sub-phases, and no heating, shearing or cooling is required! This cold-process formulation technique takes from 5 to 15 minutes per batch resulting in a significant improvement in productivity and new opportunities for unconventional product distinctions. This technique can also be readily transferred to the larger scale vessels used in the production of the final product.

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13.2.1 CONTEMPORARY COSMETICS

In the late 1960s and early 1970s functional skin treatment products emerged. Product performance expanded beyond the amelioration of superficial dryness, and their benefits evolved to a higher therapeutic level. The boundary between cosmetics and dermatological products began to blur. Cosmetic problems such as aging, uneven skin pigmentation, slack skin, cellulite, sensitive skin, oily skin, and dryness were identified and agents were sourced or developed to address these conditions. These skin disorders were often associated with conditions such as sunburn, acne, and the need for topical analgesia, etc. Cosmetics turned more to

medicine for its resources, and dermatology became a source for agents that could provide either a marketing or performance advantage.

As the number and potency of functional materials increased, there arose a need to control the delivery of these agents in order to mitigate potential irritation, target their transfer to the desired location in the skin, or protect environmentally unstable materials for a commercially viable period of time. Again, the personal care market borrowed from the latest advances in medical research. *Table 1* is a summary of some of the major delivery systems currently being used in the personal care market place, the types of active they carry, and their primary benefit.

Table1: Delivery Systems Most Commonly Used in Personal Care

Classification	**Description**	**Diameter**	**Materials Carried**
Liposomes	Phospholipid-based unilamellar or multilamellar bilayer vessicles.	100-500 nm	Hydrophilic and hydrophobic actives
Nanodispersions	Phospholipid-based micellular dispersion.	100-500 nm	Hydrophobic actives
Vesicles	Surfactant-based vesicles or micelles	100-500 nm	Typically hydrophobic actives
Polymeric	Crosslinked acrylate or allyl methacrylate polymer	10-500 micron	Typically hydrophobic actives
Microencapsulates	Aldehyde crosslinked protein	100-5000 micron	Typically hydrophobic actives
Encapsulates	Crosslinked guar, alginate or other carbohydrate polymer	5-500 micron	Typically hydrophobic actives
Entrapment/ Clatharate	Clatharate of cyclodextrin	N/A	Hydrophobic actives
Linked	Active ingredient is covalently or ionically linked to polymeric support	N/A	Hydrophilic and hydrophobic actives

a. The Future

The trend toward even greater performance in personal care products will continue as we move further into the 21st century. New, more potent therapeutic agents are

being sourced from the fields of biotechnology, pharmaceuticals, and botanicals. Materials such as enzymes, growth factors, antioxidants, cytokines, DNA, genetic promoters, epigenetic modifiers and other sophisticated materials are already being evaluated in the research facilities of leading manufacturers and suppliers. These agents, though more effective than previously used actives, are often sensitive to environmental conditions such as oxygen, heat and light. Thus, there will be a need for equally sophisticated delivery systems that will maintain or enhance the efficacy of these sensitive actives from their formulation and environmental surroundings.

While drug delivery systems have received much attention because of their potential, what is often overlooked is the vehicle into which these delivery systems are incorporated. The latter part of this chapter will focus on the proper selection of the vehicle into which the delivery systems are added, in order to ensure their performance is not compromised. The chapter will also explore a new approach to vehicle development that is complementary to most, if not all, delivery systems. This new approach is actually a true innovation in formulation science. The technology employed offers virtually unlimited aesthetic and form modification capabilities that will enable the user to truly enjoy the experience of applying the product. This new approach is called Sub-micron Micelle-Based Formulating.

b. Properties of Emulsions

Clearly, the preferred cosmetic and personal care vehicle for topical application contains both aqueous and anhydrous phases. Such products have virtually unlimited aesthetic properties and can be applied in many forms such as serums, lotions and creams. However, these components are generally incompatible with one another unless an agent is added that more significantly reduces the interfacial tension between the oil and the water phases. To do this, it is necessary to change the native properties of the oil phase and the water phase to make them more compatible with one another. This phenomenon allows the formation of a two-phase system in which one of the phases (e.g., the "oil") is suspended in the other (e.g., the water). Such ingredients are called "surface active agents" ("surfactants"). A special sub-category of surfactants is called an emulsifier. These materials not only lower the interfacial tension at the oil/water interface but, with the input of shearing energy, they enable the formation of droplets of one phase within the other. Such emulsifiers have a wide range of surface-active properties. When carefully selected, they can stabilize the incorporation of oil into a water phase or water into an oil phase. The resulting product is called an emulsion. In many cases such emulsions are prepared by heating the oil and water phases to a temperature of 70° C or greater before combining the two phases. The purpose of heating the phases is to ensure that all waxes used are melted, and that the two phases have a low enough

viscosity so the two phases can mix freely. The oil and water phases are typically mixed together until they achieve a homogeneous appearance. Thereafter, they are slowly cooled to ensure the formation of appropriately small-sized droplets. It is essential that the droplets be very small in order to ensure the stability of the emulsion since, in these cases, Brownian motion will retard sedimentation. Such emulsions typically have a homogeneous, opaque, white appearance. They provide a smooth, pleasant feel upon application to the skin, hair, or other epithelial surfaces. In fact, the fields of surfactant chemistry and emulsion science have become a major disciplinary area that a competent cosmetic chemist must master in order to be a successful formulator. The proper use of surfactants to form all of the various types of useful emulsions can become a totally engaging, lifelong pursuit. The vehicle formed by the combination of an aqueous phase with an anhydrous phase will be the primary focus of the remainder of this chapter.

c. Issues with Emulsions

The introduction of surfactants to the cosmetic industry has provided a "double-edged sword" for formulators. Although the many different types of surfactants have yielded a vast array of cosmetics with very desirable aesthetic properties, the issues associated with their use have not been properly addressed. To the formulator, the development of emulsion-based products is replete with problems. Such development is a time consuming process. Further, these issues are generally limiting towards the goal of achieving desirable aesthetic properties. These issues can produce thermodynamically unstable, non-reproducible and difficult-to-scale emulsions in the manufacturing process.

It is easy to understand, therefore, why the time to develop a traditional emulsifier-based product is so lengthy. Seldom does a formulating company's Marketing department or Business Development function request exactly the same formulation. Generally, new marketing concepts will necessitate a change in composition from prior art. This change can cause a cascade of undesirable events. Different aesthetic properties are also frequently requested by Marketing in order to generate new products with new claims. When changes to either the aqueous phase or oil phase are made, the emulsifier blend, which was effective in previous systems, generally must be altered. Altering the emulsifier blend may result in a change in one or more aesthetic, performance, or safety properties. Immediate stability of the composition is often compromised as a result and, worse, such instability is not usually identified until the second or third month of accelerated stability testing. This behavior may indicate a potential problem with the long-term shelf life of the product, and it is insidious since it requires either re-balancing of the emulsifier ratios or a change in the emulsifiers selected. This has forced the industry to develop methods to predict the degree of hydrophobicity of the oil phase in order to marry

it with the necessary changes to the water phase in order to permit the formation of a stable emulsion. Systems such as the HLB (Hydrophile-Lipophile Balance) have been devised. This assigns a relative numerical value to the degree of lipophilicity of a given oil, which enables the formulator to select the necessary emulsier, or blend of emulsifiers required. This approach was first developed by William Griffin who was employed by Imperial Cosmetic Industries (ICI). (1) This method has proven to be invaluable when preparing standard emulsion products. However, it becomes less predictive as the lipophilic phase becomes more complex through a mixture of soluble oils and is less relevant where blends of insoluble lipophilic agents are used.

Generally, a sequential approach to problem resolution will result in an extended time to develop a product. This will result in a delay in the projected launch date that can cost the organization millions of dollars in anticipated revenues. As a consequence, the formulator is best served by trying to anticipate potential issues. Multiple batches, having several ingredient variations, are typically prepared in order to address any unexpected contingencies. This process is filled with unnecessary redundancy and is generally unacceptable in commercial practice. Compounding the above mentioned issue is the effect that processing can have on the outcome of a batch. Emulsion stability is dependent on a variety of parameters such as the zeta potential, particle size, crystal formation, and water-binding activity (2) of the ingredients employed to achieve the desired rheological properties of the product. These parameters are dependent on the temperature to which the oil and water phases are heated, the rate of heating, the method and rate of mixing of the phases when combined at elevated temperatures, and the rate of cooling. Most emulsions require heating to insure that all higher melting point materials, such as waxes and butters, are completely melted, dissolved or dispersed in the appropriate phase.

Further, many raw materials with unique aesthetic properties cannot be emulsified easily, if at all. Useful molecular weight silicones, silicone and hydrocarbon-based gels, and fluorinated compounds are all very difficult to incorporate into a stable emulsion system.

Traditional emulsion systems also create difficulties in manufacturing. The need for heating and cooling systems, specialized high and low-shear mixing, and assorted additional processing devices makes the manufacture of emulsion systems very capital intensive. Further, the equipment specifications and energy requirements will vary from country to country. This situation will cause a modification in the processing variables thereby making it almost impossible to have a truly “global” manufacturing protocol. The energy needed to process such products can be significant and undoubtedly will add to the final cost of the finished unit. This is especially true in Europe and Asia where the price of energy is very

expensive. Similarly, there is a long duration of time required to prepare a batch. It can take from 5 to 24 hours, or more, to complete the processing of emulsions depending on the batch size and number of sub-phases required. This reality requires intensive labor and energy that adds to cost to the final product.

The need for high temperature water or steam to heat the phases of the batch can cause damage to heat sensitive actives such as retinoids and proteins. Prolonged heating of certain materials can accelerate the reaction of the active agent with other components in the emulsion, or with air, if the material is oxygen sensitive. For example, the exposure of unsaturated hydrocarbons, such as vegetable oils, to air at high temperature can cause oxidation and lead to both rancidity and an undesirable color change. This is especially true where there is a growing consumer demand for products that are naturally-based from sustainable/renewable sources.

As indicated above, the complexity of the manufacturing procedure for personal care emulsions, and its dependence on many processing variables, leads to frequent quality issues. This is especially true with respect to the product's final textural and rheological properties. If any factors such as the heating, cooling or mixing rates are not carefully duplicated, the material prepared may have different properties than the preceding batches of the same product! As a result, the stability of the emulsion may vary from batch to batch.

Often, the difference of a single parameter is significant enough to cause the product to be outside the established optimum specifications. Inevitably, batches have to be either discarded or reworked. The lack of reproducibility is especially problematic when the product contains a physiologically active agent. Lack of reproducibility, due to manufacturing variations, can affect product performance and decrease consumer satisfaction. It also results in products having undesirable aesthetic properties that the user may perceive as a lack of quality. This will ultimately lead to consumer dissatisfaction, or reduced compliance in product use.

Some emulsions can be made without heating but these systems preclude the use of higher melting point materials that can add richness to the aesthetics of the final product. Further, if the rate of mixing is high, there is a chance that air can be entrapped in the emulsion. This phenomenon causes an undesirable decrease in the specific gravity of the product and an increase in product viscosity. Any variability in processing can lead to a range of undesirable rheological and textural properties. This issue can occur even if the formulation is not modified. The term "product by process" is well known in the patent art and describes this phenomenon. Often, if two or more formulators prepare the same product, the resulting compositions may vary considerably. This surprising variation can occur even though each person utilized the same lots of raw ingredients. The unsettling phenomenon occurs because it may be very difficult to exactly reproduce all of the processing parameters used

to make an emulsion. If any of the processing variables is modified unexpectedly, particle-size variations may occur, or the crystalline properties of the emulsion can be compromised. *Table 2* is a chart containing the results from an experiment to determine the effect of processing on the final properties of a 5% petrolatum-containing cream. All preparations contained the same lots of ingredients. The data demonstrate that the viscosity and specific gravity can vary dramatically depending upon the processing parameters employed to make the batch.

Table 2: Petrolatum Cream (5%) - Standard Emulsion Test Results

Sample 1: Optimum Manufacturing Procedure				
Sample 2: Overheated Phases				
Sample 3: Forced Cooling				
Sample 4: Ambient Cooling				
Sample 5: Paddle mixing				
Sample 6: Rapid homogenization				
Sample 7: Underheated phases				
Sample	*Specific Gravity*	*Initial Viscosity (cP)*	*Viscosity (cP) 24 Hr @ 25oC*	*Viscosity (cP) 24 Hr. @ 50oC*
1	0.912	99,970	140,580	26,560
2	0.937	85,910	93,720	29,690
3	0.952	93,720	109,340	29,700
4	0.941	51,550	96,840	131,200
5	0.959	62,480	85,910	78,100
6	0.803	112,460	124,960	20,620
7	0.931	51,550	96,840	20,600
Note: Viscosity measurements were taken with a Brookfield LVT model viscometer				

Since there is so much uncertainty at the "bench" level in the laboratory, there is often concern that a typical 500-g to 2000-g lab preparation will not translate directly to a manufacturing environment. This concern is often well founded. Compounding this scale-up problem is the fact that equipment used in the laboratory generally does not correlate with that used in the plant. There is usually a

need for an intermediate phase during scale-up that facilitates this transition. Some equipment is engineered to mimic plant conditions but at a fraction of the size. Even so, scale-up issues abound. To deal with the vagaries of scale-up, the product may be subjected to a wide range of processing variations in order to optimize the conditions of manufacture. Products made at each level of scale-up are typically subjected to accelerated stability testing in order to ensure the integrity of the product for its anticipated shelf- life. When one adds the processing variability and the need for scale up to the uncertainty of the selection of the emulsifier system, it is almost a wonder that any product ever makes it to the market on time. As a consequence, most formulators tend to stay with the tried and true approaches of the past. A significant alteration to these systems or the development of an entirely new system is often laced with unknown issues that can severely jeopardize the introduction of a new product.

13.2.2 FACTORS DRIVING THE SEARCH FOR ALTERNATE APPROACHES

The current practices for making emulsified formulations have a number of limitations in their use. The main factors are 1) the difficulty in creating new aesthetic and textural experiences; 2) the issue of delivering the sophisticated physiologically active agents to enhance product efficacy; 3) Marketplace confusion; 4) the expansion of regulatory restrictions; 5) the desire to utilize more natural and sustainable materials.

a. Textural Diversity

Current formulation practices have produced a number of unique products with a variety of textural properties, however, it is becoming increasingly more difficult to satisfy the current market demand for new approaches to expand the experiential elements and maximize performance. The traditional approach has been to create different types of surfactants and emulsifying agents, but these have generally produced different ways of providing a similar effect. Today's market is demanding more than the minimalistic change being provided by the current formulating paradigm. It is seeking dramatic effects, not subtle nuances.

b. Enhanced Performance

Because the use of emulsifiers and other surface-active agents changes the physical and chemical properties of the oil and water phases to permit emulsification, they also modify the beneficial properties of the hydrophilic and lipophilic functional ingredients added to the composition to deliver product performance. As a result, usually only modest improvement in skin condition, wear properties, fragrance impression, cleansing efficacy, etc. can be realized. Ironically, the very

agents that permit the formation of viable oil-in-water or water-in-oil micelles may be responsible for the suppression of product performance. Further, the environment produced is hostile to liposomes and other delivery vehicles that could help maximize the active ingredient's benefit.

c. Marketplace Confusion

The proliferation of marketed products that have seemingly contradictory positions is creating consumer confusion. As a consequence, consumers have taken it upon themselves to learn more about the products that they are using. They are becoming more product savvy and want to know more about the chemicals to which they are being exposed. While much of this information is useful, some of it is either incomplete or inaccurate. Unfortunately, this has not stopped the propagation of Internet sites advocating a particular position.

d. Regulatory

Expanding consumer knowledge about products is causing the general population to pressure regulatory agencies to take action in order to maintain or preserve the safety and environmental compatibility of the materials that they are applying to their skin. Consumers are banding together to influence organizations and lobbyists in this area, which has resulted in an increase in the number of regulations that have been directing the cosmetics industry. This, in conjunction with the pressure from regulations abroad, has complicated the formulating environment to develop products that are universally acceptable.

Some of the more important regulatory factors are; the EU Directives, REACH, The Cosmetic Act of 2010, Japanese ingredient restrictions, Proposition 65, The California Green Chemistry Initiative (3), increased oversight by the FDA as well as emerging Chinese regulatory policy. While many of these legislations have similarities in their restrictions, they continue to remove materials from use and differ enough in scope that it becomes difficult to create "Globally Acceptable" formulations.

To compound the problem of globally formulating, these evolving regulations make it virtually impossible from an economic perspective, to introduce new, technologically advanced reagents into the industry. The amount of data that is required for new products to be allowed into the marketplace becomes prohibitively expensive to obtain, and causes stagnation in both product performance and aesthetics. Combined with the continuous removal of available cosmetic reagents from the positive lists, formulators are being forced to find new and unique uses for older, less controversial materials.

e. Resource Availability and Sustainability

The availability of resources and the sustainability of materials that are used in formulations has become increasingly important in recent years. There is a growing concern about the impact of synthetic chemicals on consumer health, and they are embracing the use of natural materials for their perceived nutritional and functional benefits. However, these materials require special handling to maintain their integrity. Further, natural materials are subject to shortages and unexpected price increases, which may require rapid reformulation.

Even raw materials that are in abundance have become more expensive to obtain, as the costs of energy have consistently increased and have been added on to the price tag. In fact, traditional materials that were once abundant and used ubiquitously in the marketplace, are now being replaced by less expensive, more sustainable options.

The production of conventional emulsion systems requires a tremendous amount of energy input. The entire process may rely upon extensive heating, mixing or cooling when combining raw materials into finished formulations. Unfortunately, the demand and costs of energy are increasing, which are being passed along to the consumer in the form of a more expensive product.

These and other factors have been a driving force in the search for alternate approaches to the current formulating and manufacturing paradigms.

13.2.3 SUB-MICRON MICELLES

There are alternatives to the traditional emulsification process. One of these is the formation of products utilizing the unique properties of micelles with a small particle size. Sub-micron micelles can be made with virtually any hydrophobic material by carefully controlling the selection of sub-micron production and the processing conditions during manufacture. Since the micelles of each hydrophobic material are made the same way, they are less dependent on the type of emulsifier or surfactant used, or lack thereof, and because they have approximately the same small particle size, there is much less tendency for the micelles to coalesce. This phenomenon is explained by Stokes' Law, which is illustrated in an equation relating the terminal settling or rising velocity of a smooth sphere in a viscous fluid of known density and viscosity to the diameter of the sphere when subjected to a known force field (*Table 3*). Using this equation, with all other factors being constant, a 200 nm hydrophobic agent particle has a velocity of fall that is 680 times slower than one of identical composition having a 5-micron particle size of a standard emulsion.

Further, depending upon the method of manufacture, field strength/zeta potential interactions increase the propensity of the particles to repel one another. Zeta

potential is a measure of the magnitude of the electrostatic or charge repulsion or attraction between particles, and is one of the fundamental parameters known to affect stability. Its measurement brings detailed insight into the causes of dispersion, aggregation or flocculation, and can be applied to improve the formulation of dispersions, emulsions and suspensions. Therefore, there is a greater desire to keep the particles apart, rather than coalesce. (4)

Additionally, the small particle size and field effect permit lipophilic phase concentrations of up to 60% w/w or more, without the high viscosity that is typically obtained when the particle size is larger. As a result, a concentrated sub-micron system will rapidly disperse into water, thereby spontaneously expanding to fill the volume of the vessel in which the water is contained. The micelles will remain in this configuration indefinitely, unless acted upon by an outside force that destabilizes the system. Further, micelle systems with separate lipophilic materials can be freely interspersed and will mutually coexist. Additionally, depending on the method of manufacture, the particle-size distribution can be very tight when properly controlled. In practice, the smaller the particle, the greater the potential benefit.

a. Benefits of Being Smaller

Small particles have a series of benefits depending on their size and uniformity. One interesting property of these systems is that they can alter the aesthetic properties of virtually all materials. This feature results in the opportunity to create new sensations with familiar lipophilic ingredients. Conventional materials such as petrolatum, lanolin, waxes and natural oils are given a new "life" and purpose. Further, the aesthetic properties can be adjusted by simply mixing lipophilic systems with different aesthetic properties to create a new and unique textural experience.

Independent of the method employed to create the sub-micron micelles, manufactured concentrates of various low polarity lipophilic agents (lipophiles) mix together readily, without issue. The practice of balancing the hydrophilic and lipophilic emulsifiers (HLB) depending on the composition of the lipophilic phase that is used so commonly in the preparation of standard supra-micron emulsion systems is now rendered insignificant. Thus, a virtually infinite array of lipophilic dispersions can be mixed, in any proportions, without creating any instability in the final blend.

The combination of individual systems of lipophilic micelles can be performed without the need of any extraordinary processing conditions. This attribute permits the rapid formulation of compositions with the desired aesthetic and performance properties. When it is applied to manufacturing, a significant reduction in both the processing time and the utilization of energy is achieved. A further explanation of these benefits will be covered in subsequent sections.

Systems of sub-micron micelles also have regulatory benefits. Since their particles size is greater than 100nm, they do not fall under the current regulatory definitions of nanotechnology, which is presently being subjected to consumer concerns and legislative action regarding their impact on health and the environment.

Table 3. Stokes' Law

- An equation relating the terminal settling or rising velocity of a smooth sphere in a viscous fluid of known density and viscosity to the diameter of the sphere when subjected to a known force field.

$V = (2gr^2)(d1-d2)/9\mu$

- V = velocity of fall (cm sec^{-1}), g = acceleration of gravity (cm sec^{-2}), r = "equivalent" radius of particle (cm), dl = density of particle (g cm $-^3$), d2 = density of medium (g cm^{-3}), and μ = viscosity of medium (dyne sec cm^{-2}).
- **200 nm particle is 680 times more stable than 5 μ particle of standard emulsion.**

13.2.4 METHODS OF PRODUCING SUB-MICRON MICELLES

There are many methods of producing sub-micron micelles. Some of the more commonly practiced methods are sonication, precipitation, high-pressure/high shear, stirred-kettle, freeze-drying, extrusion, and high surfactant loading with high-shear exposure. Any of these methods are suitable for the creation of sub-micron micelles to be used in the formulation of finished products.

Sonication can be used to create sub-micron micelles through the use of a high-energy probe that transfers its energy to the sample, and breaks up the sample into smaller and smaller particles. Sonication can be direct, applying the probe directly to the sample; or can be indirect, which would transfer the initial burst of energy into an ultrasonic bath, and then the sample would be immersed into this bath to receive the energy and break up the particles.

Precipitation is another method that may be employed to create sub-micron micelles. The hydrophobic phase is dissolved in an organic solvent and a water-soluble surfactant is dissolved in water. These two solutions are mixed and an emulsion is formed into small droplets in the aqueous phase. The water-soluble surfactant decreases the tension at the interphase between the water and the organic solution, allows emulsification to take place, and stabilizes the droplets formed against aggregation or coalescence. The final step involves removal of the organic solvent by evaporation. The hydrophobic phase precipitates and one particle is formed from each droplet. A suspension of small spherical hydrophobic particles is formed. The particle size is dependent upon the surfactant concentration and on the emulsification energy imparted.

High-Pressure/High Shear can be used to create sub-micron micelles as well. This methodology combines a hydrophilic and a lipophilic phase with the help of a facilitating agent and then processes them together at pressures between 5,000 and 50,000psi. Facilitating agents may include any class of surfactant or emulsifier. Generally, lower amounts of surfactant or emulsifier are required when pursuing this method of sub-micron micelle creation.

In freeze-drying, two available methods are thin film freezing and spray freeze-drying. In spray freeze-drying, for example, an aqueous solution containing active ingredients is atomized into the cold gas above a cryogenic liquid. The atomized particles adsorb onto the gas-liquid interface and aggregate there as sub-micron micelles.

Sub-micron micelles can also be created using high surfactant loadings in combination with high shear. In these cases, an appropriate surfactant is used at an atypically high level, in combination with high shear mixing. The method of imparting high shear is unimportant, and resulting particles can vary in size, but are below 1000nm.

13.2.5 FORMULATING WITH SUB-MICRON MICELLES

Traditional methods of formulating products rely on a multitude of factors in order to achieve success. These traditional systems require the separation of lipophilic and hydrophilic phases, the selection of an appropriate surfactant/emulsifier, an extended period of high temperature/high energy processing, and then an extended period of cooling/force-cooling under specific processing conditions. Each of these steps create an opportunity for mistakes and promotes an environment that is rife with variability. Formulating with sub-micron micelles removes many of the troublesome steps in formulation.

Formulations using sub-micron micelles utilize cold-process conditions. Lipophilic and hydrophilic phases are pre-formed into high concentration sub-phases, where they are incorporated together to form finished formulations. The need for high temperature exposure and high energy processing is removed, and the consistency between batches is increased substantially.

Perhaps one of the greatest benefits of formulating with sub-micron micelles, is the speed at which formulations can be created. Without the heating and specialized processing requirements of traditional formulations, several modifications of an existing formulation can be prepared within a very short period of time. This allows the formulator to quickly perform range-finding techniques when working to match a new benchmark. The increased productivity reduces the time needed to get to a final marketed product.

Another benefit of sub-micron micelles is their formulating flexibility. All sub-micron concentrates are compatible with each other. Normally the combination of incompatible lipophilic materials can present a daunting challenge to the formulation when using standard emulsification processes, the separate formation of lipophilic sub-micron systems can be readily mixed without the concern of instability. Such classes as silicones, hydrocarbons, natural oils, alkyl esters, fragrances, waxes, butters, fluorocarbons, and other lipophilic agents can be combined without issue.

Although traditional formulations require rebalancing of HLB when additional hydrophobes are incorporated into the formulation, this is not necessary when working with sub-micron micelles. Sub-micron micelle concentrates are highly compatible with each other. When the particles are made small, concepts such as HLB become far less consequential, and materials that are typically unable to be combined, are suddenly compatible.

One of the biggest problems faced by formulators is the need to deliver unique aesthetics in their new products. This task has become increasingly difficult as regulatory pressures and companies' internal negative lists grow on an almost daily basis. The ability to mix and match highly concentrated sub-micron micelles with one another allows for a virtually limitless variety of unique aesthetic properties. Since each lipophilic sub-micron micelle system has a different aesthetic profile they can be blended to achieve an intermediate aesthetic experience. In fact, having sub-micron micelle systems of just 13 materials with different aesthetic properties permits the formation of a 13 (6.227×10^9) factorial number of possibility of different aesthetic benefits which represents a potentially different aesthetic experience for everyone in the world.

Concentrated dispersions of sub-micron micelles can be prepared and then characterized by their aesthetic, tactile and rub-in qualities. When prepared in this method, the dispersions can be assigned semi-quantitative values that correspond to their characteristics. A numerical value may be assigned from 1 to 1000 or some iteration thereof. With the numbers corresponding to specific characteristics, the combination of sub-micron concentrates can be meaningfully used to alter the final aesthetics of finished formulations, and their complete compatibility allows for limitless combinations, creating unique finished formulations.

Given all of the aforementioned benefits, the resulting formulations have incredible consistency. Without the variation in particle size that is typical of traditional formulations, products made with sub-micron micelles impart consistent and predictable aesthetics into the finished formulation each time. A suitable rheological modifier is added to the water phase and adjusts for viscosity and texture, then the sub-micron micelle systems are added with minimal agitation to complete the formulation.

The challenge of transferring the laboratory preparation and processing procedure into a manufacturing environment can often be very difficult with a traditional emulsion approach. This is because the engineering properties of the equipment in the laboratory often are not the same as engineering properties of the equipment used in manufacturing. Thus the particle size and stability of a standard emulsion can vary tremendously between the laboratory and manufacturing. Therefore it is often necessary to change the processing conditions in manufacturing to achieve an analogous product to one made in the lab. This can often require an additional Process Development step to optimize. Thus, in standard emulsification situations the process is equally as important as the formula. However, since the process of producing separate lipophilic sub-micron micelles imparts so much energy in producing the fine particle size, they can be combined readily without the need of sophisticated shearing and heat transfer equipment. The use of sub-micron micelles makes the transfer from the laboratory to manufacturing a rudimentary task.

13.2.6 MANUFACTURING BENEFITS

Sub-micron micellular formulations have distinct advantages in the manufacturing area. Without the need for extended periods of heating, the ingredients of the formulation remain intact and are not subjected to any degradation due to excessive heat exposure. The process conditions are uncomplicated. They are significantly less expensive to produce, and without the need for sophisticated manufacturing equipment and/or conditions, can be successfully and reproducibly made anywhere in the world.

a. Protection of Key Materials

Manufacturing with sub-micron micelles employs a cold-process technique. Removing extended heating steps from the formulation allows for more confident use of sensitive materials in the product. The risk of promoting oxidation or degradation of these materials is completely removed, and results in a more pristine manufactured product.

b. Consistency and Reproducibility

Formulations made with sub-micron micelles are often far more simple to compound and do not require as many separate ingredients as their traditional counterparts. There are few if any critical steps in the manufacturing process that could affect the outcome of the final product. Thus they are less “compounder dependent”. There are typically fewer materials to incorporate into the batch, and there are no separate oil and water phases which must be heated, combined and properly

sheared to produce the final emulsion. Quality is dramatically improved since it is much easier to ensure batch-to-batch reproducibility. There is little waste, and virtually no "rework" of a batch is required. Thus, it eliminates the cost associated with reworking, salvaging, and disposing of out-of specification product as well as avoids the cost of lost opportunity while personnel/equipment are tied up in the salvage operation.

c. Reduced Manufacturing Cost

Labor, overhead, and processing time can be reduced from 50 to 75%. This improvement in production efficiency results in plant capacity increases without any additional capital investment. If capital equipment is needed, it will generate savings of about 70 to 80% as compared to processing equipment needed for the manufacture of conventional emulsions. Since neither heating nor cooling is required, energy savings can be greater than 90%. Kettle dwell time is greatly reduced, and the product can be transferred directly to the filling line once ingredient additions are completed. In fact, continuous processing is possible. A summary of the cost savings that can be realized when manufacturing with sub-micron micelles is found in *Table 4* below.

Table 4: Summary of Manufacturing Benefits

Energy Savings	> 90%
Capital Savings	> 70%
Overhead Savings	> 50%
Process Time Reduction	> 50%
Efficiency Enhancement	> 50%
Revenue Improvement	> 100%
Earnings Improvement	> 100%
Margin Improvement	1–3%
Capital Savings	> 70%

d. Global Consistency

Finally, the ease of manufacturing enables the product made with sub-micron micelles to be made exactly the same in any location in the world. This allows companies the flexibility to transfer production to their manufacturing plant of choice, and potentially save resources that would otherwise have been exhausted on import and export.

13.2.7 CONSUMER BENEFITS

The use of sub-micron micelles in the formulation of cosmetics and OTC products has many benefits to the consumer. Some of these include: improved product efficacy, unique aesthetic properties, reduced environmental impact, consistent quality, and greater consumer safety.

While consumers continue to demand more and more from their products from an experiential as well as a quality standpoint and performance perspective; continuously increasing regulatory constraints have tempered innovation and tied the hands of the formulators' abilities to deliver on these desires. Through the use of sub-micron micelles in the formulation of products, the limitations of current practices are lifted, and a new conduit to unique and effective formulations is created.

The availability of detailed information about cosmetics and the chemicals that they are comprised of has given rise to changes in both consumer perceptions and expectations. The new face of the consumer can be seen through the multitude of activists, bloggers, press and beauty websites.

a. Enhanced Product Efficacy

Consumers would also benefit from an increase in product performance, associated with the use of sub-micron micelles. This performance enhancement is due to an increase of surface area, resulting in a greater diffusion of functional agents into the skin. The reduction or elimination of emulsifiers may permit the use of advanced delivery systems that can both facilitate the movement of the active ingredients into the skin and protect them from the harsh environmental conditions that can deactivate their benefits. Additionally, the cold-process conditions used to make the individual micelle systems and their ultimate assembly into a final product does not expose the functional agents to the severe high-temperature processing conditions of standard emulsions. With less surface-active agents required in the formulations, the potential for active ingredients to become denatured or otherwise diminished in their activity is greatly reduced.

b. Unique Aesthetic Experiences

Small particles provide a new tactile experience to the same, commonly used reagents in the laboratory. Formulations can have the same ingredient listing on the package, yet feel significantly different when applied to the skin. This phenomenon relates to the increased packing capacity of the particles, while avoiding the increased viscosity and undesirable tactile properties of a standard emulsion of the same composition. The consumer has the additional benefit of having higher levels of hydrophobic actives and other ingredients loaded into their products without sacrificing the experience.

c. Consistency and Reproducibility

In accordance with Stokes' Law, the rate of creaming or sedimentation becomes exponentially slower as the particle size is decreased, and consequently, the more stable the suspension becomes. The lipophilic materials are processed in a manner to create a particle-size distribution that is extremely narrow. These systems can be readily mixed in the final composition to produce a consistent, highly reproducible product. The consumer experiences the same benefits with each use or with each new product purchased. In the case of purchasing products that have been formulated with sub-micron micelles, the consumer is benefitted with a more stable formulation that would require fewer ancillary stabilizing agents in comparison to the traditionally formulated products in the marketplace. This reduction in stabilizing agents promotes shorter ingredient lists, leading to a less intimidating experience for consumers when comparing products.

Another important benefit for consumers is quality and consistency. Traditional formulations require excessive heating/cooling and shearing conditions in order to successfully be produced. During the heating phase, there is a strong propensity for the systems to lose water, as hot water vapor escapes the production vessel. Additionally, during the emulsification phase of production, simply combining the oil and water phase at a rate of addition that is either too fast or too slow, can alter the results of the end-product. In addition to these caveats, the product must be cooled down to room temperature, while adding other materials to complete the batch. It is during this cool-down process that there is further potential for problems; such as cooling down too quickly, not cooling down quickly enough, shearing too much and shearing too little. All of these steps in the manufacturing process can lead to highly variable particle sizes of the emulsion, variable product aesthetics and a high level of inconsistency between batches.

Products formulated with sub-micron micelles are not reliant upon the same conditions as traditional products. Prior to compounding a finished product, sub-micron micelles are formed through one of the commercially available methods to have a uniform particle size with a very narrow distribution. Once accepted, these particles are simply combined into the finished formulation under ambient conditions, and mixed until uniform. There are no special processing procedures required at the time of compounding the finished formulation, and the resulting product is of high quality with exceptional reproducibility. With this simplified process, consumers are able to obtain the same product from anywhere in the world, and anytime they purchase the product.

d. Safety

While products created with sub-micron micelles are elegant and more aesthetically pleasing, some methods of producing these small particles allow for the ability to use fewer/lower levels of surface active agents. This is important to consumers that have sensitive skin, as these formulations tend to be far less irritating than their traditional counterparts.

e. Environmental

Consumers are becoming more cognizant of the need to use more sustainable materials and minimize the impact of their actions on the environment. One of the most relevant, yet unseen benefits for consumers is the fact that formulations created using sub-micron micelles are more environmentally friendly than their traditional counterparts. They can make elegant products out of sustainable, natural materials because they have pleasant textural properties. Further, the environmental impact can be minimized because, while there are many methods of producing sub-micron micelles, most of these methods result in far less energy consumption than standard products. In many cases, these products can be created completely by cold-processing conditions without the need for excessive heating and cooling during the creation of these products. As a result, their carbon footprint, energy consumption, and use of resources are significantly reduced.

CONCLUSION

Lipophilic sub-micron micelles offer many advantages versus the conventional approach to making traditional emulsifier-based cosmetic, personal care, and drug products. They can be prepared using various techniques, but all share the benefits associated with their smaller size. Unlike conventional emulsions, with typical particle sizes exceeding 3 microns, sub-micron oil-in-water micelles are 1/10th to 1/20th the particle size of conventional emulsions. They provide new properties to existing lipophilic materials, enhance product efficacy, and offer many consumer, manufacturing, and formulating benefits. This approach enables cosmetic scientists and process engineers to prepare personal care and OTC drug products more rapidly and more reproducibly. It addresses many of the regulatory and financial concerns that are associated with current practices. The compatibility of sub-micron micelle systems with delivery systems and new, emerging, therapeutic agents makes this technology an ideal formulating vehicle. The novel technical approach provided by the use of sub-micron micelles opens the door for a whole new range of possibilities for today's cosmetic chemist and end user.

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INTELLIGENT SELECTION AND MANUFACTURE OF NATURAL EXTRACTS

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ABSTRACT

Consumers and ingredient suppliers are both concerned about the *quality of personal care products* used on the hair, face, and body. In today's Internet-connected world, consumers want to know the *source of the ingredients* that are being used in the manufacture of their cosmetic and personal care products. Ingredient suppliers understand that the products containing ingredients derived from plants have more consumer appeal, and are viewed by them as being safer than those obtained synthetically.

In response to this consumer trend, many personal care products are being launched that contain natural extracts. These provide a wide variety of benefits to the user, and have claims such as being anti-inflammatory, antioxidants, skin whiteners, skin firming, etc. For these natural extracts to be successful, it is important to understand the key active molecule, or a group of molecules, responsible for the observed effect in the specified application. It is furthermore important that the right plant source and extraction method is chosen for the extraction of these actives in order to ensure that natural extracts are produced at a reasonable cost.

This chapter provides several examples of selection of the right plant source, for a variety of actives and different manufacturing processes in order to be used to produce a safe, consistent, and efficacious natural extract.

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13.3.1 INTRODUCTION

The trend for using natural products is ever increasing, primarily due to the consumer perception that natural ingredients are safe. To accommodate this consumer perception, the same trend is mirrored in product labels that claim to have natural or nature-derived ingredients. Ingredient suppliers are now focusing more on supplying natural and plant or bio-based products. The functionality of these ingredients spans a wide range from antioxidants to antimicrobials to emulsifiers. The active molecules to which these functionalities have been attributed tend to be produced in small quantities in their parent sources. Also, the complexity that Mother Nature has incorporated in these molecules makes their synthetic production economically unfeasible. Hence choosing the right source and the extraction technique plays a vital role in the optimal functionality of these ingredients in a cosmetics formulation.

Traditionally, the active components from plants or other biological sources have been extracted by using water or hydrocarbon solvents. Along with the objection of having residual solvents in the extracts as well as recent advancements in analytical and physical chemistry, several sophisticated extraction techniques have been developed. These techniques have substantially improved the extraction yields, reduced the usage of organic solvents, and reduced the time and investment that is needed to introduce commercially viable natural extracts in the market. There are several factors that need to be taken into account before choosing the right extraction technique.

- **Target Molecule** – The chemical properties of the active compound or the class of compound to be extracted. Primarily polarity and molecular weight, if known.
- **Source** – The source of the active molecule; plant, algae, microbes. Also the ability of the source to accumulate the desired molecule and any potential of impurities or nondesired molecules that might interfere with the extraction.
- **Regulatory/Certifications** – The natural certification or the regulatory approval that is desired for the obtained extract. Many extraction techniques and solvents are on the not-allowed list of regulatory and certification agencies. The list is not universal but is region-specific to the intended geographical market for the extract.
- **Efficacy** – The chosen extraction technique should not affect the efficacy of the desired molecule by degrading it or making it inactive during the extraction process.

The primary aim of this chapter is to give readers a general overview of the principles and abilities of different extraction technologies for extracting actives from natural sources. Some of the techniques discussed are Microwave Assisted Extraction (MAE), Ultrasonic Assisted Extraction (UAE), and Supercritical Fluid Extraction (SCFE). In addition to the extraction techniques, consideration should be given to the natural sources for the active molecules, which can have a major impact on the commercial value of an extract. Most often the activity of an extract could be attributed to a combination of molecules rather than a single molecule. The amount of complexity and redundancy that Mother Nature imparts to a particular functionality, such as antioxidants or antimicrobials, is immense. Usually it is more than one molecule that is responsible for these functionalities, and care should be taken that the majority of the molecules from that group are extracted for the resulting extract to provide "complete" functionality.

13.3.2 SOURCES OF NATURAL INGREDIENTS

Natural actives can be obtained from variety of sources. Since there is no firm legal definition for natural ingredients, many animal-based ingredients could also be considered as natural. This chapter focuses only on non-animal-based sources.

a. Plants

Medicinal disciplines such as Ayurveda, Yunani, and Traditional Chinese Medicines have developed practices that rely heavily on plant-based ingredients for curing various ailments. It is a common belief among all the traditional medicinal practices that Mother Nature provides solutions to all the health problems. Most of

these practices are based on empirical evidence and trial and error. It is only recently, due to advances in various scientific disciplines, we have started to understand the active compounds that are the cause for such benefits. For example, it was always known that eating green leafy vegetables such as broccoli, kale, and spinach was beneficial to the eyes. It was only recently, when these vegetables were analyzed, it was found that they were high in carotenoids such as lutein and zeaxanthin that contribute to eye health. [1] In Ayurveda, turmeric is used extensively as an anti-inflammatory and antiseptic. By analyzing the turmeric root extracts this efficacy has been attributed to a class of compound called curcuminoids. [2] [3]

Plants produce a variety of phytochemicals such as terpenes, polyphenols, lipids, organic acids, protein inhibitors, etc. These phytochemicals have variety of functionalities such as being antimicrobials, antioxidants, anti-inflammatory, and thermogenic. [4] These phytochemicals are secondary metabolites that are produced by plants for a variety of internal functions. Their production is highly dependent on the season, environmental conditions, and age of the plant. [5] [6] The choice of the right plant for extracting a certain active is crucial to the commercial viability of that extract. Several factors such as the application of the extract, market value, yield of the active on per acre basis, and extraction technology need to be taken into account.

b. Microorganisms

Microorganisms have been a source of a variety of ingredients used in foods and personal care. Both bacteria and fungi have been used to produce these ingredients. One of the most common ingredients used in cosmetics produced by fermentation is citric acid. Citric acid is also produced by extracting from citrus fruits, but this process is substantially more expensive than fermentation. In the fermentation process, *Aspergillus niger* cultures are fed on glucose or sucrose obtained from natural sources that then metabolize the sugars to produce citric acid. The obtained citric acid is then extracted from the media by using an acid and a base. [7] Similarly, *Lactobacillus* strains are used in production of lactic acid, [8] which is widely known to have several skin benefits. [9] Ingredients produced by microbes have a wide range of functionalities such as antimicrobial, flavors, emulsifiers, thickeners, or surfactants. Biosurfactants such as rhamnolipids and sphorolipids have been produced by fermentation of *Pseudomonas aeruginosa* and *Candida bomicola*, respectively. [10]

Choosing an appropriate microbial strain that can produce high yields of the desired molecule is crucial for production using fermentation. Subsequent downstream processing, to separate the actives from the broth, may involve cell separation and concentration of the extract followed by precipitation or drying of the broth on a carrier or extraction by using an organic solvent.

c. Algae

In the past decade, interest in algae as a source of novel natural compounds has been growing rapidly. Algae of many types floating on the oceans are common in various regions of the world. Some of the most common ingredients extracted from algae are alginates, carrageenan, and agar, which are used for thickening and emulsifying formulations. [11] Algae have also been found to have molecules with diverse biological activities such as being antioxidant, antimicrobial, and antiviral. [12] It is understood that many of these algae live in extreme conditions and must adapt to their environment. The result of such adaptation is production of secondary metabolites that can participate in the natural defense of these organisms, which can lead to formation of complex and diverse molecules. [11] Figure 1 illustrates some of the novel molecules that are found in algae. Mycosporine glycine belongs to a class of compounds called Mycosporine-like amino acids, which have antioxidant ability. [11] Eckol belongs to class of compounds called phlorotannins, which are also antioxidants and have photoprotective effect. [11] Fucoxanthin is a xanthophyll and has been found to promote fat burning in adipose tissues. [13]

Figure 1. Structures of unique molecules found in algae.

The process of extracting molecules of interest from algae involves the following steps; algal strain development and cultivation followed by harvesting the biomass through separation and downstream processing, which may involve dewatering, extraction, fractionation, and drying. [14]

It is important that the natural source used for extraction is consistent on a batch-to-batch basis. Drastic changes in the level of actives in the source can have unfavorable impact on the subsequent extraction method and hence can affect the quality and cost of the extract. Large genetic variation in the source can lead to accumulation of varying amounts of actives that may produce extracts with different efficacies. Hence it is important to have a good control on both the species of the source and the conditions in which they are allowed to hyper-accumulate the desired actives. Such control could be obtained, for example, by cultivating a single genetic strain of the source (plant, microbial, or algal). Several natural breeding and selection techniques can be used to obtain hyper-accumulating strains that can then be propagated and grown at industrial scale. Going into details of such technologies is beyond the scope of this chapter, and readers are encouraged to read the following references. [15] [16]

13.3.3 EXTRACTION TECHNOLOGIES

Choosing the right extraction technology is as important as choosing the right natural source for a bioactive. The extraction method can have a profound effect on the endproduct that is used in the cosmetic and personal care formulations. If a sub-optimal extraction technology is used, it can result into products that are difficult to formulate. This may be due to color, odor, or impurities that are co-extracted in the process. The following section will discuss the basics of several conventional and modern extraction technologies.

a. Conventional Solvent Extraction

Solvent extraction is the most traditional and most commonly used extraction technique for separating actives from a biomass. [17] In this method, biomass is exposed to different solvents individually or to a mix of solvents depending on the desired polarity. This technology is based on the fundamental principle of "Like dissolves like," where the polarity of the extracting solvent is closely matched to that of the target molecule or a class of molecules. Most often temperature, pressure, and pH can be tuned to optimize the yields of the extract. Once the target species is extracted into the solvent phase, the spent biomass is then separated by separating technologies like centrifugation or filtration. The obtained enriched solvent can then be used directly as is, as long as the solvent employed has no toxicity associated with it. If there is toxicity associated with the used solvent then it is removed by distillation or precipitation of the target molecule.

Traditional solvent extractions have been carried out using organic solvents such as hexane, ether, dichloromethane, chloroform, and ethanol. [18] They have been used for extracting actives with different polarities; nonpolar, mid-polar, and

polar. Most of these solvents have toxicity associated with them, hence they need to be removed to an acceptable level before the extract is used in the endproduct. [19] The choice of solvent will also depend on the regulatory restrictions imposed on the application of the extract. Solvent extraction may be advantageous due to the low processing cost, but the use of toxic solvents that requires their removal, most often by heat, can result in degradation of the active molecule.

Solvent-extraction techniques have been improved immensely in recent years, and have been made more efficient by use of less solvent, recycling of solvents, and concomitant use of heat and pressure during the extraction phase. All of these improvements contribute to higher yield of extraction, which in turn decreases the cost of the extract. [20] However, none of these improvements change the consumer perception about solvent-processed ingredients. The more well-informed consumers of today, relying on the Internet and social media, are more aware of the source and processing of the ingredients used in their product, and they demand that eco-friendly processes be used in the production of ingredients. This trend has led to an increase in the use of more efficient and improved modern extraction technologies for natural ingredients.

b. Microwave Assisted Extraction (MAE)

Microwaves have been applied in many fields such as: communication, navigation, astronomy, spectroscopy, and heating. [21] For extraction purposes, it is the heating aspect of microwaves that is used. In the electromagnetic spectrum, microwaves are between infrared and x-ray regions. Their frequencies are between 300 MHz to 300 GHz. [22] However, for most general applications frequencies, between 915 MHz to 2.45 GHz are used. [23]

Microwaves induce two kinds of molecular motions: ionic conduction and dipole rotation. Ionic conduction is the electrophoretic movement of a molecule under an applied electromagnetic field. If the solution offers resistance to this flow of ions, the solution is heated due to the friction generated between the molecular movement and the surrounding solvent. Dipole rotation is the realignment of the dipoles in the applied field. At 2450 MHz the dipoles align and randomize at 4.9×10^9 times per second, which causes friction between the molecules and generates heat in the system. [24] [25] [23] When a sample is irradiated with microwaves, the rate of absorption of energy is dependent on its dissipation factor (tan d):

$$\tan\delta = \varepsilon'\varepsilon/$$

where e' is dielectric loss, which is the efficiency of converting microwave energy into heat and e is dielectric constant which is the measure of the ability to absorb microwave energy. A high dissipation factor indicates less penetration of microwave energy and high absorption, which results in better heating of the samples. Table 1 illustrates dissipation factors of some of the common solvents.

[24] Due to the higher tan d values, polar solvents are used more often in MAE. Nonpolar solvents like hexane and acetone are transparent to microwaves and produce no heat during MAE.

Table 1. Physical constants of common solvents used in MAE

Solvent	Dielectric constant, e	Dipole moment	Dissipation factor, tan d ($\times 10^{-4}$)
Acetone	20.7	-	-
Acetonitrile	37.5	-	-
Hexane	1.89	-	-
Ethanol	24.3	1.96	2500
Methanol	32.6	2.87	6400
Water	78.3	2.30	1570

The basic principle of extracting actives from bio-sources, using MAE, entails heating of trace amounts of water in the plants, algae, or microbes using microwaves of appropriate power and frequency. This heating results in evaporation of the water, which induces tremendous pressure on the cell wall and causes it to rupture and release its internal components. The yields of the actives can be increased if the bio-source is soaked in a solvent with high tan d values. [25]

An MAE system has four major components: A microwave generator called a magnetron, a wave guide, which is used to guide the propagating microwaves from magnetron to a microwave cavity; a third component known as the applicator, where the sample and the sample holder are placed; and a final component—the mode stirrer, which distributes the propagating waves in various directions to heat the samples. In some systems there are also turntables for homogenous heating.

MAE systems can broadly be divided into two forms; closed and open system. In a closed system, the solvent is heated above its boiling point in a closed vessel to improve the extraction efficiency. The major advantages of this technique are temperature control and high throughput; several samples can be extracted under same conditions at the same time, no loss of volatiles. Some of the drawbacks of this technique include thermal degradation of the actives at high temperature; only materials that can handle the high temperature and pressure can be used for vessel construction. In an open system, the highest temperature at which the extraction can be conducted is determined by the boiling point of the solvent used for extraction. The major advantages of this technique are that large samples can be processed in an open vessel and the instrumentation is relatively less expensive than closed systems. Some major drawbacks are less control on the temperature and larger amounts of solvents needed for high extraction efficiency. [24] [25]

c. Factors affecting efficiency of MAE

Solvent: The solvent chosen for extraction has a tremendous impact on the extraction efficiency of the molecule of interest. The chosen solvent should have optimal solubility and high selectivity for the active under the extraction conditions. The solvent should also exhibit high dissipation factor (tan d) when irradiated with microwaves. Nonpolar solvents like hexane and chloroform are transparent to microwaves, whereas polar solvents like methanol, water, and ethanol are excellent microwave-absorbing solvents. However, mixing hexane with acetone can result in microwave heating that can extract target analytes. [26]

Temperature: Temperature is also an important factor that dictates the solubility and selectivity of a target analyte in the solvent. The ability of a solvent to solubilize a solute increases with temperature. Care should be taken in choosing temperature for thermally labile compounds. [27] Higher temperature also improves the diffusivity of the solvent in the matrix, which in turn will increase the extraction yields.

Extraction Time: The extraction yield of the analyte can be increased with time. In general, extraction times for MAE are shorter than those for the conventional solvent extractions, which reduces the risk of degradation of the labile compounds.

Microwave Power: An optimal balance needs to be reached between the microwave power and irradiation time to prevent any loss or degradation of the compound of interest.

Matrix: The physical and chemical characteristics of the matrix also play a vital role in the extraction efficiency of the analyte. Smaller particle-size matrix will yield better extraction efficiency than the one with larger particle size. Pretreatment of the matrix can also assist in improving the efficiency of extraction. Pretreating the matrix with microwave-absorbing solvents will cause the solvents to impregnate the cells and cause heating of the solvent inside and outside of the cells when irradiated with microwaves. [28]

MAE has been used for extraction of several kinds of actives such as polyphenols, essential oils, pesticides, and organic pollutants. In most cases the biggest advantage that MAE has over conventional solvent extraction is lesser consumption of solvents and shorter extraction times. [19] [24] [29] The current trend of "Green Chemistry" has led to an increase in the use of green solvents such as water in MAE. Water at ambient conditions has low Dissipation Factor, Table 1. But when subjected to heat and pressure it can act like an "organic solvent" due to decrease in dielectric constant. [24] This behavior of water has been exploited to extract a variety of flavonoids, terpenes, and polyphenols from plants. [24]

d. Ultrasonic Assisted Extraction (UAE)

Ultrasound has been used in extraction of natural ingredients as an alternative to traditional solvent extractions. UAE provides several distinct advantages over conventional extraction techniques such as use of less solvents, faster extraction times, and higher yields. [19] In UAE ultrasound frequencies range between 20 kHz to 2 MHz; in this range ultrasound is able to produce physical and chemical effects in the medium and can facilitate extraction of actives from natural sources. [30]

Unlike electromagnetic waves, ultrasound waves need an elastic medium to travel. They travel by expansion and compression cycles through the medium. A medium, which could be the extracting solvent, consists of molecules held together by certain attractive forces. When an ultrasound wave passes through this medium it causes molecules to displace from their original position, which results in successive compression and expansion phases in the medium. During the compression phase, the molecules collide with their surrounding molecules. During the expansion phase, the molecules are pulled apart and microscopic negative pressure is generated in the medium, which results in formation of a void called a cavitation bubble. This bubble is formed from dissolved gases in the medium. Successive compression and expansion cycles cause the cavitation bubbles to grow continuously until they reach a critical size beyond which they collapse during the compression cycle. This collapse results in generation of heat and formation of a transitory "hot spot" in the medium. These "hot spots" are known to have high temperature and pressure. They are able to accelerate chemical reactivity in the medium. When the cavitation bubbles collapse on the surface of solid matter in the medium, microjets and high-energy shock waves are generated. These jets and shock waves are able to destroy the cell walls of any biological material and release their contents in the extracting medium. [30]

All ultrasonic systems are composed of a transducer that converts electrical energy into sound energy by vibrating at ultrasonic frequencies. The generated ultrasound is then irradiated by an emitter. There are two common emitters used for UAE: bath and probe. In a bath system, the bio-source is exposed to a solvent in a container and then subjected to ultrasonic waves. As the system goes through successive expansion and compression, the targeted molecules are extracted in the solvent. The obtained enriched solvent is then separated from the spent biomass by centrifugation or filtration. The separated mixture is then subjected to the appropriate downstream processing to separate the analyte from the solvent. In the probe system, ultrasonic energy is irradiated by a small surface at a tip of a probe, where the probe is immersed in the extraction system. Both setups have their pros and cons. In the bath system the generated energy attenuates over time but is easy to set up and has low implementation cost. The probe system, although considered powerful and more reproducible, can cause a rapid increase in temperature in the surrounding regions of the probe. [30]

e. Factors Affecting Efficiency of UAE

In UAE, enhancement due to the use of ultrasonic waves is attributed to several mechanisms such as cell membrane disruption, improved penetration of the solvent, enhanced swelling, and capillary effect. Factors that improve the above-mentioned effects will have an impact on the efficiency of extraction. [31]

Power and Frequency: Generally, increase in power and frequency of the ultrasound waves improves the efficiency of extraction of the target analyte. [31] But it is also known that high-powered ultrasonic waves can cause a detrimental effect on the integrity of the extract by inducing high-shear forces. [32] [33]

Shape and Size of Ultrasonic Reactors: Shape of the reaction vessel is critical in determining the efficiency of extraction during UAE. As ultrasound waves are reflected and attenuated at a solid surface it is important that the right shape and size vessel is used for extraction. In the case of a probe system, the shape and the size of the tip are important and are chosen based on the extraction system. [30]

Dissolved Gases: Cavitation bubbles are formed due to the presence of dissolved gases in the solvent, where the dissolved gases act as nuclei for new cavitation bubbles. Absence of dissolved gases would make it difficult to form cavitation bubbles. On the other hand, if too many cavitation bubbles are formed, they might grow faster and result in boiling of the solvent because they would have no time to collapse. [30]

Parameters such as solvent, temperature, time, and matrix have a similar impact as they would in conventional solvent extraction. It's the efficiency of extraction that would change with the use of ultrasound.

UAE has found application for extraction of essential proteins and bioactives from a variety of bio-sources. [34] UAE improved the extraction of carnosic acid from rosemary using ethanol as the extracting solvent. The extracting yields were comparable to that obtained from the use of ethyl acetate and butanone. [35] UAE also reduced the extraction time of anthraquinones from *Morinda citrifolia* by 75% in comparison to traditional solvent extraction. [36] UAE has also been used for extraction of carbohydrates and polysaccharides. UAE was found to improve the extractability of hemicellulose from sugar cane by the cell wall and cleaving the linkage with lignin. [37] UAE has also been used with other extraction technologies such as MAE. In this example, in which natural pigments were extracted from strawberries, the optimal extraction process involved treating the biomass with MAE followed by UAE. [38]

f. Supercritical Fluid Extraction (SCFE)

SCFE is considered to be a fast, efficient, and clean method to extract actives from bio-sources. Due to these advantages, SCFE is gaining popularity over other

conventional and modern extraction technologies. It utilizes supercritical fluids, which above their critical point have both liquid and gas-like properties.

When a gas is pressurized sufficiently it converts into a liquid. On the other hand, if the gas is heated beyond a certain temperature, called critical temperature, no amount of pressurization will cause it to liquefy. The corresponding vapor pressure at the critical temperature is called critical pressure, Figure 2. The junction of the two is called critical point (CP), which is unique to a given gas/solvent, and the state of the substance in the region above the critical point is called supercritical. [39] [40] Table 2 illustrates critical properties of several common solvents. [41]

Figure 2. Pressure – Temperature diagram for a compound

Table 2. Critical properties of a few common solvents

Solvent	Critical Temperature, ˚C	Critical Pressure, MPa
Water	374.0	22.1
Methanol	–34.4	8.0
Carbon Dioxide	31.2	7.3
Ethane	32.4	4.8

This supercritical fluid has many properties of both gas and liquid. In this region minute changes in temperature and pressure can affect several solvent properties quite drastically. For extraction purposes, it can offer ideal diffusivity, viscosity, and surface tension properties. The diffusivity of this fluid is several orders of magnitude higher than the liquid solvent, which facilitates rapid mass transport. Also, low viscosity and surface tension allow for easy penetration of the biomass for extraction of actives. [41] The strong temperature and pressure dependence of solubility of active molecules in the supercritical fluid is the phenomenon that is exploited in SCFE.

The most common solvent used for SCFE is carbon dioxide; it is nonflammable, nontoxic, inexpensive, environment-friendly, inert, and nonpolluting. Processing with CO_2 results in a solvent-free extract since CO_2 is a gas under ambient conditions. Also, the critical temperature of 31.2°C for CO_2 makes it an attractive candidate for extraction of thermo-labile compounds. The efficiency of extraction of actives using CO_2 depends on the molecular weight, functional group, and polarity of the molecule of interest. In general, hydrocarbons and compounds with relatively low molecular weight can be extracted at low pressures. Nonpolar molecules with hydroxyl or carboxyl groups require moderately high pressures for their extraction. Polar compounds are poorly soluble in CO_2, and for extraction of these molecules a more polar co-solvent is often used. [40]

Figure 3 shows a schematic of a simple SCFE system. The system consists of an extraction vessel, a separation vessel, and a CO_2 source. A typical SCFE process involves the following steps: 1) extraction of the target active from the biomass; 2) separation of the active molecules from the solvent; and 3) recycling of CO_2.

Figure 3. A schematic of SCFE – CO_2 process.

During extraction phase, the solid or liquid biomass is charged into the extraction vessel, where the biomass is then exposed to CO_2 at the desired pressure and temperature above the CP of the solvent. The target actives are extracted in the solvent phase. The resulting mixture is then sent to the separation vessel where the reduction in temperature and pressure below the CP occurs. This change causes the extracted material to precipitate. The CO_2 is then recycled to the extraction vessel where it flows through a pump that compresses the gas. The extracted material can then be further reprocessed to meet the quality parameter of the final material, which then can be used in formulations. Depending upon the complexity of the source, several modifications can be made to the process by adding multiple extraction vessels and separation vessels that can depressurize the extracted stream in a gradient that can cause selective precipitation of certain molecules based on their solubility. [40]

g. Factors affecting efficiency of SCFE

In general, parameters that improve the solubility of the active molecules in the extraction solvent tend to improve the efficiency and yield of extraction. Some of the major factors that influence the efficiency of extraction are listed below. Kinetic modeling for optimizing extraction using SCFE has also been developed by several researchers. The provided reference is an excellent review of such modeling. [42]

Biomass: It is important that the feed from which the active is to be extracted is processed optimally for extraction. Parameters like particle size, shape, porosity, and morphology play an important role. Smaller particle size provides larger surface area for the solvent matrix interaction and hence can improve the yield of extraction. But it is also important that the solids are not ground too fine because that may lead to packing of the solid material and hence channeling of the solvent. The moisture content of the biomass also plays an important role and can often compete with the extraction solvent to remove the solute from the biomass. However, in many cases removal of water to the desired level may not be possible, and in such cases other parameters would need to be optimized. [40]

Temperature and Pressure: Both temperature and pressure change the solvation power by changing the solvent density. At a constant temperature, increase in pressure results in increasing the solvent density and hence the solvating power, while at a constant pressure, increasing the temperature results in decreasing the solvent density and hence the solvation power. [39] Lower density actives such as essential oils can be extracted at low temperature and pressure. On the other hand, higher temperature and pressure are needed for extraction of larger-molecular-weight compounds such as waxes. [41]

Solvent Flow Rate: Increasing the solvent flow rate usually improves the extraction yield due to high solvent to solute ratio. This drives the solute into the solvent by keeping the solubility below the saturation limit. However in many cases it can also decrease the extraction efficiency due to reduction in contact time between the solute and the solvent. [43]

Co-Solvent: SC-CO_2 has limited ability to dissolve highly polar molecules. A co-solvent is often used to improve the polarity of SC-CO_2 for extraction of polar molecules. The choice of the co-solvent and the ratio used often depends on the active that is being extracted. Water and ethanol have been the most common co-solvents that have been used in SCFE. [44] [45] The improvement shown by the use of co-solvents can be attributed to increase in solute-co-solvent interactions and matrix swelling by the co-solvent. [40]

The versatility of the SCFE has led to it being used in many applications with a wide variety of bio-sources. For example, SCFE has been used for extraction of lipids and carotenoids from microalgae such as *Botryococcus braunii* and *Chlorella vulgaris*. [46] Essential oils have traditionally been extracted using steam distillation or hydrodistillation; however, higher temperatures used in the process can degrade the actives in the essential oil. SCFE has been used for successful recovery of variety of essential oils, without the loss in activity. [47] SCFE has also been used for extraction of natural pigments and dyes, and turmeric extract rich in curcuminoids was extracted using SCFE and ethanol as a co-solvent. The obtained extract was used as a natural yellow colorant in dairy foods. [48]

CONCLUSION

An increase in the use of natural ingredients has led to the development of several modern extraction technologies that utilize the principles of Green Chemistry. Some of the improvements achieved by these new extraction technologies are use of nontoxic solvents, use of less solvents, and generation of little to no waste from the extraction process.

In addition to the extraction method, it is equally important to choose the right source for the desired extract. Substantial variability in the amount of accumulated active(s) occurs in the plants that are harvested at different times of the year or grow in different regions. Hence it is important to obtain the biomass that exhibits little variability in target molecule(s) so that the subsequent extraction process behaves consistently. Sources that produce actives on a sub-microgram level per kilogram of the biomass will always be more expensive to extract than the source that produces them in a gram scale per kilogram of the biomass. This is true regardless of the efficiency of the extraction technology or the cost of the raw material. This can be addressed by using an optimized bio-source that hyper-accumulates the desired molecules.

The extract that is obtained by these natural processes should then be standardized so that it will behave in a consistent manner within a formulation. To achieve this, it is important to know the molecule(s) that will contribute to the efficacy of the extract. By combining the right source, optimal extraction technology and standardization, one can obtain an extract that is not only efficacious but also cost-effective, consistent, and safe.

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PACKAGING

Part 14.1
Emerging Strategies for Sustainable Packaging: Balancing Materials, Design and Appearance
Part 14 Packaging
Part 14.2
Aerosol Containment and Delivery

EMERGING STRATEGIES FOR SUSTAINABLE PACKAGING: BALANCING MATERIALS, DESIGN, AND APPEARANCE

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ABSTRACT

"Plastic" has become the most widely used medium for packaging virtually every type of product today. It has become particularly pervasive in the cosmetic, personal care, food, and disposables industries. Unfortunately, plastic has become a victim of its own success because of its almost universal use and the consumer push to greener products and more sustainability. There is an increasingly heightened global awareness of the fragility of the planet's resources, and a rapidly escalating commitment to conserve those resources, even in the face of their value in desirable products.

Packaging professionals, realizing this trend, have been actively searching for and finding ways to make their products more sustainable. To many, sustainable simply means using a renewable source material, but in fact there are several ways to improve the sustainability of packaging besides this approach.

Packaging manufacturers, and the customers they serve, are utilizing numerous strategies beyond simply looking for or developing materials that employ a renewable source material. Some of these include light-weighting, changing package design, reducing overall packaging, and investigating alternative materials—all in an effort to make the package more sustainable. However, at the same time, designers and marketers must preserve brand identity, which is often associated with the plastic package their products appear in on the consumer shelves.

One certainty that has emerged from this quest is that the field of cosmetics and personal care must continue to expand its research in order to find renewable materials, but at the same time, must meet appearance expectations and the ability to be processed into the unique shapes and designs required in order to serve these industries. While this search for new materials is still in its infancy, the industry must deal with how to modify currently used "plastics" so companies can move

along the sustainability-green path that has emerged and from which there is no turning back.

Packaging professionals will find in this chapter a concise overview of:

1. What *renewable plastics* are commercially available and their functional advantages and disadvantages
2. Developments in design to reduce waste and packaging and the resultant advantages and pitfalls
3. The visual, cost, regulatory, and functional limitations when radically changing a package design or material
4. The sustainability cycle—all the factors that must be considered when determining the sustainability of your package
5. A review of the latest FTC Green Guidelines referring to what needs to be proven when the manufacturer makes sustainability claims

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14.1.1 PLASTIC: MATERIAL OF CHOICE FOR A GENERATION

Today it seems hard to believe that just a generation ago plastic packaging was a novelty and barely existed. Today it is the most widely used material for packaging everything from cosmetics to food to water and pharmaceuticals. There are some very compelling reasons for plastic's popularity. It can be molded or formed into virtually any shape; it can be decorated to look like almost anything but plastic; it can be colored or designed in almost every way possible to promote brand identity. Plastic is durable, sustainable, and relatively inexpensive.

But plastic has become a victim of its success. Today it is the subject of web attacks and non-government organizations (NGOs). It has been attacked for its lack of sustainable content and vilified in any number of ways.

One of the reasons that plastic packaging is such an easy target is that consumers have no emotional ties to packaging like they do for the product in the package. Plus, consumers view plastic as an oil-based product that is wasteful at the end of its life cycle.

Yet, in spite of these challenges, plastic packaging is projected to grow at a rate that outpaces inflation and general GDP for the next several years.

Since the question of sustainability is so diverse and expanding every day, it is difficult to even begin to offer any definitive recommendations on what makes a specific material or design the most sustainable package. Rather, we offer a view of material options currently being used effectively, their relative pluses and minuses, and a view of where different plastic packaging materials fit within the overall sustainable product value chain.

First, there are a few misconceptions that need to be addressed. As we have said, it is generally thought that plastic is a derivative of oil when in fact, most plastics manufactured in the U.S. are a by-product of natural gas that otherwise would be burned off at the wellhead. Therefore, most of the widely used conventional resins such as polyethylene, polypropylene, polystyrene, and polyethylene teraphthalate (PET) are not necessarily depleting the earth's oil resource. Also, they can be easily recycled and don't necessarily end up in landfills.

A few years ago, the Society of Plastics Industry commissioned a study to determine public opinion on plastics, and the results were interesting. Most of the "millennial" generation, those consumers between the ages of 18 and 28, have very little opinion as to the good or bad of plastic, just a somewhat negative view of the disposal, or end-of-life aspect that fills up landfills or litters highways.

This study would suggest that some of the public outcry the industry has been facing has been a matter of a few "loud voices" versus universal public opinion. Nonetheless, the industry has known for several years that it needs to address the

sustainability issue, and for a number of years has been developing new resins; designing packaging with a smaller carbon footprints; funding recycling programs; starting recycling companies; and enhancing public awareness of the benefits that plastic packaging offers, such as strength, durability, product protection, cost, design, etc. . . .

Perception has become reality for the plastics packaging industry, and sustainability is a very real concern for future generations; so the resin manufacturers, packaging designers, and end-product manufacturers are working diligently to make their products more sustainable—in the use of resources, reuse, and end-of-life scenarios.

14.1.2 MATERIAL OPTIONS

Consumers generally view sustainable packaging as packaging that is recyclable, biodegradable/compostable, or made from recycled materials or renewable resources.

One of the most visible developments in plastic raw materials has been the commercialization of polylactic acid (PLA), marketed as Ingeo® by Natureworks, and polyhydroxyalkanoates (PHA), marketed as Mirel by Metabolix/Telles.

Two more recent bio-based raw materials that hold much promise are a bio-based PET and bio-based high-density polyethylene (HDPE). Both of these resins are based on sugar rather than hydrocarbons. Both resins process just like their hydrocarbon-based equivalents, can be easily recycled, but will not degrade.

There are a number of other polymers and hybrid copolymers that have been developed for primarily automotive applications and are based on renewable resources, i.e., sugar or corn. They are not typically used in any packaging applications, so it doesn't make sense to expand on them at this time. These resins include bio-based Nylon 6, cellulose acetate and copolymers of PLA, plus polycarbonate and PLA plus ABS. The hybrid resins partially replace a part of the raw material with a more renewable source, but are not compostable or biodegradable.

a. Bio-Based Resins

To begin, we first need to define the term Bio-Resin or Bio-Plastic. As of this writing, a bio-resin is defined as "a plastic resin that either is manufactured from a biorenewable source of raw material, and/or will completely degrade in either a composting or landfill environment."

If you manufacture a packaging and want to claim on the package that it is compostable, it will have to meet at the very least ASTM 6400.[1] It is recommended that you review the FTC Green Guidelines before making any claims of compostability or biodegradability, and that you have the testing data to back up your claim.

Two of the most commercially active bio-based resins have different advantages and limitations, so they will be discussed separately and then later compared to the most widely used conventional resins.

INGEO® PLA ADVANTAGES

- In its natural state it is appealing.
- Its main raw material is corn, so it is a sustainability poster child and claims to emit lower greenhouse gas emissions during production.
- It meets ASTM 6400 standards.
- It is approved for all current FDA applications including food contact.
- It has also been approved for all typical applications in the EU.
- It molds and extrudes quite well.
- Natureworks, the oldest and largest manufacturer of PLA, markets their product under the tradename Ingeo. Natureworks is committed to providing a high level of technical and production support to companies committed to switching to Ingeo® PLA.
- INGEO® (PLA) is gaining market acceptance, and improvements and additives are developing on a continuing basis.

INGEO® PLA LIMITATIONS

- It has a low vicat softening point, so it can lose its shape when exposed to temperature conditions such as hot coffee, or in trucks parked in the summer sun in warm climates.
- However, there are products being commercialized virtually every month to modify PLA in different ways. A company called Purac now has a modified PLA that is claimed to be stable to 365°F.
- It is not biodegradable; however, it is compostable, so it must be collected and disposed of in a composting facility.
- Finished product is limited to 1.5-mm wall thickness to compost well or to be certified compostable.
- It is not recyclable with other resins; it is a contaminant.
- Very few colors and additives are FDA approved for specific applications, so the color range for any FDA application is limited.
- It cannot be used with dyes, so crystal tints are not available.
- Certain metal stearates reduce molecular weight dramatically.
- Solubility in certain solvents is a concern.

When a designer works on a package, a key design element is centered around exactly what that package will contain. Therefore it is extremely important to know that the resin used in manufacturing the package will not be attacked or degraded

as a result of coming into contact with a chemical in the cosmetic, perfume, or other personal care product.

The chart below is provided by Natureworks and outlines the solubility of Ingeo® PLA.

SOLVENT	% D	% SOLUBLE
1,2 DICHOLORETHANE	10	99.8
1,2 DICHOLORETHANE	2	99.9
DMF	10	99.8
DMF	2	67.5
HEPTANE	10	8.6
HEPTANE	2	27.5
ISOPROPYL ALCOHOL	10	0
ISOPROPYL ALCOHOL	2	0
MIBK	10	20.6
MIBK	2	0
OCTANOL	10	1.3
OCTANOL	2	5.2
THF	10	99.8
THF	2	99.9
TOLUENE	10	99.8
TOLUENE	2	0.4

MIREL PHA ADVANTAGES

- Sugar-based, so it has a similar carbon footprint to PLA
- More heat-stable than PLA
- Certified biodegradable in aquatic and landfill environments
- FDA approved for food contact packaging
- Resistance to solubility appears more widespread than PLA.
- PHA is soluble in chloroform, methylene chloride, and N-methylpyrrolidone, so this resin can be widely utilized in a variety of cosmetic and personal care packaging applications.

MIREL PHA LIMITATIONS

- Beige opaque in color, so colors are very limited, especially pearl effects
- Biodegradability limited to wall thickness of the finished part
- Costly compared to PLA and others

- PHA has not gained the market acceptance of PLA as of this writing, so the number of additives etc. available is very limited.
- Compared to PLA and PHA, more conventional packaging materials exhibit the following solubility characteristics:
- ***Polystyrene:*** Higher aromatics will solubilize
- ***Acrylic:*** Similar to PS
- ***PP & PE:*** Not really resistant, and cloudy in color
- ***PET:*** Resistant to almost everything except ortho-chlorophenol

b. Bio-Based Pet

A relative newcomer to the world of Bio-Based Resins, Bio-Based PET is projected to grow at the fastest rate of all the bio-resins. This resin in its finished form is PET, and it processes and recycles just like conventional PET. The difference between the two is the feedstock used to create the monomers. As with PHA, sugar is the major raw material source that is converted to ethylene and then into a monomer used in the manufacture of the resin. It is claimed that this resin is chemically and functionally identical to conventional PET.

To date, the resin is not generally commercially available and nearly all of the production is being used in the manufacture of beverage bottles, coined "plant bottles," in Coca Cola products. PepsiCo has also announced that they are introducing a line of bio-based PET bottles as well.

BIO-BASED PET ADVANTAGES

- It processes just like PET, so it is a quick drop-in where conventional PET is being used.
- Colors like PET, which is clear, so many color effects can be achieved.
- It has the same barrier and chemical resistance properties as PET, so it is a good candidate for packaging.
- It is recyclable with PET in the normal recycling stream, which has one of the highest rates of post-consumer recycling in the U.S. at this time.

BIO-BASED PET LIMITATIONS

- PET has a relatively high molecular weight, so more resin by weight is used to fill a mold cavity than with a relatively "lighter" resin such as PP or PE.
- Like conventional PET, it is not compostable or degradable in any way.
- It has to be dried before processing, just like conventional PET.
- PET has not been widely used in the cosmetics packaging industry, so changeover from other resins could be very costly to processors.

c. Bio-Based Hdpe

Introduced in 2010 by Braskem, this product is similar to bio-based PET in that its raw materials are based on renewable resources, and the actual polymer is virtually identical to conventional HDPE in terms of processing and performance. In addition, the product can be recycled in the normal PE recycling stream.

BIO-BASED HDPE ADVANTAGES

- It is made with raw materials that come from renewable sources.
- It will process like conventional HDPE.
- HPDE is used in many packaging applications, so this resin could be an easy drop-in.
- It is recyclable in the normal PE recycling streams.
- Its functional properties are reported the same as conventional HDPE.

BIO-BASED HDPE LIMITATIONS

- The cost will be higher than conventional HDPE.
- The plethora of different grades for conventional HDPE don't exist, so there may be some limitations of use.
- HDPE is not clear, but neither is conventional HDPE. However, a clarifier can be added that may help somewhat.
- This product will not degrade in any environment; therefore, with regard to disposal, it can be recycled in the normal polyethylene recycling stream, or it can be diverted to a co-generation plant and used as fuel.

d. BASF Eco-Flex and Ecovio

This is a line of degradable resins from BASF that utilize PLA and other degradable components. Eco-Flex is the line of resins that are used for film and flexible packaging applications. Ecovio is a PLA-based product that is reported to exhibit very good heat stability either when used by itself or when blended with PLA. As of this writing, not enough has been in commercial use to be able to comment on its advantages or limitations.

e. Biodegradable Additives

There are a number of products on the market that claim, when added to polyethylene plastic, to make the product biodegradable. There is quite a bit of controversy surrounding these products. Most of the biodegradable claims are based on degradation of PE film, and as of this writing the Bioplastics Institute, the Society of Plastics Industry Bioplastic Council, and Walmart are not recognizing these additives as effective additives that truly degrade the entire product. Steve Mojo,

President of the Bioplastics Institute (BPI), which is widely considered the authority on certification of compostable and biodegradable resins, uses the phrase "bio-extrapolation" to explain the claims made. The way the test is run is: the product is subjected to normal degradation conditions, and 10%, which is the additive, degrades within a week or less. The biodegradable claim is based on extrapolating that curve forward to claim complete degradation. BPI claims that they have run a number of tests with these products and that this is not the case, as the degradation curve in fact flattens out after the initial additive degrades.

Our recommendation is, if you want to investigate the use of these products, investigate the wall-thickness limitations on the degradability claims and search BPI for certification of degradability.

f. Bio-Resin Design Limitations

If you are concerned with end-of-life disposal of containers for cosmetics and personal care, and want to design a product that will compost or degrade, there are a few basic limitations to keep in mind:

- The wall thickness of your package typically will have to be 1.5 mm or less.
- If it is PLA it must go to a composting facility to degrade, as it will not degrade in a landfill.
- Colorants and additives used to color bio-resins are limited due to metal components in pigments.
- Certain appearance effects such as clear bright tints in PLA or pearlescent colors in PHA will not be achievable.
- Certain physical impact limitations exist as they do with conventional resins.
- The addition of modifiers to these resins may or may not affect their ability to degrade.

g. Bio Advantages

There are really two driving advantages for bio-resins, and they are both very compelling arguments: bio-resins are partially made from renewable sources; and if not modified, they either degrade in a composting facility or biodegrade depending on the resin.

h. Bio Limitations Summarized

Bio-resins have definite appearance limitations for producing containers for cosmetic and personal care products, especially if you want to have clear tints and

in some cases pearlescent effects. This issue of colors is particularly important to designers of cosmetic or personal care packaging, since color is very often used to maintain brand identity. As mentioned earlier, opaque resins such as PHA make it very hard to create pearlescent color effects, which are extremely popular in cosmetic and personal care packaging. Additionally, clear bright tint colors that are equally popular cannot be created in either PLA or PHA.

Bio-resins are not compatible with conventional resins in the normal recycling streams, and there are very few recycling streams in place to handle bio-resins.

There is limited composting infrastructure in place to handle compostable resins. In order to meet composting or degradation standards per ASTM 6400, the wall thickness of the finished product is usually limited to 1.5 mm or less.

Bio-resins typically require drying before processing and modifiers to obtain the properties needed for a package.

Realizing these design limitations, bio-resin manufacturers are now marketing their products noting the renewable nature of the feedstock being used as the major advantage, with less emphasis on the disposal, compostability, biodegradability, or recycling aspect of the package. In fact, most of the development work being performed now is in the area of making the bio-resins very tough and acceptable for automotive-type applications, with no degradability at all!

i. Conventional Resins

The main resins that will be addressed are the ones most widely used in packaging of cosmetic and personal care products. These resins are: polyethylene, polypropylene, PET, and polystyrene.

These conventional resins are: Proven, Strong, FDA, EU, and Mercosur (South America) certified for virtually all uses including food, cosmetic, and personal care packaging, generally recyclable, and reusable in many cases. As their major raw material, most of these resins today use natural gas that would otherwise be burned off.

j. Advantages

COST: Because there are several manufacturers of these resins, the law of supply and demand exists and competition has kept these conventional resins' pricing relatively low as compared to alternative materials and bio-based materials. This pricing differential may reduce in the future as bio-based resin production volumes increase and economies of scale permit the producers to reduce prices.

DESIGN FLEXIBILITY: These resins' performance capabilities are well documented, so designers are now working on reducing package weight with minimal concern for breakage.

APPEARANCE: These resins' appearances and capability to accept color, tint, and special effects are all well known by colorant manufacturers. Since bioresins are relatively new, colorant manufacturers are still developing colorants that will not negatively impact compostability or degradability of the resin.

RECYCLABILITY: All of these resins are readily recyclable. PET and PE are well established in normal *municipal recycling facilities* (MRFs) and have intrinsic value in the recycling stream. The other conventional resins are also recyclable and a number of initiatives are currently underway to increase their recycling streams, including foamed polystyrene.

WASTE DIVERSION: In many areas of the country, conventional resins that cannot be recycled for a number of technical or infrastructure reasons are being diverted from landfills to *co-generation power plants*. Co-generation power plants burn a combination of natural gas and municipal waste to create energy. Because conventional resins are excellent sources of BTUs, they have become a preferred type of waste to be used as fuel in these facilities.

k. Limitations

DISPOSAL: When a conventional resin ends up in a landfill, it is going to stay there.

SUSTAINABLE SOURCE: Conventional resins are typically made from natural gas or oil.

BULK DENSITY: Some conventional resins are "heavy," so they will automatically contribute more to the waste stream.

PERCEPTION: The public perceives plastic packaging not from renewable resources or degradable as wasteful.

14.1.3 DESIGN STRATEGIES

Packaging designers are faced with a number of challenges when developing a sustainable package. In order to achieve maximum sustainability a package should:

1. Utilize the minimum amount of material to adequately protect your product, from the time the package is filled until it is completely used by the consumer.
2. Either be made of renewable sources that can be disposed of in a way that minimally impacts landfills, or made of a product that is easily recyclable.
3. Be formed and decorated in such a way that the package projects your brand identity and encourages consumers to purchase the product inside

the package. Depending on what is used to project brand identity, resin choice could be determined by how well you can color the resin.

a. Choosing the Material

The first decision that needs to be considered is what material will best protect your product and can be utilized in such a way that sustainability is optimized.

The single most important element in any package is that it must adequately protect the product throughout the product's life. Second, the appearance must be attractive, and/or promote brand identity. Third, the tactile quality of the package has to be considered. Different resins give different sensory experiences to the product. For instance, if a heavier glass-like feel is desired, many times PET is the resin of choice due to its high molecular weight. If the package needs to be flexible, then very often polyethylene is the best choice.

One strategy widely used today is multilayer packaging. The multilayers exist to perform different functions, such as a barrier layer and an appearance layer. However, these packages usually contain noncompatible resins and cannot be recycled.

There are also some packages that use an inner layer that is composed of recycled material in an effort to reduce costs and carbon footprint. In many of these cases, the layers are not compatible since they are each serving a functional purpose, such as an oxygen barrier, oil-resistant, low-cost inner filler, etc.

This lack of compatible layers is a very serious challenge to sustainability because any package with noncompatible layers will not be recyclable in any traditional sense. Current technology limits the disposal to either a co-generation facility or a landfill.

Once the key design elements are decided upon, the designer can then begin the process of evaluating the best material for the package.

b. Bio-Resins

New developments in bio-resins are coming to market virtually every day. However, as of this writing there are four types that are commercially available and currently in use. They are: Ingeo® PLA, Mirel PHA and their alloys, and bio-based PET. Of these three, PLA is the resin that has achieved the greatest market penetration.

The most prominent use of bio-resins in packaging today is in disposables: PLA is widely used in sheet-extrusion applications such as baked goods trays and lids. It is not widely used in injection-molded parts due to larger wall thicknesses, which will negate the degradability of the product. Additionally, none of the bio-resins are widely recycled today. PLA is recyclable, but to date there is no commercial value to it. If you want to use a bio-resin in your package, you will likely need

to accept that it is sustainable from the raw-material source end, but not necessarily in the end-of-life or disposal scenario. The reason for this is that the majority of personal care and cosmetic packages that could be made from a bio-based resin such as PLA will be packaged in containers that exceed the 1.5-millimeter wall thickness that is dictated by ASTM 6400 for compostability of PLA and PHA.

Additionally, if the product is to be packaged in a film-type of outer wrap, PLA has another interesting quality to it. One of the most written-about failures in the use of PLA in packaging was the use of PLA film in the manufacture of potato chip bags. The performance of the product was fine, but it was intolerably noisy when crinkled or handled. After a few months, the product was withdrawn from the shelves.

If the designer envisions using General Purpose Polystyrene, then PLA may be a good replacement if a bio-sourced resin is desired. If high-impact polystyrene is to be considered, then an impact-modified PLA or PHA can be considered as a replacement resin.

To date, PLA garners a relatively small market share, and the question of "light weighting" still needs to be addressed.

c. Bio-Resin Alloys

Alloys are resins that are typically PLA plus another resin that are alloyed together in order to improve upon the physical properties of the PLA. The most widely available alloys are PLA/ABS and PLA/polycarbonate alloys. These resins typically exhibit similar properties to the conventional resin but also include a bio-source component.

Alloys have been most widely used in automotive applications because they typically offer more performance than is needed in packaging applications, and the costs are somewhat high for these applications as well. Personal care and cosmetic packaging designers are well advised to avoid using these alloys simply because the packaging manufacturer would be paying an elevated material cost to gain the functional resin performance that is required in automotive or similar applications but certainly not for cosmetic or personal care packaging.

d. Conventional Resins

Conventional resins have been in use for several years, and their use has pretty much matured. If one is considering the carbon footprint from a "cradle to grave" viewpoint, conventional resins may actually have some advantages. Consider the weight of the package versus the volume of product it holds. In this case, polypropylene has the lowest molecular weight and is recyclable, so you may be putting the least product into the landfill at its end of life.

As already mentioned, PET and polyethylene are being widely recycled in existing infrastructures, and PS is following.

If one considers the most potentially sustainable life cycle of a conventional resin, it can appear as very "earth friendly." Its main raw material is natural gas that would otherwise be burned off. The resin is then converted into a package, and it can be recycled several times into other packages or other products. Finally, it ends up being used to create energy at a co-generation facility as discussed earlier, which it would have been used for in the first place had it not been diverted into manufacturing a resin. In fact, there is a company, Terracycle, that has created a network called the "Beauty Brigade" that collects and recycles personal care and cosmetic packaging.[2]

14.1.4 SUSTAINABILITY

When a designer is challenged to make his package more sustainable, with current conventional materials available, the most obvious solution that comes to mind is to lessen the weight. However, this can have some unintended results. Some years ago, Walmart introduced its Packaging Scorecard, which outlined ways to minimize the carbon footprint of packages of products sold in Walmart stores. After a period of time, Walmart experienced tremendous water bottle breakage. This attempt to reduce the carbon footprint of water bottles actually resulted in added plastic in the landfills, and the loss of one of our most precious resources, water.

So in this case, a few added grams of PET or polyethylene resin would have likely strengthened the water bottles to the point that there would have been very little if any loss of packaging or product.

In another example of how to view packaging's role in overall sustainability, Bob Lilienfeld, editor of the Use Less Stuff Newsletter (ULS),[3] offers the example below.

Lilienfeld uses dairy as an example and counts the gigajoules per person that are used during the product's lifecycle:

Food supply	9.0
Primary packaging	1.3
Transport	0.6
Factory to shop trans	0.6
Retailing	0.5
Consumer shopping	1.2
Consumer freezing	3.0
Consumer cooking	2.5
Total	**18.4**

So, consider that the packaging makes up about 7% of the total energy used in the product's lifecycle.

Assume that one adds 2 grams of plastic to a 50-gram package that assures no breakage in all but the most extreme cases. This addition added 0.28% energy consumption to the entire product. If one package is broken and lost due to over-lightening the package, this would waste 400 times the energy in the product life cycle that was added to strengthen the package.

In another example, years ago the polystyrene clamshell was abandoned in favor of paper—thinking that the use of waxed paper was more sustainable.

The Garbage Project Research resulting in the book *RUBBISH!*[4] *The Archaeology of Garbage* by William Rathje and Cullen Murphy found that in most cases the switch to paper resulted in less fully consumed hamburgers, since the hamburger was not protected as well and was less appealing. So while packaging waste may have decreased, the overall environmental impact was 10 to 20 times greater. The public tends to forget that product waste is a waste of our resources.

From Keep Odessa Beautiful, an extensive study of what makes up a landfill:

- 40% is paper
- 17.5% is yard waste
- 8% comes from plastic
- 7.5% is food waste
- 7% comes from glass
- 11.5% comes from everything else

As you can see, food waste is one of the larger contributors to landfills. Plastic in total is 8% and personal care packaging is likely less than 5–8% of the total plastic, since single-use disposable items are one of the larger contributors.

a. Measuring Sustainable Claims

There are a number of different types of sustainability claims that can be made. The Society of Plastics Industry[5] research finds that the claims below typically resonate well with consumers.

1. Made from renewable resources, i.e., Ingeo® PLA, Mirel PHA, etc.
2. Package is compostable, i.e., Ingeo® PLA
3. Package is recyclable: conventional resins

Claim 1 is self-evident; you make the product from PLA and it is accepted that corn is a main raw material in the resin. However, it must be remembered that the raw material it replaces is not oil. Rather, it is natural gas that would have been burned off instead of being converted to resin. So how much natural resource is saved is a very complex question, indeed.

Claim 2 is more measurable in nature. If you want to claim that your package is compostable, you need to make sure it conforms with ASTM 6400: 3.1.2 compostable plastics, 5.1.1 disintegration during composting, 5.1.3 adverse impacts on ability of compost to support plant growth, 6.2 disintegration during composting.

These regulations will impact your design abilities in the areas of wall thickness, colorants used, and additives used to improve package functionality.

Finally, if you want to have the package end up in the "right place," it has to be made clear to the consumer that the package must not go to a recycling bin, but rather to a composting facility.

Claim 3 is more complex than one would think at first. There are only a few conventional resins that have inherent value to recyclers: polyethylene, polypropylene, PET, and polystyrene (limited areas). If multilayers are used to meet functional needs, then those layers must consist of compatible resins if they are to be recyclable.

Before you make any specific claims about the environmental friendliness of your package, it is recommended that you consult the FTC Guide on Greenwashing, which is available in book form under the title "FTC Green Guides."[7]

The Guide has laid out specific guidelines that need to be met before you can make certain sustainable claims. This will be discussed further at the end of the chapter.

At the end of the day, the most sustainable package is one that fully protects your product and encourages that the product be completely used before the package is discarded.

b. The Big Picture

When package design is being contemplated, designers can take the lifecycle approach. The most sustainable thing the designer can do is consider what design will minimize the waste or loss of the product that is being packaged. Consumers rarely see this, and as a result, brand owners are making demands of packagers that while quieting some packaging critics, in reality may very well be counterproductive to true overall sustainability due to loss of product and/or packaging.

c. State Your Message

While it is important to state our message that we are using a material that is friendlier to the environment, it is also very important that the message not be misleading in any way. As noted earlier, the FTC recently released an updated version of the Green Guide, which is similar to the previous one but differs in some significant ways.

The language that can be used when making environmental claims has been modified quite a bit, especially in the areas of both compostability and biodegradability claims.

Also of significant note is in the area of recycling claims. If a claim for either recycling or composting is made, the FTC also wants it to be noted that composting or recycling facilities for that resin may not exist in your area.

It should also be noted that the FTC bought suit against a cup manufacturer who claimed the cup was degradable when in fact it was compostable. The suit is based on the premise that if the cup is disposed of in a landfill it will not degrade and will contribute more to landfills since the public thinks the cup will degrade, when in fact it will not.

The updated rule also addressed "Free Of" claims. In all cases it is highly recommended that the packager review the new guidelines carefully before asserting any claims of "eco-friendly" or otherwise on the package.

At the end of the day, the most sustainable package is one that fully protects your product and encourages that the product be completely used before the package is discarded.

When you decide to create or utilize sustainable packaging material and design, communicate the rationale for your decision on the package. Communicate your reasons to the brand owners and then tell consumers that "this plastic package is the most sustainable package available with existing technology, and why."

Quoting Bob Lilienfeld, who authored *Use Less Stuff: Environmental Solutions for Who We Really Are*, "The entire value chain needs to help consumers, the media, legislators, and others understand that by protecting the resources contained within, packaging plays a very positive and important role in our efforts to build a more sustainable society."

REFERENCES:

1. ASTM D6400: This specification covers plastics and products made from plastics that are designed to be composted under aerobic conditions in municipal and industrial aerobic composting facilities, where thermophilic conditions are achieved.

   This specification is intended to establish the requirements for labeling of materials and products, including packaging made from plastics, as "compostable in aerobic municipal and industrial composting facilities."

   The properties in this specification are those required to determine if end items (including packaging), which use plastics and polymers as coatings or binders, will compost satisfactorily in large-scale aerobic municipal or

industrial composting facilities. Maximum throughput is a high priority to composters, and the intermediate stages of plastic disintegration and biodegradation should not be visible to the end user for aesthetic reasons.

2. Terracycle and Beauty Brigade: Members of the Beauty Brigade collect empty cosmetic and personal care packaging and send it to Terracycle for recycling into other plastic products. Members of the Beauty Brigade collect points for sending the packages to Terracycle; the points can be used for selected personal care and cosmetic merchandise.

   Information about Terracycle and the Beauty Brigade can be found at www.terracycle.com.

3. Bob Lilienfeld: President of the Cygnus Group. Authored Use Less Stuff: Environmental Solutions for Who We Really Are (Random House, 1998). Collaborated on RUBBISH! The Archaeology of Garbage by William Rathje and Cullen Murphy. Illustrated. 250 pp. New York: HarperCollins. Currently writes the Use Less Stuff Newsletter.

4. The Garbage Project: The Tucson Garbage Project is an archaeological and sociological study instituted in 1973 by Dr. William Rathje in the city of Tucson Arizona.[1]

   This project is sometimes referred to outside of academic circles as the "garbology project."

   Dr. Rathje studied the contents of Tucson residents' waste in order to examine patterns of consumption. Quantitative data from bins were compared with information known about the residents who owned them. The results have shown that information people freely volunteered about their consumption habits did not always tally with the contents of their waste bins. For example, alcohol consumption was proven to be significantly higher in reality than in the questionnaires completed by the people studied. Such findings have highlighted the difference between people's self-reported and actual behaviors.

   Such findings cast doubt on the reliability of the historical record when applied to archaeological sites in general and follow a processualist approach, stressing the benefits of scientific analysis.

   The project has since expanded to other American cities and has undertaken excavation of landfill sites. Findings are also being used to study what makes up a landfill and what degrades to what extent over time.

   The research resulted in the book RUBBISH! The Archaeology of Garbage by William Rathje and Cullen Murphy.

5. Keep Odessa Beautiful: A 1995 study aimed at reducing landfill use in Odessa, TX was commissioned to determine what percentages of waste comprised a typical landfill.
6. Society of Plastics Industry (SPI): Leading industry association supporting all sectors of the plastics industry. Website: www.plasticsindustry.org.
7. FTC Green Guide: Updated guide to outlining sustainability, recycling, and "earth friendly" claims that can and cannot be made. Information can also be found in the Federal Register under 16 C.F.R. Part 260: Guides for the Use of Environmental Marketing Claims: Adoption of Revised Guides FTC File no. P954501.

AEROSOL CONTAINMENT AND DELIVERY

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ABSTRACT

This chapter is intended to outline the status of aerosol technology in cosmetic preparations, at the time of this writing, with no attempt to provide details of product formulation. A listing of cosmetic aerosol products follows; it excludes aerosols intended to deliver over-the-counter (OTC) drugs and other drug products.

The aerosol industry has adopted some terminology from industries unrelated to cosmetics; an effort will be made to acquaint the reader with these concepts. The chapter is unique in the sense that it not only is comprehensive but it focuses the reader's attention on the concept that aerosol can parameters are chosen not only because of the mechanical aspects needed but also because of the properties of the contents. Thus, the can and the product inside represent a *system*. Finally, the chapter ends with a section based on extensive experience in this field and provides guidance to formulators of typical products delivered by the aerosol approach.

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14.2.1 HISTORY

Aerosols for delivery of personal products had their beginning early in the twentieth century but achieved popular acceptance only about 40 years later. At that time, bug sprays based on refrigerant gases were adapted to cosmetic applications. Starting about 1950, aerosols for personal use experienced significant growth.

Table 17.1 Cosmetic Aerosol Products

Antiperspirant/Deodorant
Perfume
Hair Spray
Shave Foam
Shave Gel
Styling Mousse

The aerosol industry experienced significant growth until 1978, when the use of chlorofluorocarbons (CFCs) for non-drug applications was essentially banned, at least in the United States.

The subsequent introduction of propellants with reduced ozone-depletion potential provided some impetus to the marketing of cosmetic aerosols. In recent years, concerns about the introduction of volatile organic compounds (VOCs) into the atmosphere have created additional restrictions on the aerosol industry.

14.2.2 DEFINITION

The word *aerosol* is a generic term in colloid chemistry for finely subdivided liquid or solid particles dispersed in, and surrounded by a gas. The particles/droplet size should be smaller than about 50μ and usually is less than 10μ *in view of an inhalation concern.* The Consumer Specialty Products Association CSPA (formerly known as the Chemical Specialties Manufacturers Association) broadly defines an aerosol product as a self-contained sprayable product in which the propellant force is supplied by a liquefied/pressurized gas. Scientifically, the term "aerosol" refers to a suspension of liquid or solid particles in a gas that is the result of dispensing a product from a container under pressure. Nevertheless, the commonly used

terminology defines aerosols as delivery systems for foams, pastes, powders, and the like from a pressurized container.

14.2.3 PRINCIPLE OF AEROSOL TECHNOLOGY

The concept of dispensing a product from a container under pressure is illustrated in Figure 14.2.1. Depression of the actuator opens a valve that otherwise seals the contents of the container from the lower ambient external pressure. The pressurized, liquefied, or gaseous propellant then expands and forces the product up into the dip tube for dispensing into the surroundings [1].

14.2.4 AEROSOL SYSTEMS

a. Homogenous systems

Are those in which the concentrate (product) *and* the propellant are dissolved in each other to form one single liquid phase. Examples of homogenous phase systems include, but are not limited to: anhydrous hair spray, spray cologne, etc.

b. Heterogeneous systems

Are those where the concentrate and the propellant form a temporary dispersion or uniform mixture liquid phase for a period of time. Such systems may separate upon prolonged standing into both a liquid and a vapor phase. Examples for heterogeneous systems include hair-styling mousse, antiperspirant, etc. Heterogeneous systems require a “Shake Well Before Use” statement on the label.

c. Barrier pack systems

Are those in which the concentrate and the propellant are filled into separate chambers within the aerosol container. In such systems, the two components are not mixed and only the concentrate is dispensed, in a manner similar to a post-foaming shave gel. Examples include ShaveGel and sunscreen spray.

Later in this chapter there is a brief discussion on the barrier pack alternative aerosol system.

14.2.5 COMPONENTS OF AN AEROSOL CONTAINER

A finished aerosol product includes the product, the propellant, the container, the valve with attached dip tube or with an inverted body that only dispenses when actuated in the upside-down position. In common practice, the typical arrangement of these components is as shown in Figure 14.2.1.

Figure 14.2.1: Components of an aerosol dispensing system; the propellant in this case is lighter than the product and is not miscible with it. Headspace is not specifically shown. (reprinted from Harry's Cosmeticology 8th Ed.)

Aerosol containers must be designed to contain the product and the propellant. This means that the shape of the container must be maintained despite the pressure differential across the inside and the outside wall. The container must resist deformation and assure product integrity even after prolonged contact with the propellant and the product. The materials of choice are tin-plated steel, aluminum, glass, and—in some countries—polyethylene terephthalate.

a. Three-Piece Tin-Plated Steel

For the manufacture of this type of container, steel plate of the desired thickness is plated with tin to produce a shiny nonporous coating. Three-piece steel containers

are decorated and coated flat before coiling and assembly. A variety of organic coatings may be applied to enhance the stability of the metal against corrosion. Such cans are available in different sizes and in varying levels of tin coating.

In the United States, steel-can height dimensions are described in inches. For example, a can designation of 202 × 509 identifies a can diameter of 2 and 2/16 inches, and a can height of 5 and 9/16 inches.

b. Two-Piece Tin-Plated Steel

These cans are manufactured by impact extrusion and wall ironing. As a rule, protection by the tin plating is less reliable than that obtained in three-piece cans. As increasing attention has been paid in recent years to ozone-layer depletion, regulatory changes have aimed at reducing the Volatile Organic Content (VOC) of solvents used in such cans. As a result, there has been a significant shift in development of products packaged in aerosol cans towards more water-based products. DS Container (www.dscontainers.com) now offers a two-piece extruded steel can with a nylon-type internal lining. This approach is quite useful since the aerosol contained is more compatible with a variety of water-based products.

c. Aluminum

Most aluminum cans are produced from 99.5% pure aluminum by impact extrusion. Aluminum cans have aesthetic appeal, and with today's technology, shaped cans are readily available. With the current rising cost of steel, the price for aluminum aerosol cans is now comparable to that of steel cans.

Most experts agree that aluminum cans are better suited for products that are water-based than tin-plated steel cans.

Examples of such aqueous products include those with a pH range of 5.0 (acidic) to 7.0 (neutral) and include, for example, mild cationic hair-styling mousses.

Aluminum cans, like tin-plated steel cans, are shatterproof and can withstand a wide range of pressures. Aluminum can be provided with outside or inside curls and various shoulders to accommodate a variety of overcaps or spray-through domes. They require corrosion-resistant internal coatings such as organosols (recently banned in the EU) for water-based products, epoxy phenolics, or polyamide imide (for water-alcohol and dimethyl ether, i.e., DMF-containing formulas). DMF/water-based formulas have been patented for their ability to reduce flammability. Regardless of the can type, all metal cans shipped in the United States must conform to regulations concerning buckle pressure and burst pressure. In the United States these regulations are formalized and regulated by the Department of Transportation.

d. Glass

Both plastic-coated and uncoated glass aerosol containers are available. Uncoated glass containers must withstand pressures up to 15 to 20 psig at 21°C. They are useful for fragrances in hydro-alcoholic media and employ *n*-butane as propellant. If they are formed from clear glass, they are not useful for products sensitive to ultraviolet light, since the ingredients might react, degrade, or change color.

Color-coated glass containers provide ultraviolet light protection and—when fractured—retain glass fragments in the plastic coating. Though safer at higher pressures, the end-product pressure should not exceed 40 psig at 38°C. The thickness of the plasticized or polyvinyl chloride coating is about 0.035" to 0.055".

Regardless of the type of coating employed, glass containers have aesthetic appeal and are rarely plagued by corrosion problems. They are available only with 20 mm openings, which restrict the valve-mounting cup to 20 mm as compared to the one-inch openings on metal cans.

e. Polyethylene Terephthalate

Presently (2013) in the United States, the use of polyethylene terephthalate (PET) for aerosol containers is not permitted because of the tendency of propellant permeation through the container wall; however, laminated PET containers are under consideration. The use of PET bottles is allowed in some other jurisdictions.

14.2.6 VALVE

Without a fully functional valve, a marketable aerosol cannot be prepared. As a result, many types of valves are available for controlling the flow of product from the filled container. Aerosol valves may be vertical-acting, toggle-acting, or inverted acting. They may also have metering capability, and may be capable of delivering foam liquids like shaving creams or dry antiperspirant sprays.

a. The Male Valve (see Fig. 14.2.2)

The male valve includes the stem, stem gasket, spring, body or housing, dip tube, and mounting cup. The valve must not only seal the product from contamination from the environment but also preclude loss of the propellant. The stem is commonly made of a rigid plastic (nylon, acetal, or polyester); it is slotted or barbed to hold the actuator. The stem gasket is made of a flexible polymer, such as buna, neoprene, or butyl rubber. The shape of the stem can be varied to allow rapid filling of propellant "through the stem." The spring is 302 or 304 stainless steel, with five to

seven spirals. The housing may be provided with a vapor tap that allows dispensing in a position in which the dip tube does not contact the product. The dip tube is commonly prepared from polyethylene or polypropylene and is firmly attached to the housing. Finally, the mounting cup, which is crimped to the aerosol container curl, is made of the same metal as the container. The mounting cup may include a cut gasket or other sealing mechanism, such as a polyethylene sleeve gasket, to ensure a hermetic seal when the valve is crimped to the container. If the product is dispensed only when the container is inverted, a dip tube may not be required, as, for example, in some shaving gels and styling mousses.

Figure 14.2.2: Diagrammatic view of a typical aerosol valve, showing the key components (Courtesy of Precision Valve Co., Yonkers, N.Y. reprinted from Harry's Cosmeticology 8th Ed.)

b. Female Valve (see Fig 14.2.3)

The female valve has similar components as the male valve except without the valve stem. The stem portion of the valve with various orifices is fabricated into the actuator configuration to control the flow of products. Together with different inserts, they govern the spray rate and pattern. Without the valve stem, products and propellant can be filled through the wide-open body at very high speed.

	PARTS LIST
1	MOUNTING CUP
2	GASKET
3	SPRING CUP
4	BODY
5	SPRING
6	DIP TUBE

	SUGGESTED STARTING CRIMP DIMENSIONS
LAMINATED CUP ON 3-PIECE TINPLATE CAN	Ø 1.065-1.075 [27,05-27,31] HEIGHT . 180-.190 [4,57-4,83]

1 DIMENSIONS IN BRACKETS ARE IN MILLIMETERS
2 TOLERANCE FOR ORIFICES LESS THAN .060" [1,52 MM] DIA.: ±.002" [0.05 MM]
3 ALL OTHER TOLERANCE ±.005" [0,13 MM] UNLESS OTHERWISE SPECIFIED

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Title	AR-74 VALVE ASSEMBLY, STANDARD HEIGHT TAPERSEAL			Approved	Date
				Oral Mgl	CAC 2010.05.17 14:43:00 - 05'00'
Drawn By RJS	Date 02-Dec-91	Chkd By TFE	Date 10-May-10	R & D	RLC 2010.05.18 15:04:09 - 05'00'
				Lab Ngr	MER 2010.05.17 17:23:39 - 05'00'
Material -	Scale 3:1	Dwg No. C99 - 9500 - 01		Rev Ltr	B

Seaquist Perfect DISPENSING

Figure 14.2.3: Female Valve
(*Courtesy of Seaquist Perfect Dispensing*)

The selection of the stem gasket is critical for proper operation of the aerosol unit. For the sake of compatibility and functionality, the formulator has to make a selection from nitrile, neoprene, buna, butyl, or viton rubber. *This choice is as important as the selection of propellants or of the solvent used and may require extensive stability testing.*

14.2.7 TYPES OF VALVES

a. Standard valves

Are designed to deliver the product at a rate acceptable to the consumer and appropriate to the product. The criterion is the spray (or delivery) rate, which must remain inviolate throughout the life of the product. *Foam valves* provide unobstructed product passage through the body and stem orifice.

b. Powder valves

With self-cleaning stem-wiping action allow smooth passage with no hold-up of solids to interfere with valve operation.

c. Spray valves

For water-based products or products require finer breakup of particle size, and they may require two entrances to the chamber in the valve housing; one of these taps the vapor phase, which admits only propellant vapor, and the other provides access via the dip tube. This type of valve, known as a vapor tap valve, helps to break up particles when the product is actuated. Such valves are sometimes provided with a so-called Aquasol piece, in the valve body; the exiting propellant/product mixture spins the piece, which helps breakup of larger particles.

Vapor tap valves are required for spraying in the upright and inverted positions.

d. Vapor tap valves

Reduce the product discharge rate and deliver a drier product. Formulators should recognize that such valves increase the discharge of propellant and may modify the spray pattern. It is essential to make certain that there is sufficient propellant in the unit to ensure uninterrupted discharge until empty.

e. Metering valves

Include a holding chamber in the valve body for delivery of a predetermined quantity of the product. They are employed primarily for costly perfumes and therapeutic inhalers.

f. Crimping

Tight attachment of the valve to the container is essential if leakage is to be avoided. Latent leakers are those cans that pass the water bath test but may begin to leak after storage. Successful crimping requires adherence to tight dimensional specifications for different valve cups attached to different cans.

Large-diameter aluminum from 50 mm and larger sizes might have "eye-lashing" from the curl-forming stage. A lathe-cut gasket is used in combination with a shave curl. The curl is shaved at an angle to form a sharp edge so that the lathe-cut gasket has a sure sealing point, which will ensure tight leakproof crimping.

The crimp diameter and the crimp height are critical dimensions. A workable set is shown in Table 17.2.

14.2.8 THE ACTUATOR

The actuator is the final mechanical device required for a functioning aerosol product. As shown in Figure 14.2.2, the actuator or button fits on the valve stem and is available in a variety of sizes and shapes. It determines whether the product is dispensed as a foam, spray, or steady stream. The terminal (0.025–0.30 mm) orifice in the actuator may range from 0.01 to 0.06 inches. The actuator may include an insert that determines the nature and the spreading of the escaping product.

Table 17.2: Crimping Dimensions in mm; Allowable Variations Are ±0.13mm

	3-Piece tin plate can		Aluminum can outside curl		Aluminum can inside curl	
Cup Style	**Crimp Diameter**	**Crimp Height**	**Crimp Diameter**	**Crimp Height**	**Crimp Diameter**	**Crimp Height**
Taperseal tin plate	27.18	4.70	27.3	4.7	27.3	4.70
Tin plate with cut gasket	27.18	4.83	27.18	4.83	27.18	4.83
Tin plate laminate with cut gasket	27.18	5.08	27.18	5.08	27.18	5.08
Aluminum with cut gasket	26.92	4.95	26.92	4.95	26.92	4.95

14.2.9 PROPELLANTS

The aerosol propellant provides the energy for driving the product out of the container and through the valve. The propellant may be a liquefied gas, which vaporizes at atmospheric pressures and ambient temperature, or a compressed gas. The propellant may act as a diluent or solvent for components of the contained product formulation.

The key requirement for a propellant is the ability to deliver a product at an acceptable rate throughout the life of the product [2]. Pressure drop, in the case of common compressed gases such as nitrogen, carbon dioxide, nitrous oxide, and the liquefied gases such as hydrocarbon, DME, and 152a, are more likely to provide reliable product delivery. Table 17.3 lists the propellants currently used in aerosol technology.

As of the current time period, all of the liquefied hydrocarbon and dimethyl ether have been classified as Volatile Organic Compounds, and the percentage that can be used in different product categories has been regulated by government agencies.

a. Hydrocarbon Propellants

The once-popular chlorofluorocarbon (liquefied gas) propellants were identified by Molina and Rowland in 1974–77 as ozone-depleting chemicals that increase the intensity of solar radiation through the earth's atmosphere and have been shown to increase skin cancer potential. Measurements are made of the size and depletion of the ozone layer, which has been a significant driving force towards the development of water-based systems with lower and lower VOC content. CFC systems have been banned in the United States since 1978 and are rarely used in other countries. The liquefied propellants shown in Table 17.3 are odorless and have low levels of toxicity on inhalation. They produce the same constant pressure at any time as long as propellant remains in the container. Hydrocarbons such as propane, *i*-butane, and *n*-butane are FDA-approved food additives.

Table 17.3 Aerosol Propellants

Liquefied Gas Propellants
Hydrocarbons
Propane
i-Butane
n-Butane
i-Pentane
n-Pentane
Ethers
Dimethylether
Hydrofluorocarbons
Difluoroethane

Liquefied Gas Propellants
Tetrafluoroethane
Hexafluoroethane
Compressed and Soluble Gas Propellants
Carbon dioxide
Nitrous oxide
Nitrogen
Air

After the Montreal Protocol, and the ban of chlorofluorocarbons in 1978, hydrocarbons found in crude oil and natural gas fields became the dominant aerosol propellants. They are marketed at levels exceeding 95% after purification by fractional distillation.

Major impurities include hexanes, unsaturated hydrocarbons, and some sulfur-containing compounds. Hydrocarbons belong to the group of volatile organic compounds, the VOCs. The vapor pressures of the principal liquefied hydrocarbons at 70°F (21°C) are as follows: propane—108 psig (7.6 bars), *i*-butane—31 psig (2.2 bars), *n*-butane—17 psig (1.2 bars). Hydrocarbon propellants have low specific gravities, and the liquids generally float on the product unless they are miscible. They are soluble in many organic solvents and are entirely noncorrosive to contacting metal surfaces. Their flash points and explosive limits are low and they can be blended to achieve various desirable pressures. Hydrocarbon blends exhibiting 46 psig pressure at 70°F (3.17 bars at 21°C) are particularly useful as aerosol propellants and may be prepared by weight as follows:

A. Propane (A 108) 15.1% and *i*-butane (A 31) 84.9%

B. Propane (A 108) 26.0% and *n*-butane (A 17) 74.0%

C. Propane (A 108) 27.3% and *i*-butane (A 31) 28.9% and *n*-butane (A 17) 43.8%

Despite their identical vapor pressures, blend A produces a drier foam shaving cream than blend B; blend C yields intermediate results.

Work with hydrocarbon propellants must be conducted with care, taking into account the following:

The propellants are heavier than air; explosion-proof exhaust hoods are required; static electricity must be avoided; and confined areas should be examined for hydrocarbon residues with infrared detectors.

b. Dimethyl Ether

Dimethyl ether (DME) exhibits a vapor pressure of 63 psig (4.34 bar) at 70°F (21°C) and is quite soluble in water (35%). DME is flammable and has global-warming

potential but no ozone-depletion potential. Its water solubility makes it particularly attractive for use in high-water systems. It develops a high pressure but is much less flammable than hydrocarbons when mixed with water; nevertheless, the precautions described for handling hydrocarbons should be followed. DME forms azeotropic mixtures with hydrofluorocarbons; such blends are briefly discussed later in this chapter. DME is a solvent for many of the gasket rubbers used in aerosols. Butyl and Viton gaskets are likely to perform well, but swelling tests with specific formulations are required. All equipment handling DME must meet Division 1 Group C requirements.

DME is not corrosive, but in blends with aqueous systems, attack on metal surfaces exposed to liquid or gas phases is possible. Thus the need for using corrosion inhibitors exists. This is particularly important if a solvent such as ethanol is present. As noted earlier, DME is a VOC as defined by the California Air Resources Board (CARB) and the Environmental Protection Agency (EPA). DME and water mixtures have been patented as useful for reducing or even eliminating the ability of the propellant to flash as it is sprayed from the aerosol can.

c. Hydrofluorocarbons

Table 17.3 identifies three hydrofluorocarbons (HFCs) that are important propellants for the aerosol industry *HFC 152a* has no ozone-depletion potential and is exempted by the CARB and the EPA from VOC regulations. Structurally it is CH_3CHF_2 with a vapor pressure of 63 psig (4.34 bar) at 70°F (21°C). The material has a high vapor pressure and has only slight solubility in water. Its use requires containers that can withstand high pressures. HFC 152a can be blended with DME or the commonly used hydrocarbons to provide a variety of pressures. The pressures developed vary and are best obtained from propellant suppliers. In practice, the blends of pure propellants may form azeotropes, but the pressures developed in diverse products containing surfactants, solvents, and water should be determined experimentally.

HFC 152a is not subject to hydrolysis; can corrosion tests and inhibitors are, nevertheless, required to prevent attack on steel or aluminum containers in the presence of products. *HFC 152a* is slightly soluble in water and this must be taken into account when using water-based formulations such as hair mousses, lotions, and creams. It yields a creamy, glossy, and wetter foam. In some products this sensory effect is preferred over the foams produced by hydrocarbon propellants.

Even nonflammable blends may fractionate and should be handled as flammable components. *HFC 152a* is not an aggressive solvent and is compatible with most valve gaskets; butyl rubber is preferred over buna N or neoprene. *HFC 152a* exhibits a low order of toxicity.

HFC 134a is a nonflammable propellant exhibiting a pressure of 70 psig or 4.8 bar at 21°C; its specific gravity is 1.22. Because of its relatively high global-warming potential, regulatory agencies discourage its use in personal care aerosol packages. It forms azeotropes with DME and hydrocarbon propellants and its solubility in water is low. This material is currently used as a replacement for chlorofluorocarbon propellants in metered-dose drug inhalants because of its low ozone-depletion potential.

HFC 227a has a low ozone-depletion potential and a low global-warming potential. Its vapor pressure is only 40 psig (2.76 bar) at 21°C. It too, is used in inhalation drug products.

d. Compressed Gases

The most commonly used gases, CO_2, N_2O, and N_2, are injected as gases into the aerosol product and produce pressures as high as 140 psig at ambient temperatures. The most serious problem with their use is loss during repeated actuations. Liquefied gases produce pressures near 45 to 50 psig and maintain this pressure during discharge up to 100%. In contrast, compressed gases may start at pressures near 100 psig but create a pressure of only 30 to 25 psig after 50% of the product is discharged. They are low-cost, nontoxic, nonflammable propellants with no adverse effects on ozone levels. These propellants cannot be used with vapor tap valves, and CO_2 may have an accelerating effect on corrosion. During use, the spray rate should be expected to drop.

Ostwald solubility coefficient for ethanol in carbon dioxide is 2.84, i.e., 1 ml of pure ethanol will accommodate 2.84 ml of CO_2. Using a saturator, CO_2 can be pre-dissolved in SDA (Special Denatured Alcohol) and used to fill into SDA base formulas as liquid and maintain pressure about 70 psig without too much differential pressure drop throughout the usage of the product.

14.2.10 FILLING

Only three commercial procedures for filling aerosols are practiced: cold filling, under-the-cup filling, and pressure filling. Of these, cold filling is rare as of this writing and will be described only in passing.

a. Cold Filling

In this process, the propellant is chilled and can be handled as a liquid. The product (concentrate) is also chilled and metered into the container. Next, the cold propellant is added. Finally, the assembled valve is then crimped on the container. In this filling sequence, the evaporation of some of the propellant before sealing expels most of the air from the open container. Today, EPA/VOC regulations have

critically reduced this type of filling. In addition, the costly steps of chilling and transferring cold product and propellant have been replaced by alternative filling procedures.

b. Under-The-Cup Filling

In this procedure, the concentrate is filled into the open container at room temperature. Next, the assembled valve is placed loosely on the aperture of the container, and the filling head is lowered to seal around the container. After slightly lifting the valve assembly, a vacuum is drawn on the container headspace, and the propellant is injected between the container and the valve unit. Finally, the valve is crimped to the aperture while the filling head recedes. This filling method is practiced primarily in the United States; it is fast, but some loss of propellant is unavoidable.

c. Pressure Filling

This is the most widely used method of filling aerosols, even though under-the-cup filling remains popular in the United States. The propellant losses are much lower than in under-the-cup filling. The concentrate is added to the container, followed by removal of air from the headspace. After crimping of the valve to the container, the propellant is pressure filled through the valve.

Variations are sometimes necessary to bypass the button or to attach the button to the filled container.

d. Hot Water Bath Testing

Before or after the filled unit is examined by check weighing, it is passed through a hot water bath (50–55°C). This exposure is intended to identify and allow removal of leakers by observation of bubbles escaping from the filled unit.

e. Headspace

The need for adequate headspace in a filled aerosol container arises from the fact that upon warming, the contents inside the package must have room to allow for content expansion. Without adequate headspace, the expansion of the liquid and propellant in the can will raise the internal pressure and volume precipitously, resulting in bursting of the package. *A sensible headspace is about 20% of the container overflow capacity.* The computation requires data on the specific gravity of contents (propellant plus concentrate).

The volume of the fill is then easily computed. Some minor correction is made for the space occupied by the valve. A level of about 20% of the overflow capacity

not occupied by product and propellant is sufficient to allow for expansion during normally expected temperature exposure.

14.2.11 OPERATION

The operation of a typical aerosol product is pictured in Figure 14.2.4.

Figure 14.2.4: Diagram showing the operation of a conventional aerosol product (Courtesy of Precision Valve Co., Yonkers, N.Y. reprinted from Harry's Cosmeticology 8th Ed.)

14.2.12 ALTERNATE SYSTEMS

Over the years, systems have been developed that use principles of pressure dispensing that differ from conventional aerosol technology. The most important of these are attempts to reduce or eliminate the dispensing of propellants into the atmosphere with every actuation. Some of these devices are designed to replace the propellant with a compressed gas. In a second type, the use of a propellant is replaced with a pressure-generating device (pump) activated by the consumer.

	POUCH SIZE	F1R	F2R	F3R	F4R	F5R	F6R	F7R	F8R	F11R
OH	OVERALL HEIGHT	104	116	139	142	146	174	125	160	148
OW	OVERALL WIDTH	97	97	97	97	97	97	97	97	97
GH	GUSSET HEIGHT	30	30	30	30	30	30	30	30	30

Figure 14.2.5: Bag-On-Valve System
(*Courtesy of Aptar Company*)

a. Bag-On-Valve

Bag-On-Valve (BOV) is a system in which a laminated bag is attached and sealed onto an aerosol valve. The unit is then crimped on an aerosol can and pressurized outside the bag to about 30–40 psig with nitrogen or compressed air. After the product is filled and as the bag expands, the internal pressure increases to about 100 to 120 psig. The product is dispensed through the valve by the external pressure outside the bag when the actuator is activated. (See Figure 14.2.5)

Up to four different materials are typically used to make the laminated bag. The most commonly used materials are: polypropylene, nylon, aluminum, and PET. Depending on the product in the can, polyethylene may also be used. It is important that product integrity and delaminating of the bag are thoroughly tested after elevated temperature storage for up to 90 days.

The BOV approach gained popularity after the successful launch of sunscreen spray and shave gel by several major marketers. Other products now using the BOV system include: saline solutions, makeup spray, facial spray, body bronzer, tooth gel, etc. The main advantage of BOV is that the product can be sprayed/dispensed at any position. Further, there is no VOC issue, and the aerosol is more eco-friendly without any propellant. It is useful for sensitive consumer health products, and there is no concern about can corrosion.

On the negative side, the products for BOV have to be homogenous and stable at all temperatures; and the pressure will be different at the beginning, middle, and end of the product cycle. BOV manufacturers are presently working on improving the design of this intriguing approach so as to minimize these issues.

b. Bag-In-A-Can System

This trademarked Advanced Barrier System provides a pouch for the formulation, which is completely separated from the propellant system in the unit. The pouch is a laminate of polypropylene (which is in contact with the product), with a central aluminum foil and an outer layer of nylon. It is compatible with aqueous and solvent-based formulations. The propellant, either compressed air or nitrogen, surrounds the bag. When activated, the gas exerts pressure on the pouch for discharge. No propellant gas is released into the atmosphere.

c. Sepro Can System

In this system the product is contained in a polyethylene bag that is separated from the propellant system. The driving propellant, which may be a hydrocarbon liquid, is injected through a plugged hole in the bottom of the can. The propellant is retained in the container after use.

d. Lechner System

This system relies on a thin flexible aluminum bag that is fitted into an aluminum can. This approach has been employed for hair-coloring products and drugs; the aluminum bag prevents permeation. The driving propellant is usually a liquefied hydrocarbon and is injected into a plug on the bottom of the unit. In this case, too, the driving propellant remains in the can.

e. Piston System

In this approach a plastic cup-shaped piston forms a barrier that fits inside an aluminum or three-piece steel can. The piston separates a thick viscous product from a hydrocarbon liquid gas that is inserted through a hole in the base of the container. When the actuator is depressed, the propellant raises the piston to effect product discharge. The product has to be viscous enough to prevent seepage between the can well and the piston. Several systems have been designed to avoid the use of any propellant. Some typical propellant-free dispensing systems are described briefly below.

f. Atmos Dispensing System

This system consists of a package within a package—a thin-walled durable PET bottle surrounded by a rubber sleeve. As the inner bottle is filled, the rubber sleeve expands to provide a self-pressurized driving force that expels the product when the valve actuator is opened. The viscosity of deliverable products includes liquids, gels, creams, and the like.

g. Pump-Activated Systems

Some dispensing devices combine a pump activator with a container for the product and a valve unit. Devices for dispensing products in droplet form by mechanical agitation are well known. Some more sophisticated approaches are briefly noted here:

1. Dry Spray Dispenser

The ambient air pumped through the valve into the package provides internal pressure. The dry spray valve includes a vapor tap, which when actuated helps to atomize more viscous fluids. The components are refillable.

2. F-Z Finger Pump Foamer

This device pumps a combination of air and liquid through two fine-meshed screens located in the actuator to create a dense foam. The foaming liquids should have low viscosity and may include hair fixatives, facial cleansers, body emollients, tanning products, and shaving creams.

3. Co-Dispensing Systems

A co-dispensing pump assembly separates two components up to the point of discharge. It is constructed to dispense two products at a 10:1 ratio by using a larger outer pump and a smaller inner pump. The containers are fed by two dip tubes. The design is such that the filled assembled package resembles a single-dose dispenser.

14.2.13 FORMULATING AEROSOL PRODUCTS: THE VOICE OF EXPERIENCE

Products in aerosol format are in sealed packages with internal pressure typically about 30 to 70 psig at room temperature. Any type of failure with the sealed package due to the external environment impact such as moisture, heat, or internal reaction between the products and the cans will result in leakage and in the worst case—explosion, causing damage to the surrounding objects and/or people. When formulating an aerosol product, we first have to consider integrity, safety, and where the product will be used, taking into account that the aerosol components are part of the formula.

The other factors taken into consideration are: how the product is dispensed out, performance criteria, and application of the product, etc. Following are some guidelines to select the aerosol components and general physical and chemical property requirements of the products that need to be packaged in aerosol form.

a. Aerosol Containers:

Start with the aerosol containers, the tin-plated cans and aluminum cans most commonly used in personal care products. Both of them come with different internal lining. There is a general misconception that the linings are there to protect the products from the metal cans, but the truth is that the function of these linings is to protect the cans from the products. The physical and chemical composition of the product itself affects the integrity of the lining. Linings on the tin-plated cans are applied on the tin-plated sheets before the cans are fabricated, and the linings do not have 100% coverage. On the other hand, linings on aluminum cans are sprayed on and cured after the cans are formed, thereby providing better protection to the cans.

With anhydrous products, such as professional hair spray and silicone-based shine spray, both tin-plate and aluminum are widely used and acceptable. For products containing water, tin-plated cans are more compatible with products having pH above 7.0 to 9.0; aluminum cans are more compatible with products having a pH range of 5.0 to 7.0. Depending on the other solvent systems in the products, selecting the type of linings is just as important. An aggressive solvent will soften the lining and eventually lift off the can and fall into the product, and may end up

clogging the dip tube and/or the orifices in the valve body and the stem. With the lining lifted off, product comes into contact with the exposed metal; in some cases, corrosion will occur, resulting in perforation and leakage.

For some water-based products, the propellant dimethyl ether (DME) is needed to achieve certain spray characteristics. DME is not compatible with the common lining materials such as epoxy, whereas the PAM lining which is more compatible with DME is required. For tin-plated cans that do not have the option of polyamide-imide (PAM) lining, plain cans without any lining are used in conjunction with the proper liquid and vapor-phase corrosion inhibitors. Stability study is absolutely necessary for these formulas to ensure product integrity.

b. Aerosol Valves:

The aerosol valve, along with the internal pressure, controls the flow and dispensing rate of the product coming out from the can. The male valve has two orifices, at the valve body and at the valve stem. For products like shave foam and body mousse, which require an internal pressure of about 30–46 psig, a wide-open body orifice and slightly restricted stem orifice are used to control the flow and expansion of the foam coming out. For products like hair spray, the internal pressure is usually around 50–70 psig. Such products require a more controllable spray rate; a more restricted orifice in the body and at the stem are used to control the amount of product dispensing out. A female valve has a wide-open body orifice; the spray rate and spray characteristic are controlled by the actuators.

c. The Actuators:

How the product dispenses out from the aerosol package is governed by a combination of many factors. The actuator is one of the most predominant factors of all. For a particular formula, the performance, spray character, and particle size can easily change by simply switching from a larger orifice insert on the actuator to a smaller opening, from a mechanical breakup to a nonmechanical breakup actuator, even a different design of the channel behind the insert of the same dimension will affect the spray pattern and/or particle size. Selecting the proper actuator for the optimum performance requires some trial-and-error work.

14.2.14 PHYSICAL & CHEMICAL PROPERTIES OF THE PRODUCT

a. Viscosity:

As a rule of thumb, for spray products, the lower the viscosity of the product, the finer the particle size will be. Try to avoid using high-molecular-weight polymers, especially in water-based products, as the high viscosity (at the high shear rates produced at the exit of the can) can make it difficult to break the product into the desired spray droplet configuration.

b. Suspension System:

Products like antiperspirants or dry shampoo contain fine solid particles suspended and dispersed in a solvent system. Anti-caking agents and specially designed powder aerosol valve and actuator are required to function properly.

c. Solvent System:

Single-phase aerosol formulation will provide uniform spray rate and spray pattern and maintain constant internal pressure throughout the lifetime of the unit. Using a solvent that is compatible with the propellants is preferable.

d. pH Value:

As mentioned previously, tin-plated cans are more compatible with alkaline formulas containing water, and aluminum cans are more compatible with slightly acidic formulations. There are always exceptions, and care must always be taken along with thorough stability testing which is absolutely a must before introducing the aerosoled product into the stream of commerce.

e. Foam Products:

Most of the lotion-type emulsion-based formulas once aerosolized with hydrocarbon propellants will create rich and creamy foam. Use of higher-pressure hydrocarbons and higher-percentage ratios will result in a stiffer foam body. However, these foams usually lack required wetting properties. By using slightly water-soluble propellants like 152a and DME, wetter and faster-dissipating foam can be achieved.

f. Sprayable Products:

As mentioned previously, the viscosity of the concentrate determines the characteristic of the spray. The ideal system for a sprayable product should be one phase where the concentrate and the propellant are in homogenous solution. With the single-phase system, no shaking is required before dispensing the products. For a multi-phase system where the propellants separate out upon standing, ratio and the type of propellants have to be carefully selected to ensure that after shaking, the concentrate and the propellant stay in uniform suspension for the duration of the product's use. "Shake Well Before Use" should be clearly stated on the label.

14.2.15 STABILITY TESTING

The stability testing of filled aerosols differs from that of other products because corrosion and mechanical failure are responsible for product instabilities.

Formulators must make sure that the product is stable and unaffected by storage in the container in the presence of the propellant. In addition, corrosion of the container and swelling of valve components require monitoring.

For practical purposes, tests are best conducted on filled units under various conditions. Devices exist that accelerate corrosion by electrical means, but the repeated actuation of the filled container reveals all types of defects to the examiner, including product changes. Can corrosion and gasket deterioration may require destruction of stored samples at scheduled intervals. Marketing of newly formulated aerosol products should be delayed until all test requirements have been completed satisfactorily. Every company has its own stability requirements. Typically, products are stored at 110°F, room temperature, and 32°F for periods up to 12 months. The aerosol units are opened at intervals of 1, 2, 3, 6, 9, and 12 months to examine for any sign of corrosion or issue. One to three units are required for each opening and for each storage condition.

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