



NINTH EDITION

# Harry's Cosmeticology

## **Harry's Cosmeticology**

9<sup>th</sup> Edition

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# About the Editor-in-Chief



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CChem, CPC, CChE, CFEI, DABFE, DABFET, FAIC

Meyer R. Rosen is President of Interactive Consulting, Inc. ([www.chemicalconsult.com](http://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 International Safety, Regulatory and Certification Symposia.



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# Acknowledgements

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

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# 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 6<sup>th</sup> editions as well. The 7<sup>th</sup> edition was published in 1982 and co-edited by J.B. Wilkinson, MA, BSc, CChem, FRSC, and R.J. Moore, BSc, CChem, MRSC, MIIInfSc; followed by the 8<sup>th</sup> edition in 2000 by Martin M. Rieger, PhD, and now, the 9<sup>th</sup> 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 9<sup>th</sup> 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 9<sup>th</sup> edition of *Harry’s* is to support that yearning in the best way I know how.

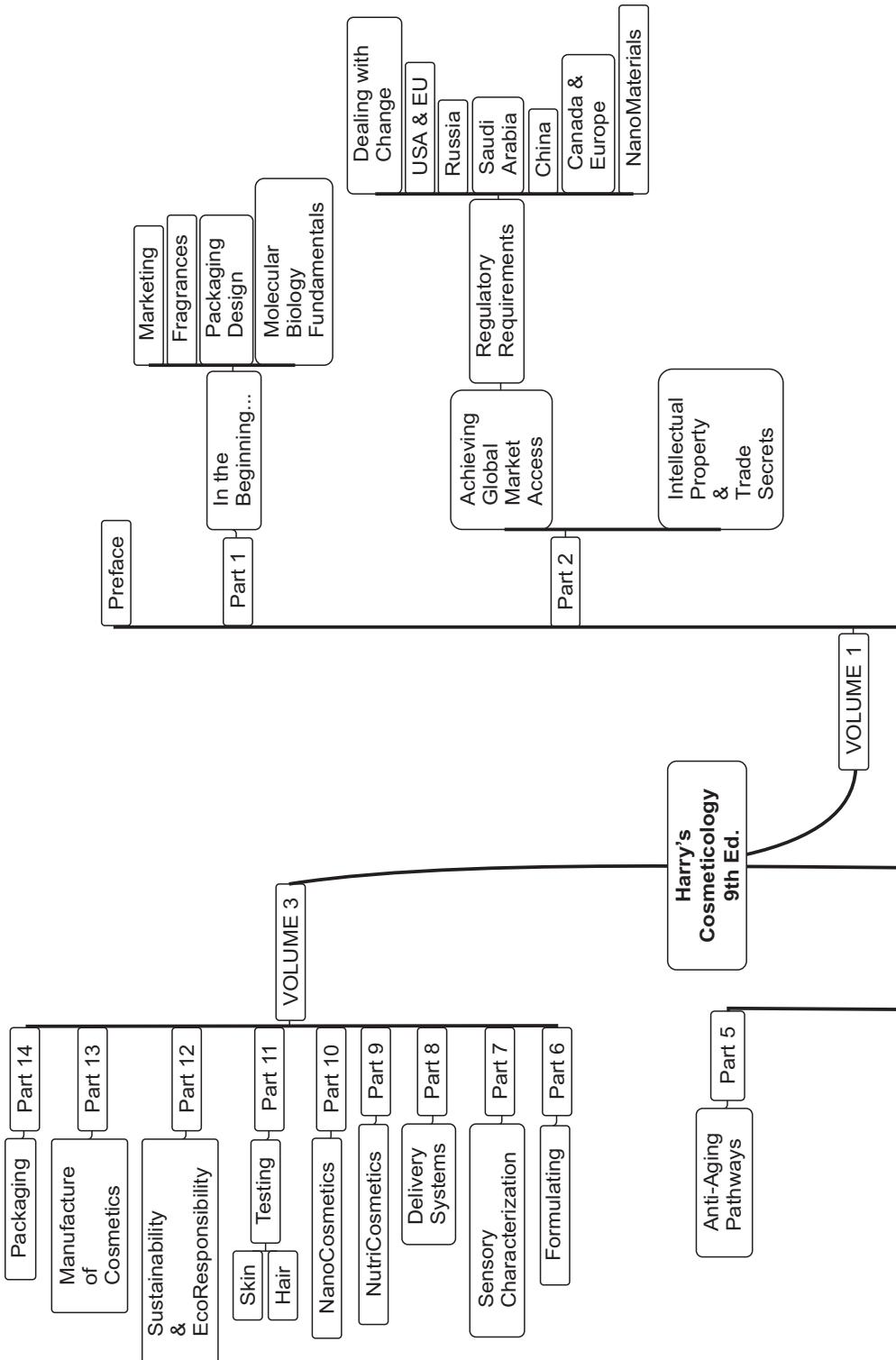
I believe we are at a tipping point in fulfilling this eternal search for youth. With the breakthroughs in understanding genetics, epigenetics, and biochemistry—as well as the enormous growing consumer pull, the need to develop approaches that really fulfill the longing to be young again is the opportunity of our lifetimes.

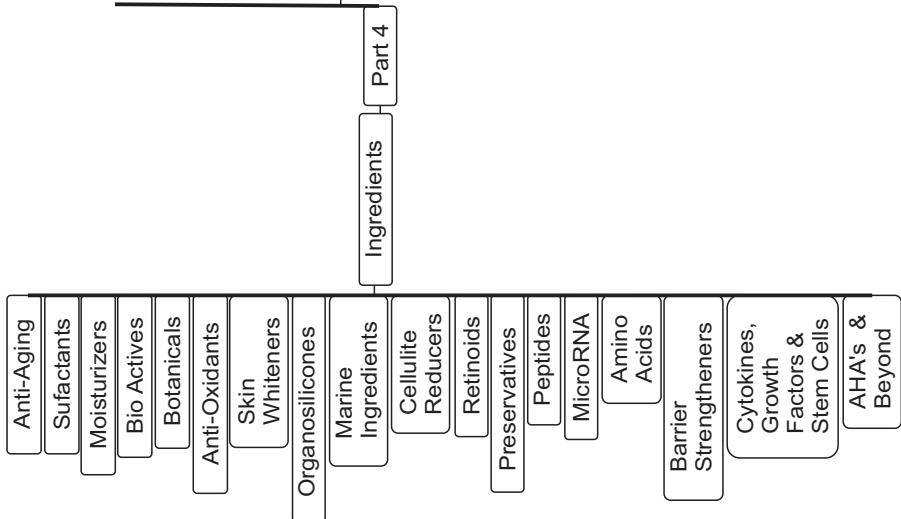
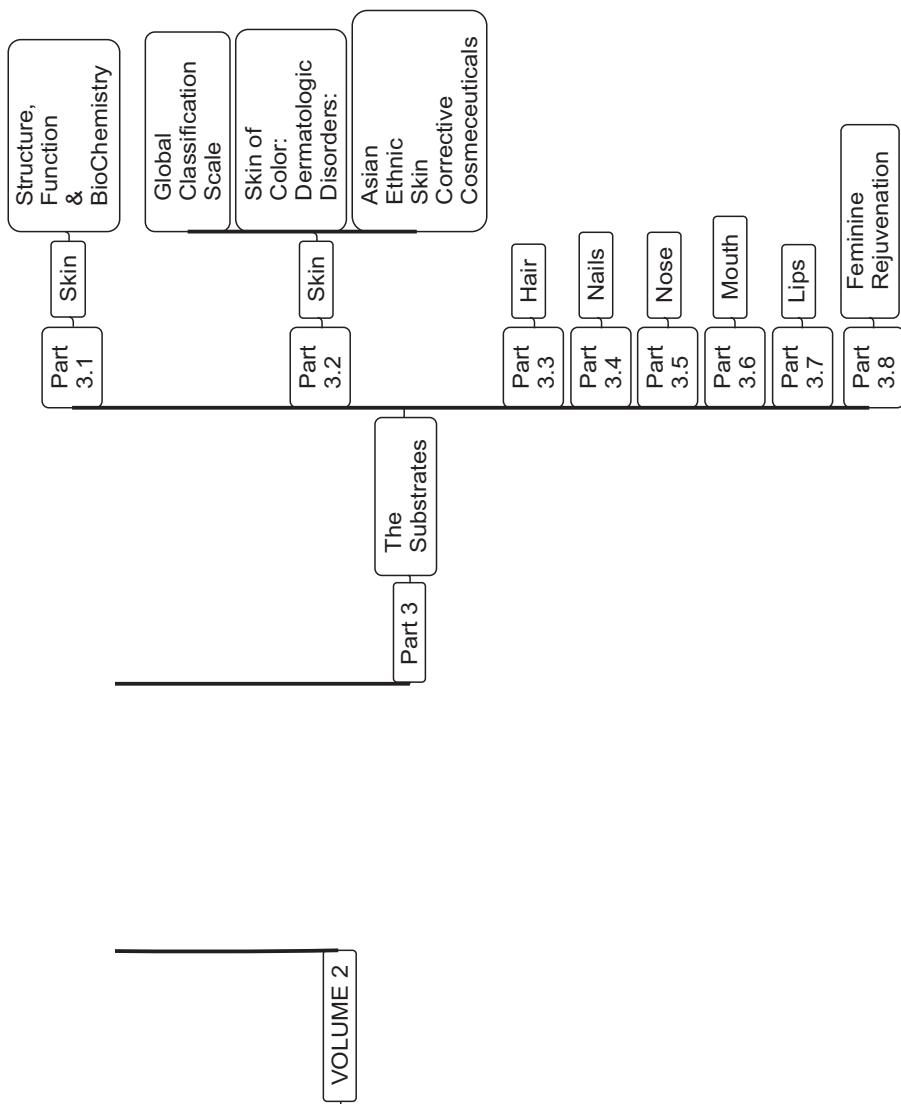
It is my pleasure to give you, in the best tradition of sixty years of *Harry's Cosmeticology*, the knowledge described and documented in this book. I give it to you for your study and to mentor future generations of beauty/youth-seekers in moving towards this goal.

Meyer R. Rosen

FRSC, FAIC, CPC, CChE

Editor-in-Chief: *Harry's Cosmeticology*, 9<sup>th</sup> Edition





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# **Volume 1**

## **PART 1 IN THE BEGINNING**

### **PART 1.1**

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Darrin C. Duber-Smith, MS,  
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## PART 2.3.1

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**PART 3 THE SUBSTRATES****PART 3.1**

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**THE SKIN:  
STRUCTURE, BIOCHEMISTRY, AND FUNCTION**

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Editor: Manuel Gamez-Garcia

## Ashland Specialty Ingredients

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**SKIN WHITENER INGREDIENTS**

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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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## PART 5 ANTI-AGING

### **PART 5.0**

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## FUNDAMENTALS OF SKIN ANTI-AGING OVERVIEW

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### **PART 5.1**

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## THEORIES OF AGING SKIN ANTI-AGING: AT THE TIPPING POINT

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### EPIGENETICS OF SKIN AGING

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AGING PROCESS**

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# Volume 3

## PART 6 FORMULATING COSMETICS AND PERSONAL CARE PRODUCTS

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## SKIN CARE OVERVIEW

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## FORMULATING WISDOM CATEGORY BY CATEGORY

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### PART 6.2

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**HAIR COLORANTS AND PROTECTION**

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AND PRACTICES FOR HEALTH AND BEAUTY**

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## SENSORY SIGNALS—THE APPLIED SCIENCE OF SENSORY PERCEPTION AND ITS VALUE

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EFFECTS OF CLEANSING PRODUCTS**

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# BIOPHYSICAL MEASUREMENT AND EVALUATION OF SKIN ELASTICITY AND TOPOGRAPHY

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# **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**

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**A GLOBAL APPROACH FOR THE COSMETIC  
AND PERSONAL CARE INDUSTRY** **1990**

Editor's Overview  
Alban Muller (President, Alban Muller Group)

**PART 12.1**

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## PART 13.0

# MANUFACTURE OF COSMETICS SECTION OVERVIEW

Meyer R. Rosen

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**PART 13.1****COSMETIC MANUFACTURING PROCESSES**

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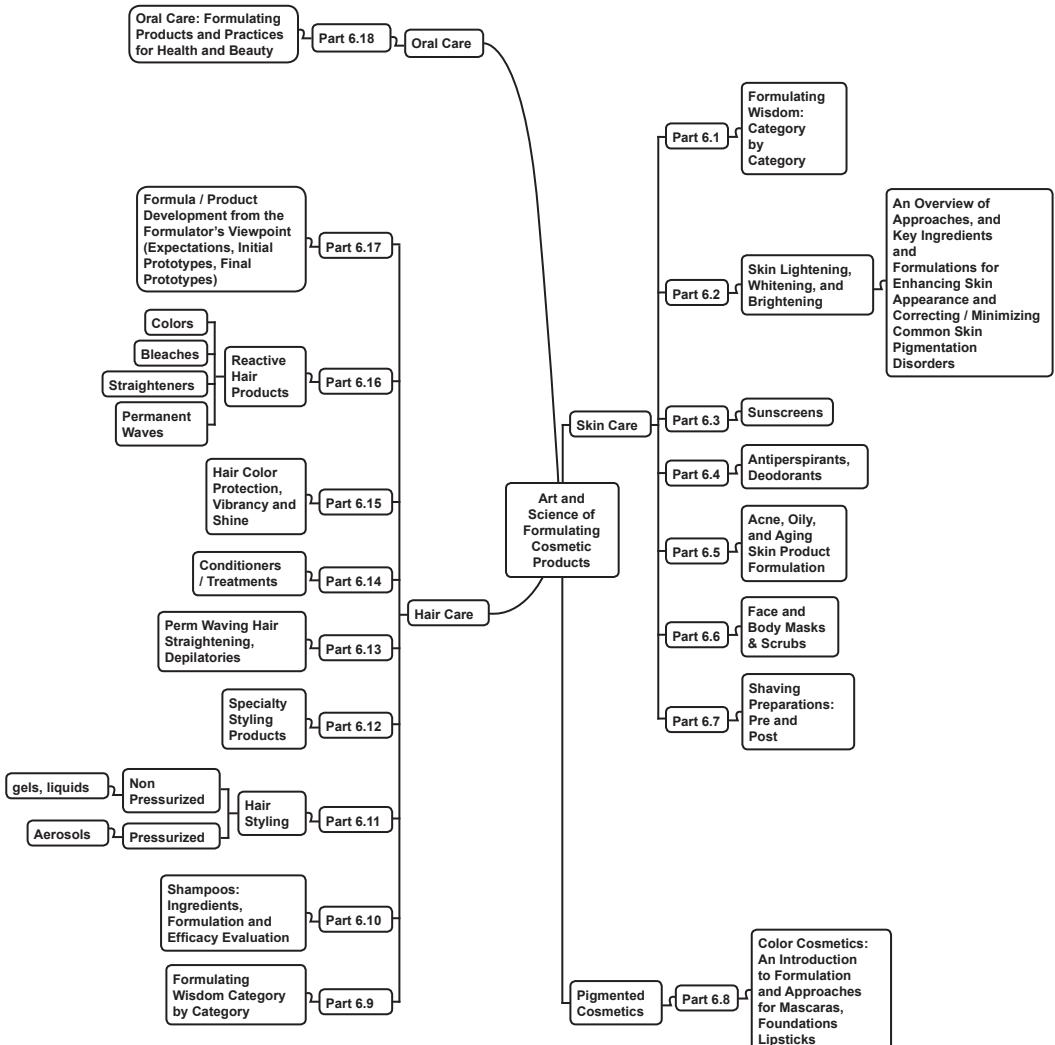
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# ART AND SCIENCE OF FORMULATING COSMETIC PRODUCTS



## 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 hyperpigmentation, 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

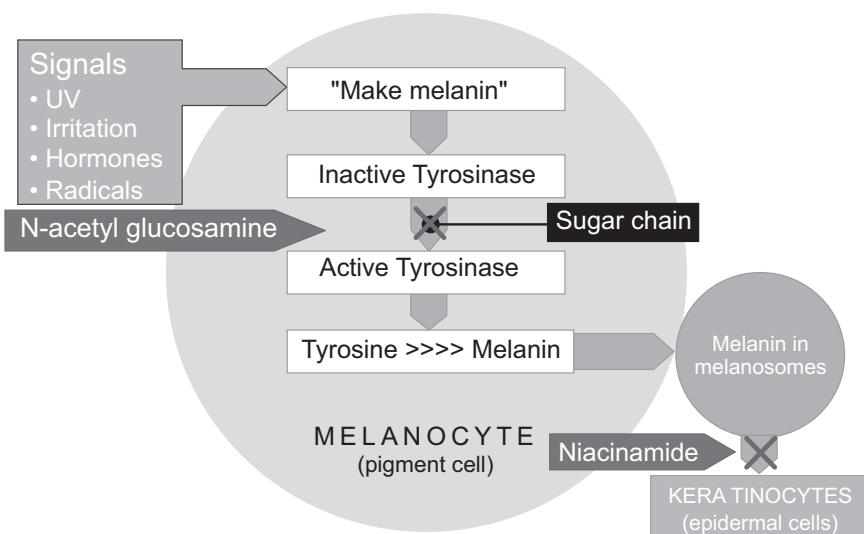
1. Sun (UVA/UVB rays, environmental damages, free radicals)
2. Hormones (internal unbalance, pregnancy, menopause)
3. Inflammation (blemishes, acne, injury, laser treatment, heat, medical procedures, chemical procedures)
4. Oral Medications (diuretics, birth control pills, antibiotics, anti-depressants, 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.

## Inhibition of Pigmentation process



**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 (TiO<sub>2</sub>-Mn) to instantly and gradually brighten the skin. Lumisphere, the Diacetyl Boldine (DAB) stabilizes tyrosinase in its inactive form. It also contains TiO<sub>2</sub>Mn, 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 biotransformation 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 macrocephala, 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.

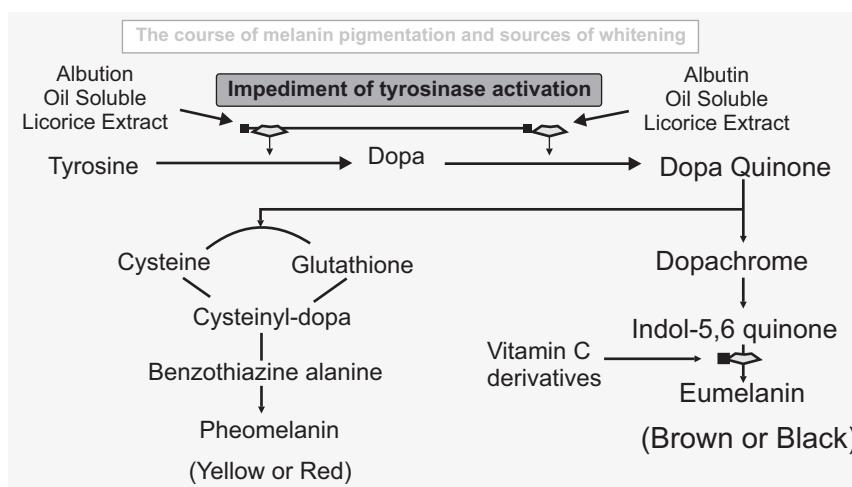
*Supplier: Lucas Meyer Cosmetics*

**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
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### 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**

<b>Phase A</b>	<b>wt/%</b>
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

<b>Phase B</b>	<b>wt/%</b>
Ammonium Acryloyldimethyltaurate & VP Copolymer (Arisoflex AVC/Clariant)	1.20

<b>Phase C</b>	<b>wt/%</b>
Deionized Water	68.90
Glycerin (Rita Glycerin USP/Rita)	5.00
DISMUTIN-PF	0.20
DIOCIDE	0.70

<b>Phase D</b>	<b>wt/%</b>
Deionized Water	10.00
ALPHA-ARBUTIN	1.00

<b>Phase E</b>	<b>wt/%</b>
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**

<b>Phase A</b>	<b>wt/%</b>
Parsol HMS (DSM) (Homosalate; USAN)	10.00
Parsol 1789 (DSM) (Butyl methoxydibenzoylmethane) (Avobenzone; USAN)	3.00
Parsol 340 (DSM) (Octocrylene) (octocrylene; 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-Butyleneglykol (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
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**Phase D**

Alpha-Arbutin (DSM)	2.00
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**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**

	wt/%
Phase A	
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

**PROCEDURE:**

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 (Salix alba [willow] bark extract) (Active Concepts)	2.00
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
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**Phase D**

ACB Lemon Peel Extract (Active Concepts)	2.00
(Lactobacillus/citrus medica limonum [lemon] peel ferment extract [and] butylene glycol)	
AC DermaPeptide Micro C (Active Concepts)	0.50
(Saccharomyces/capsicum annuum fruit ferment filtrate)	
ACB Yogurt Dermal Respiratory Factor	5.00
(Active Concepts) (Lactobacillus bulgaricus ferment filtrate)	
Stabilized Vitamin C (Active Concepts)	
(Ascorbic acid)	0.50

**Phase E**

Glycerin	5.00
AC Leucidal (Active Concepts)	0.50

(Leconostoc kimchii [radish] root ferment filtrate)

**Phase F**

Lavender and Vanilla Sage 302669	0.25
(American Flavors and Fragrances) (Fragrance)	

**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

<b>Phase A</b>	<b>wt%</b>
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
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### 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

<b>Phase A</b>	<b>wt%</b>
Cremophor A6 (Ceteareth 6, stearyl alcohol)	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 isomerase)	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
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**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.

<b>Age Spot Defense</b>	<b>wt/%</b>
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.

## REFERENCES

1. Schallreuter KU, Moore J, Tobin DJ, Gibbons NJ, Marshall HS, Jenner T, et al. alpha-MSH can control the essential cofactor 6-tetrahydrobiopterin in melanogenesis. *Ann NY Acad Sci.* 1999; 885: 329–41.)
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<sub>12-15</sub> 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/isobutane 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 Stearyl Stearate Octinoxate Octisalate Oxybenzone Panthenol

<b>Common Comedogenic Ingredients</b>			
<b>Highly Comedogenic (4-5/5 or 5/3)</b>	<b>Moderately Comedogenic (3-4/5 or 2/3)</b>	<b>Mildly Comedogenic (2-3/5 or 1/3)</b>	<b>Noncomedogenic</b>
		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 & 1 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 pledge 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 pledge 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**

#### **Charles Warren**

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<sub>12-15</sub> 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 not 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  $\alpha$ - and  $\beta$ -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 bases 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 consulting 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 hydroalcoholic-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., isopropyl palmitate), 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**

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## **COLOR COSMETICS: AN INTRODUCTION TO FORMULATION AND APPROACHES FOR MASCARAS, FOUNDATIONS AND LIPSTICKS**

### **Authors**

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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).

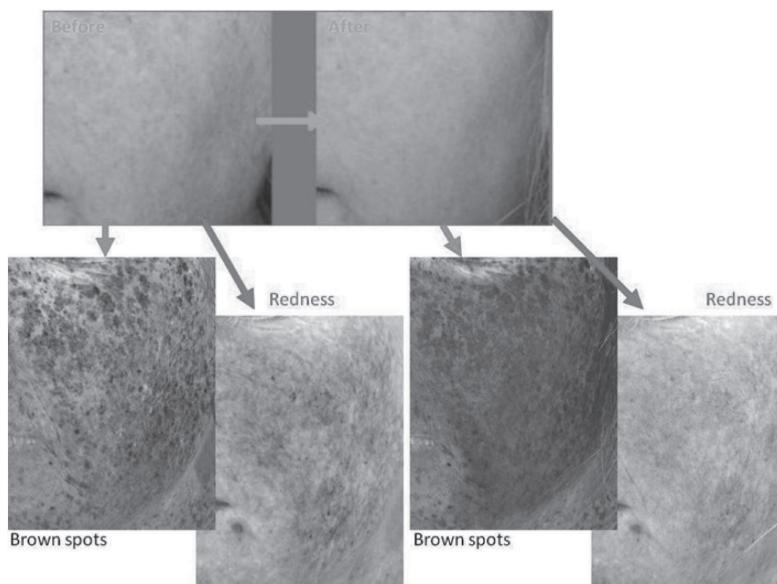
In all these areas, products often combine optical benefit (color, sparkle, diffusion) with skin appearance improvements (even toner, concealers, wrinkle fillers, and active ingredients such as firming or healthy nail growth).

## 6.8.2 FOUNDATIONS

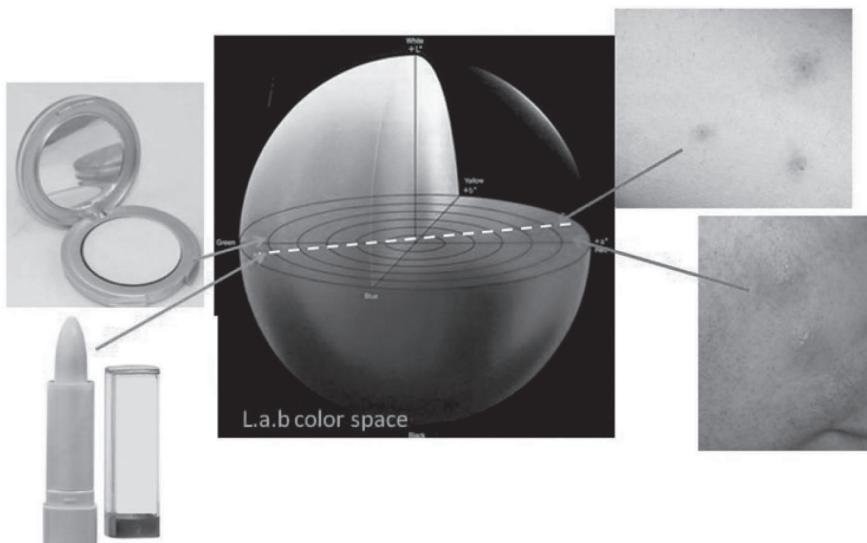
Three main types of foundations can be differentiated: water based, oil based, and silicone. Foundations are intended to provide desired coverage of skin inhomogeneities as well as color shades while maintaining the skin's natural breathing.

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<sub>12</sub>–C<sub>22</sub> 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
<b>A</b>	Water Glycerin disodium edta triethanolamine Acrylic Acid/VP crosspolymer	50.95 2 0.1 0.1 0.1
<b>B</b>	SB700 Silica Beads BTD-401 ITT Treated TIO2 BYO-12 ITT Treated Yellow Iron Oxide BRO-12 ITT Treated Red Iron Oxide BBO-12 ITT Treated Black Iron Oxide O-13 ITT Treated Sericite	1 5.08 0.48  0.18  0.1  1.56
<b>C</b>	GLYCERYL Stearate (and) Laureth-23 Ceteareth-20 Decyl Oleate Isocetyl Stearate Octocrylene Ethylhexyl salicylate Avobenzone Dimethicone Trimethylsiloxyphenyl Dimethicone Triacontanyl PVP VP/Eicosene Copolymer	2.3 1.3 1.5 0.75 3 3 2 3 7 3 2
<b>D</b>	Cyclopentasiloxane	6
<b>E</b>	Acrylates/C12–22 Alkylmethacrylate Copolymer Caprylyl Glycol, Phenoxyethanol	2 1.5
	<b>Total:</b>	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 =  $7 \times 800 = 5,600$  cps PH = 6.53

24-hour viscosity: LVT-TB @ 5rpm @ 25c:  $10 \times 800 = 8,000$  cps PH = 6.54

## Formula 26.2. Silicone Foundation

Phase	INCI	% W/W
<b>A</b>	Ethylhexyl Palmitate	3.00
	Isodecyl Neopentanoate	4.00
<b>B</b>	Cyclopentasiloxane, PEG/PPG-18/18	5.00
	Dimethicone	
	Lauryl PEG-9, Polydimethyl Siloxyethyl	2.30
	Dimethicone	
	Cyclopentasiloxane, Dimethicone	5.00
	Vinyltrimethylsiloxy silicate crosspolymer	
<b>C</b>	Cyclopentasiloxane	6.00
	Silica	1.00
	Titanium Dioxide (and) Isopropyl Titanium	6.70
	Triisostearate	
	Iron Oxides (and) Isopropyl Titanium	0.87
	Triisostearate	
	Iron Oxides (and) Isopropyl Titanium	0.33
	Triisostearate	

Phase	INCI	% W/W
<b>C</b>	Iron Oxides (and) Isopropyl Titanium Triisostearate	0.14
	Mica (and) Isopropyl Titanium Triisostearate	1.10
<b>D</b>	Water	60.31
	Sodium Chloride	0.50
<b>E</b>	Propylene Glycol	3.00
	Phenoxyethanol, MethylParaben, Butyl Paraben, EthylParaben, Propylparaben	0.75
	<b>Total:</b>	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:  $42 \times 2000 = 84,000$  cps, PH = 4.94

## Formula 26.3. Radiance Skin Illuminating Foundation

Phase	Ingredients	% W/W
<b>A</b>	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>B</b>	SB700 Silica Beads	1.00
	BTD-401 ITT Treated TIO2	6.78
	BYO-12 ITT Treated	0.87

Phase	Ingredients	% W/W
<b>B</b>	Yellow Iron Oxide BRO-12 ITT Treated Red Iron Oxide BBO-12 ITT Treated Black Iron Oxide O-13 ITT Treated Sericite	0.33 0.20 1.82
<b>C</b>	GLYCERYL Stearate (and) Behenyl Alcohol (and) Palmitic Acid (and) Stearic Acid (and) Lecithin (and) Lauryl Alcohol (and) Myristyl Alcohol (and) Cetyl Alcohol C12–15 Alkyl Lactate Decyl Oleate Isocetyl Stearate Ethylhexyl Methoxycinnamate Ethylhexyl Salicylate Dimethicone	5.00 3.00 1.50 0.75 7.00 3.00 1.00
<b>D</b>	Acrylates/C12–22 Alkylmethacrylate Copolymer	1.00
<b>E</b>	Cyclopentasiloxane	7.50
<b>F</b>	Diazolidinyl Urea (and) Iodopropynyl Butylcarbamate	0.50
<b>G</b>	Styrene/Acrylates Copolymer	10.99
<b>H</b>	Mica (and) Titanium Dioxide	3.00
	<b>Total:</b>	100

## PROCEDURE

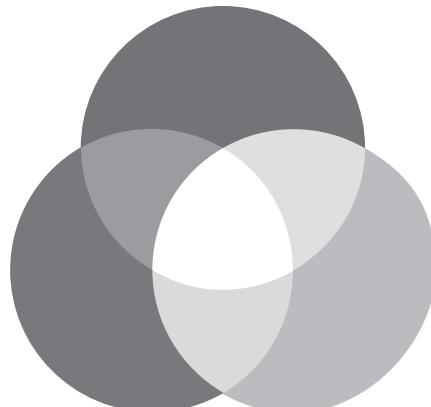
1. Heat water to 75°C, add remaining ingredients of phase A (pre-wet gums with Butylene Glycol).
2. Mix well until gums are completely hydrated.
3. Pre-blend phase B (pulverize). Add phase B to phase C at 75°C using homomixer.
4. Add phases B & C (70–75°C) to phase A (75°C) using homomixing. Homogenize 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

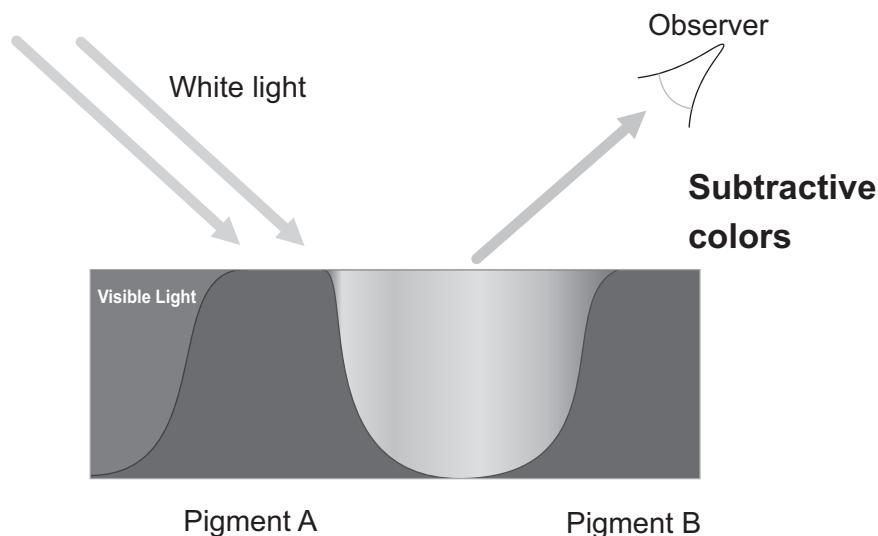
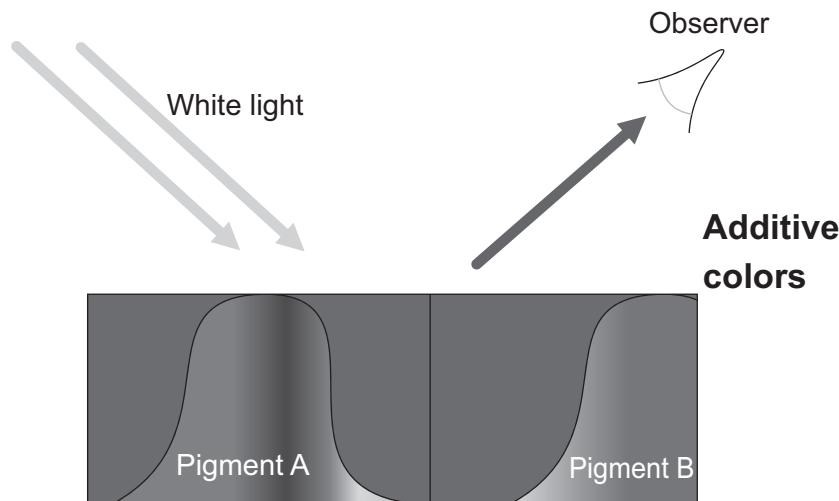
Pigments impart color to the final formulation. They can be of two types: absorption pigments or interference pigments. Absorption pigments give color by absorbing part of the visible light spectrum. Their observed color results from reflection of the nonabsorbed light spectrum. Combining absorption 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<sub>12</sub>–C<sub>22</sub> 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 <sub>12-15</sub> 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) Iron Oxides (C33-8073 Yellow Iron Oxide) Titanium Dioxide (White) Iron Oxides (C33-5198 Black Iron Oxide) Isocetyl Stearyl Stearate Tricontanyl PVP	1.00 1.00 1.20 0.35 3.45 0.10
D	Mica Mica (and) Iron Oxides (and) Titanium Dioxide	13.00 4.00
	<b>Total:</b>	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
<b>A</b>	Myristyl Lactate	9.00
	Tribehenin	3.00
	Beeswax	3.00
	Tocopheryl Acetate	0.10
<b>B</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
<b>C</b>	Mica (and) Iron Oxides (and) Titanium Dioxide (Gemtone Tan Opal)	8.00
<b>Total:</b>		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<sub>12–22</sub> 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

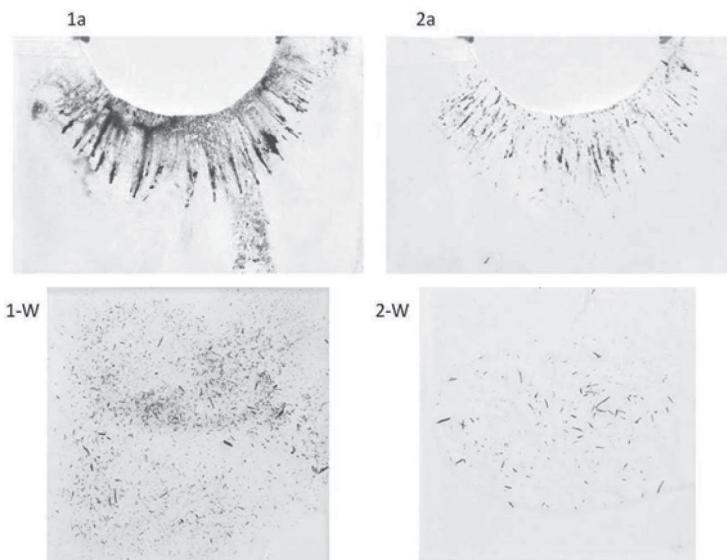
Phase	Ingredients	% (wt)
A	D.I. WATER Disodium EDTA (Versene Na2 Crystal) Triethanolamine (TEA 99%) Acrylic acid/VP Crosspolymer (Ultrathix p-100) Xantham gum (Keltrol T)	45.42 0.1 0.38 0.5 0.3
B	Water Polynvinyl pyrrolidone (PVP-K30)	10 1
C	Iron oxide (Unipure black LC989)	12
D	Glyceryl stearate and Laureth-23 (CERASYNT 945) $C_{18-36}$ acid triglyceride (Synchrowax HGLC) White beeswax Stearic acid Copernicia cerifera (Carnauba) wax SP63 Euphorbia cerifera (Candelilla) wax PEG-20 stearate (Lipo PEG10-S) Tricontanyl PVP (Ganex WP-660)	3 5 5 2 3 1 2 0.5
E	Triethanolamine (TEA 99%)	1
F	Water Allantoin Vitamin E (Tocopheryl acetate) Acrylate/alkylmethacrylate copolymer (Allianz OPT)	5 0.1 0.2 1
G	Phenoxyethanol, octanediol (optiphen)	1.5
	<b>Total:</b>	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 watery 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<sub>12</sub>–C<sub>22</sub> alkyl methacrylate copolymer on water resistance: Test 1 (Top): Bending under wet conditions and Test 2 (Bottom):

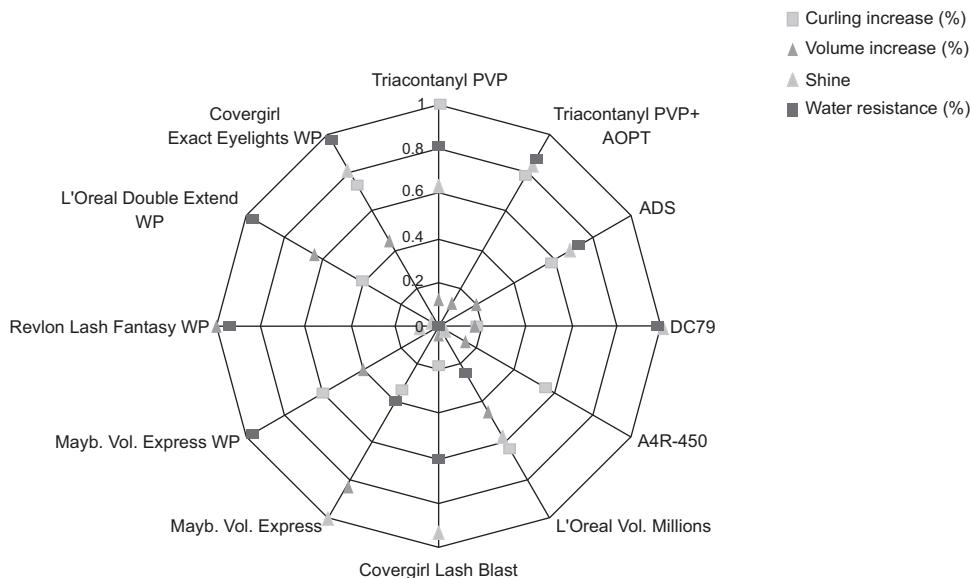
particles released after 60 seconds of eyelash immersion in deionized water. (1) Mascara base without polymer; (2) mascara with 1% acrylate/C<sub>12</sub>–C<sub>22</sub> alkyl methacrylate copolymer. In all cases, mascara was applied on eyelashes and given 20 minutes drytime at air prior to tests.

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 (macadamia, 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

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#### 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 ether 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<sub>12</sub> fatty alcohols. This chain length is chosen to maximize the foaming and wetting traits of the final surfactant. Various mixes of C<sub>12</sub>–C<sub>14</sub> 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 to 16 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-nitrosamines. 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.

### **Amphoteric**

Amphoteric 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 amphoteric 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 amphoteric 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, micellar 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 micellar 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<sub>4</sub>OH, 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 campestris* 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 cinnamido-propyltrimonium 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
Ketoconazole	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 sulfocuccinate (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 8<sup>th</sup> 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,  $\gamma$  is the interfacial tension between any two surfaces, P represents the particle, H represents the hair surface, while W represents water. For hydrophobic particles,  $\gamma HW$  would be larger and  $\gamma PH$  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  $\gamma HW$  and, therefore,  $Wa$ . Similarly, binding of surfactant to a hydrophobic soil reduces  $\gamma PW$ , 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 dependency 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  $\gamma$  is the interfacial tension between two phases, H represents hair, O represents the oil phase, and W represents water.  $\theta$  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  $\gamma HW$ , will increase the contact angle of the oil droplet. For sufficiently large increases in  $\gamma HW$ ,  $\theta$  will increase to  $180^\circ$ , 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 micellar 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  $5 \times 10^{-3}$  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 micellar 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
<b>Average % removal</b>	<b>95.9</b>	<b>85.9</b>

*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, dicetylmonium 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**

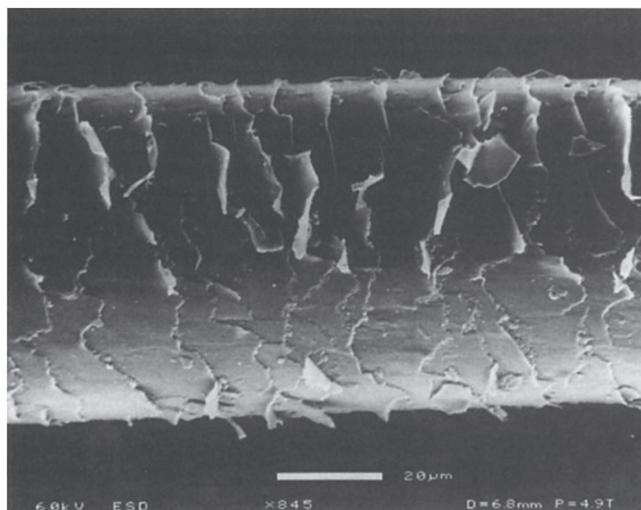
The use of shampoos can damage hair indirectly as a result of fiber abrasion occurring when hairs are rubbed against each other during cleaning. More important to the damage process, however, is the removal of sebum from the fiber surface during shampooing. This is because sebum acts as a natural lubricant 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.

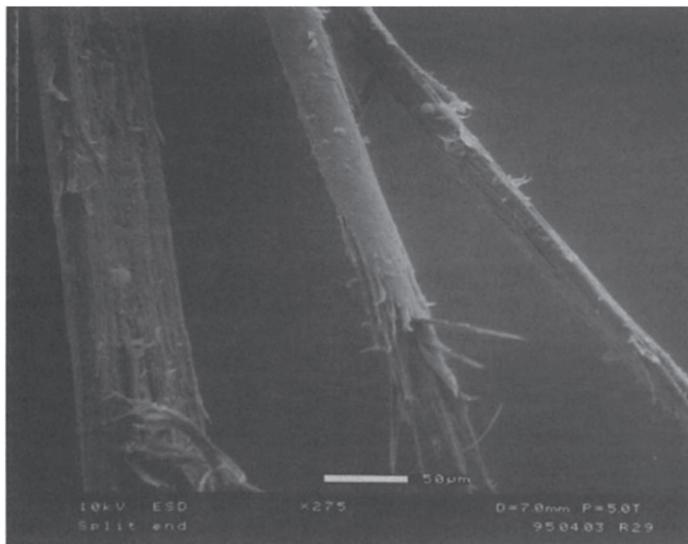
### Damage to Chemically Treated Hair

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  $\alpha$ -1,4 and  $\alpha$ -1,6 linkages. Makes up 76% of dent corn starch and 100% of waxy corn starch.

**amylose** – a linear polysaccharide with  $\alpha$ -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**

### **Author**

**Charles Warren**

#### **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 a 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 isobutane). 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**

### **Author**

**Charles Warren**

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.

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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.<sup>1</sup> 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 Lefaudoux 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.**

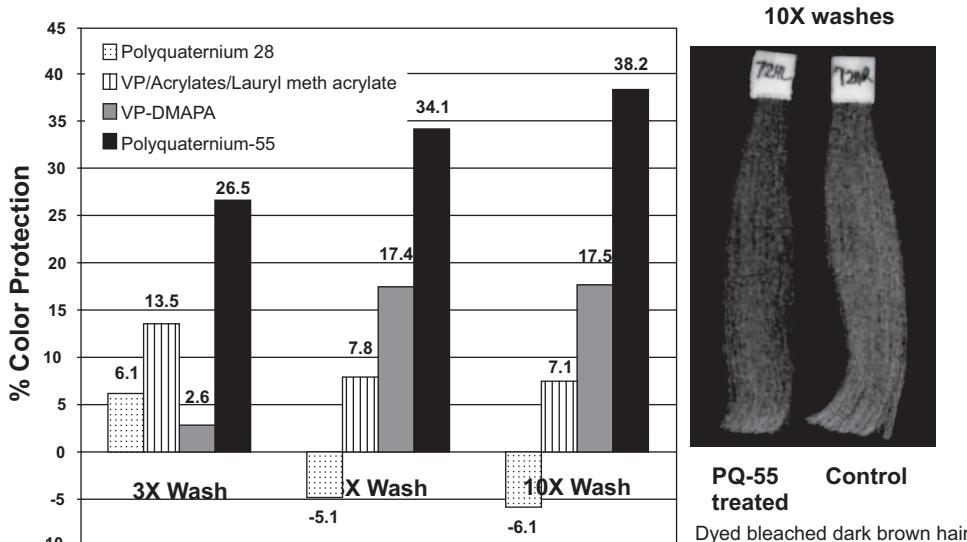
Red shades tend to fade more than brown or black shades due to their relatively small molecule size, which can diffuse from the hair and wash away more quickly than other shades. UV exposure also breaks down the red tone more easily, while pure browns and blacks resist fading because their color molecules 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.

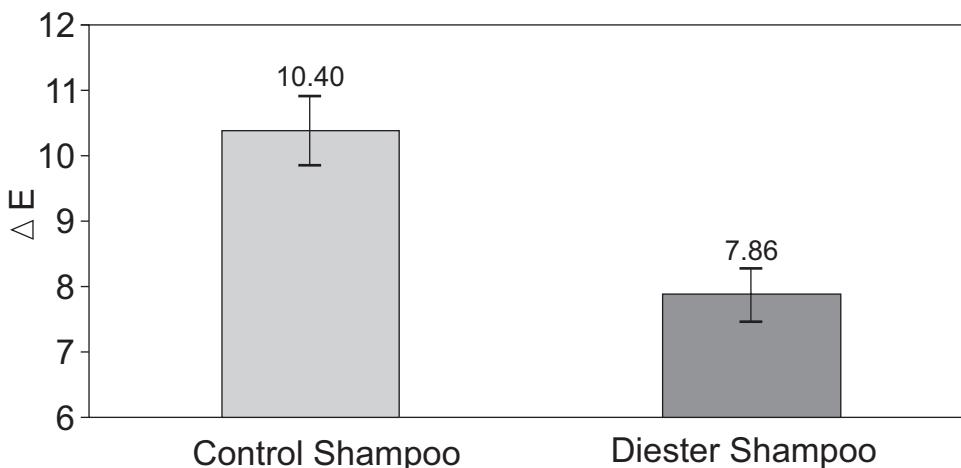
Studies by X et al. showed that color fading primarily occurs through physical removal of dyes by rinsing with water. These authors have investigated the physical and chemical factors that influence hair-color removal during the washing process by testing hair dye dissolution in water from dyed hair 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-MAPTA copolymer) leave-in treatment provides the highest color protection with 38% higher color improvement at the end of 10 $\times$  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 $\times$  SLES washes and leave-in treatment of 2% of various polymers.

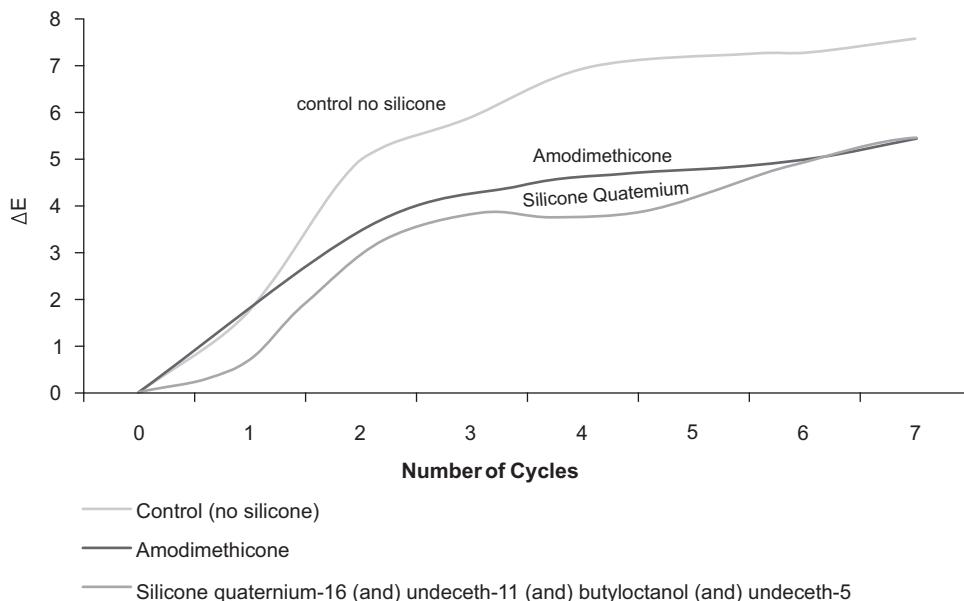
Both visual and instrumental measurement results indicate that Polyquaternium-55 provides a high level of color protection when formulated in a hair-color protection regimen with up to 50% color protection. Conditioning shampoos and conditioners with cationic surfactants and water proofing amino silicones 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  $\Delta E$  value, the silicones significantly reduced color loss compared to the control (the same rinse-off conditioner without silicone). The lower  $\Delta E$  value for hair treated with the silicones indicates they help maintain color intensity and depth.



**Figure 3.**  $\Delta E$  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

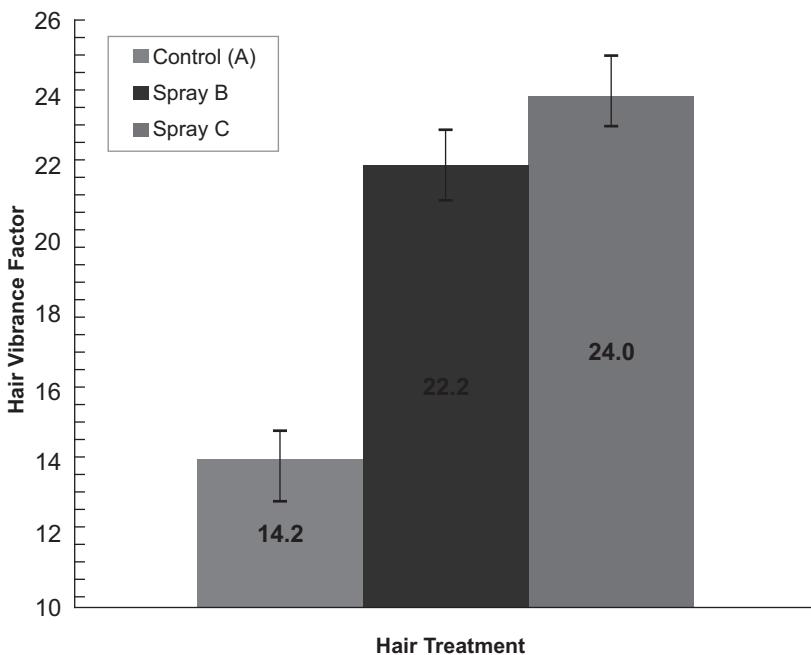
be used in personal care applications where a need exists to form a protective barrier on the hair cuticle. This protective barrier phenomenon can be strongly applied to products claiming hair-color protection. The film formed on the hair is believed to protect hair color from losing its vibrancy as 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.<sup>21</sup> 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<sup>TM</sup> UV-HPP from Croda and PARSOL<sup>®</sup> 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**

**Author**  
**Charles Warren**

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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.<sup>1</sup> 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%.<sup>2</sup> Fluoridated water supplies in the U.S. have saved more than \$4.6 billion annually in dental costs.<sup>2</sup> 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.<sup>2</sup> Assessments to determine risk for dental caries are being utilized (Caries Assessment and Management By Risk Assessment: CAM-BRA), 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.<sup>3</sup> For over a decade we have had the scientific evidence that dental sealants do work, particularly those applied to children in school programs.<sup>4</sup> 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.<sup>5</sup> 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.<sup>6</sup> 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,<sup>7</sup> 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.<sup>8</sup>

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<sup>9</sup> 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<sup>10, 11</sup> 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.<sup>12–14</sup> 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.<sup>10</sup> 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.<sup>15</sup>

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
Cetylpyridinium 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.<sup>16</sup>

- 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.<sup>17</sup> 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<sup>18</sup>

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<sup>21</sup>

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.<sup>18, 19</sup> 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.<sup>20</sup>

**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.

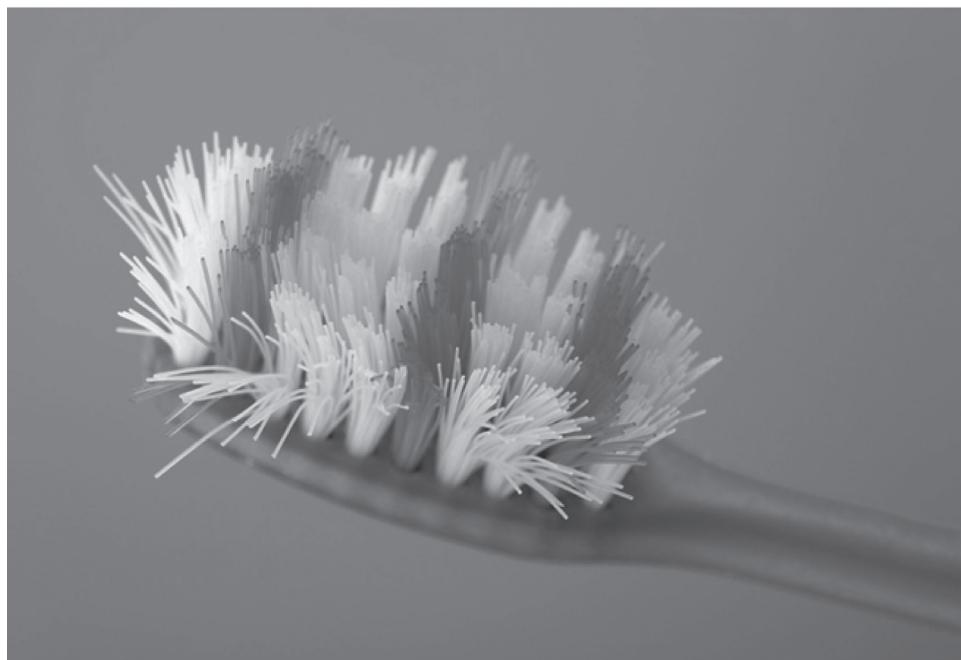
Calcium phosphates include diverse chemicals, such as dicalcium phosphate dihydrate, dicalcium phosphate, tricalcium phosphate, calcium pyrophosphate, and synthetic apatites, all of which are used as toothpaste abrasives. *Dicalcium phosphate dihydrate* (DCP-D) is the calcium phosphate most commonly 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, non-toxic, 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.<sup>20</sup> 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<sup>21</sup> 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.<sup>21</sup> 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.<sup>22</sup>

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 Bränström's hydrodynamic theory, tooth sensitivity<sup>23</sup> 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.<sup>23</sup>

## ***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.<sup>24</sup> 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),<sup>25</sup> reduce oral discomfort, strengthen enamel, provide moisture to oral tissues, and fight bad breath.<sup>26</sup> 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 to 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.<sup>27–29</sup> 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.<sup>30,31</sup>

**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.

*Cetylpyridinium 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.<sup>32</sup>

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.<sup>33</sup> There were fears that patients would brush too vigorously. Early clinical research revealed no difference in brushing with a manual versus an 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.<sup>34</sup>

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 non-keratinized (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.<sup>34–37</sup> 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.<sup>36</sup>
- There is insufficient evidence that dental floss reduces plaque.<sup>40</sup>
- There are no studies that indicate that the use of dental floss reduces the risk of dental caries.<sup>35</sup>
- The quality of evidence to support the theory that flossing and toothbrushing reduce gingivitis is very low.<sup>36</sup>
- When dental professionals flossed patients' teeth, significant benefits were seen that were not seen when patients flossed their own teeth.<sup>37</sup>
- 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.<sup>35, 36</sup>

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.<sup>38</sup>

## **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:

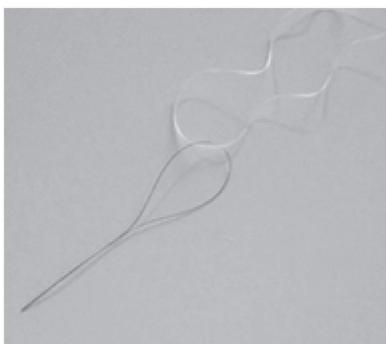
- End-tufted brushes
- Floss threaders
- Interdental cleaners with elastomeric “fingers”
- Interproximal (interdental brushes)
- Powered flossers
- Rubber-tip stimulators
- Soft wooden sticks
- Textured flosses
- Toothpicks
- Waterflossers (oral irrigators)



**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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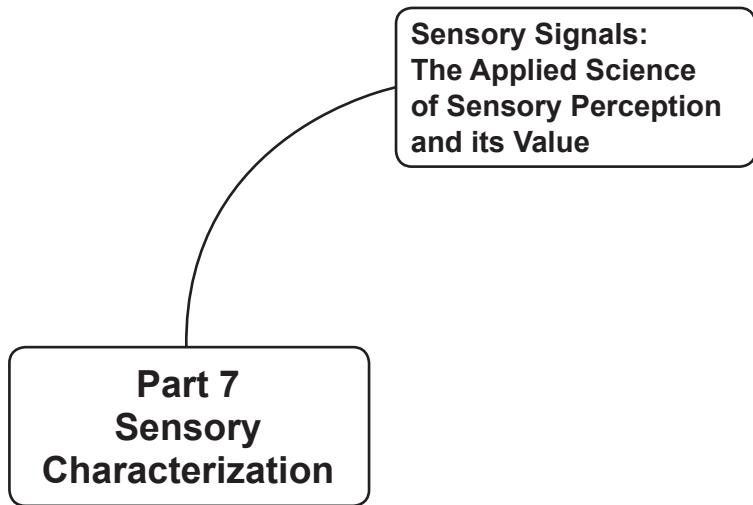
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## SENSORY CHARACTERIZATION



**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](http://www.astm.org)) and the International Organization for Standardization (ISO) ([www.iso.org](http://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:**  
Volatile 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<sup>TM</sup> 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<sup>TM</sup> 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<sup>TM</sup> 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).

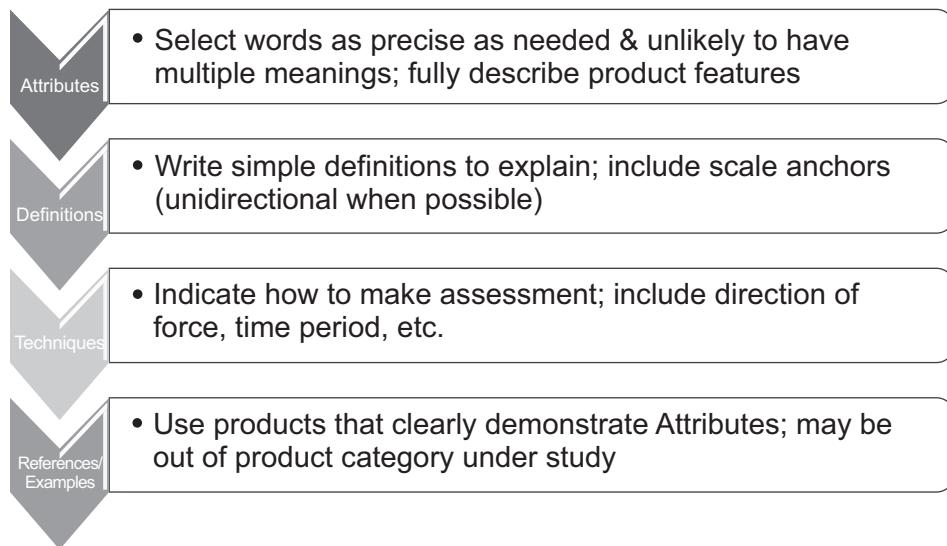
While hundreds of lexicons exist in the literature, it is the responsibility of the user to assess the quality of any lexicon considered for use. ASTM International ([www.astm.org](http://www.astm.org)) prescribes five steps for development of a well-designed lexicon, based on use of an established panel 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		
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)

<b>Attribute</b>	<b>Intensity Reference</b>
Wetness Intensity	
0	Talc
22	White Petrolatum
60	Vaseline Total Moisture Original Lotion
100	Water
<hr/>	
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:*

*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 petroleum
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  $35 \pm 2^\circ\text{C}$ . 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 tactiley 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  $35 \pm 2^\circ\text{C}$ . 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" x 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: <i>soap film, oily, waxy, greasy, powder, silicone, other (list).</i>

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) <i>Evaluate by stroking finger across site.</i> [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 PROTOCOL  
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

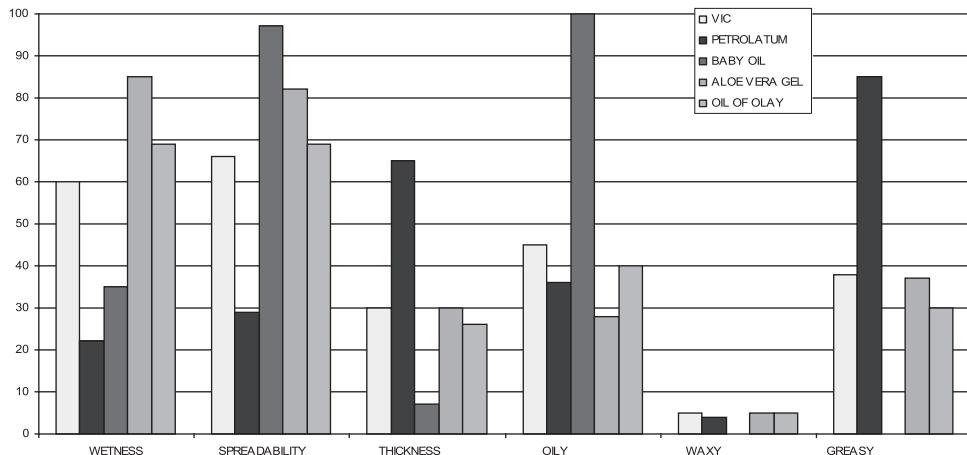
Descriptive Attribute	Sample 489	Sample 784	P-VALUE	LSD	sig
<b>Rub Out</b>					
Wetness	44.6 A	39.7 B	<0.001	0.98	***
Spreadability	51.0 A	46.0 B	<0.001	0.95	***
Thickness	36.0 B	40.2 A	<0.001	1.64	***
Oil	37.7	37.2	0.589	--	
Wax	6.5 B	7.7 A	<0.001	0.37	***
Grease	42.8	43.8	0.109	--	
Rubs to Absorbency (Count)	83.6	86.6	0.361	--	
<b>Immediate Afterfeel</b>					
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	
<b>20 Minute Afterfeel</b>					
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
<b>20 Minute Afterfeel</b>					
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	--	
<b>Pick-Up</b>					
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	--	
<b>Appearance</b>					
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	*

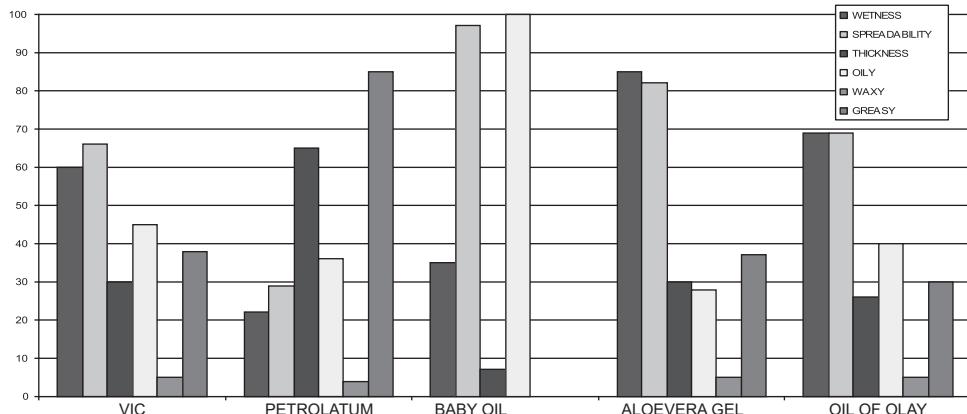
Many audiences prefer graphical presentation of data. Figure 2 provides histograms of the same data presented in two different ways: by product attribute and by sample. Data should be presented in the way that best answers the questions posed prior to the study.

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.

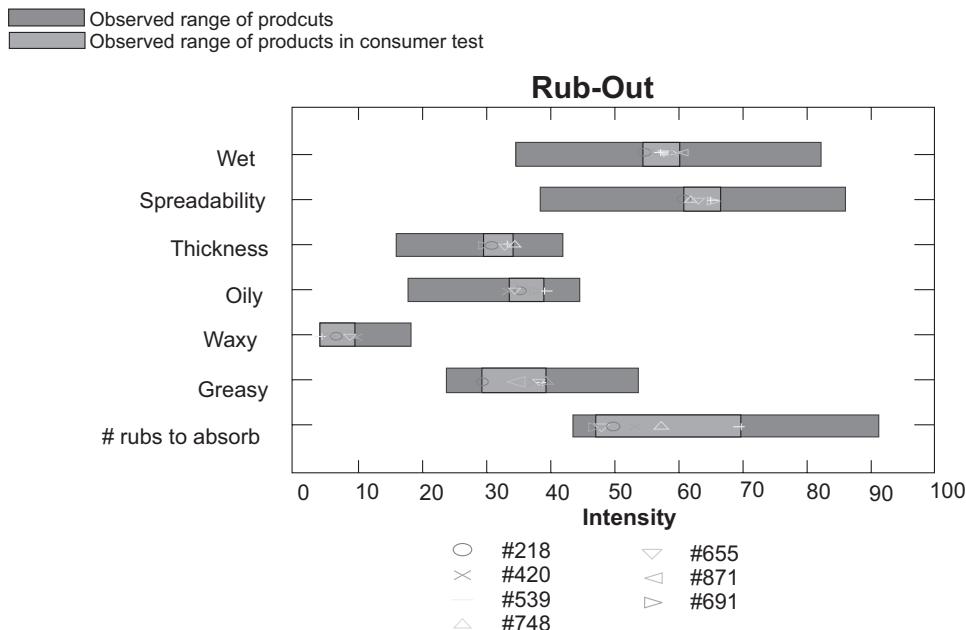
### Rubout Profiles of Lotion References: By Attribute



### Rubout Profiles of Lotion References: By Sample



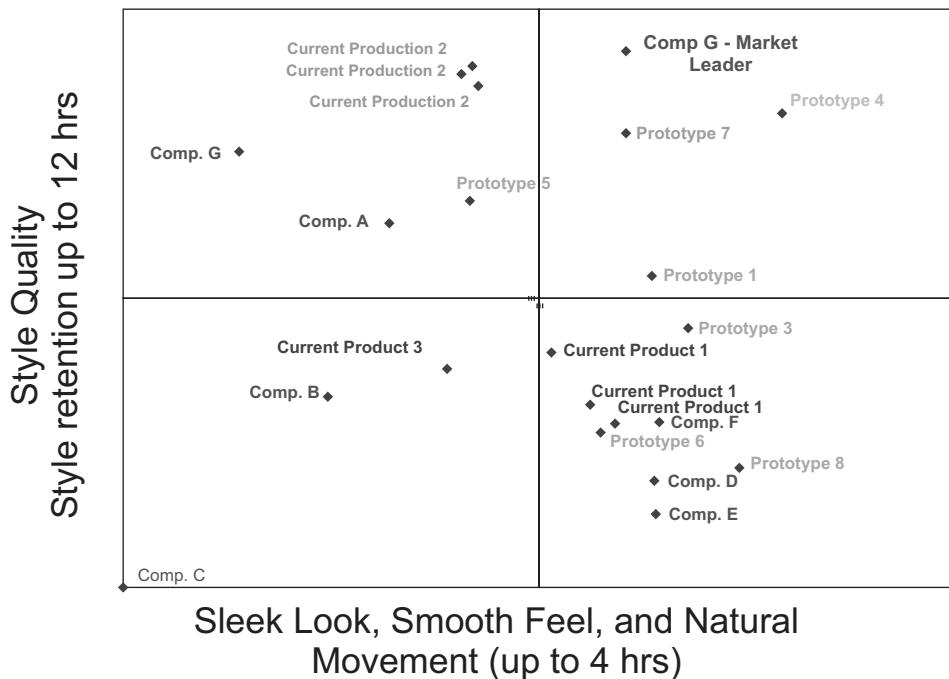
**Figure 2:** Use of Histograms to Present Data by Attribute and by Sample



**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

	<b>Control 1-Gel with aloe and vitamins</b>	<b>Control 2-Instant Foam (pump)</b>
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

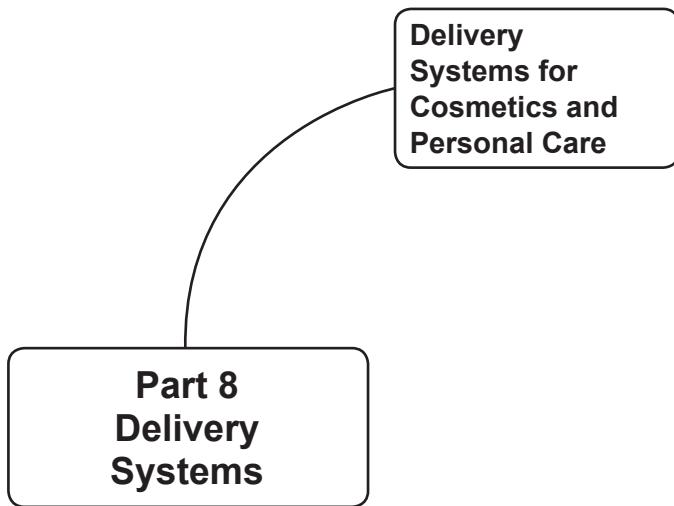
	<b>Control 1-Gel with aloe and vitamins</b>	<b>Control 2-Instant Foam (pump)</b>
Phase Change	Happens at about 25–30 seconds; moderately high sticky-tacky feel between palms and fingers; noticeably taut	Happens at about 20 seconds; low moderate sticky-tacky feel between palms and fingers
Immediate Afterfeel	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<sup>TM</sup>, Polytrap<sup>TM</sup>, Macrobead<sup>TM</sup>, Tagravit<sup>TM</sup> microspheres, microencapsulated fragrances, and MultiSal<sup>TM</sup>
2. **Lipid-based encapsulation systems**, consisting of liposomes, solid lipid nanoparticles (SLN), SalSphere<sup>TM</sup>
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,<sup>1</sup> 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,<sup>2</sup> 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<sup>TM</sup> 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<sup>3</sup> or nanoporous silica spheres.<sup>4</sup> 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.

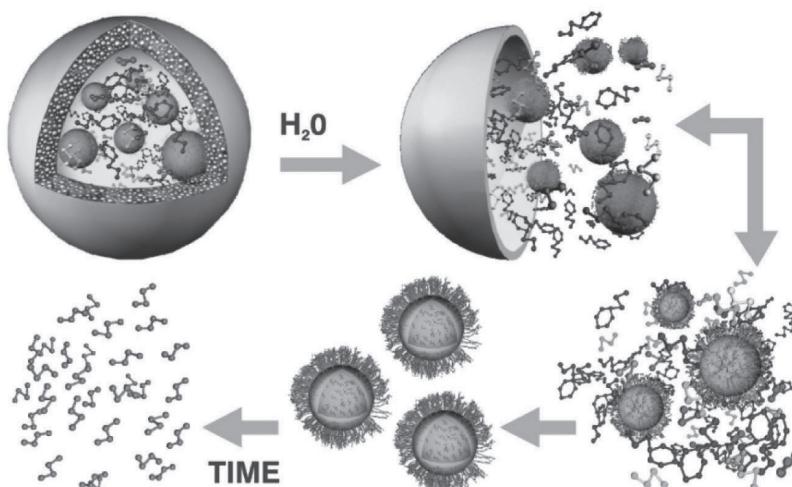
### a. Powder Technologies:

**Starch-based encapsulation systems**

**MultiSal<sup>TM</sup> MicroSphere<sup>TM</sup> Technology**

MultiSal<sup>TM</sup> is a multi-compartment encapsulation that allows the release of multiple functional ingredients at the same location. This technology incorporates

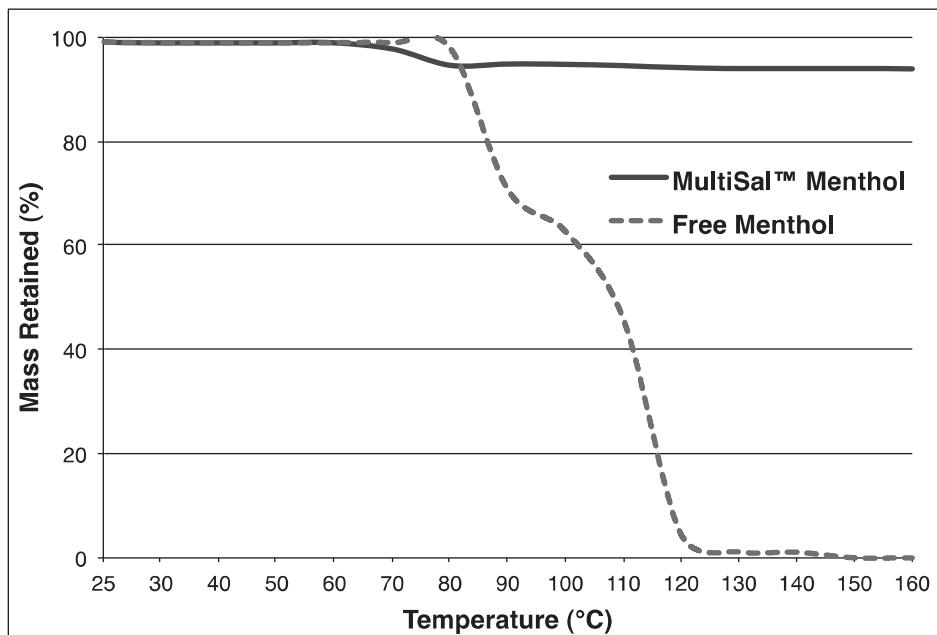
the sub-micron hydrophobic spheres discussed in the previous section into a larger, water-sensitive microsphere that averages 20–30 microns in diameter.<sup>5</sup> The outer microsphere can be constructed out of various ingredients. These include: modified starches, gums, and other natural or synthetic 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 appearance 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.<sup>6</sup> 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.<sup>7</sup> 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, rejuvenation/regeneration, 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 Resveratrol	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,<sup>8</sup> 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<sub>17</sub>H<sub>24</sub>O<sub>6</sub> and molecular weight of 324.37 g/mol. It is also applicable as a carrier vehicle for essential oils.<sup>8,9</sup>

**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, makeup, facial masks	<ul style="list-style-type: none"> <li>• Alcohol free</li> <li>• Oil free</li> <li>• Carbomer gel formulations</li> <li>• Sebum adsorption and control</li> <li>• No preservatives/no surfactants</li> </ul>
3	Poly-Pore 120RE*	Allyl Methacrylate Crosspolymer (and) Polysorbate 20 (and) Retinol (and) BHT	Retinol	20	Anti-aging, skin-renewal creams, makeup, powders, skin care formulations	<ul style="list-style-type: none"> <li>• Stability of retinol</li> <li>• No irritation</li> </ul>

**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 ( $\Phi$  20–40 micron) is stored at  $-1$  to  $4^\circ\text{C}$ . Poly-Pore RE should be added to finished product batch during cooling ( $40^\circ\text{C}$ ). There is a delayed release of salicylic acid from Poly-Pore formulation indicated in the literature.<sup>10</sup>

**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	<ul style="list-style-type: none"> <li>• Sebum control</li> </ul>
2	Polytrap 665		Vitamin E	65	Sunscreens, powders, and liquid makeup	<ul style="list-style-type: none"> <li>• Sustained delivery</li> <li>• Stability of vitamin E</li> </ul>
3	Polytrap 650	Lauryl Methacrylate/Glycol Dimethacrylate Crosspolymer (and) Butyrospermum Parkii (Shea Butter)	Shea Butter	50	Anti-aging products, moisturizers, facial masks	<ul style="list-style-type: none"> <li>• Sebum control</li> </ul>

#	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	<ul style="list-style-type: none"> <li>Exceptional sebum adsorption capacity</li> </ul>
5	Polytrap 6500	Dimethicone (AND) Petrolatum (AND) Lauryl Methacrylate/Glycol Dimethacrylate Crosspolymer	Dimethi-cone/Petrolatum	70	Gels, powders	<ul style="list-style-type: none"> <li>Sebum control</li> <li>Long-lasting performance</li> </ul>
6	Polytrap 680	Lauryl Methacrylates Glycol Dimethacrylate Crosspolymer (and) Melaleuca Alternifolia (Tea Tree) Leaf Oil	Tea Tree Oil	80	Skin care, gels, lotions	<ul style="list-style-type: none"> <li>Ease of formulation</li> </ul>

### 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 Methacrylate/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<sup>11</sup> 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<sup>12</sup> 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,<sup>12</sup> 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	acidophilus/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<sup>13</sup> 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.<sup>14</sup> A good description of these and other gums may be found in the *Rheology Modifier Handbook* by Braun and Rosen.<sup>15</sup>

### b. Solid Lipid Nanoparticles (SLN)

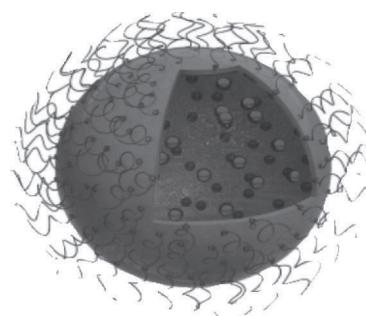
Solid lipid nanoparticles are comprised of organic free, oily droplets of lipids that are solid at room temperature and stabilized by surfactants.<sup>16</sup> SLNs can protect the encapsulated ingredients from degradation. They can be used for the controlled delivery of cosmetic agents such as perfumes, 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.<sup>17</sup> 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.

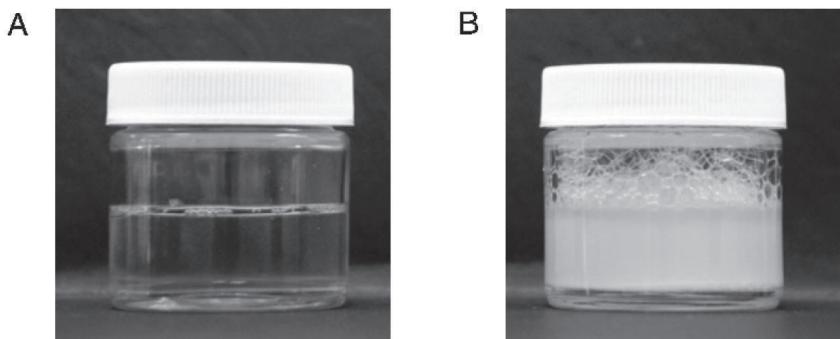


**Figure 3:** Structure of the sub-micron spheres

#### 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**)

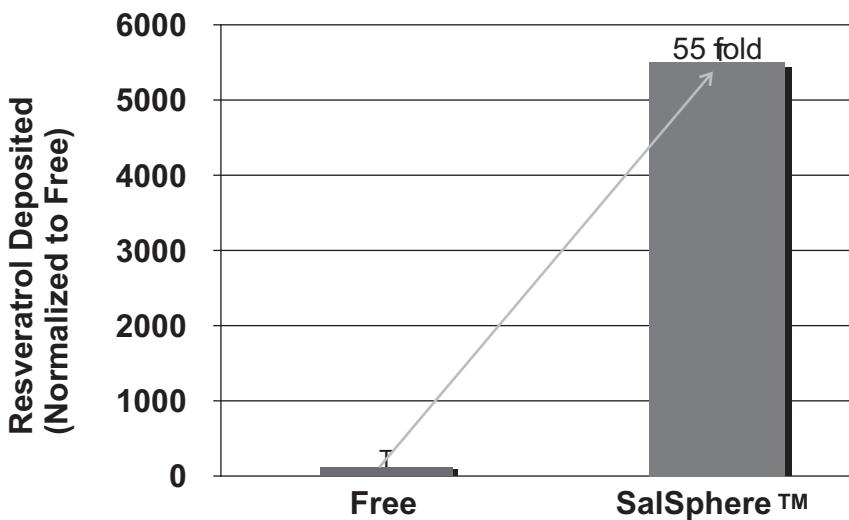
### Improving Performance

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<sup>2</sup> 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.<sup>18</sup> 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 Laurate	10%	Sensory	Long-lasting, odorless, refreshing sensation

#	Product Name	Functional Ingredients	Loading (%)	Functional Applications	Effect
14	SalSphere™ Aqua Skin	Fucus Vesiculosus 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-cytotoxic, shine reduction, skin-friendly pH

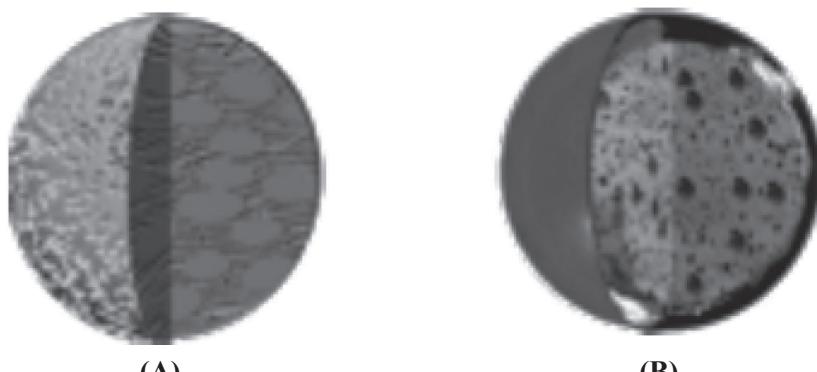
**Lipo Technologies** manufacture a number of delivery systems that are comprised of a lipocapsule made up of Gelatin and Polyoxymethylene urea (PMU). Methoxymethyl Methylol Melamine (MMM) capsule size is of 5–3000 microns. The products employ precipitation of synthetic polymers around a hydrophobic 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.



**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.<sup>19</sup>

#### **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.<sup>20</sup> 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.<sup>21</sup> Data on clinical efficacy and stability have not been found in the literature.

MultiSal™ retinol is composed of microspheres with sub-micron spheres (Sal-Spheres™) 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 Multi-Sal™ 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.<sup>22</sup>

## **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.<sup>22</sup>

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?

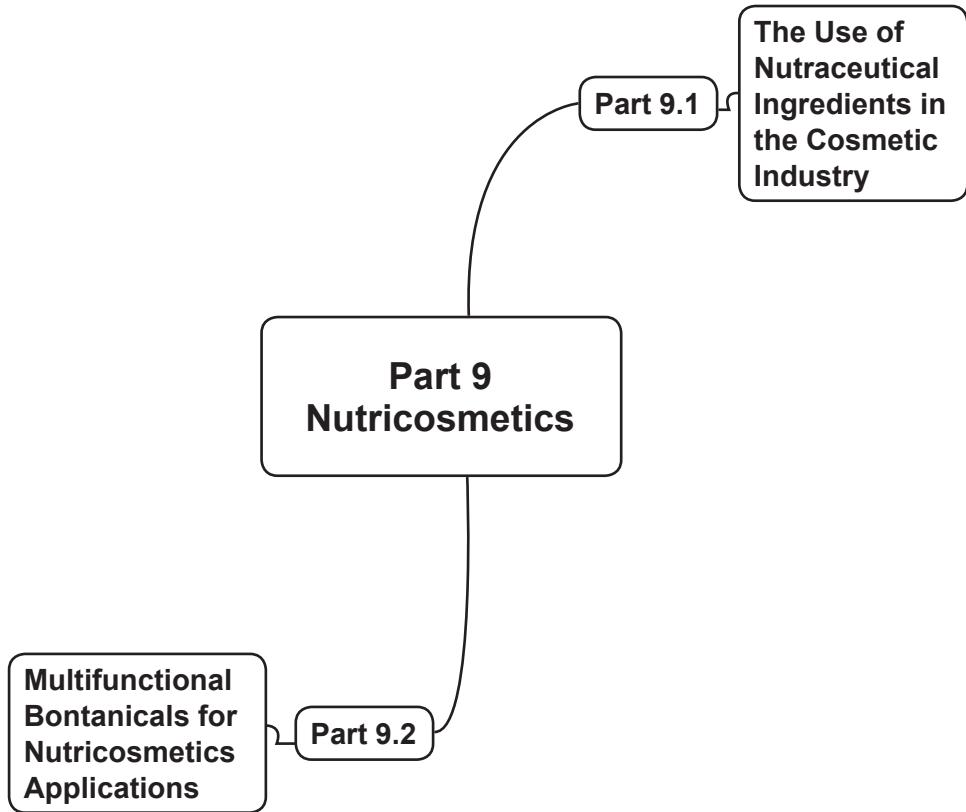
3. Co-polymeric systems for sustained release—e.g., HPMC and Eudragit for API formulations
4. Different trigger responses for cosmetics
5. Adapting pharma API tablet technologies to cosmetics and topical formulations: is it possible?
6. Surfactant-free systems; oil-water emulsion stabilization with natural/or-  
ganic 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? Encap-  
sulation is not applicable to all actives. What is the alternative: a) chemi-  
cal modification, or b) micelle-based encapsulation systems?
11. Leakage of hydrophilic actives into continuous phase during encapsula-  
tion-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.<sup>1</sup> Since introduction of the terms nutraceuticals and cosmeceuticals<sup>2</sup> 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.<sup>3</sup> 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;<sup>4</sup> 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 SUITABLE 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.<sup>5</sup> 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.<sup>6</sup> 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 ultra-violet 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  $\alpha$ -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.<sup>7</sup> Nutritional ingredients promoted as antioxidants include vitamins (i.e., VC, VE, VB,  $\beta$ -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.<sup>8</sup>

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<sup>9</sup> and vitamin E even at very high dosages.<sup>10</sup> 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.<sup>11</sup> 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.<sup>12</sup> 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.<sup>13</sup> Natural polyphenols have a great structural diversity with antioxidation capacities higher than vitamin C and E.<sup>14</sup> 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,<sup>15</sup> 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,<sup>16</sup> and reduction of hyperpigmentation<sup>17</sup> with procyanidins found in apples, pine bark, and grape seeds.

Carotenoids are the most extensively studied and utilized antioxidants in nutraceutical products. Carotenoids include different types of lipophilic natural compounds such as  $\alpha$ ,  $\beta$ ,  $\lambda$ , and  $\delta$ -carotenes; lycopene found in fruits such as tomato, watermelon, grapefruit, and red bell pepper;<sup>18</sup> 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.<sup>19</sup> Carotenoids in skin are strongly associated with the influence of stress factors such as fatigue, illness, smoking, alcohol consumption, and daily dietary intake.<sup>20</sup> 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 ( $H_2O_2$ ).<sup>21,22</sup>

Oral supplementation of carotenoid-enriched diets can quickly increase the measured carotenoid level in skin.<sup>23</sup> Oral supplementation of isotretinoin for photo-aging has also been documented.<sup>24</sup> 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,  $\alpha$  &  $\beta$ -carotene,  $\alpha$ -tocopherol, and organic selenium.<sup>25</sup> 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.<sup>26</sup>

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.<sup>27</sup> 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.<sup>28</sup> 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.<sup>29</sup>

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.<sup>30</sup> 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 administered 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.<sup>31</sup>

### **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.<sup>32</sup> 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.<sup>33</sup> 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.<sup>34</sup>

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.<sup>35</sup> The source of the dietary polyunsaturated fatty acids are plant-based 18-carbon fatty acids with three to nine double bonds such as  $\alpha$ -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- $\alpha$ , IL-1 $\beta$ , and IL-6.<sup>36</sup> 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.<sup>37</sup>

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.<sup>38</sup> 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.<sup>39</sup> 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.<sup>40</sup>

Polyphenols are a widely distributed group of natural products that have been reported to have anti-inflammatory, antiallergic, antimutagenic, antibacterial, anti-viral, 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.<sup>41</sup> 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- $\kappa$ B and mitogen-activated protein kinase (MAPK).<sup>42</sup> 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.<sup>43</sup> 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.<sup>44</sup> Polyphenols 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.<sup>45</sup>

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.<sup>46</sup> Curcumin is well reported with potent inhibition of NF- $\kappa$ B that regulates the expression of a large number of genes—such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, cyclooxygenase-2, chemokines, inducible nitric oxide synthase, and matrix metalloproteinases (MMP)—which are involved in UV-induced skin inflammation and skin aging.<sup>47</sup> Curcuminoids and other diarylheptanoids were also characterized as dual inhibitors of arachidonic acid metabolism by reducing PGE<sub>2</sub> production in the cyclooxygenase pathway<sup>48</sup> and down-regulating matrix metalloproteinases (MMPs).<sup>49</sup> 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.<sup>50</sup> Recent development of formulation and delivery platforms such as liposomal, phospholipid complex, encapsulation, nanoparticulation, and delivery with metabolism adjuvant significantly improved the pharmacokinetic profile of curcumin.<sup>51</sup>

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.<sup>52</sup>

### 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.<sup>53</sup> Further, there is no good correlation between sunburn protection factor (SPF) and an immune protection factor (IPF) from sunscreens.<sup>54</sup> 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.<sup>55</sup> Many natural products, such as polyphenols,<sup>56</sup> epigallocatechin gallate (EGCG) from green tea,<sup>57</sup> and plant-derived oligosaccharides,<sup>58</sup> 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 antioxidant properties.<sup>59</sup> 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.<sup>60</sup> Oral supplement of immunomodulation composition containing β-glucan, melatonin, and vitamin E also provided reduction of wrinkle, age spots, and photoprotection benefits.<sup>61</sup>

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.<sup>62</sup> 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.<sup>63</sup> It has been shown to prevent UVB-induced contact hypersensitivity (CHS) suppression and, importantly, has demonstrated inhibition of UVB-induced TNF- $\alpha$  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<sup>64</sup> and UVB-induced suppression of accessory cell function of Langerhans cells.<sup>65</sup> The immunomodulatory activity of aloe polysaccharide is also reported to have induced differentiation of immature dendritic cells.<sup>66</sup> 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.<sup>67</sup>

#### 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 mono-unsaturated 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.<sup>68</sup>

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,<sup>69</sup> genital herpes, dermatitis, psoriasis,<sup>70</sup> or internal application for irritable bowel syndrome, immune deficiency, hypercholesterolemia, and type 2 diabetes.<sup>71</sup> 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.<sup>72</sup> 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).<sup>73</sup> Such hormone reduction also leads to altered extracellular matrix and elasticity, decreased skin moisture and skin thickness, and increased skin wrinkles.<sup>74</sup> Oral supplement natural phytoestrogenic isoflavanoid for human health is well established with much less understanding of potential use in skin aging.<sup>75</sup> 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.<sup>76</sup> Oral supplement soy isoflavone aglycone in food for 12 weeks significantly improved fine wrinkles and malar skin elasticity compared with control group.<sup>77</sup>

Hydrolyzed collagen from bovine type I collagen or chicken sternal type II collagen, collagen-derived peptides,<sup>78</sup> 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.<sup>79</sup> 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.<sup>80</sup>

### 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.<sup>81</sup> In recent decades, more and more clinical evidences have been published to support topical efficacy from natural ingredients,<sup>82</sup> such as polyphenols from green tea for skin immune restoration, resveratrol from grape seeds for skin protection, Co-Q10 for wrinkle reduction, etc.<sup>83</sup> 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. Imeedeen 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.<sup>84</sup>

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.<sup>85</sup> 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 glycosaminoglycans, 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.<sup>86</sup> 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.<sup>87</sup> 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.<sup>88</sup>

### **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.<sup>89</sup>

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.<sup>90, 91</sup> Natural nutrients such as polyphenols generally have poor absorption, rapid metabolism, and rapid systemic elimination after oral supplement.<sup>92</sup> Resveratrol offers a perfect example<sup>93</sup> 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.<sup>94</sup> 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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Sabinsa**

### **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).

Anticarcinogenic 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 *Emblica 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.

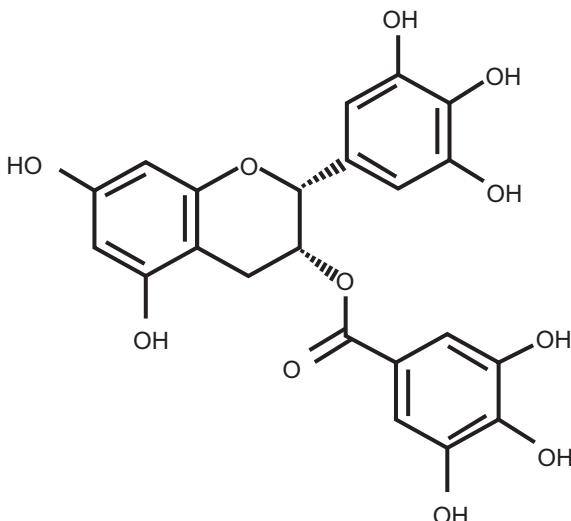
**Table 1:** ORAC activity assay on Saberry®

ORAC Hydro ( $\mu\text{mol TE/g}$ )	ORAC Lipo ( $\mu\text{mol TE/g}$ )	ORAC Total ( $\mu\text{mol TE/g}$ )	HORAC ( $\mu\text{mol CAE/g}$ )	NORAC ( $\mu\text{mol TE/g}$ )	SORAC (Kunits SODeq/g)	SOAC ( $\mu\text{mol VItE/g}$ )
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.

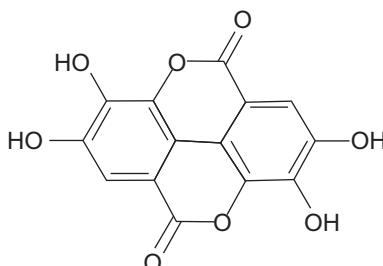
The study results show that oral consumption of green tea catechins may contribute to protection against sunburn, erythema, and perhaps even long-term UV radiation-induced damage to skin (L.E. Rhodes et al. 2013).

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 tumorigenesis (P. Lesca 1983; R. Chang et al. 1985), H. Mukhtar et al. (1986) designed a study to investigate whether the parenteral 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.

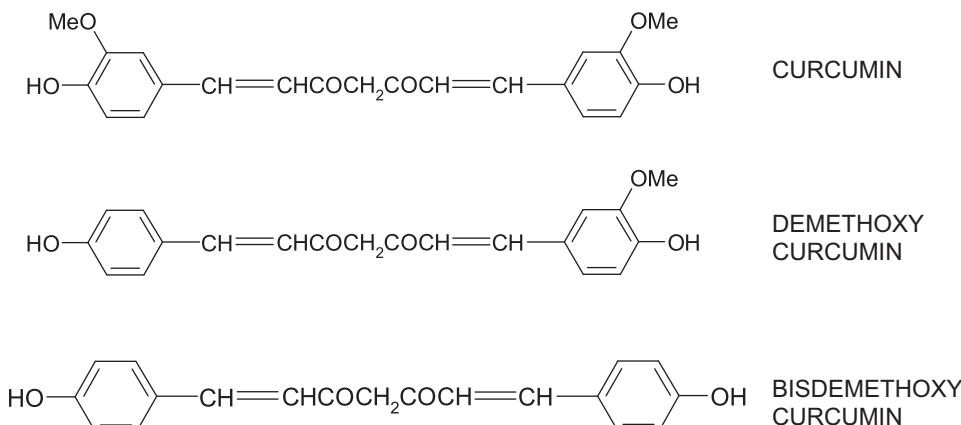
The whitening or protective effect of ellagic acid against UV-induced pigmentation was studied using a spectro-colorimeter for luminance in skin and a mexameter for measuring melanin and erythema in the skin.

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).

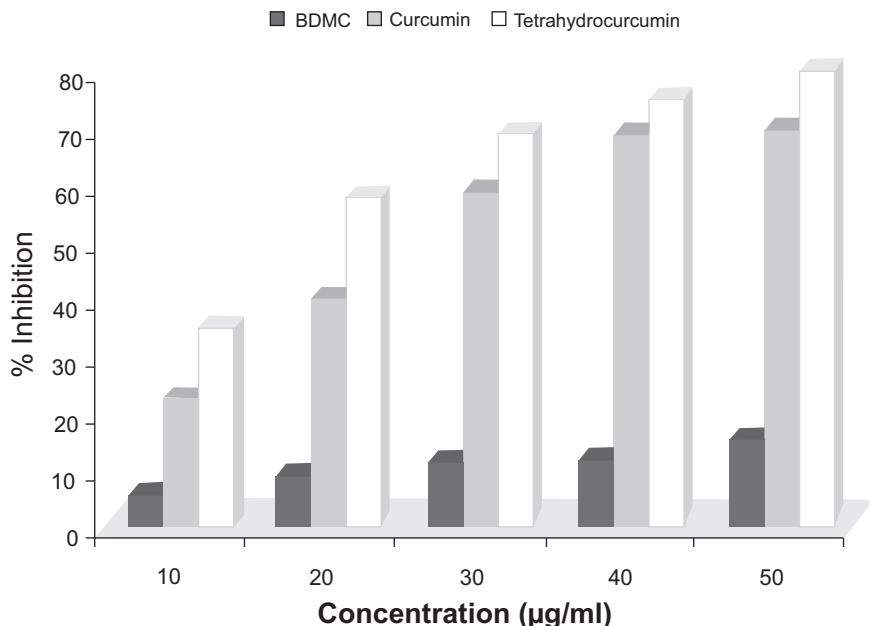
#### d. Curcumin C3 Reduct® (Turmeric)

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  $\alpha, \beta$  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<sub>3</sub> 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<sub>3</sub> Complex®, and tetrahydrocurcuminoids are available as Curcumin C<sub>3</sub> 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.

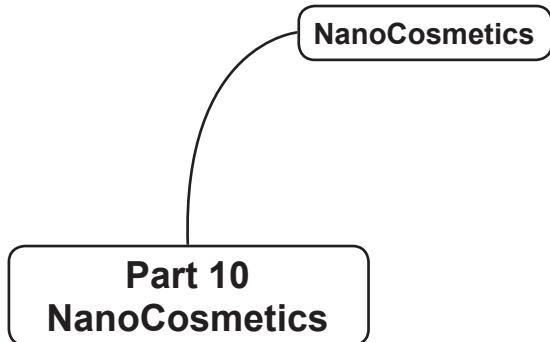
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## NANOCOSMETICS



## 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

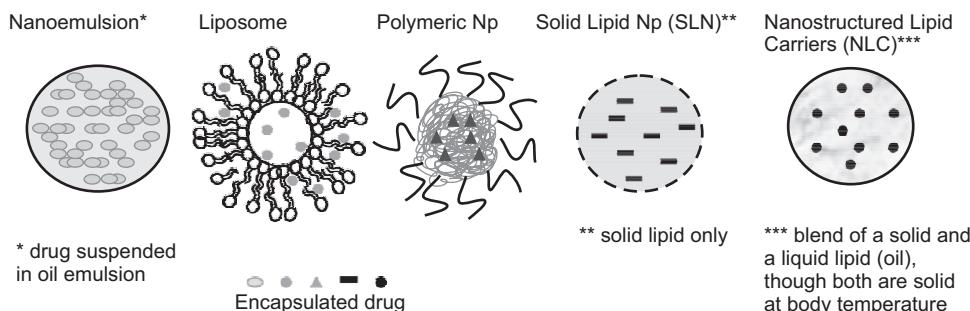
### 10.1.1 INTRODUCTION

In its broadest sense, Nanotechnology is the purposeful design and manipulation of nanoscale materials for applications in a variety of disciplines.<sup>1</sup> 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.<sup>2-4</sup> 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<sup>2</sup>.

Nanoparticles are increasingly used in sunscreens, cosmetics, electronics, sporting goods, tires, paints, and foods.<sup>5</sup> In the medical field they are being utilized for diagnostic modalities, imaging studies, and drug delivery.<sup>3,5</sup> 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.<sup>2,6</sup> 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.*<sup>108</sup>

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.<sup>7</sup> 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.<sup>7</sup>

### 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.<sup>3</sup> 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.<sup>3</sup> The presence of ultra-small droplets confer special properties to nanoemulsions, including transparency and a pleasant texture and sensory experience when applied topically.<sup>5</sup> 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.<sup>8</sup> A wide variety of cosmetic products have been synthesized utilizing nanoemulsions: lotions, milks, and clear gels, all with slightly varying, and controllable, physical behavior.<sup>8</sup>

### 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.<sup>3</sup> While liposomes are primarily composed of phospholipids, niosomes are mostly generated from nonionic surfactants, such as alkyl ethers or esters.<sup>9</sup> 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.<sup>10,11</sup> When applied topically, liposomes and niosomes tend to break into their individual components, similar to nanoemulsions.<sup>3,5</sup> 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.<sup>12,13</sup>

### 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.<sup>3</sup> 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*.<sup>14</sup> 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.<sup>15</sup> Nanoencapsulation can also increase the bioavailability<sup>16</sup> and the stability of the topically

applied compounds.<sup>17,18</sup> Moreover, nanoencapsulation can protect the substances from degradation in storage, as well as photodegradation.<sup>14</sup>

### Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLN) were developed in the 1990s as the first generation of lipid nanoparticles<sup>19</sup> and served as an alternative carrier system to other nanomaterials such as emulsions, liposomes, and polymeric nanoparticles already being utilized.<sup>12</sup> 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.<sup>12</sup> 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.<sup>20</sup> 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.<sup>20</sup> Formulation parameters and compound concentrations can be varied to yield different release profiles from SLN.<sup>20</sup> Cosmetic ingredients and active pharmaceutical ingredients (APIs) can subsequently be incorporated into these carriers, providing the potential for a wide variety of topical applications.<sup>19</sup>

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.<sup>21</sup> 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).<sup>21</sup> 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.<sup>12,22</sup> 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.<sup>12</sup> 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.<sup>21</sup> 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.<sup>22</sup> 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.<sup>13</sup> 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.<sup>21</sup> 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.<sup>23</sup> 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.<sup>21</sup> Adding a higher fraction of liquid lipid will modify the solid matrix to allow for greater solubility of active ingredient in the lipid regions.<sup>24</sup> These liquid regions, known as oily nanocompartments, prevent expulsion of the active ingredient, while maintaining the solid matrix structure of the NLC.<sup>24</sup>

Both SLN and NLC are appropriate for use on inflamed or damaged skin because they are composed of non-irritant and nontoxic lipids.<sup>25,26</sup> SLN and NLC have been investigated using a variety of encapsulated compounds, including Vitamin E,<sup>20</sup> tocopherol acetate,<sup>27</sup> retinol,<sup>15</sup> and ascorbyl palmitate.<sup>28</sup> 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,<sup>29</sup> 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.<sup>19</sup> 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.<sup>6,30</sup> 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.<sup>6</sup> 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.<sup>30</sup> Despite their benefits, molecular sunscreens can penetrate the skin and can cause photo-allergies, skin irritation, and phototoxic reactions.<sup>31</sup>

Titanium dioxide ( $\text{TiO}_2$ ) and zinc oxide ( $\text{ZnO}$ ) are two of the most common inorganic UV filters utilized in sunscreens.<sup>2,32</sup> These inorganic sunscreens are effective filters of UVA and UVB light<sup>2</sup> and have long been considered safe and effective methods of photoprotection because of their photostability and low photo-allergic potential.<sup>32,33</sup> Yet historically, their use was limited because of poor aesthetics: the large size of the  $\text{TiO}_2$  and  $\text{ZnO}$  particles caused a white film to remain on the skin after application.<sup>2,6</sup> Additionally, these inorganic particles did not disperse well in their carriers and left a grainy feeling on the skin when applied.<sup>6</sup> 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.<sup>31</sup> 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.<sup>31</sup> 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.<sup>31</sup> 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.<sup>31</sup>

$\text{TiO}_2$  and  $\text{ZnO}$  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<sub>2</sub> and ZnO enabled sunscreens to be transparent upon skin application, resulting in increased consumer acceptance and use.<sup>5</sup> Nano-sized TiO<sub>2</sub> and ZnO were first patented in the 1980s; the United States Food and Drug Administration (FDA) approved nanoparticle use in sunscreens in 1999.<sup>2</sup> Today, one of the largest applications of nanoparticles is in sunscreen products.<sup>3,5</sup> The proposed benefits include not only improved aesthetics, but also superior UV protection<sup>6,30</sup> when compared to micron-sized particles.<sup>34</sup> Their enhanced UV blockage properties are thought to be due to their ability to scatter light efficiently.<sup>30</sup>

TiO<sub>2</sub> 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<sub>2</sub> 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.

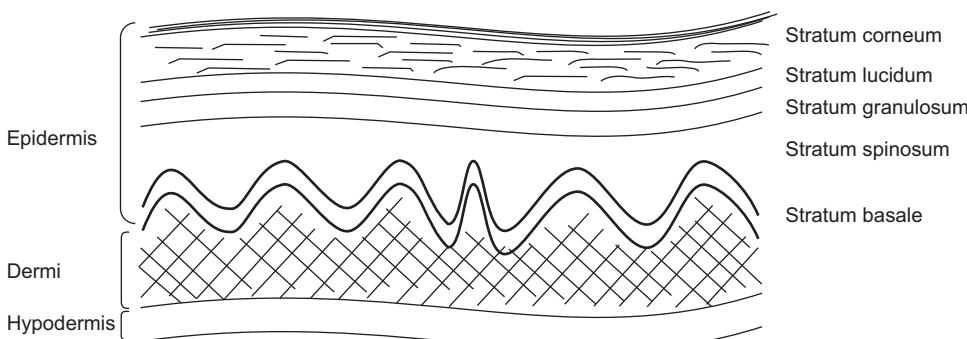
The degree of UV absorption by inorganic nanoparticles such as TiO<sub>2</sub> and ZnO depends on the size of the aggregates<sup>3,6</sup>; SPF will vary significantly if different sunscreen formulations are made with varying size of inorganic nanoparticles, even if the concentration of those particles is the same.<sup>6</sup> Large agglomerates of TiO<sub>2</sub> 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.<sup>3,5,6</sup> 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.<sup>6</sup> Regulations around the world vary as to the acceptability of organic versus inorganic UV filters.

Despite the cosmetic benefits of nano-sized TiO<sub>2</sub> and ZnO in sunscreens, which include dispersal in a less viscous vehicle, increased transparency, and skin blending,<sup>2</sup> safety concerns have emerged. While particle size is fundamental to their utilization, size is also the cause for concern regarding their potential toxicity,<sup>4</sup> including the potential for reactive oxygen species (ROS) generation upon UV exposure,<sup>2,6</sup> percutaneous penetration of the particles,<sup>6</sup> their ability to evade the host's immune defense mechanisms and complex with host proteins, and the potential respiratory tract consequences resulting from inhalation.<sup>2</sup>

Overall, both  $\text{TiO}_2$  and  $\text{ZnO}$  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  $\text{TiO}_2$  and  $\text{ZnO}$  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.<sup>2</sup> Several *in vitro* studies have suggested that  $\text{TiO}_2$  and  $\text{ZnO}$  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.<sup>35</sup>

Another study failed to describe its methodology and therefore the results cannot be appropriately analyzed or reproduced.<sup>36</sup> A commonly cited study by nanotechnology proponents refutes the potential dangers of nano-sized  $\text{TiO}_2$  and  $\text{ZnO}$ . Investigators applied nano-sized  $\text{ZnO}$  in the dark, either under simultaneous irradiation with UV light, or to cells pre-irradiated with UV light. Pre-irradiated cells later treated with  $\text{ZnO}$  and cells given concurrent  $\text{ZnO}$  and irradiation both demonstrated the same type and incidence of chromosomal aberrations. Researchers therefore concluded that  $\text{ZnO}$  is not phototoxic, and that the DNA damage resulted from UV-mediated enhanced susceptibility to  $\text{ZnO}$ .<sup>37</sup> 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  $\text{TiO}_2$  and  $\text{ZnO}$  exposure to UV radiation should only be considered a valid concern if the nanoparticles are proven to penetrate the epidermis.<sup>2</sup> In healthy human skin, nanoparticulate  $\text{TiO}_2$  or  $\text{ZnO}$  do not penetrate further than the upper layers of the *stratum corneum*.<sup>2,38-44</sup> 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.<sup>5</sup> 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<sub>2</sub> nanoparticles have been detected within hair follicles after topical application of sunscreen; however, this does not represent nanoparticle penetration into living skin layers.<sup>39</sup> Furthermore, sebum flow from the pilosebaceous glands and follicular epithelium turnover effectively eliminate insoluble particles collected in follicular orifices.<sup>3</sup> Thus, despite the safety concerns regarding the toxic effects demonstrated by TiO<sub>2</sub> and ZnO nanoparticles on living cells, available evidence demonstrates that when applied to healthy, intact skin, TiO<sub>2</sub> 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.<sup>2</sup> However, more research is warranted to examine the effect of nanoparticle application to damaged skin, as no conclusive evidence exists in the literature.<sup>2,6</sup>

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.<sup>5,45</sup> 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.<sup>3</sup> Similarly, sunburned skin slightly enhances TiO<sub>2</sub> and ZnO nanoparticle penetration in sunscreen formulations in *in vitro* and *in vivo* models, but transdermal absorption was not detected.<sup>46</sup>

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.<sup>6</sup> Oxybenzone is an aromatic ketone that provides broad-spectrum UV coverage and was first approved by the FDA in the early 1980s.<sup>33</sup> Despite its effectiveness as a sunscreen agent, it is the most common cause of photoallergic contact dermatitis<sup>19</sup> and systemic absorption of oxybenzone following its topical application has been reported.<sup>47</sup> Sanad et al. developed NLCs of oxybenzone to enhance its photoprotective efficacy as well as safety.<sup>48</sup> 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.<sup>48</sup> Today, it is one of the most widely used organic UV filters used in sunscreens produced in the United States.<sup>33</sup>

#### 10.1.4 NANOTECHNOLOGY AND HAIR CARE

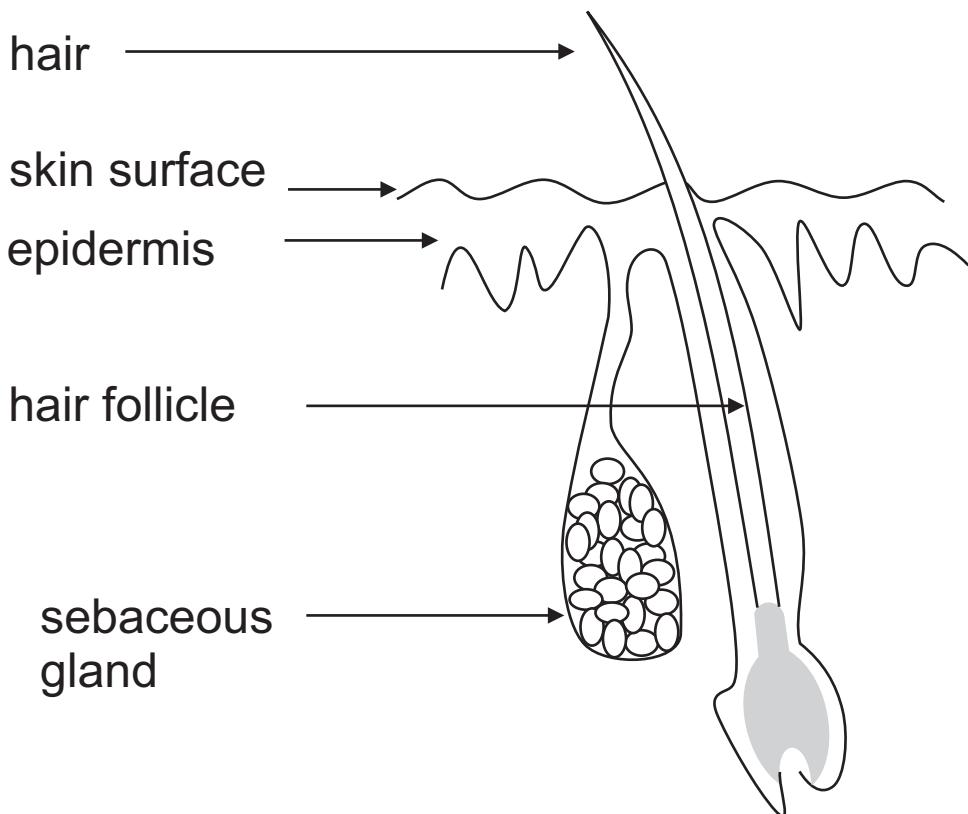
Hair covers much of the skin's surface area, with the exception of glabrous zones such as the palms, soles, and vermillion border of the lips. Appendageal hairs consist of pilosebaceous apparatus coupled to either terminal or vellus hairs. The abundance of human hair lends it to being a focus of many 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<sup>49-51</sup> and potential side effects from systemic absorption.<sup>51</sup> Hair dyes consist of a range of chemicals whose composition and duration of effect varies among dyes.<sup>49</sup> Contact sensitization to hair dyes is a well-known safety issue and is most common from professional exposure.<sup>51</sup> There has also been increasing awareness regarding *p*-phenylenediamine (PDA), a potent contact allergen found in permanent hair dyes.<sup>49,50</sup> Delayed-type (and rarely, immediate) hypersensitivity reactions have been reported following exposure to PDA and its related compounds.<sup>49,50</sup> When tested in murine models, PDA-containing hair dyes induced local inflammation, Th1 cell proliferation<sup>50</sup> and increased levels of inflammatory cytokines such as interferon-gamma and interleukin (IL)-17.<sup>52</sup> 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.<sup>49</sup>

Hair-coloring products generate \$12 billion per year with an estimated 50% of adults using hair dyes at some point in their lifetime.<sup>49</sup> 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.<sup>53</sup> 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.<sup>54</sup> 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.<sup>54,55</sup> 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.<sup>54</sup> 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<sup>52,55,56</sup> in a time-dependent and size-dependent manner,<sup>55,56</sup> with smaller nanoparticles accumulating in higher concentrations within follicular openings.<sup>56</sup> 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,<sup>57</sup> and hinokitiol-containing nanocapsules, when included in shampoo and hair tonic, stimulated hair growth.<sup>58</sup> Additionally, fullerene nanomaterials have been shown to potentiate hair growth in mice and human skin sections.<sup>59</sup> 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.<sup>50</sup> 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.<sup>50</sup> This nanoemulsion demonstrated thermodynamic and physical stability during storage, suggesting it is suitable for use as a conditioner on human hair.<sup>50</sup> 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.<sup>50</sup> 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.<sup>49</sup>

### 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.<sup>2,60</sup> 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.<sup>61</sup>

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.<sup>5,61</sup> 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.<sup>3</sup>

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.<sup>61</sup> 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.<sup>22</sup>

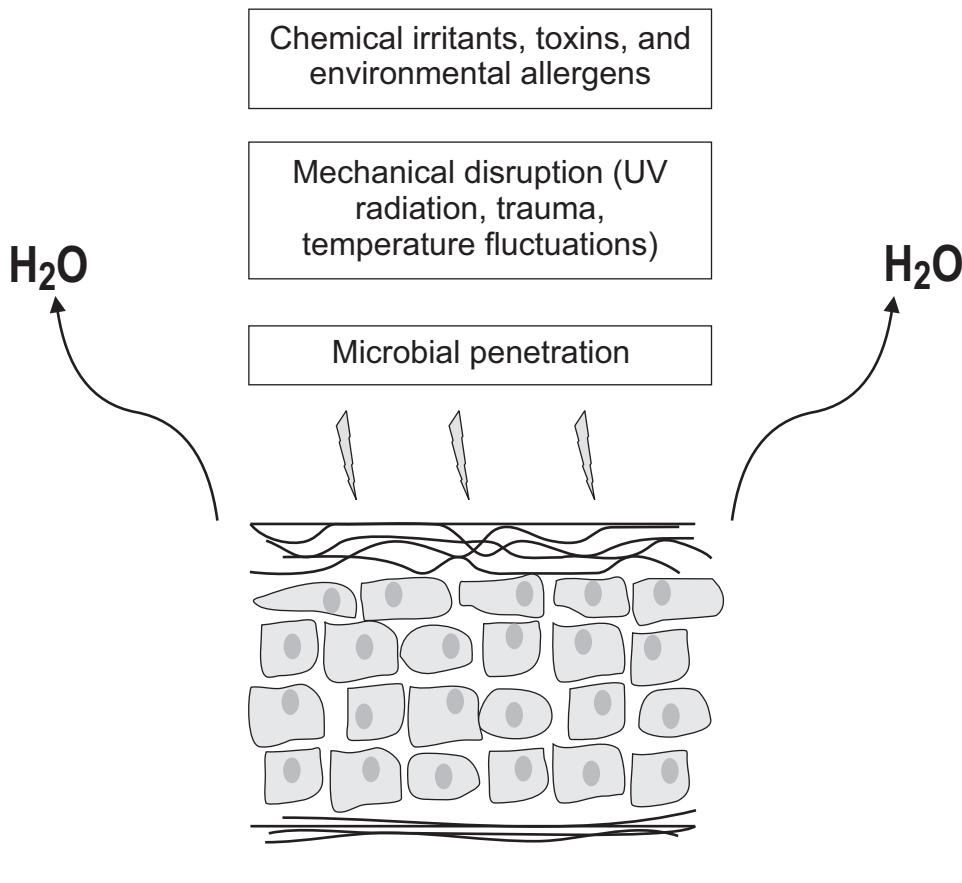
The efficiency of a cosmetic product not only depends on its active ingredient, but also on the vehicle that carries the active ingredient.<sup>30</sup> These carriers are also important determinants of the penetrating capability of the applied product. Due to increased skin penetration and efficacy, SLN and NLC are used as carriers for various cosmetic and dermatologic purposes.<sup>31,61</sup> Lipid nanoparticle carriers provide improved stability of active ingredients, controlled release of the active ingredient, and lack irritant properties and toxicity.<sup>30,61</sup> 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.<sup>22</sup> They also possess occlusive properties that help to form a film on the skin, and have the potential to block UV radiation.<sup>30</sup>

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.<sup>22</sup> 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.<sup>22</sup> Another study demonstrated that when incorporated into

NLC, Kenzo perfume demonstrated a longer release profile compared to emulsion and conventional shampoo.<sup>22</sup>

### 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<sup>5</sup> 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.<sup>5</sup> 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.<sup>22</sup> 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.<sup>13</sup> 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.<sup>22</sup> While topical therapies with occlusive properties exist, many have an undesirable appearance when applied.<sup>22,62</sup> The SLN-containing creams not only provide enhanced hydration, but also demonstrate more acceptable aesthetics.<sup>22</sup> In 2005, Cutanova Cream Nano Repair Q10 cream was the first lipid nanoparticle-based cosmetic product introduced to the cosmetics market.<sup>22</sup> Investigations have found that this Q10-containing NLC cream had superior skin hydration properties compared to a conventional oil/water cream.<sup>22</sup> This NLC dispersion provided a fivefold higher occlusive factor compared to an oil/water emulsion.<sup>22</sup> Furthermore, it achieved a similar degree of occlusion as highly occlusive liquid paraffin while avoiding a glossy appearance of the skin.<sup>63</sup> 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.<sup>63</sup>



Multiple external insults lead to disruption of the skin barrier and subsequent transepidermal water loss.

**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.<sup>63</sup> 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,<sup>63</sup> nanoemulsions containing ceramides can be utilized to restore the lipid barrier.<sup>64</sup> In part due their inherent function as part of the water-impermeable skin barrier, ceramides are exceptionally insoluble compounds.<sup>65,66</sup> 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

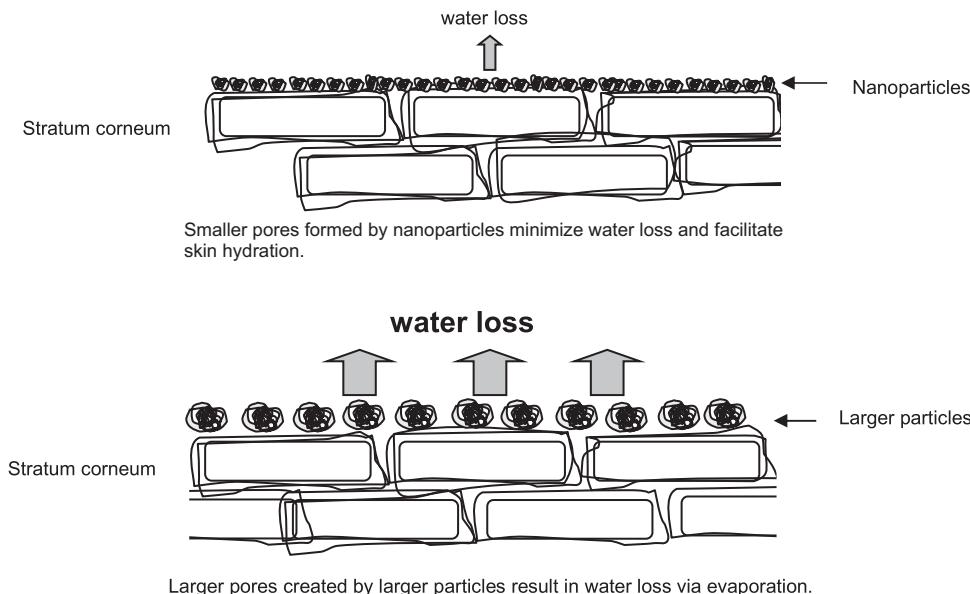
lipophilic core, nanoemulsions have the potential to carry water-immiscible active ingredients through aqueous environments and function in drug delivery.<sup>16,67</sup>

Several studies have investigated supplementing native epidermal lipids for the purposes of treating dry and aging skin.<sup>68,69</sup> 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.<sup>69</sup> 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.<sup>69</sup>

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 analogous 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.<sup>70-72</sup> 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.<sup>68</sup> Two liposome formulations, synthetic *stratum corneum* lipid mixtures (SSCL) and IWL, when topically applied to the skin limited TEWL and improved skin capacitance.<sup>68</sup> 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*.<sup>7</sup> When applied to the skin, nanoparticles form a monolayered lipid film and thus achieve better occlusion and hydration.<sup>7</sup> Figure 5.



**Figure 5:** Emollient properties of nanoparticles. Adapted from Müller *et al.*<sup>12</sup>

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.<sup>73</sup> 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.<sup>7,27</sup> 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.<sup>27</sup>

### 10.1.7 NANOTECHNOLOGY AND ANTI-AGING

Aging skin exhibits wrinkles, increased dryness, and pigmentation changes.<sup>74</sup> 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.<sup>75</sup> 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.<sup>74</sup> 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.<sup>75</sup>

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.<sup>74</sup> 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-decaprenyl-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;<sup>76</sup> it also functions as a powerful antioxidant that protects cells from ROS.<sup>77</sup> 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.<sup>78,79</sup> Because of these characteristics, coenzyme Q10 has been an appealing focus for cosmetic development.<sup>80</sup> Coenzyme Q10, however, is susceptible to photodegradation, and this chemical instability limits its utility in topical cosmetic formulations.<sup>76</sup> 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.<sup>76</sup> However, nanomaterial technologies pose a solution to these therapeutic barriers. For example, nanoemulsions containing coenzyme Q10 demonstrated improved incorporation into suspension;<sup>81</sup> while a topically applied SLN had a more consistent biphasic release profile.<sup>82</sup> A surfactant-free nanoparticle showed steady delivery of coenzyme Q10 for a two-month period.<sup>83</sup>

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.<sup>84</sup> 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.<sup>14</sup> Coenzyme Q10 was demonstrated to hamper cytokine production and MMP generation in *in vitro* studies with cultured human dermal fibroblasts.<sup>74</sup> 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.<sup>74</sup> 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<sup>85</sup> and coenzyme Q10.<sup>82</sup> Both of these active ingredients are often used in anti-aging products because of their ability to reduce photo-oxidation and wrinkle depth.<sup>7</sup> 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.<sup>82,85</sup> Nanoparticle encapsulation confers improved chemical stability and allows for the incorporation of these lipid-based ingredients into a variety of anti-aging cosmetic products.<sup>7</sup>

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,<sup>86</sup> as well as good chemical stability.<sup>87</sup> Omega-3 unsaturated fatty acids have been shown to inhibit the expression of MMP-type 1 after UV exposure.<sup>86</sup> Since matrix metalloproteases type 1 participate in the degradation of collagen and elastin, vital structural skin proteins,<sup>88</sup> the activity of the unsaturated fatty acids against this enzymatic degradation was shown to maintain skin vitality by protecting collagen.<sup>86</sup> 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  $\alpha$ -tocopherol.<sup>89</sup> Vitamin E predominantly acts to protect lipid structural components against oxidation in the skin.<sup>89</sup> The critical role of  $\alpha$ -tocopherol in protecting the *stratum corneum* makes it an ideal therapeutic agent for protecting the skin against this oxidative damage and subsequent aging.<sup>90</sup> For example, Dingler et al. studied an anti-aging formulation consisting of vitamin E-loaded solid lipid nanoparticles.<sup>20,91</sup> 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.<sup>92,93</sup> 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.<sup>92,94</sup> Various studies have investigated natural and synthetic retinoids, demonstrating histologic and clinical success in skin treated with topical tretinoin since the 1980s.<sup>95-97</sup> Since that time many cosmeceuticals have been developed utilizing tretinoin for anti-aging treatments.<sup>98</sup> 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.<sup>93,99,100</sup> This skin irritation is particularly prominent with the use of tretinoin products, as compared to other retinoids.<sup>93</sup> In addition, tretinoin demonstrates low water solubility and great instability on exposure to light, air, and heat, possibly limiting its efficacy in topical application.<sup>98</sup> 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.<sup>98</sup> 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.<sup>101-103</sup> 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.<sup>104</sup> It is known to be a powerful scavenger of free radicals<sup>105</sup> 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.<sup>104</sup> 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.<sup>106</sup> By all four metrics of assessment, there was a statistically significant improvement in the cream-treated half of the face.<sup>106</sup> 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.<sup>104</sup>

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,<sup>107</sup> 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<sup>4</sup> 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,  $\alpha$ -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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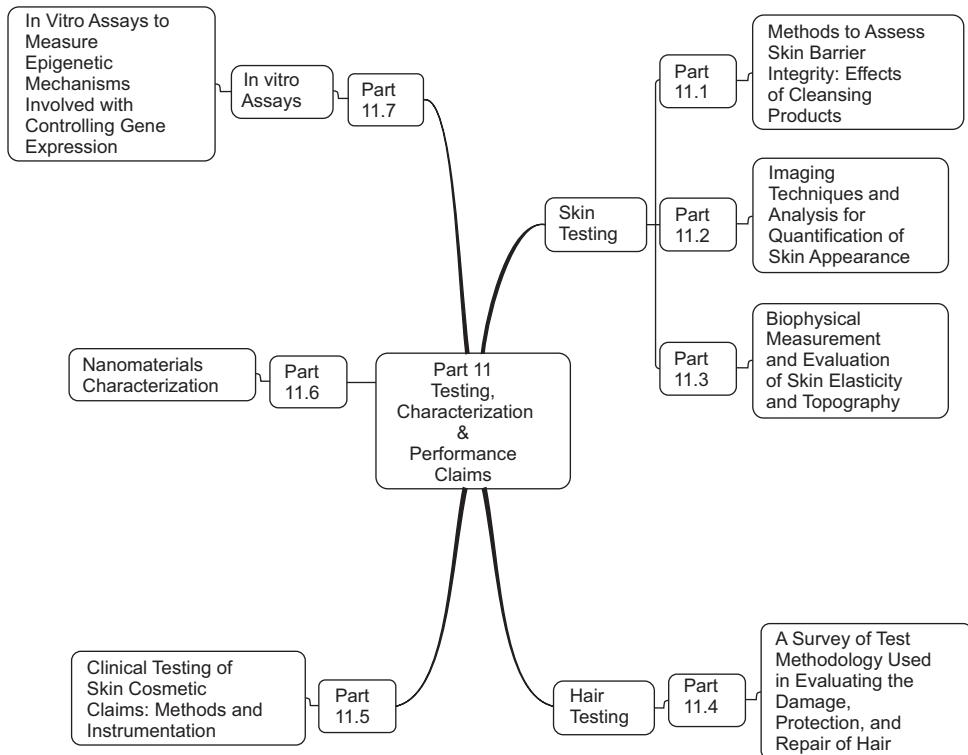
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## TESTING, CHARACTERIZATION AND PERFORMANCE CLAIMS



## **METHODS TO ASSESS SKIN BARRIER INTEGRITY: EFFECTS OF CLEANSING PRODUCTS**

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### **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<sup>1-3</sup>. 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<sup>4</sup>. A widely employed analogy for the SC organization is a brick-mortar (corneocyte-lipid) model<sup>5</sup>. 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<sup>6-8</sup>. 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<sup>9–10</sup>. 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<sup>11–12</sup>. 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<sup>13</sup>. 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<sup>14</sup>. 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<sup>15-16</sup>.

### a. SC protein binding, denaturation, dissolution, and SC swelling

Surfactant interaction with skin proteins can adversely affect skin hydration and viscoelasticity. It is widely reported that surfactants can bind to skin keratin with high affinity<sup>17-22</sup>. 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<sup>17-18</sup>. 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<sup>11, 19-22</sup>.

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<sup>23</sup>. 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<sup>24</sup>. 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)<sup>25</sup>. 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<sup>24, 26-29</sup>. In one study, Loden concluded that lipid extraction/solubilization was caused by surfactant micelles and therefore is concentration dependent<sup>30</sup>. 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<sup>21, 31</sup>.

The solubilization and removal of SC lipid by a large amount of absorbed surfactant molecules has been reported in lipid films<sup>32</sup>. Lipid content in the skin SC is normally observed to decrease because of delipidization after cleansing with soap products<sup>33</sup>. 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<sup>27</sup>. 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)<sup>28-29</sup>.

### 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<sup>34</sup>. It is also reported that anionic surfactants induce intercellular lipid structure disruption, disordering, and, possibly lipid fluidization<sup>32, 35</sup>. 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<sup>9</sup>.

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<sup>24, 36</sup>. 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<sup>37</sup>. Lipid composition is of paramount importance for the SC organization and its barrier properties<sup>38-40</sup>. 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<sup>41</sup>. 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<sup>42</sup>. These phase changes in turn will affect the water-holding capability and barrier properties of the SC, causing dry skin and/or skin irritation<sup>37, 43-44</sup>. Evidence indicates that diminishing amount of ceramide and increasing levels of fatty acid (nearly doubled) are related to soap-induced winter xerosis<sup>42</sup>.

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 surfactant-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 integrity by surfactants. The effect of pH on SC barrier function and skin permeability to drug molecules has been the subject of many studies<sup>9, 45–51</sup>. The results of these studies demonstrate that the environmental 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 irritancy 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<sup>52</sup>.

Since the pH of a healthy SC is acidic (generally around pH 5), the attraction 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<sup>35</sup>. 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<sup>53</sup>.

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<sup>52</sup>. 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<sup>9, 45, 54</sup>.

### 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<sup>55–56</sup>, skin hydration by electrical methods<sup>57</sup>, TEWL in cosmetic sciences<sup>58</sup>, skin surface pH<sup>59</sup>, tensile functional properties<sup>60</sup>, skin color<sup>61</sup>, skin microcirculation<sup>62</sup>, and skin topography<sup>63</sup>.

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<sup>35, 64</sup>. 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<sup>65–66</sup>. 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<sup>67–68</sup>.

### ***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<sup>69</sup>. 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<sup>70</sup>. 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<sup>24, 36, 42, 71–74</sup>.

### ***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)<sup>75</sup>.

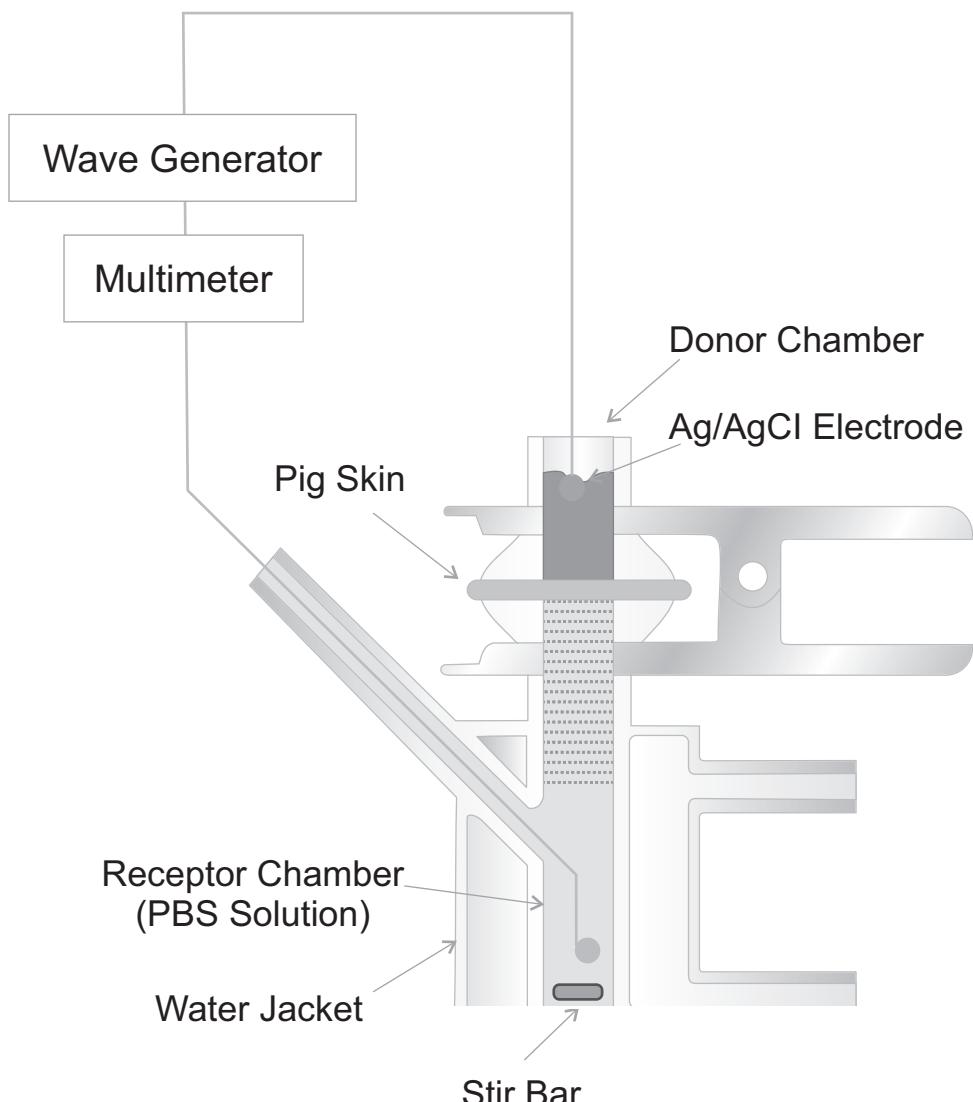
Skin's electrical impedance can be used to evaluate skin integrity and skin-barrier damage and recovery<sup>12</sup>. Skin-barrier perturbation due to exposure to different chemicals can be quantitatively expressed by measuring changes in skin impedance. When the skin barrier is perturbed, skin electrical 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<sup>12, 21, 76</sup>. Thus, for example, Ghosh and Blankschtein<sup>21</sup> 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.<sup>12</sup> 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<sup>77</sup>. 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.

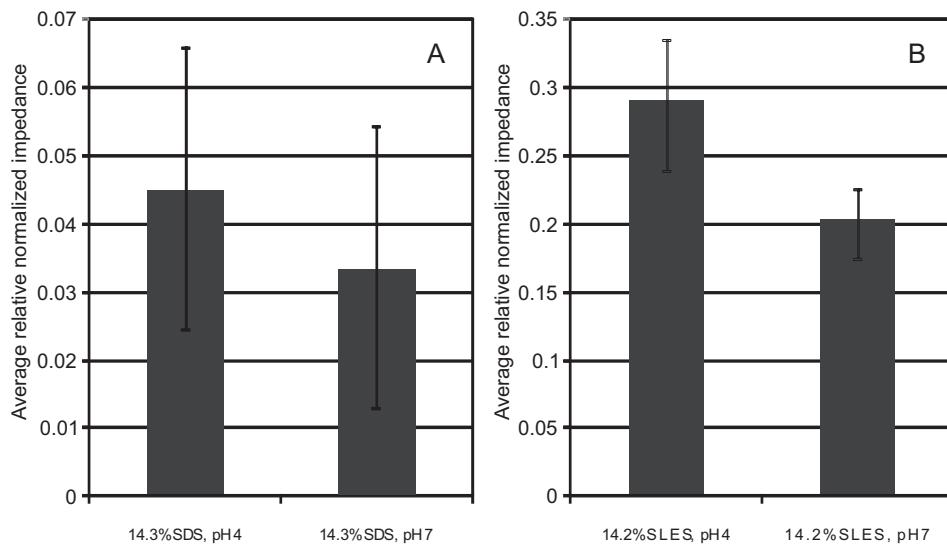
In a recent study, *in vitro* skin electrical current (impedance) measurements were conducted in a Franz diffusion cell (Figure 1) to quantitatively monitor changes in skin-barrier integrity as a function of surfactant chemistry and concentration<sup>54</sup>. In addition, time of exposure, temperature, 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<sup>78</sup>. 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*<sup>54</sup>. 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<sup>54</sup>. 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<sup>1</sup>. 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<sup>79–81</sup>. 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<sup>82–83</sup>. Suction systems, on the other hand, were designed to

measure the elasticity perpendicular to the skin surface<sup>84</sup>. 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<sup>85</sup>. A device based on shockwave propagation (Reviscometer from Courage & Khazaka) was developed to study skin viscoelasticity (specifically in the SC) by measuring the resonance running time of an acoustical shockwave<sup>86</sup>. This approach is very sensitive and able to differentiate between cosmetic treatments, and has been correlated to consumer perception<sup>87</sup>.

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<sup>88–89</sup>. 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<sup>90–91</sup>. 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<sup>92</sup>. 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<sup>93–95</sup>. 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<sup>96–97</sup>. 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<sup>66, 72, 98–100</sup>.

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<sup>37, 41, 66, 69, 98</sup>. 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<sup>29, 101–103</sup>.

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<sup>24</sup>. 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<sup>33, 104</sup>. 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<sup>105</sup>.

### **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<sup>106</sup>. 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<sup>107–111</sup>. 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.

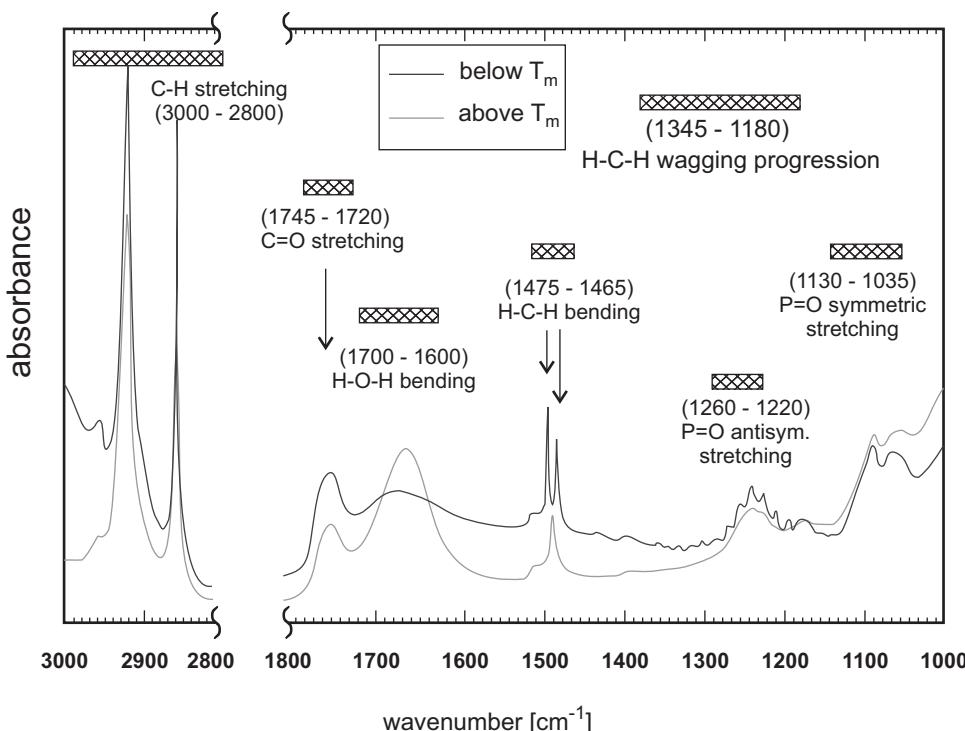
#### **1. Differential scanning calorimetry (DSC)**

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<sup>112–114</sup>.

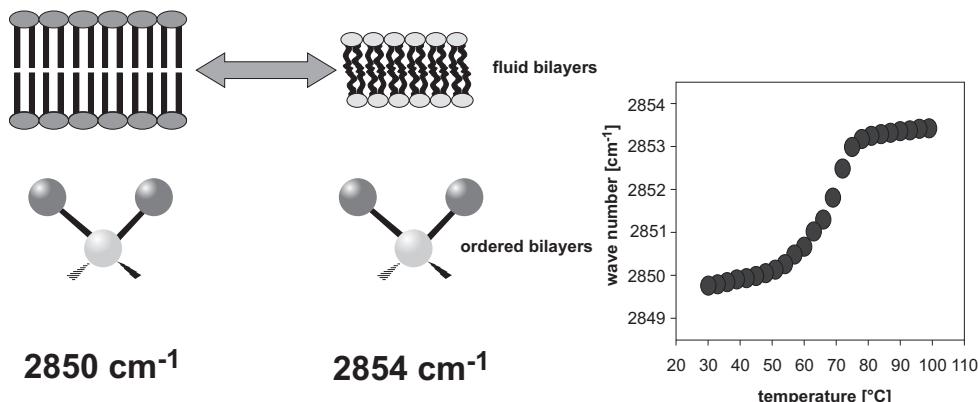
#### **2. Vibrational spectroscopy (FT-IR)**

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*<sup>115–117</sup>. 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  $\text{CH}_2$  stretching/rocking (vsCH) around wavenumber  $2850 \text{ cm}^{-1}$ , and/or  $\text{CD}_2$  symmetric stretching/rocking (vsCD) parameters for fatty acid around wavenumber  $2090 \text{ cm}^{-1}$ . For illustration, Figure 4 contains a plot of the position of the  $\text{CH}_2$  stretching band as a function of temperature, allowing us to elucidate the phase behavior of a selected lipid system. The vsCH 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<sup>43</sup>. The vsCD 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<sup>118</sup>.



**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<sub>2</sub> stretching mode of the lipid chains from the IR spectra of the SC<sup>9, 119</sup>.

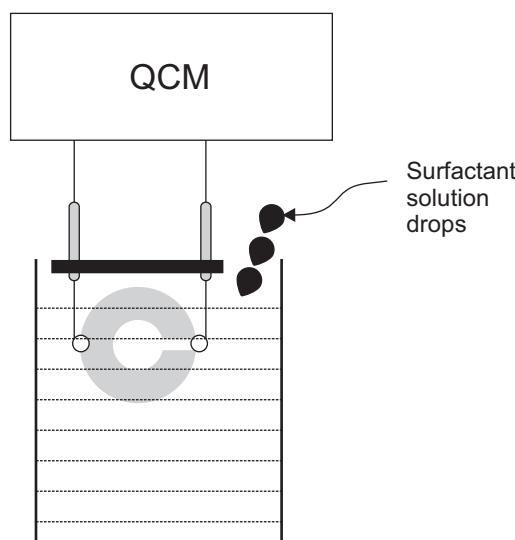
FTIR spectroscopy has been applied extensively to study the interactions between surfactants and *stratum corneum* lipids<sup>9, 24, 69, 120</sup>. *In vivo* and *in vitro* attenuated total reflectance Fourier-transform infrared (ATR-FTIR) and transmission-mode IR spectroscopy of skin, isolated SC, or 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<sup>9, 24, 120–122</sup>. 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<sup>41, 69</sup>. 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*<sup>109, 123</sup>.

### 3. Quartz crystal microbalance (QCM)

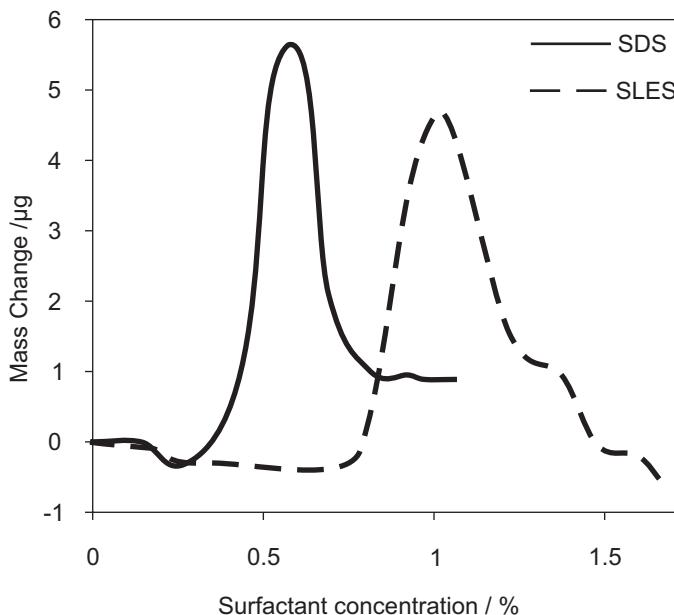
The quartz crystal microbalance (QCM) detects very small changes in mass via a piezoelectric mechanism and has been broadly utilized as a physical, chemical, electrochemical, and biochemical sensing tool in various applications<sup>124</sup>. As a simple mass measuring technique with nanogram resolution, 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<sup>19–20, 32</sup>.

Recently, water vapor uptake and surfactant sorption onto SC lipid model layers cast on a quartz crystal were studied using the QCM technique<sup>32, 125–126</sup>. In a very recent article, *in vitro* water vapor uptake and surfactant absorption onto skin lipid model films were quantitatively studied using the QCM<sup>111</sup>. 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<sup>111</sup>. 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<sup>127</sup>. 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

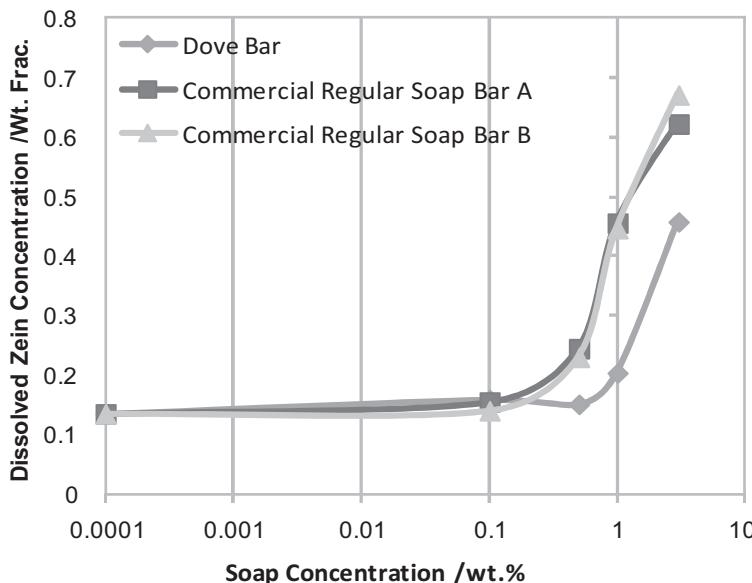
surfactant-induced BSA denaturation and skin roughness, after exposing to detergent solutions, was demonstrated<sup>128–129</sup>.

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<sup>78, 130–131</sup>. 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<sup>78, 132–133</sup>. 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<sup>134–135</sup>. 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<sup>130, 132, 136</sup>. *In vitro* assays based on these parameters (e.g., pH rise) have been developed to predict potential of surfactant-induced skin irritation<sup>137</sup>.

## 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<sup>138</sup>. 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<sup>137, 139–140</sup>.

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.<sup>78, 141</sup> One example is shown in Figure 7, where dissolved zein concentration is plotted as a function of soap concentration<sup>78</sup>. 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<sup>142</sup> and IR imaging with tape stripping<sup>143</sup>.

By IR spectroscopic imaging and confocal Raman microspectroscopy<sup>25, 144</sup>, 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<sup>82</sup>. 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.<sup>1</sup> 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 glyptic patterns. Glyptic patterns provide the skin with the necessary flexibility to accommodate various ranges of motion, especially in the joint regions. Glyptic patterns should be distinguished from wrinkles, which are genetically determined and develop during adulthood as a result of dermal matrix deterioration.<sup>2</sup> 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.<sup>3,4</sup> 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.<sup>5</sup> 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.<sup>6</sup>

**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.<sup>6</sup>

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 Fitzpatrick 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

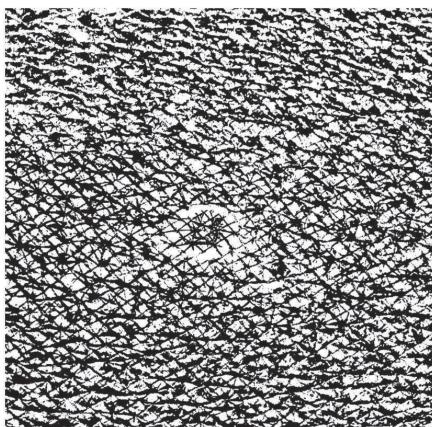
and directionality of the furrows in a given image. Usually, computer-generated algorithms or image-processing and analysis software are utilized to isolate the furrows from the original image. In Figure 2, several typical steps are shown using commercially available software (Image Pro Plus and Adobe Photoshop) in order to isolate the furrows so that further measurements can be made.



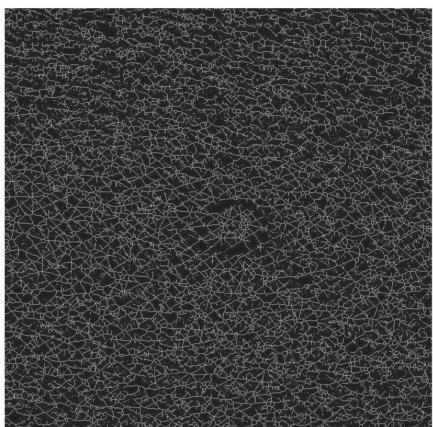
(a)



(b)



(c)



(d)

**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](http://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

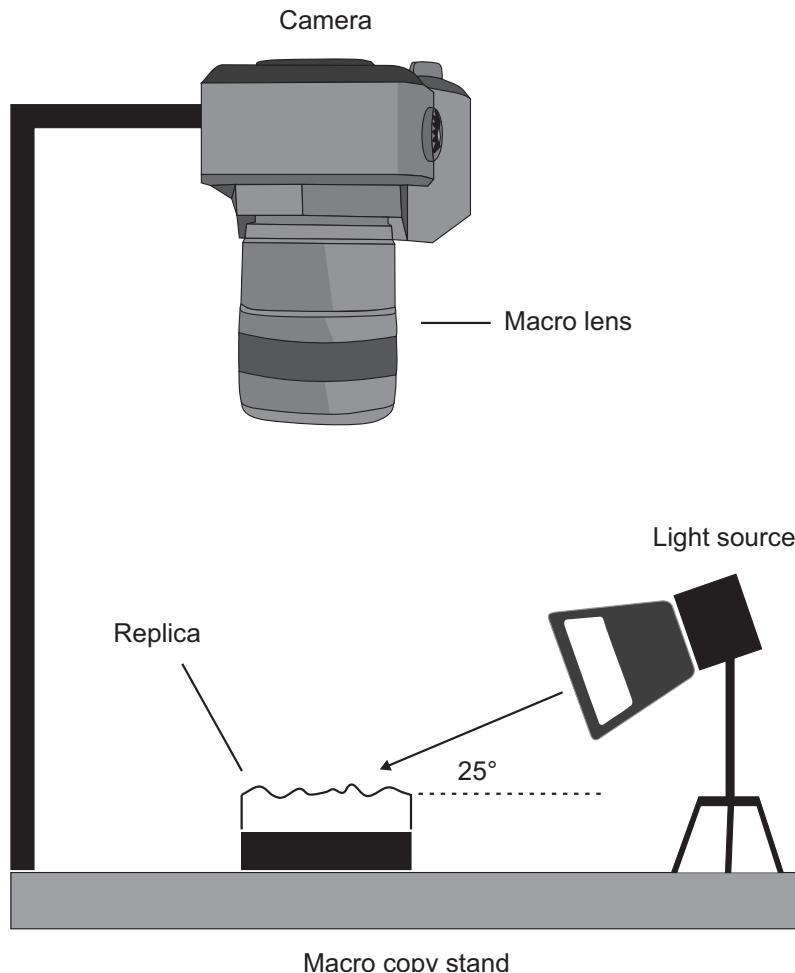
**Skin replica techniques** are among the oldest of the trade.<sup>7</sup> They are relatively quick procedures that are easy to carry out, and they provide very useful information about the surface structure of the skin. To a large extent, innovative instruments capable of noninvasive (noncontact) three-dimensional 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.<sup>8,9</sup> Skin replicas can capture topographic features such as deep wrinkles, furrows, pores, and, even, droplets of sweat beads.<sup>10,11</sup> 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](http://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.<sup>12,13</sup> 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).<sup>14</sup> Depending on the desired level of magnification, scanning electron microscopy could also be used to capture surface details of replicas.<sup>15,16</sup> 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  $25^\circ$ . 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.

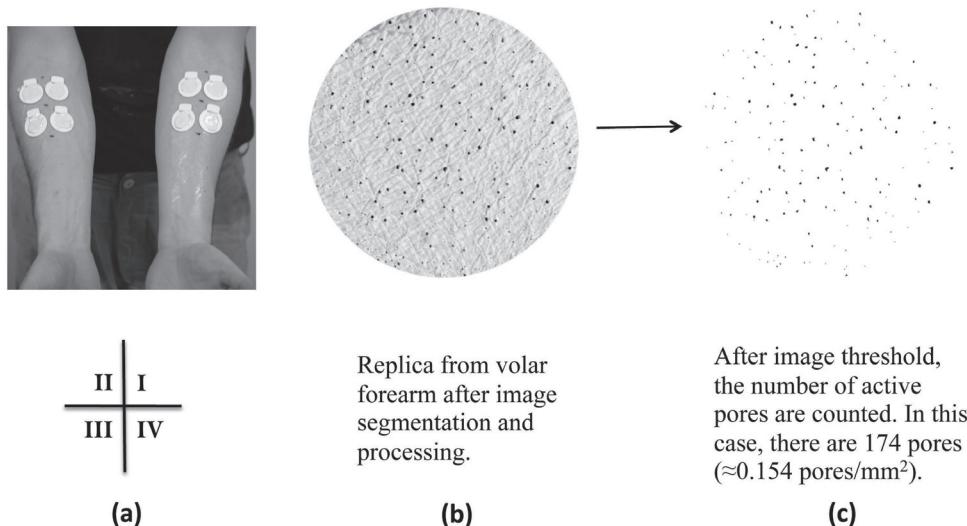


**Figure 4:** Illustration of a macro copy stand that includes a camera, macro lens, and illumination source to provide oblique lighting of a skin replica.

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.<sup>9,10</sup> A typical replica analysis would be carried out on the periorbital 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.<sup>9</sup>

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 ( $45^{\circ}\text{C}$ ) 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.

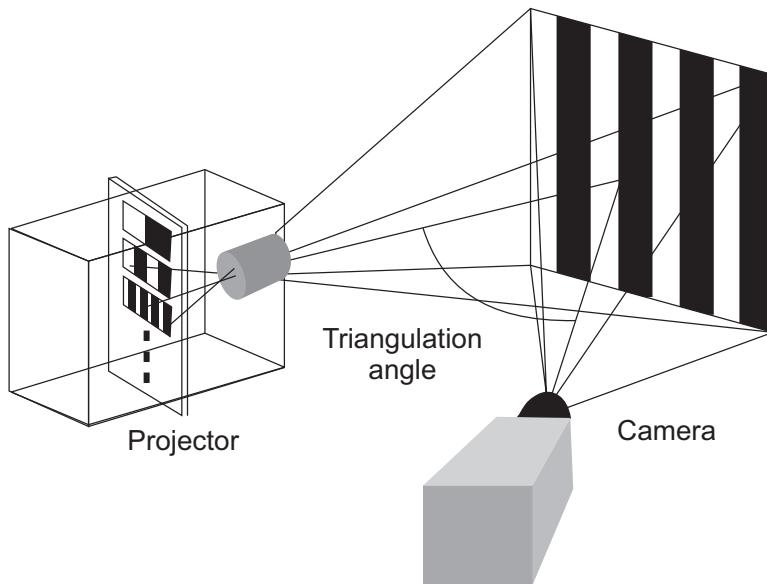


**Figure 5:** (a) Photograph of the volar forearms of a subject containing replica rings arranged in a four-quadrant layout. (b) Digital image of a cast from the volar forearm region along with (c) its image-processed counterpart containing isolated sweat profusions on a white background (counted). Reprinted from McMullen et al. with permission from the Society of Cosmetic Chemists, © 2013.<sup>17</sup>

### 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.

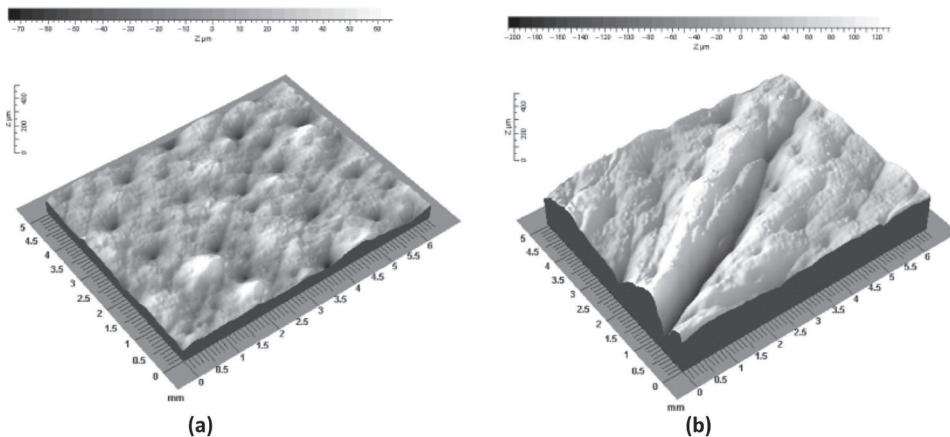
Meanwhile, an image of the fringe pattern on the three-dimensional object is obtained with a camera. Due to surface topography, the fringe pattern is deformed and can be analyzed by a computer algorithm allowing for the generation of a three-dimensional image. For evaluating the surface features of skin the two most popular commercially available instruments are the PRIMOS® (GFMesstechnik GmbH, Teltow, Germany) and DermaTOP® (EOTECH SA, Marcoussis, France) systems.<sup>19</sup> 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.<sup>18,20</sup> Optimally, the DermaTOP® FOV HE-25 system performs a measurement on a  $20 \times 15 \text{ mm}^2$  field of view with  $2 \mu\text{m}$  vertical resolution,  $15 \mu\text{m}$  lateral resolution, and 1 s acquisition time. On the other hand, the PRIMOS® premium system gathers data from a  $24 \times 14 \text{ mm}^2$  field of view with  $2.4 \mu\text{m}$  vertical resolution,  $24 \mu\text{m}$  lateral resolution, and 70 ms acquisition time.<sup>21</sup> 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.<sup>18</sup>

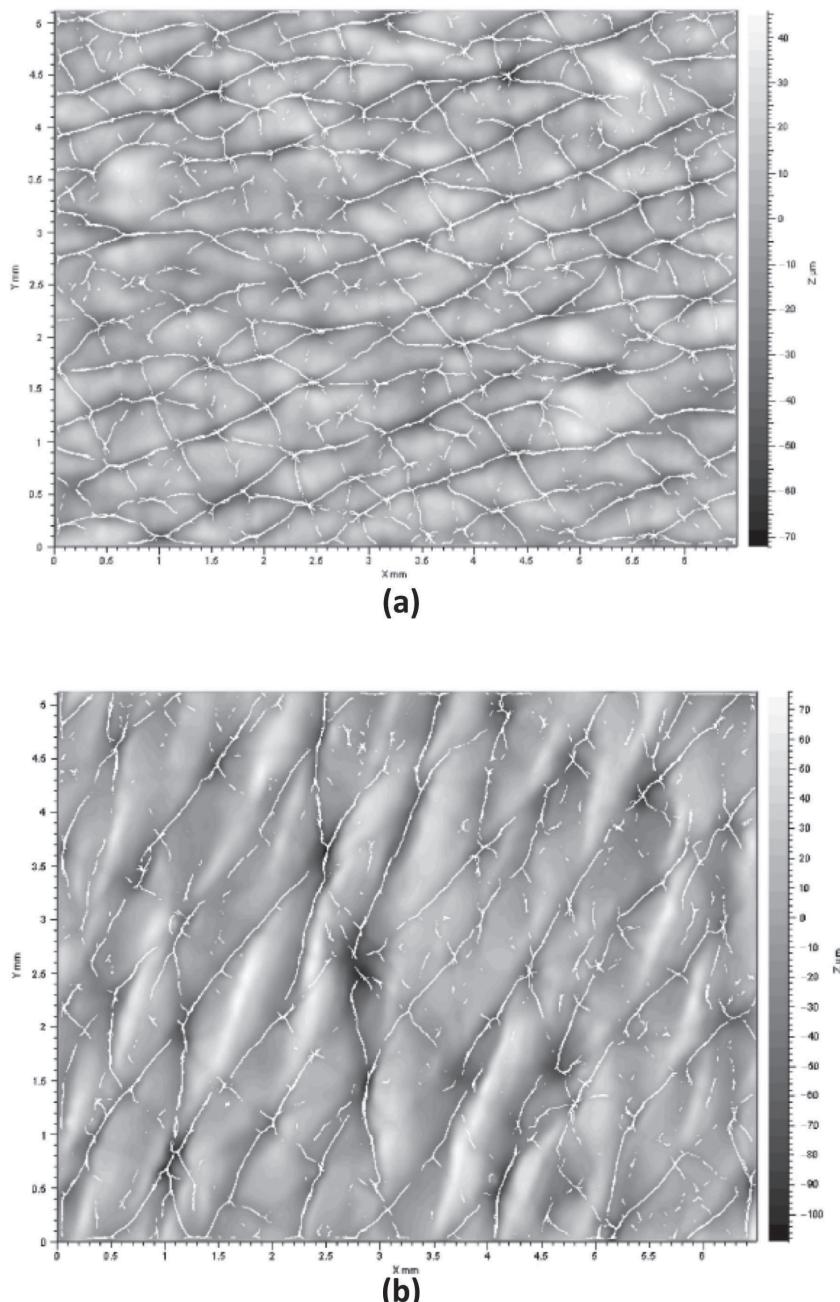
Fringe projection techniques are used to measure skin topography directly *in vivo* and also *in vitro* using skin replicas. Skin topography, also referred to as skin relief or texture, is an important feature of the skin that reveals its state of health. Most studies focus on measuring skin roughness and other topographical features,

such as wrinkles and furrows.<sup>22</sup> 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.<sup>21</sup> For illustration, Figure 7 demonstrates the use of the Derma-TOP® 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.<sup>22</sup>

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.<sup>22</sup> 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 ( $W_t$ ).<sup>23</sup> 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.<sup>22</sup>

#### 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.<sup>24</sup> 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 ( $0.1\text{--}0.6\text{ mm}^2$ ), enlarged skin pores ( $0.3\text{--}0.6\text{ mm}^2$ ), and blackhead embedded pores (tonality is lower than the rest of the skin surface).<sup>25</sup> 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 ( $< 0.04\text{ mm}^2$ ), visible pores ( $0.04\text{--}0.07\text{ mm}^2$ ), and enlarged pores ( $> 0.07\text{ mm}^2$ ).<sup>26</sup>

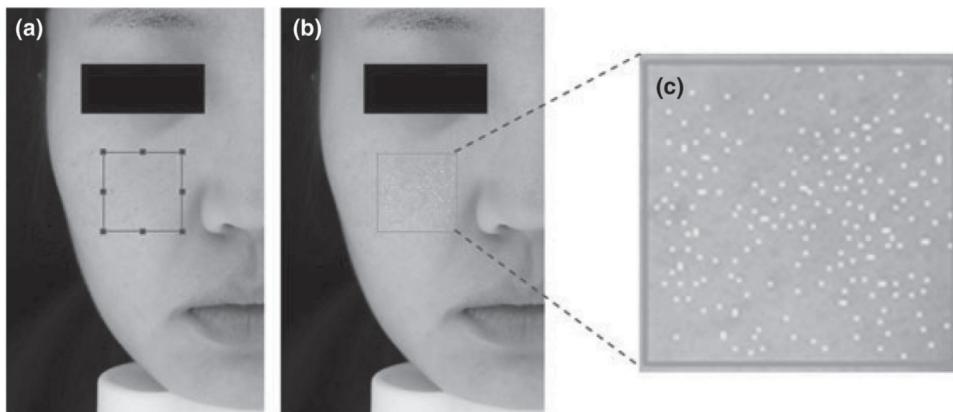
Surprisingly, there has not been a great deal of research investigating the nature of facial pores until recent years. The origin and formation of pores is dependent upon a variety of factors including hereditary factors, seborrhea, acne, comedogenic xenobiotics, aging, and chronic UV light exposure.<sup>27</sup>

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.<sup>25</sup> 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.<sup>25</sup> 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,

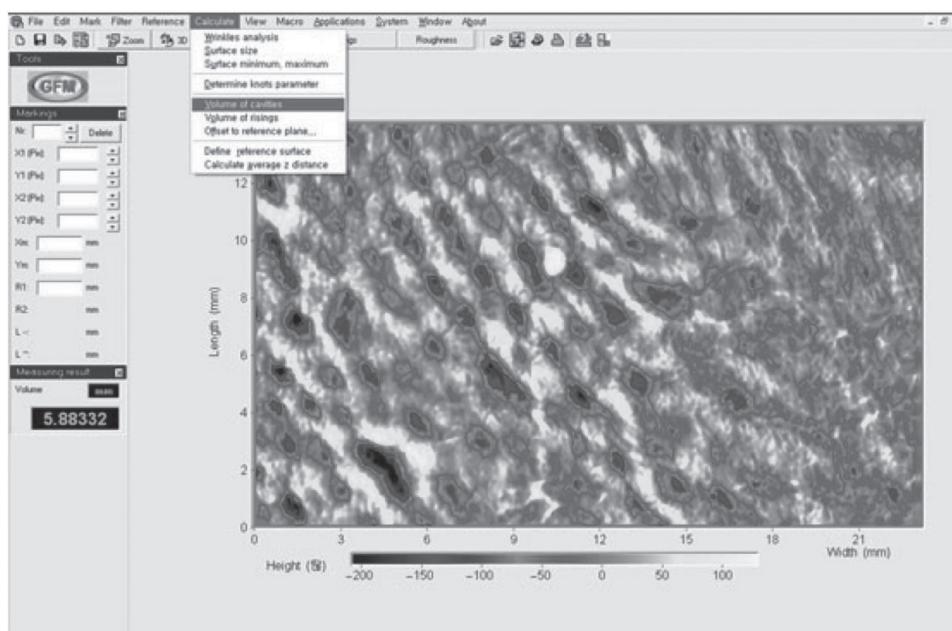
macrophotography, and evaluation of silicone replicas. Visual grading may be completed in the presence of a panelist or utilizing a photograph. In most cases, it is imperative to correlate visual grading to one of our quantitative techniques. Macrophotography is a rapid, noninvasive strategy for quantifying 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.<sup>26</sup>

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.<sup>26</sup> Stereoimaging with an optical topometer is also a viable route for quantifying pores.<sup>28</sup>



**Figure 10:** Analysis of pore volume with the PRIMOS premium system. Reprinted from Kim et al. with permission from John Wiley & Sons, © 2014.<sup>26</sup>

**Confocal laser scanning microscopy** may also be used to investigate the dimensions of pores.<sup>29</sup> 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:

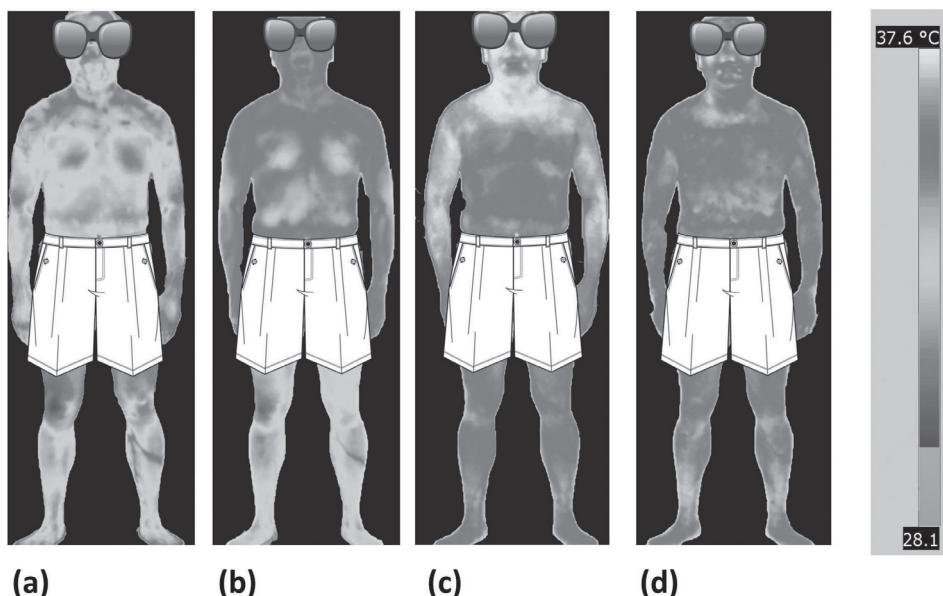
$$E+T+R=1 \quad (1)$$

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.<sup>17</sup> 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

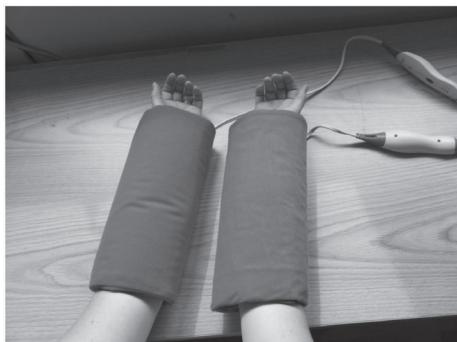
reached at the rostral region, superior torso, and lower limbs. The subject begins to sweat profusely after 10 min in the sauna, resulting in an overall cooling effect over all surfaces of the integument as shown in Figure 11D. Subsequent images were obtained at 30, 40, and 50 min, which demonstrated 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.<sup>17</sup>

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  $2 \text{ mg/cm}^2$  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.



(a)



(b)

**Figure 12:** Application of (a) hot and (b) cold pads used to induce stress in skin.



(a)



(b)

**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**.<sup>30</sup> 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.<sup>31</sup> 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.<sup>32,33</sup> 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.

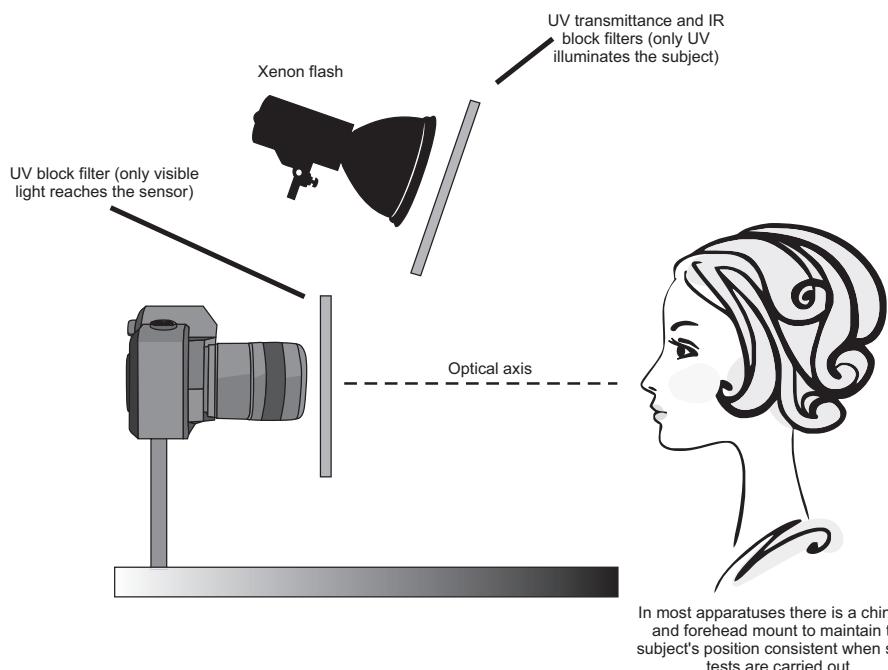
In general, UV photography is conducted with the same type of cameras employed for light photography with some modifications. In order to carry out such experiments, one should employ a camera that is sensitive to UV light. In film photography, high-speed (e.g., ISO 1600) black-and-white film is used due to 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.<sup>34</sup> 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.<sup>31</sup> 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.<sup>31,35</sup> 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.<sup>35</sup>

### **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**.<sup>36</sup> 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.<sup>4</sup> In fact, the use of cross-polarization photography has been applied to study erythematic inflammatory lesions in acne vulgaris.<sup>3</sup> 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.

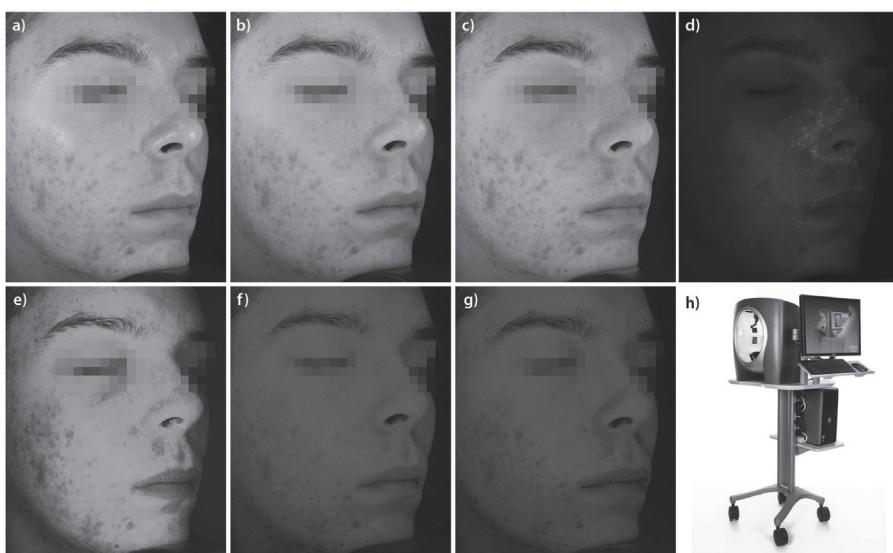
A key feature of the VISIA-CR and related technologies is the capability to resolve fluorescent images resulting from fluorescence of important biochemical components in skin in an automated fashion. Fluorescent photography has been used extensively in the evaluation of acne vulgaris. Table 1 identifies 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.

	<b>Excitation filter</b>	<b>Captured emission image</b>
Porphyrin	405 nm ± 10 nm	Long pass filter ( $\geq 620$ 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.<sup>37</sup> 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.<sup>38</sup>



**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**.<sup>39,40</sup> 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<sup>2</sup> to cm<sup>2</sup>—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 ophthalmic table (similar to the assembly in Figure 14).<sup>39,41</sup> 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.<sup>39</sup> 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.<sup>42</sup>

### **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.<sup>43</sup> 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.<sup>44</sup> Insights into the internal structure of cellulite-afflicted skin have been achieved with ultrasonography, magnetic resonance imaging, optical coherence tomography, and reflectance confocal microscopy.<sup>43</sup> 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**

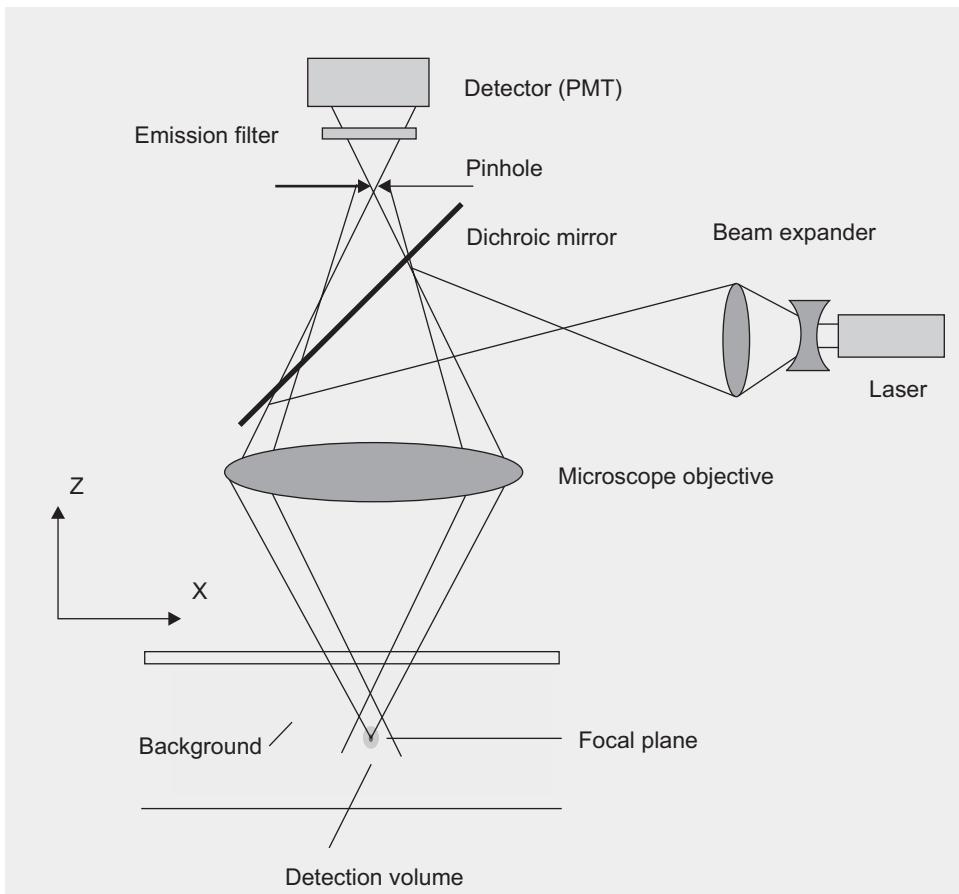
So far, most of our discussion has centered on imaging surface features of skin. There are also many important phenomena that occur below the surface of skin that we may wish to visualize. In recent years, there has been a plethora of published research demonstrating the use of sophisticated instrumentation 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).<sup>45</sup>

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

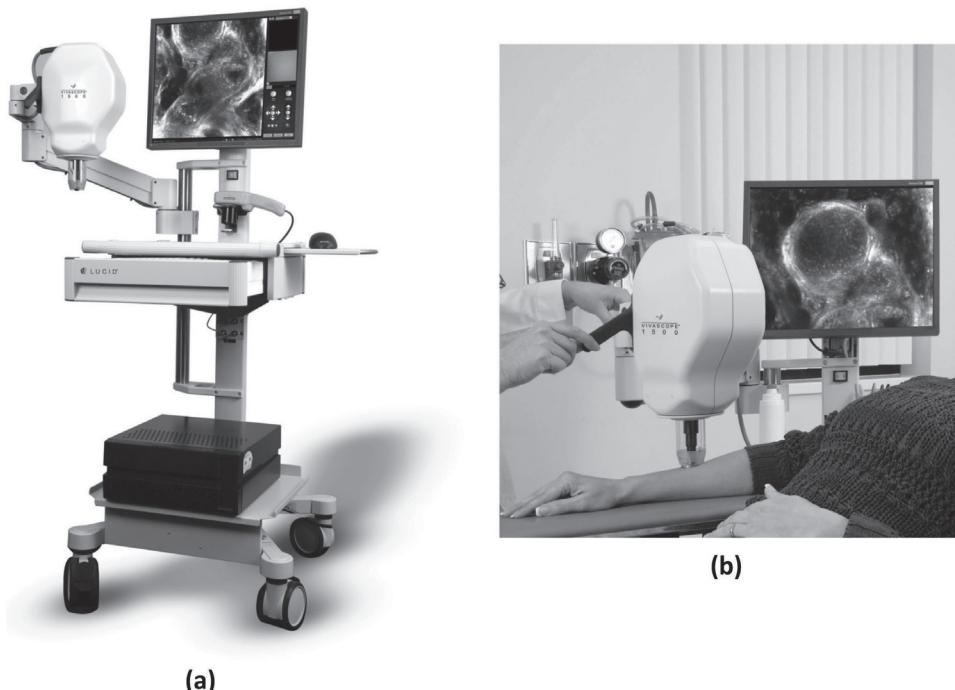
**Confocal laser scanning microscopy**, also known as reflectance confocal microscopy, allows for the collection of high-resolution optical images. These images are collected at selected depths within the sample by a process known as optical sectioning. The images are gathered *en face* (horizontal rather 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.<sup>15,46-51</sup> 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<sup>51</sup>.



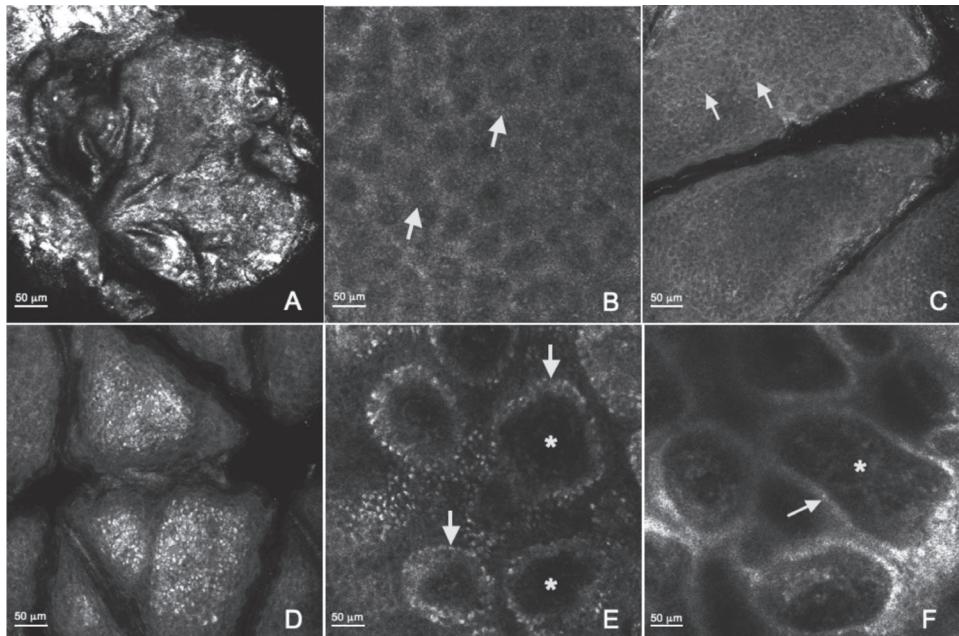
**Figure 16:** Diagram of a typical confocal scanning laser microscope. Reprinted with permission from Carl Zeiss.<sup>52</sup>

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  $< 1.25 \mu\text{m}$  while the vertical resolution (z-axis) is  $< 5.0 \mu\text{m}$ . The field of view is  $500 \mu\text{m} \times 500 \mu\text{m}$ . 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.<sup>51</sup> 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  $\mu\text{m}$ .



**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.<sup>53</sup>

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  $\mu\text{m}$ ) 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).<sup>53</sup>

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

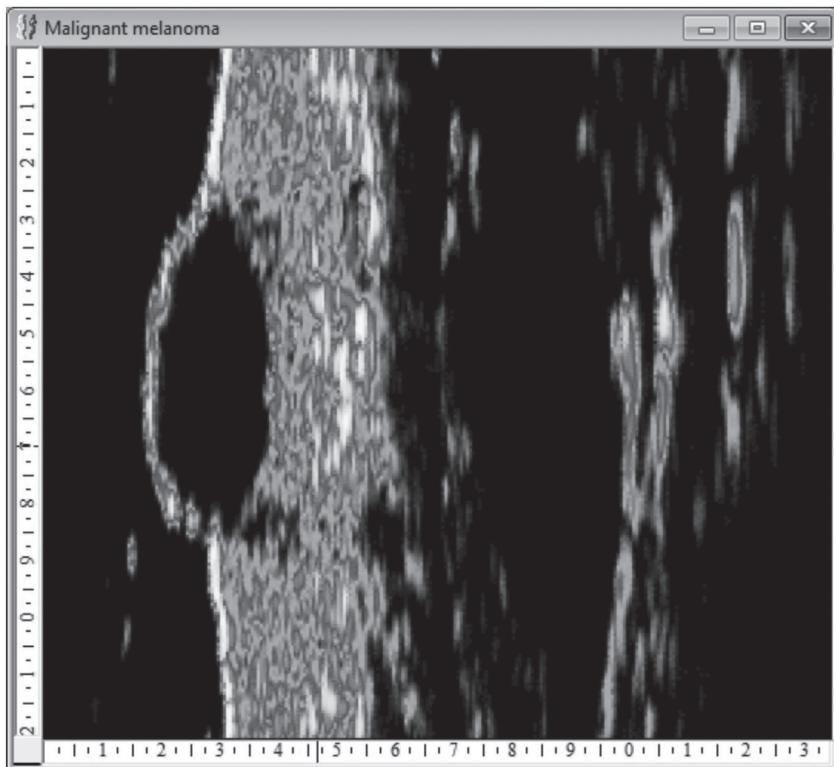
**Ultrasound** is an important diagnostic tool in general clinical medicine that allows clinicians to examine the health state of internal organs by the use of a noninvasive procedure. Over the last several decades, advances in ultrasound instrumentation have been developed allowing for the diagnosis 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).<sup>54</sup> More recently, it 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.<sup>55,56</sup> 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.<sup>57</sup> Overall, there has been a flurry of research utilizing ultrasound in the dermatologic community.<sup>58</sup> 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.<sup>59–63</sup>

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

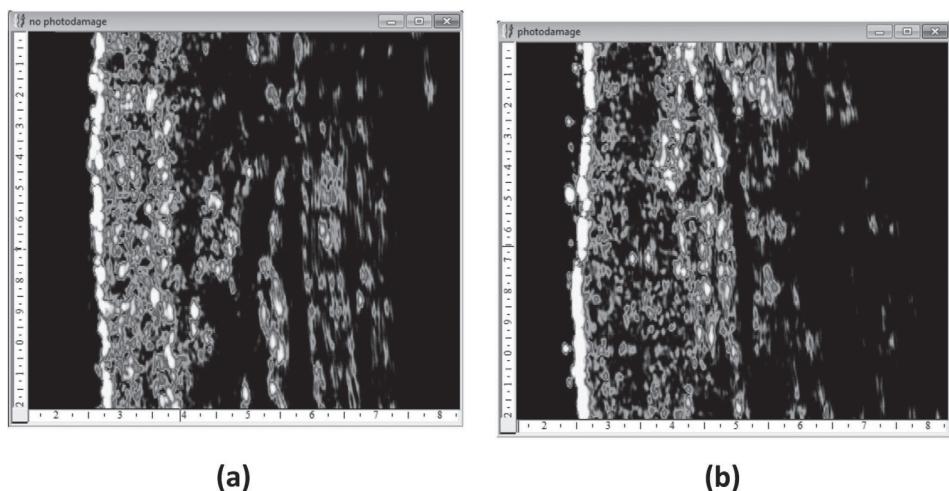
as echoes. The propagated wave is generated by a piezoelectric device, which converts electricity into sound waves, and the echo (reflecting from the tissue) is also detected by piezoelectric devices, which can also convert sound waves into electric signals. In practice, a ring is placed on the surface 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.<sup>54</sup> 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.<sup>64</sup>

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.<sup>54</sup>

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

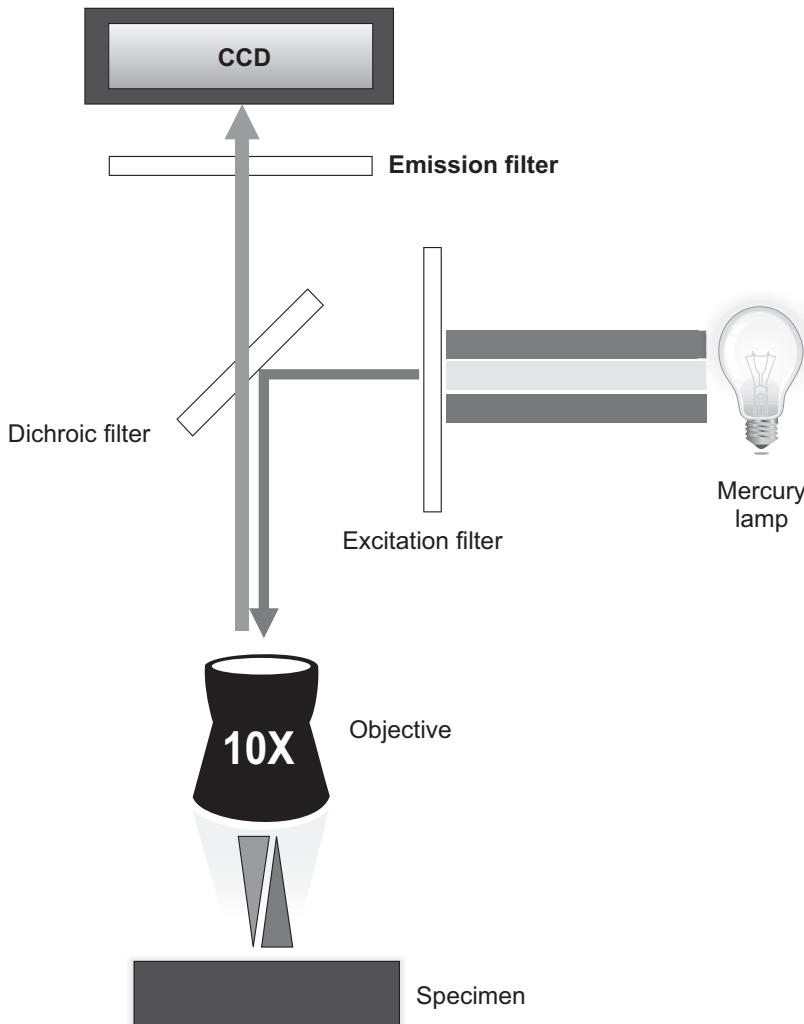
Often, in order to understand real-world phenomena one must delve into the realm of things not readily seen by the naked eye. Like most other tissues and organs, this is also true for skin. In addition to its morphological complexity, there are a number of anatomical structures that occur at the micron 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

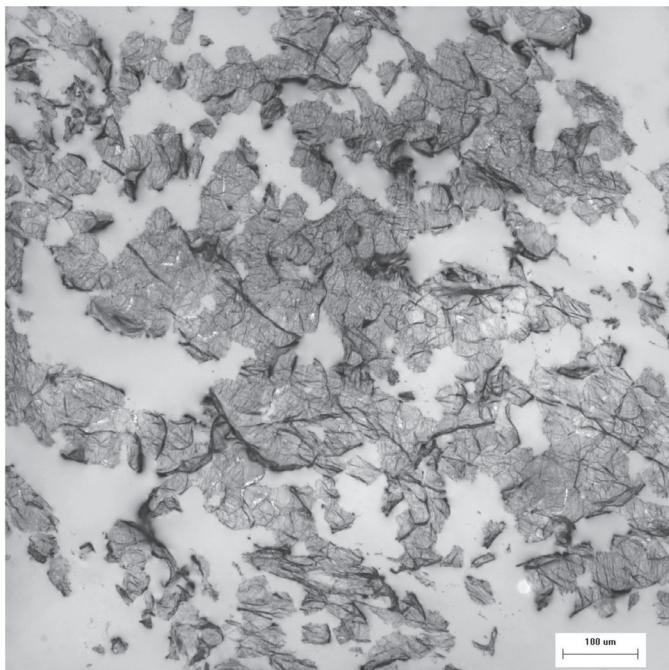
system is treated with a fluorescent dye; or a primary antibody along with a secondary antibody that contains a fluorescent probe covalently bound to its structure (the antibody technique is known as immunofluorescence). Whichever probe is utilized, the dye or the dye-antibody combination, these molecules 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).



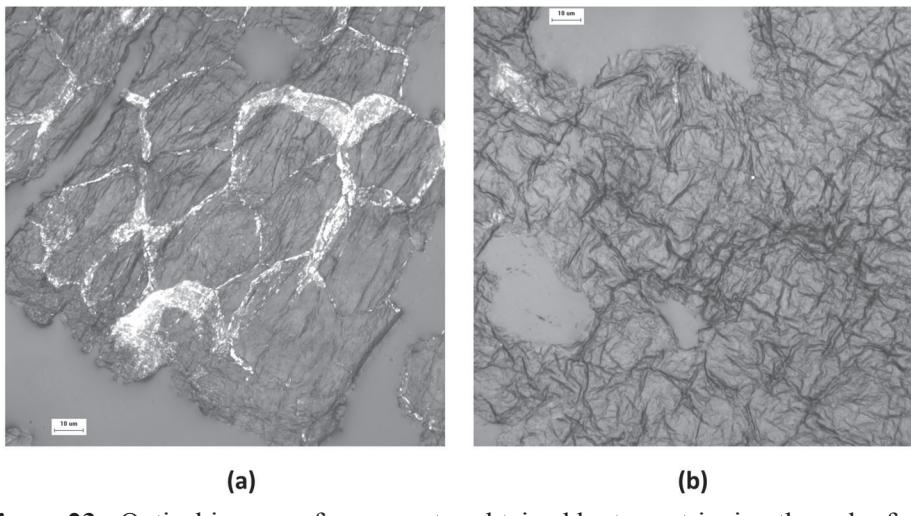
**Figure 21:** Schematic of a fluorescence reflectance microscope.

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.



**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.

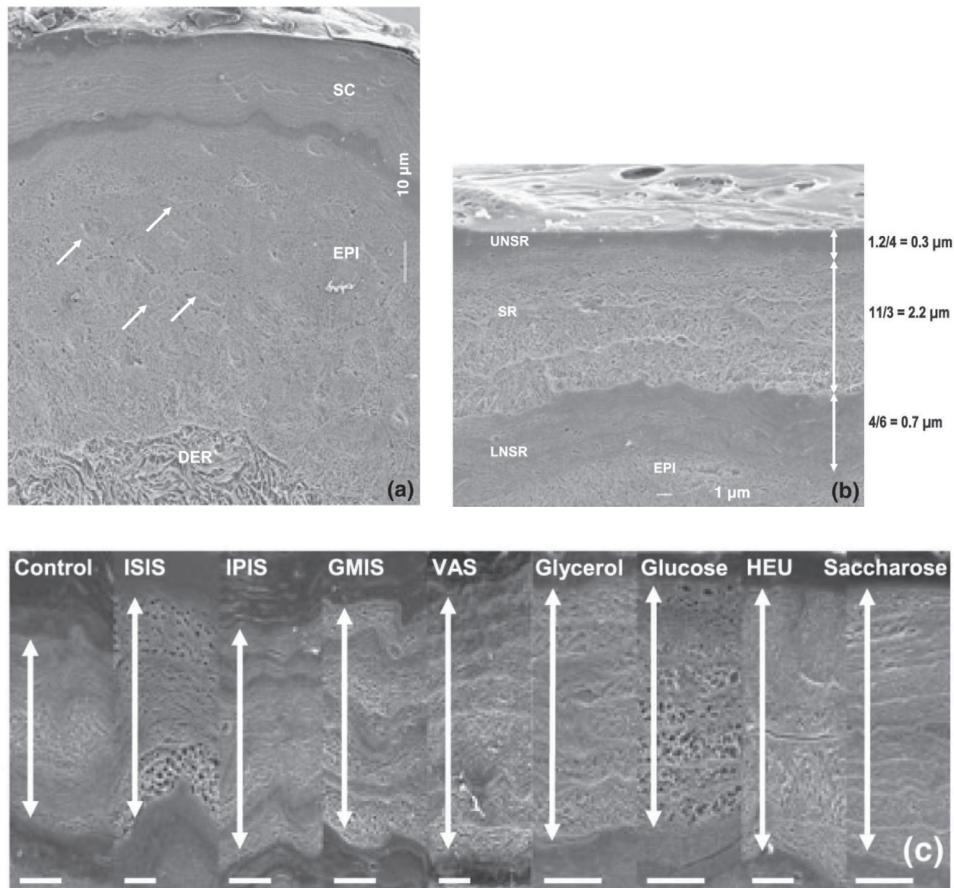
In a traditional reflected light microscope, it is not feasible to conduct *in vivo* measurements. There simply is not enough space between the translation stage and the objectives of the microscope. For this reason, many portable microscopes with the ability to capture *in vivo* readings have been developed 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 its 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.<sup>65,66</sup> In most cases, SEM images are generated from positive casts constructed from skin replicas and examined for general topology and exfoliation patterns of stratum corneum cells.<sup>67</sup> 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.<sup>68</sup> 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.



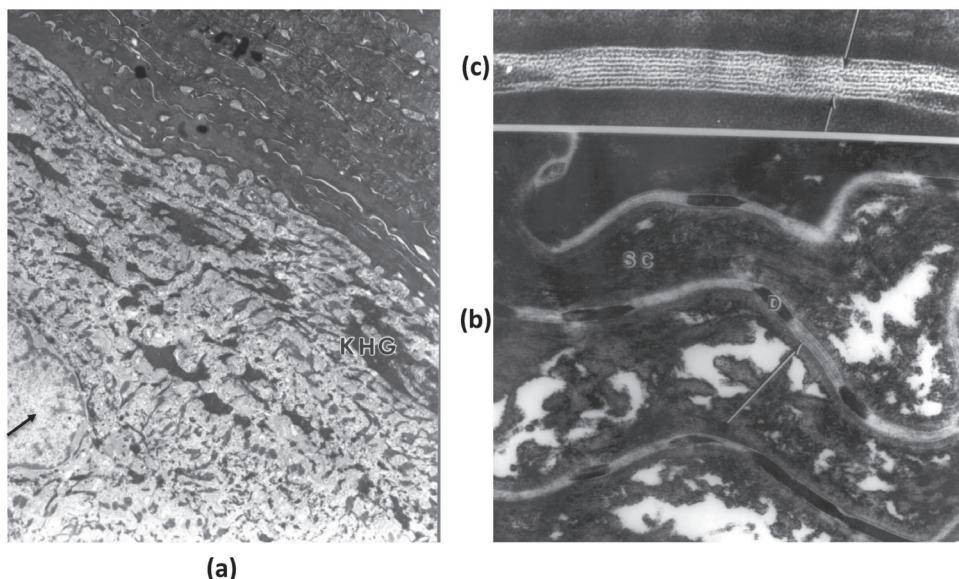
**Figure 24:** (a) Cryo-SEM image of a dermatomed skin cross-section equilibrated to 80% RH after cryo-fixation and cryo-planing. The stratum corneum (SC), viable epidermis (EPI), and dermis (DER) can clearly be distinguished. Arrows in the epidermis indicate the presence of keratinocyte nuclei. (b) 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), glycerol monoisostearate (GMIS), Vaseline (VAS – petrolatum), glycerol, glucose, hydroxyethyl urea (HEU), and saccharose. Reprinted from Caussin et al. with permission from John Wiley & Sons, © 2007.<sup>68</sup>

### c. Transmission Electron Microscopy (TEM)

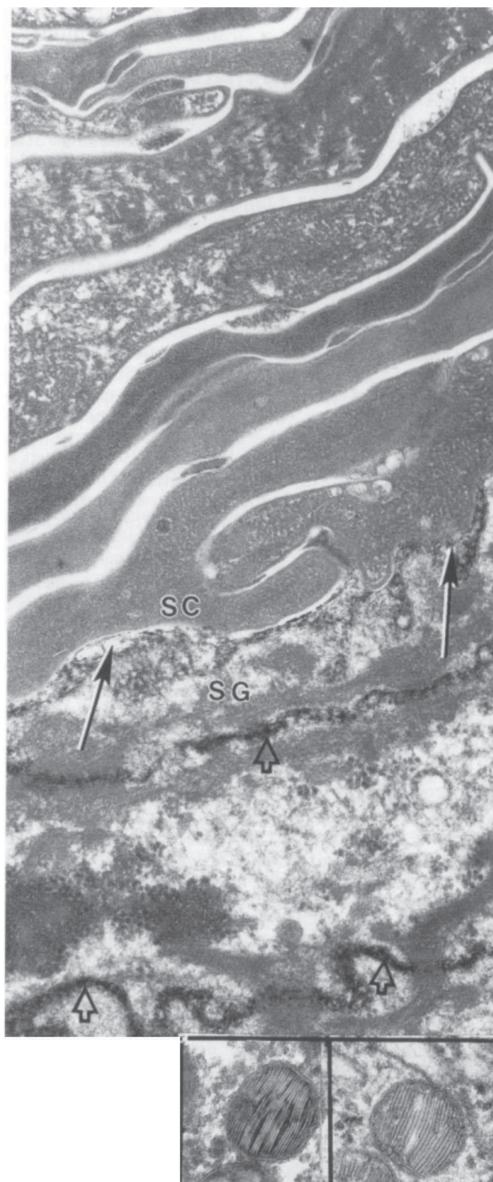
Many of the intricate details we know about skin structure have come about from **transmission electron microscopy** (TEM) studies. One need only to peruse some of the classic literature about skin structure and function to fully appreciate its contributions to the field.<sup>69–71</sup> Transmission 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.<sup>72</sup> 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.<sup>71</sup> 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.



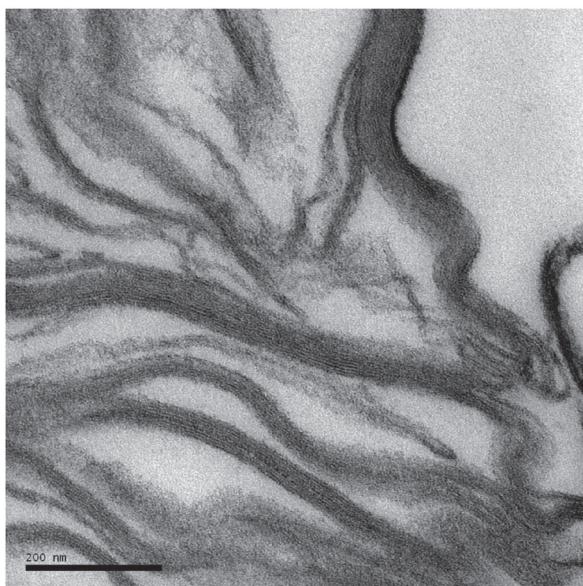
**Figure 25:** TEM images of various regions of the epidermis obtained from thin sections of human skin. In the first image (a) portions of the stratum spinosum, stratum granulosum, and stratum corneum are shown. In the bottom left corner of the image (arrow), one can discern the nucleus of a stratum 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.<sup>73</sup>



**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.<sup>74</sup>

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.

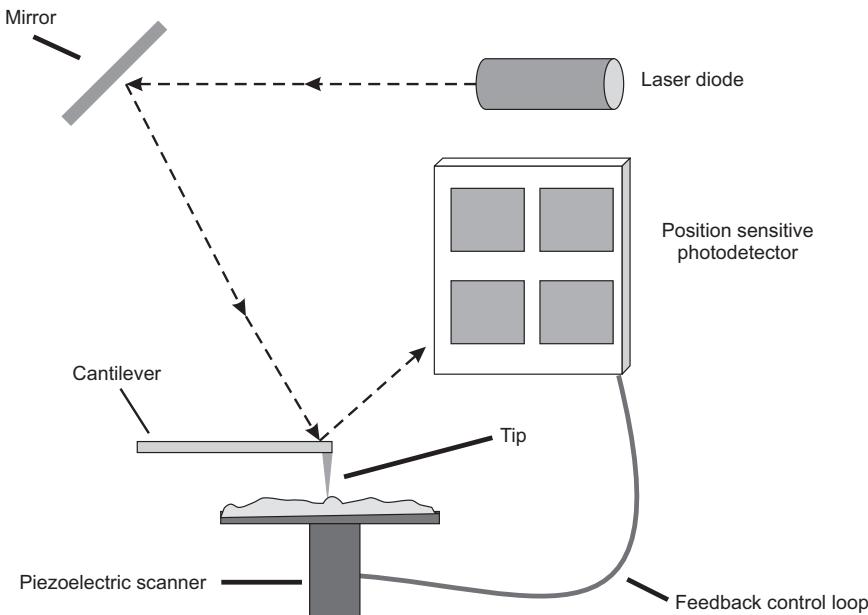


**Figure 27:** TEM image of a specialty ingredient product used in the cosmetic industry to fortify the stratum corneum lipid barrier. It is comprised of the following ingredients: glyceryl stearate, behenyl alcohol palmitic acid, stearic acid, lecithin, lauryl alcohol, myristyl alcohol, and cetyl alcohol. 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.

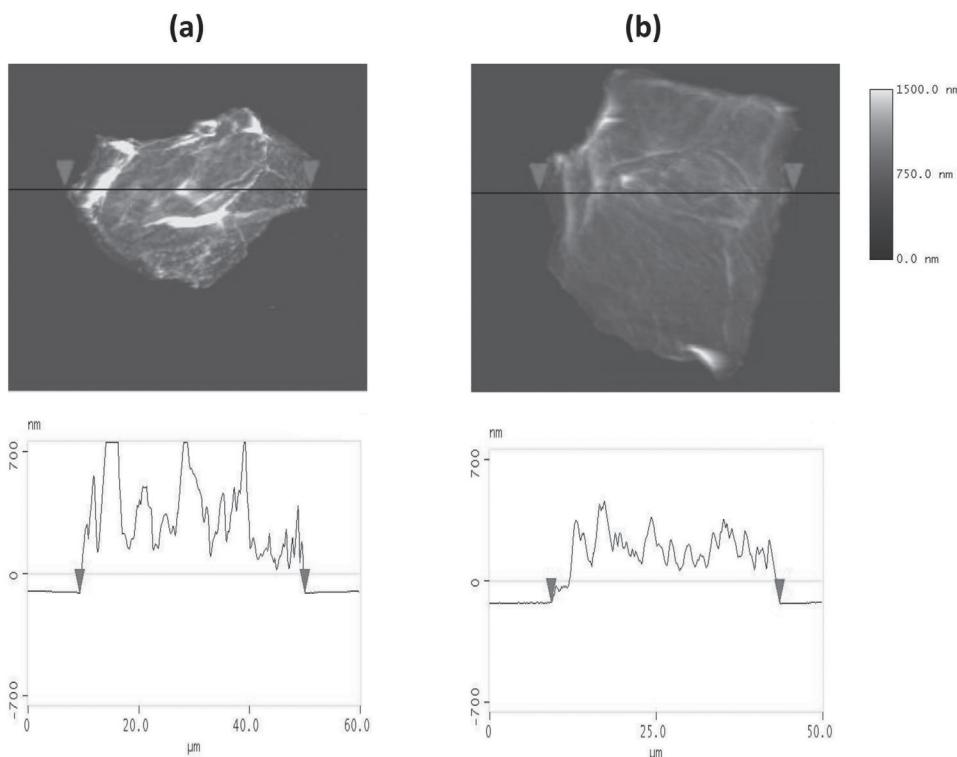
As illustrated in Figure 28, a laser is reflected on the back of the cantilever, and as it moves in the  $z$ -direction due to topography of the sample, the position of the reflected laser on the photodetector is monitored. Therefore, by scanning in the  $x$ - $y$  plane we can generate a three-dimensional 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.<sup>75-77</sup> 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.<sup>76</sup>



**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

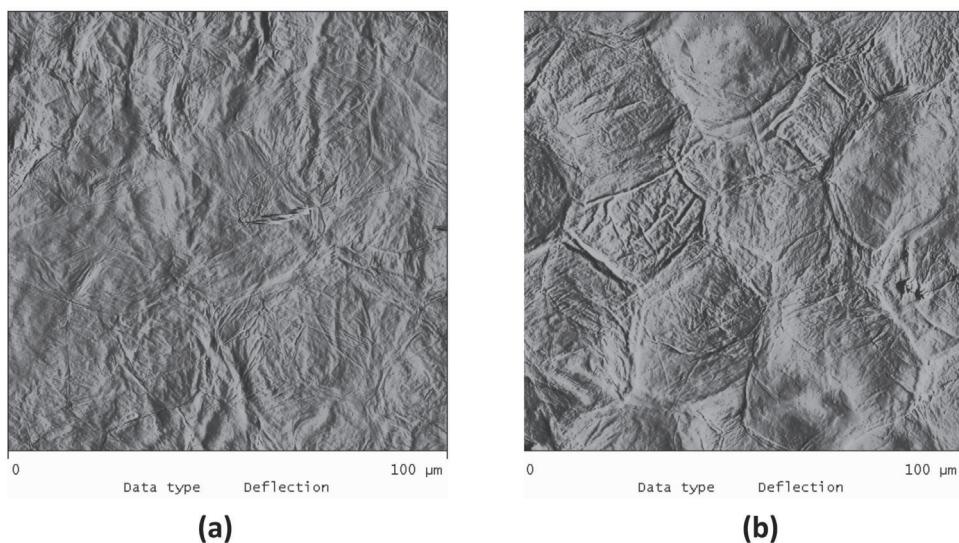
from deeper layers. Differences in corneocyte topography have also been discerned for corneocytes isolated from distinct anatomical regions as well as samples obtained from subjects representing various age groups and individuals with pathologic skin conditions.<sup>75</sup> Other AFM studies have focused on characterizing structural proteins of skin, such as collagen, in terms of adhesion and elasticity.<sup>78</sup> 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.<sup>79,80</sup>



**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.<sup>81</sup> 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)  $\text{H}_2\text{O}$  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

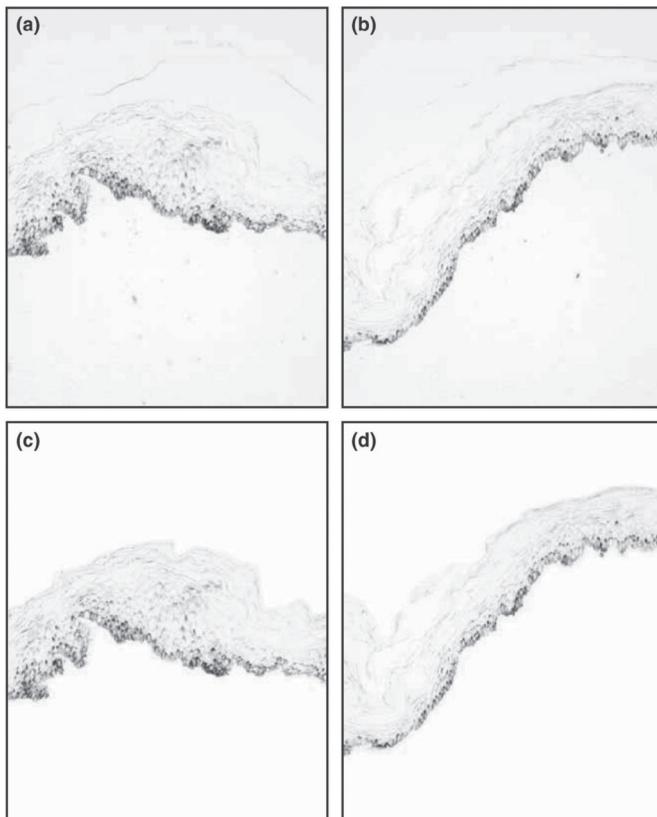
Numerous *in vitro*, *ex vivo*, and *in vivo* tests exist for the determination of expression of biological molecules as related to skin immunological status, pigmentary changes, and skin aging. Often, these tests are carried out utilizing *in vitro* cell

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.<sup>82-84</sup>

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

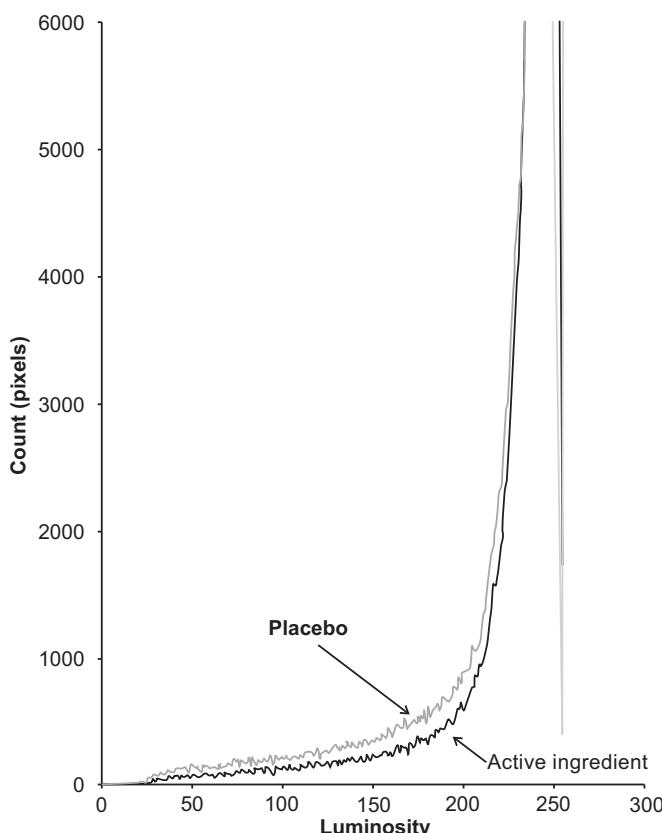
The Fontana Masson staining technique is commonly employed to measure pigmentation effects such as skin whitening, tanning, and hyperpigmentation disorders. The staining procedure consists of treating a thin histological section of skin with silver nitrate ( $\text{AgNO}_3$ ). As a result of treatment,  $\text{AgNO}_3$  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.<sup>84</sup>

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

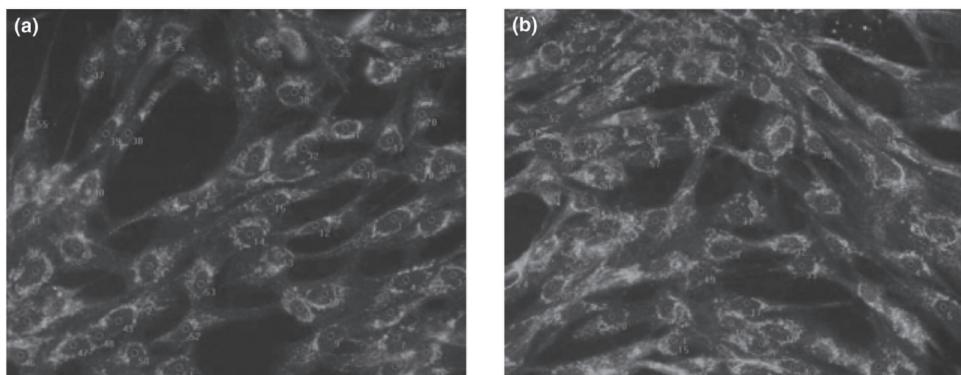
each case, a large peak at high luminosity values is apparent in the histogram and corresponds to the white background in the images. In agreement with the histogram, the Area Under the Curve value obtained for the placebo ( $40 \pm 7$ ) is greater than that for the active ingredient ( $29 \pm 7$ ) treated 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.



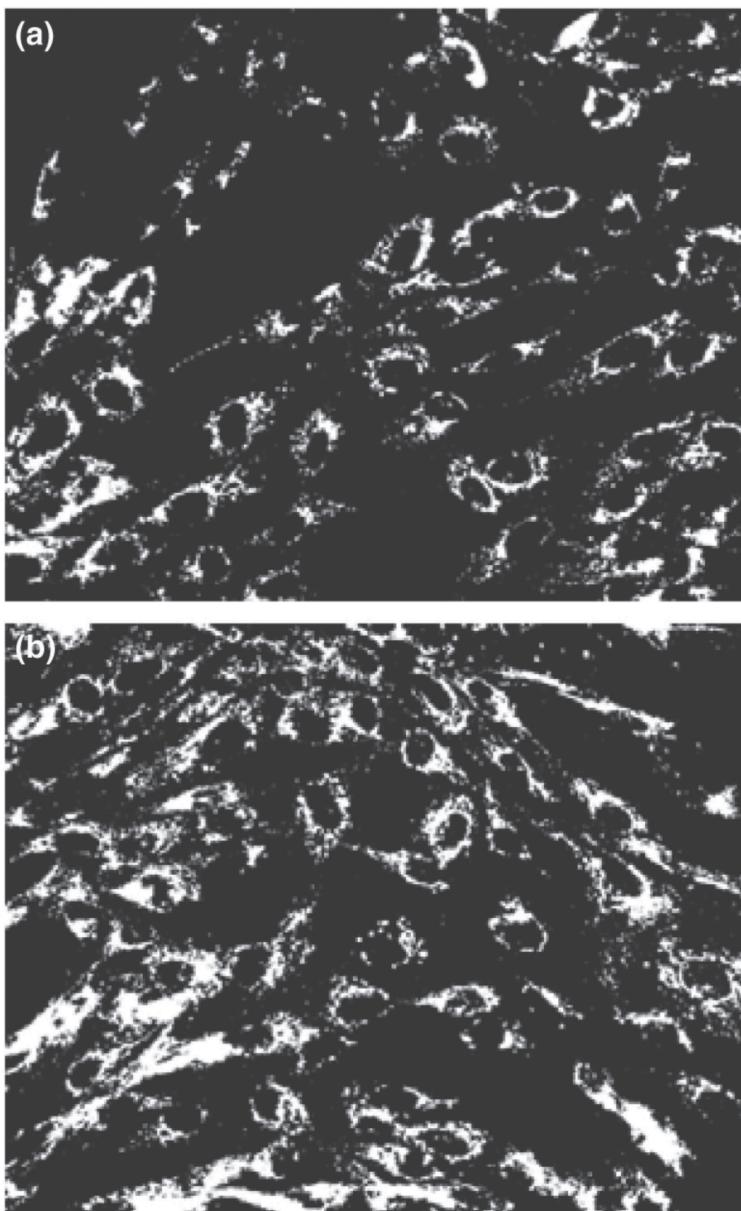
**Figure 32:** Image histograms corresponding to the processed images in Figures 31c and 31d. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.<sup>84</sup>

### 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 mitochondrial 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.<sup>84</sup>

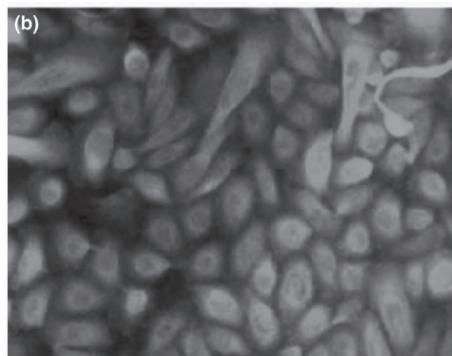
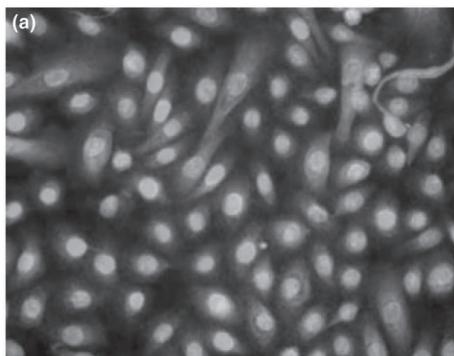


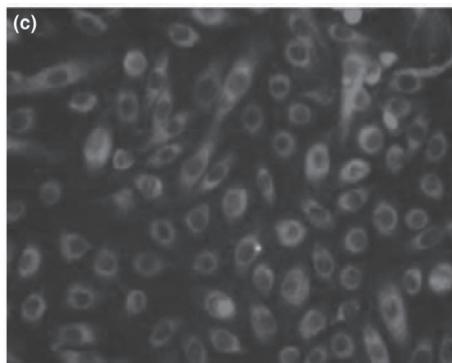
**Figure 34:** Conversion of images shown in Figure 33 to binary images corresponding to (a) placebo and (b) active ingredient treated samples. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.<sup>84</sup>

### c. Image Analysis of Triple-Stained Normal Human Keratinocytes (NHKs)

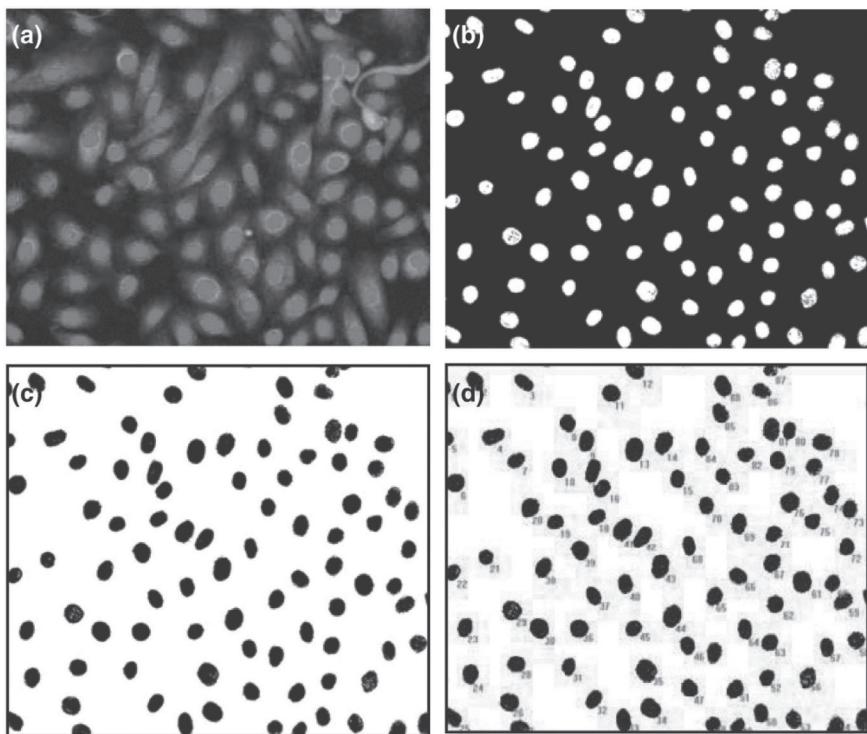
Often, one may wish to monitor the presence or expression of several different molecules in cell culture. One component that is almost always monitored is the cell nucleus, which is stained with DAPI, a molecular probe characterized by  $\lambda_{\text{ex}} = 358 \text{ nm}$  and  $\lambda_{\text{em}} = 461 \text{ nm}$ . 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.<sup>84</sup>



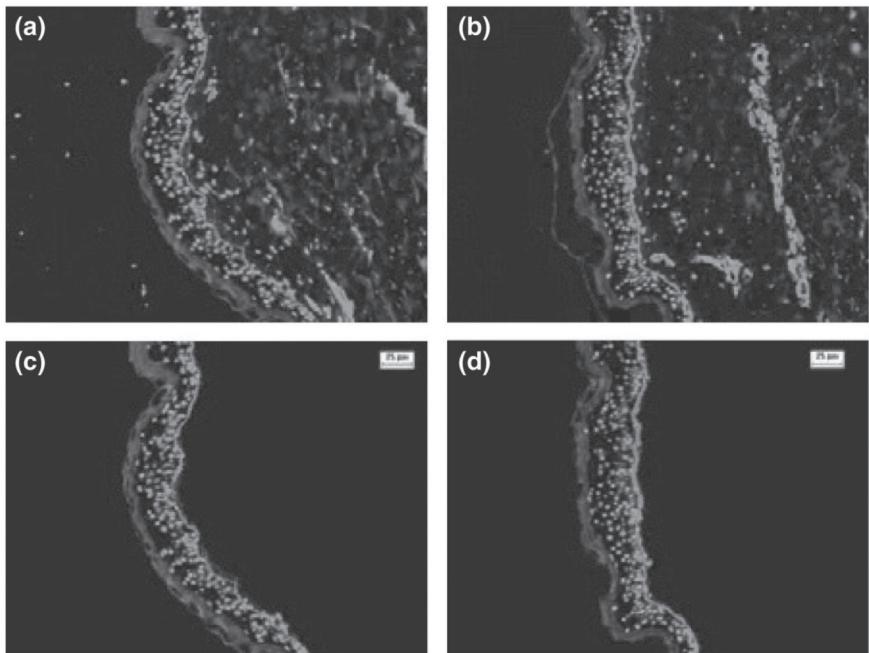
**Figure 36:** Series of segmentation steps for the analysis of DAPI staining: (a) color segmentation, (b) isolation of nuclei, (c) image inversion and (d) cell counting. Reprinted from McMullen et al. with permission from Society of Cosmetic Scientists and the Société Française de Cosmétologie, © 2010.<sup>84</sup>

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).

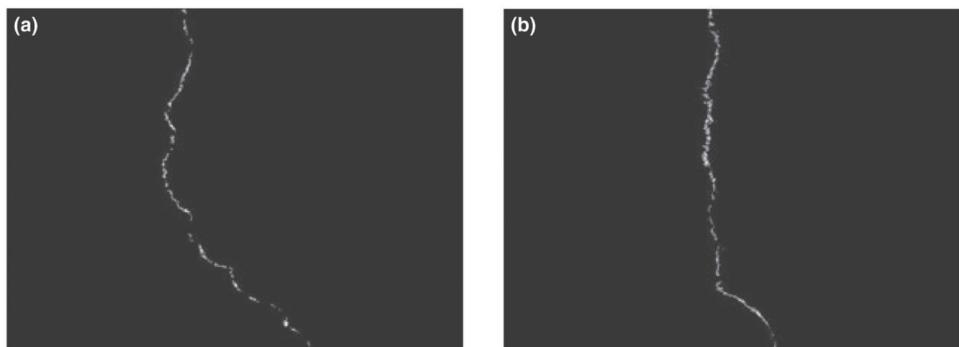
#### **d. Measurement of Collagen IV Expression at the Dermal-Epidermal Junction**

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.<sup>84</sup>



**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.<sup>84</sup>

## 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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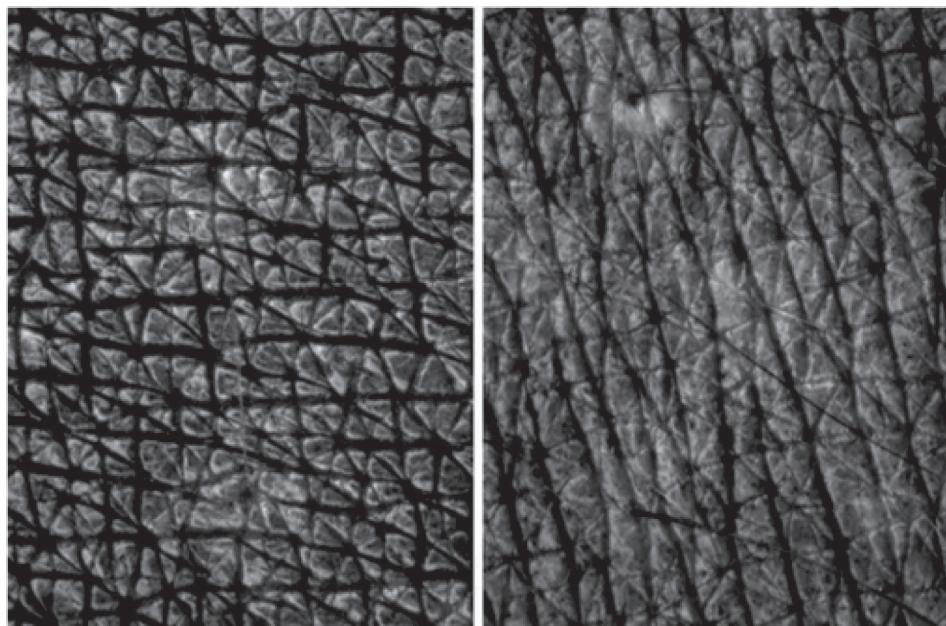
### 11.3.1 SKIN TOPOGRAPHY

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  $<0.5\text{ }\mu\text{m}$ , 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).

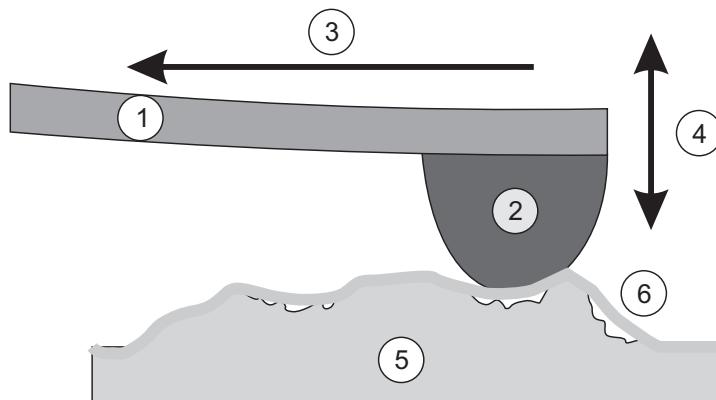
### a. Quantitative assessment of skin topography

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

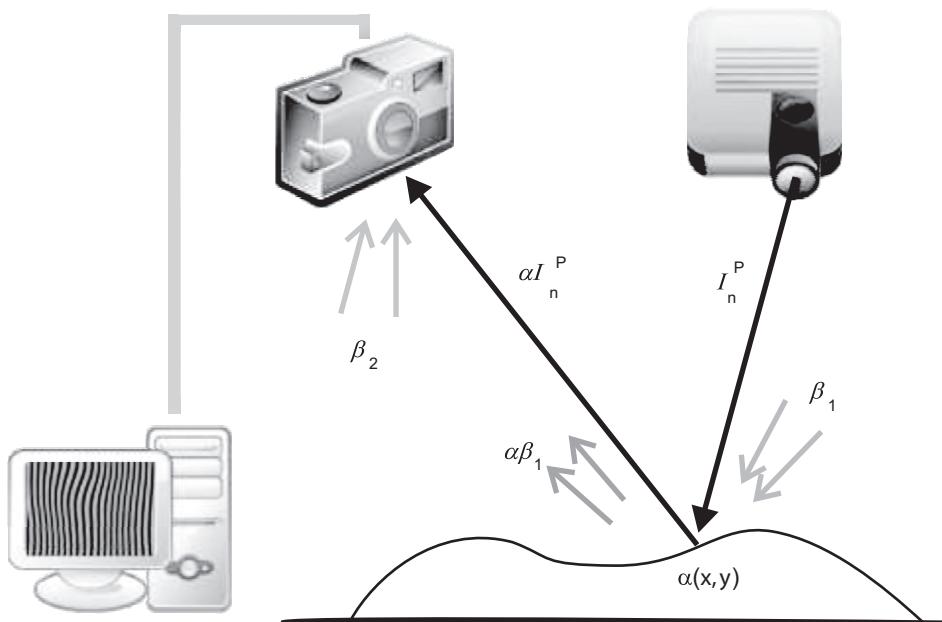
**Profilometry** [8] and shadowing method [9] represent techniques using two-dimensional analysis of replicas to evaluate skin surface topography. The most widely used material in the preparation of skin replicas is a monomeric silicone polymer (Silica®, J. & S. Davis, Hertfordshire, UK) that is mixed with a thinner and a catalyst. 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 micro-relief 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].

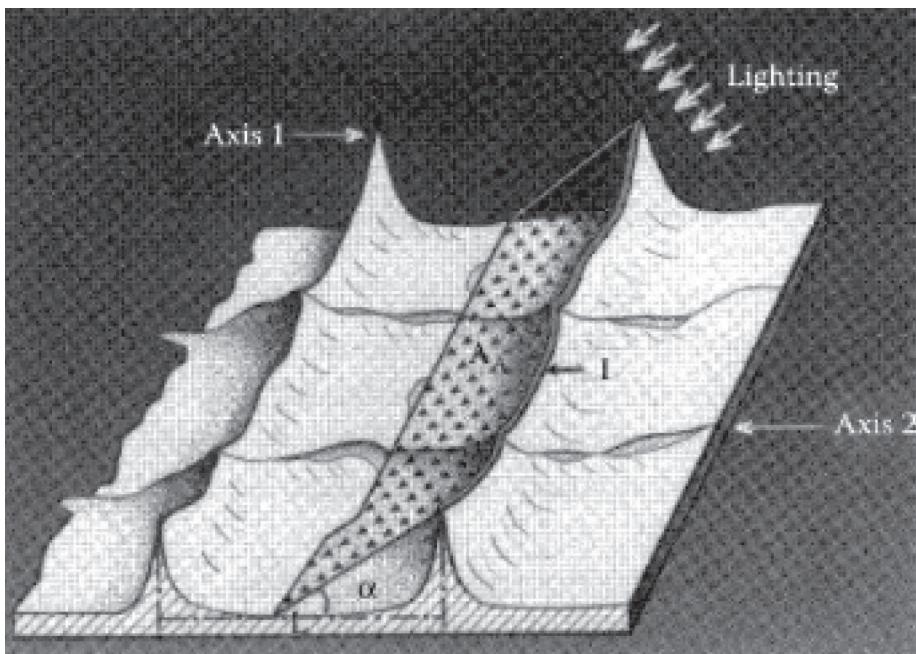
Profilometry techniques have the advantage of assessing skin topography with a high degree of accuracy and precision ( $<0.5 \mu\text{m}$ ), but the scanning of skin replica is very slow [4] and large skin structures such as cellulite cannot be evaluated. Due to the mechanical load on the skin replica, 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. Both, 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

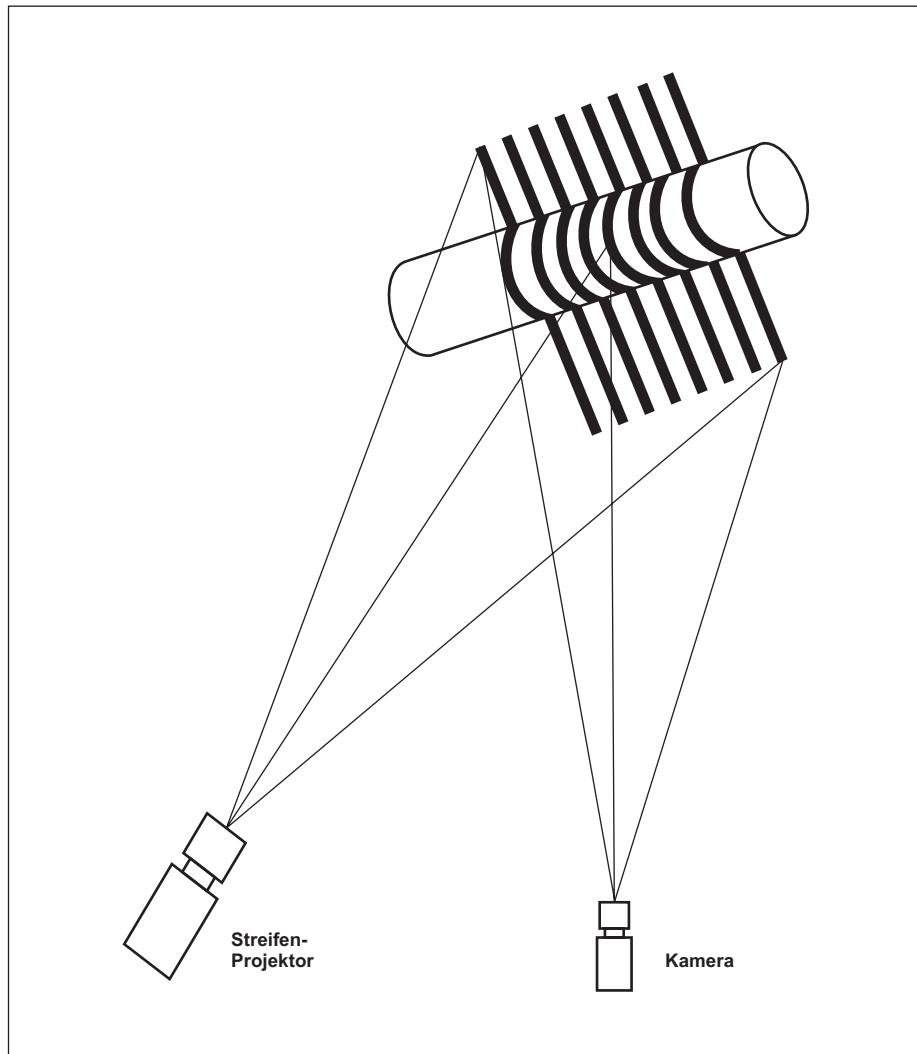
images allows the three-dimensional reconstruction of the surface, which can be quantified in terms of angles, surface areas, and volume [15; 16]. For the assessment of anti-wrinkle products, the volume parameter has become one of the most important parameters as it is most suitable to show a change 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

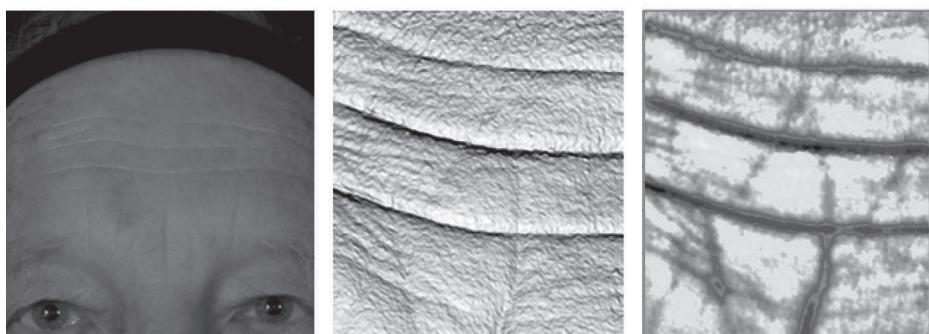
The most commonly used device in the field of cosmetic science is the PRIMOS®-System (*Phaseshift Rapid In Vivo Measurement of the Skin*) produced by GF Messtechnik GmbH (Teltow, Germany). An alternative device based on the same principle is the 3D Breuckmann Scanner (Breuckmann GmbH, Germany).

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 ( $1000 \times 620$  pixel). The DMD consists of an array of  $1024 \times 768$  square  $16 \times 16\mu\text{m}^2$  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  $+10^\circ$  (ON) or  $-10^\circ$  (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  $24 \times 14 \times 13 \text{ mm}^2$  [23] and measures wrinkle properties from a wrinkle depth of 40 to 50  $\mu\text{m}$  [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  $\mu\text{m}$ .*

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 than 40–50  $\mu\text{m}$  *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 ( $\text{max}W_d$ ), and maximum largest wrinkle depth ( $IW_d$ ). 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 ( $W_d$ )	$\mu\text{m}$
Average maximum wrinkle depth ( $\text{max}W_d$ )	$\mu\text{m}$
Maximum largest wrinkle depth ( $IW_d$ )	$\mu\text{m}$
Number of wrinkles ( $W_n$ )	no
Total wrinkle volume ( $W_v$ )	$\text{mm}^3$
Absolute wrinkle area ( $aW_a$ )	$\text{mm}^3$
Percentage wrinkle area ( $pW_a$ )	%
Wrinkle shape ( $W_s$ )	arbitrary units
Wrinkle length ( $W_l$ )	$\mu\text{m}$
Arithmetic average roughness ( $R_a$ )	$\mu\text{m}$
Maximum profile height ( $R_y$ )	$\mu\text{m}$
Arithmetic average of maximum roughness ( $R_z$ )	$\mu\text{m}$

### 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].

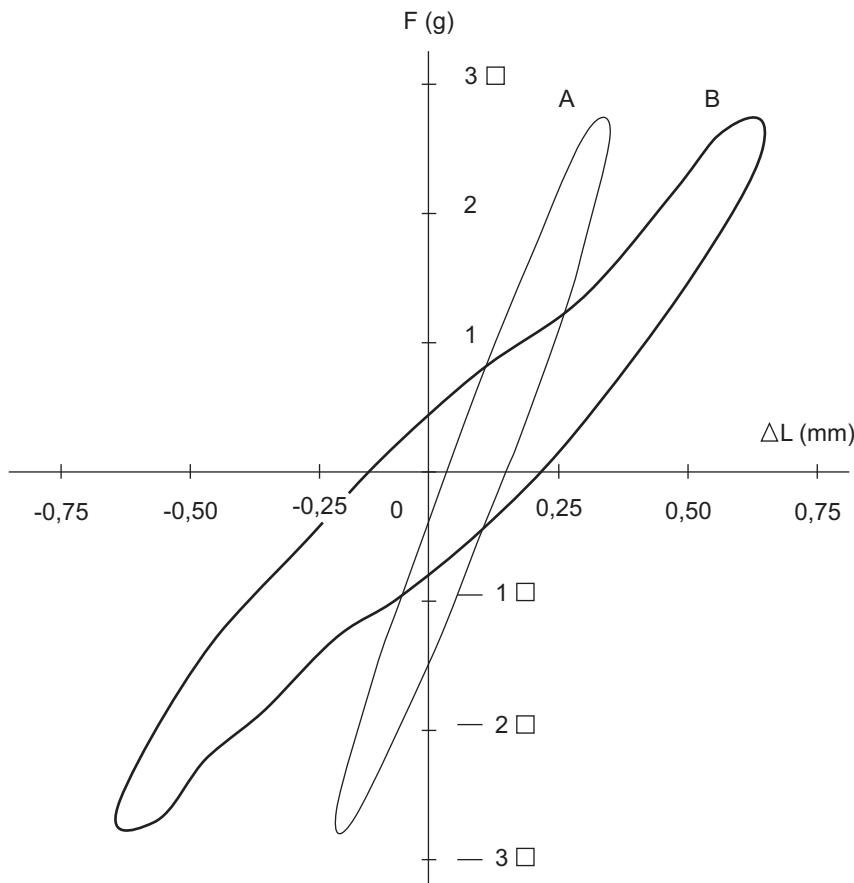
### a. Quantitative assessment of skin elasticity

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

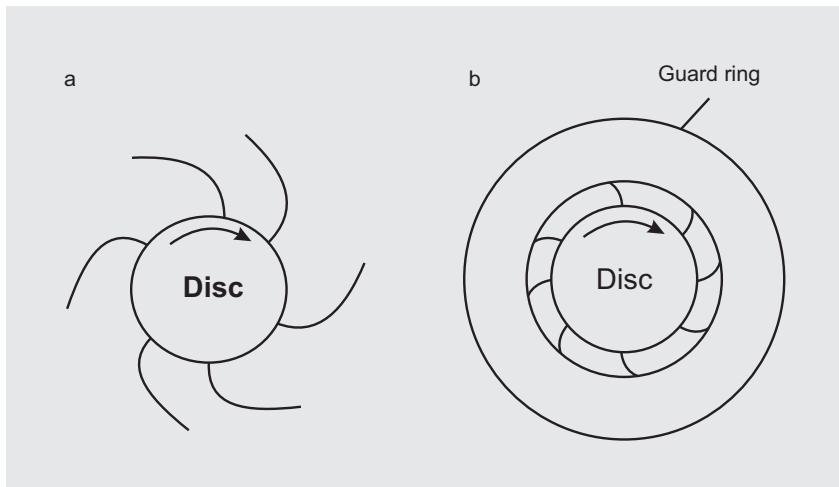
Tensile testing is based on recording skin extension following the imposition of a parallel force to skin's surface [28]. Therefore, tensile testing with electric dynamometers is the oldest commercialized device for investigating mechanical properties of the skin [38]. In this procedure, based on uniaxial 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

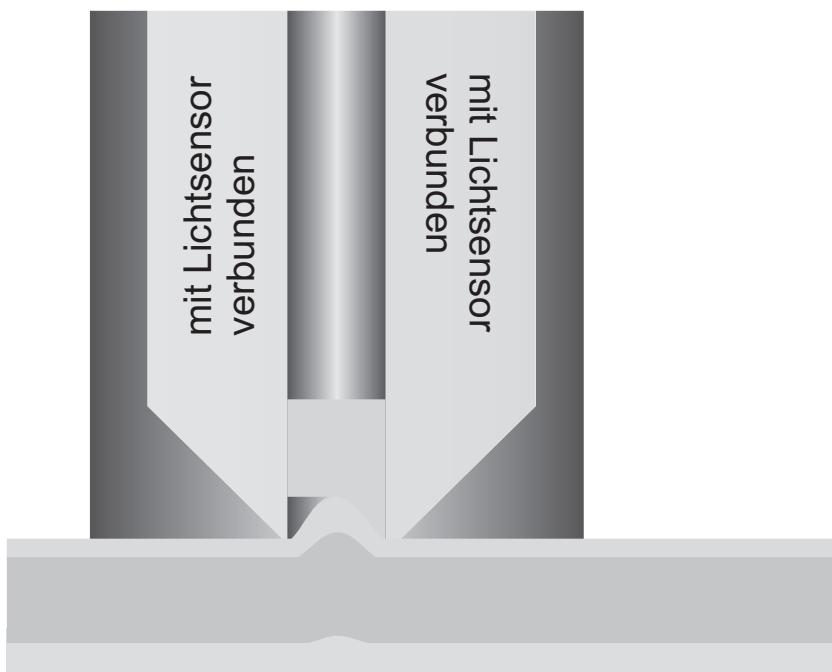
The impact testing of the human skin, also called ballistometrique technique, was designed to approach specific tensile functions of the skin involving the deeper skin layers [28]. The ballistometrique technique records the successive rebounds of a small hammer being dropped on the skin's surface with 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 ballistometrique 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].

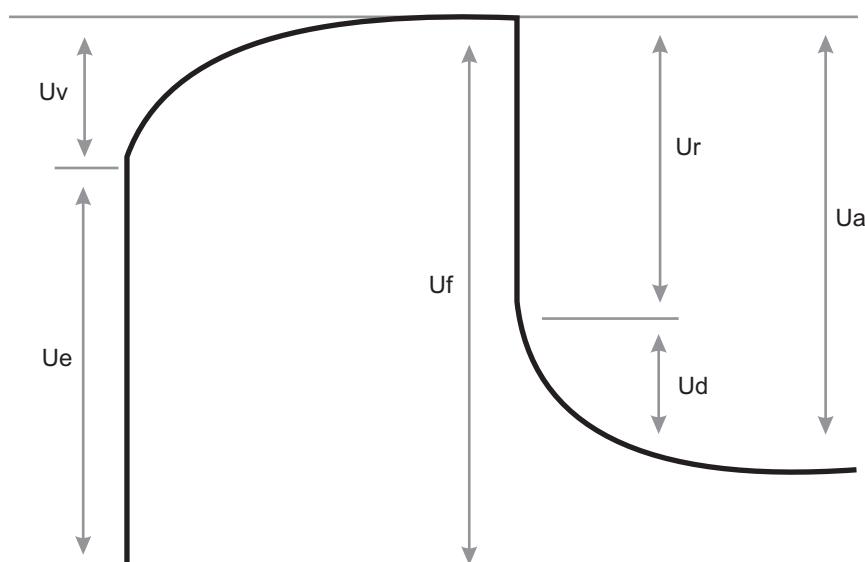


**Figure 5:** Schematic representation of the suction chamber method.

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 ( $U_r/U_f$ ), as well as the gross ( $U_a/U_f$ ) and net elasticity ( $U_r/U_e$ ) 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	$U_r/U_f$
Gross elasticity	$U_a/U_f$
Net elasticity	$U_r/U_e$
Maximum recovery	$U_a$
Immediate recovery	$U_r$
Skin distensibility of the last curve	$U_f5$
Skin distensibility	$U_f$
Immediate distensibility	$U_e$

## 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 subcutaneus, 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:** (/ɛkrən/, /ɛ, kraɪn/, or /ɛ, krɪn/; 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.

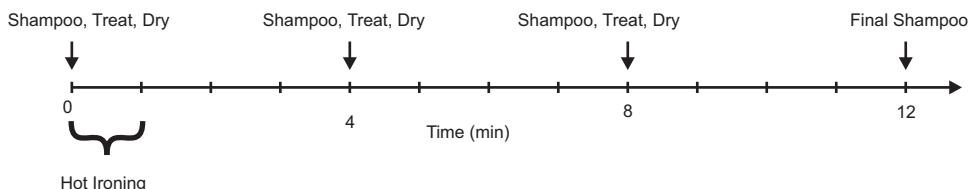
#### a. Treatment Schedules

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 its end. Alleviating damage of the hair due to the experimental composition can be determined by comparing results versus an appropriate control. A treatment regimen 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 Calorimetry (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***

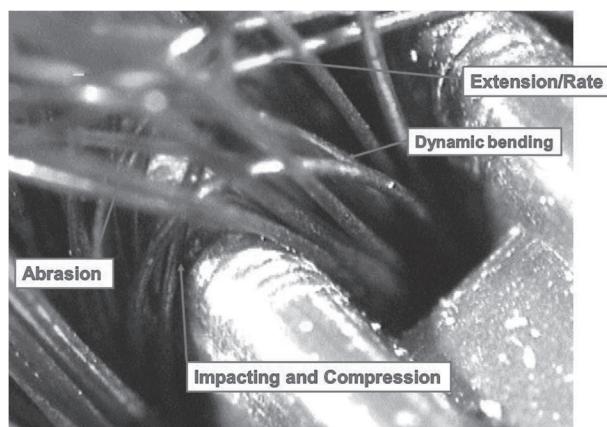
Peroxide containing oxidative hair color is designed to “lift” the natural color of the hair and at the same time react dye intermediates inside of the hair. The intentions of this process are primarily for covering gray and/or changing one’s natural color from brown, for example, to red. Although these 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 INSTRUMENTATION AND EXPERIMENTAL METHODS IN STUDYING THE DAMAGE AND PROTECTION OF HAIR

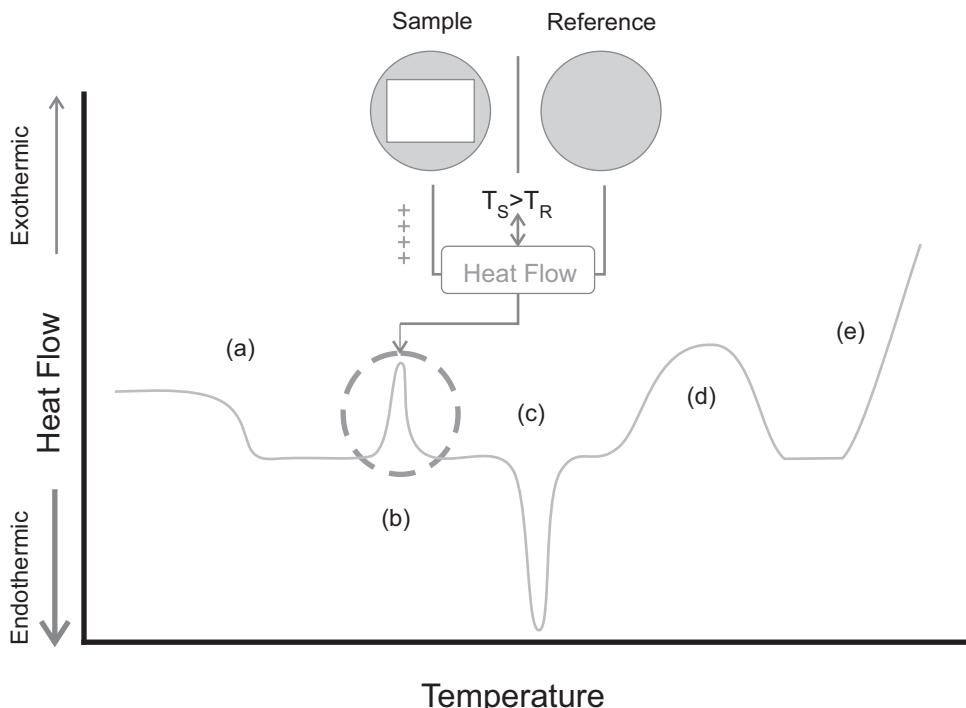
### a. Physical-Chemical

#### 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**).

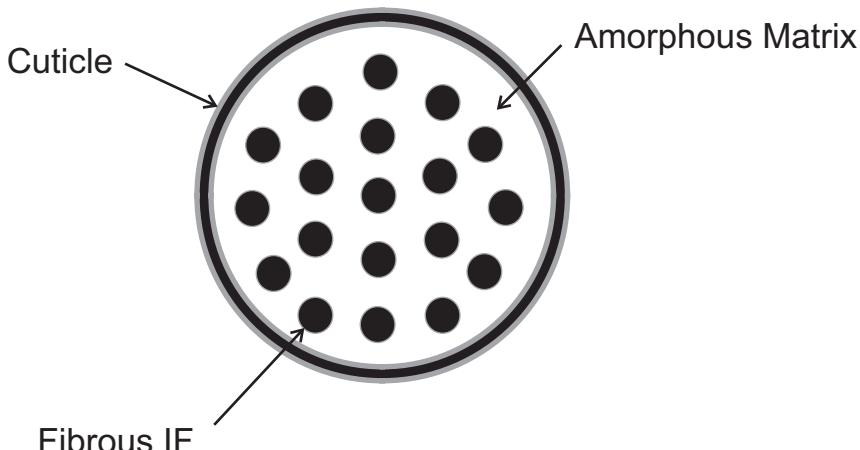


**Figure 3:** Peaks represent positive or negative heat flow (single-furnace). Characteristic DSC thermal transitions scanned from low to high temperature (exotherm points up): (a) glass transition temperature ( $T_g$ ); (b) crystallization temperature ( $T_c$ ); (c) melting temperature ( $T_m$ ); (d) 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  $\alpha$ -keratin to random coils and  $\beta$ -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  $\alpha$ -helix?

The short answer is that water plasticity, in combination with prior chemical alterations, shifts the  $T_D$  of the  $\alpha$ -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  $\alpha$ -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  $\alpha$ -keratin is kinetically controlled by the cystine-based crosslink density and the viscosity of the nonhelical *amorphous matrix*, whereas the energy to denature ( $\Delta H_D$ ) correlates with the structural rigidity of the  $\alpha$ -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].

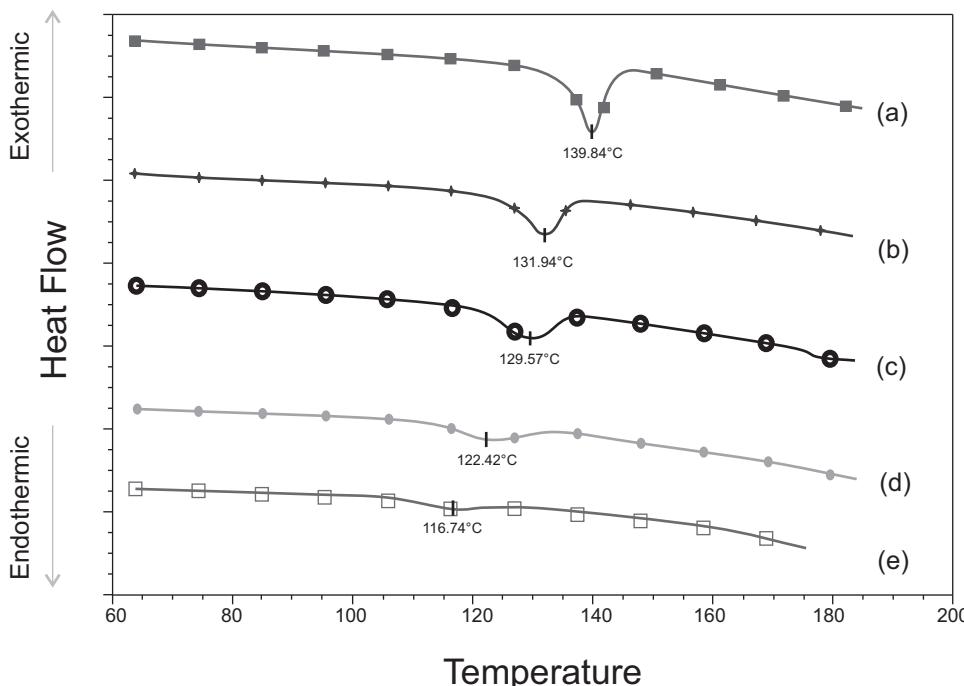


**Figure 4:** Diagram of a transverse section of hair fiber showing the traditional Feughelman (two-phase) morphological model where the composite fibrous intermediate filament(s) phase is immersed within a highly cross-linked amorphous phase.

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  $\alpha$ -keratin structure. The diminishing magnitudes of the  $T_D$  and  $\Delta H_D$ , 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  $\alpha$ -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  $\alpha$ -keratin denaturation.



**Figure 5:** Effect of thermal styling treatment (232°C) on the  $\alpha$ -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. Koelman, 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* ( $\gamma$ ) 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 ( $\gamma$ ), such as a surfactant, will have the propensity to be adsorbed onto a surface,

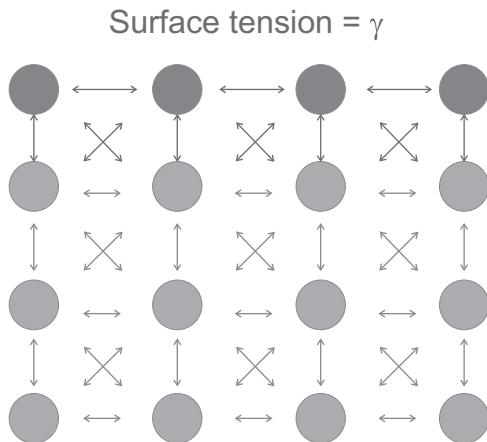
$$\Gamma_i = -1/RT (\partial\gamma/\partial \ln a_i)_{T,P} \quad (1)$$

where  $\Gamma_i$  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<sup>2</sup>), 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,

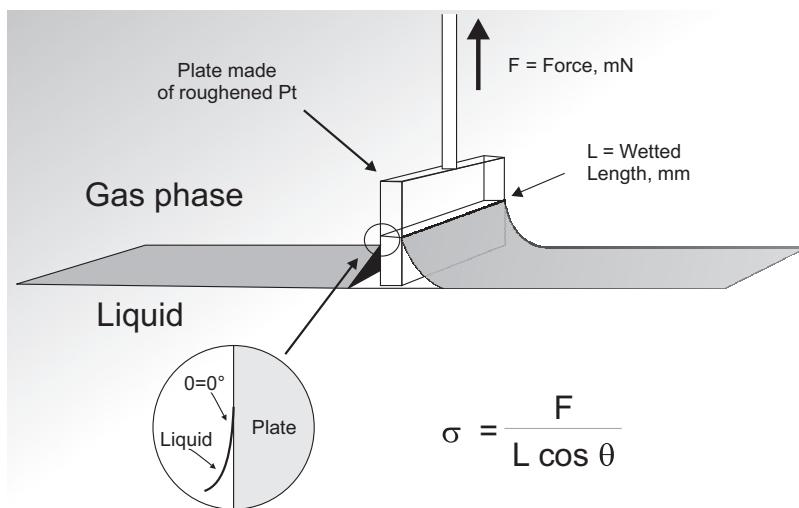
$$\gamma = F/l, \text{ or } \gamma = (\partial G / \partial A)_{T,P,n} \quad (2)$$

where, in either form, Equation 2 infers that it requires energy ( $\Delta G$ ) to extend the surface ( $\Delta A$ ) 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.  $\circ$ =interfacial molecules.  $\bullet$ =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  $\gamma$  (or  $\sigma$  in **Figure 7**).



**Figure 7:** Wilhelmy plate method for determining surface tension.  $L$ =wetted perimeter;  $F$ =force exerted on microbalance;  $\sigma=\gamma$ =surface tension;  $\theta$ =contact angle.  $\theta$  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**).

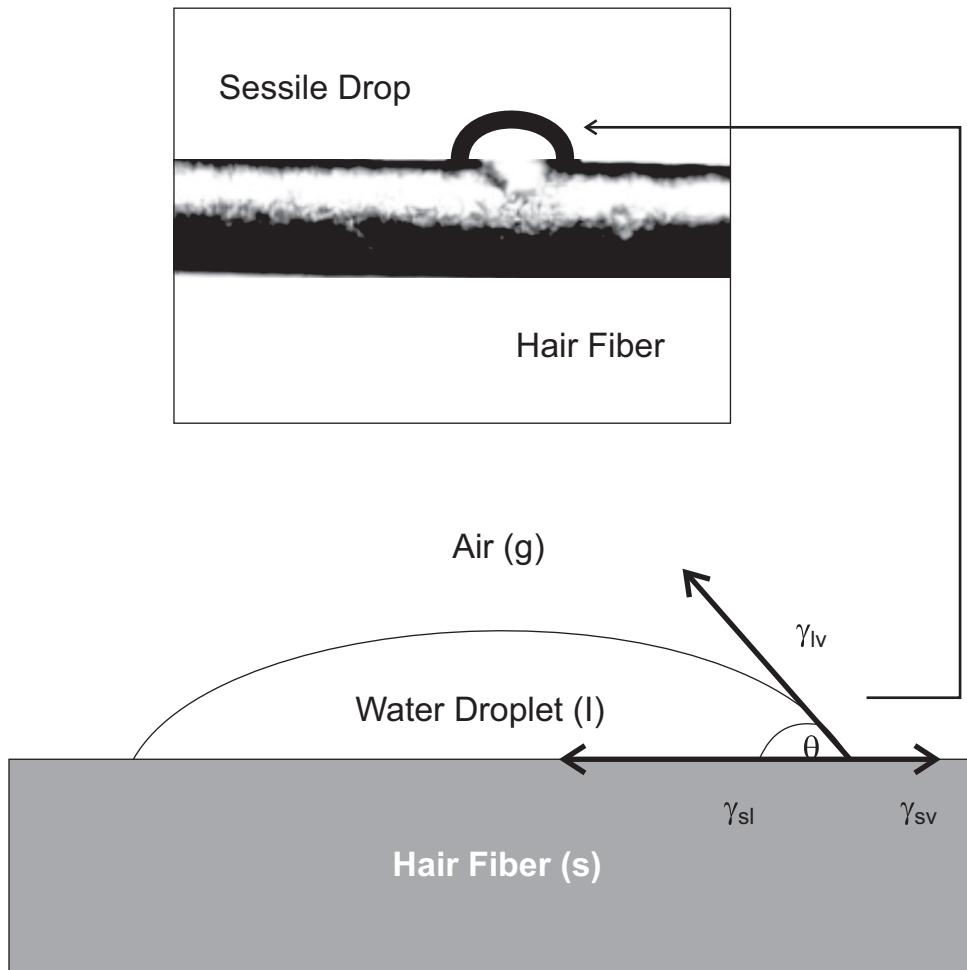
**Table 3:** Description of cohesive and adhesive properties and relation to contact angle [courtesy of ramé-hart Instrument Co.]

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*:  $\gamma_{sl}$  is the surface tension of the solid-liquid interface,  $\gamma_{sv}$  is the surface tension of the solid-vapor interface, and  $\gamma_{lv}$  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).

$$\cos \theta = (\gamma_{sv} - \gamma_{sl}) / \gamma_{lv} \quad (3)$$

As indicated in **Table 3**, at  $\theta > 90^\circ$  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  $\theta = 0^\circ$  (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 ( $\gamma_{lv}$ ), solid-liquid ( $\gamma_{sl}$ ), and solid-vapor ( $\gamma_{sv}$ ) interfacial tensions.

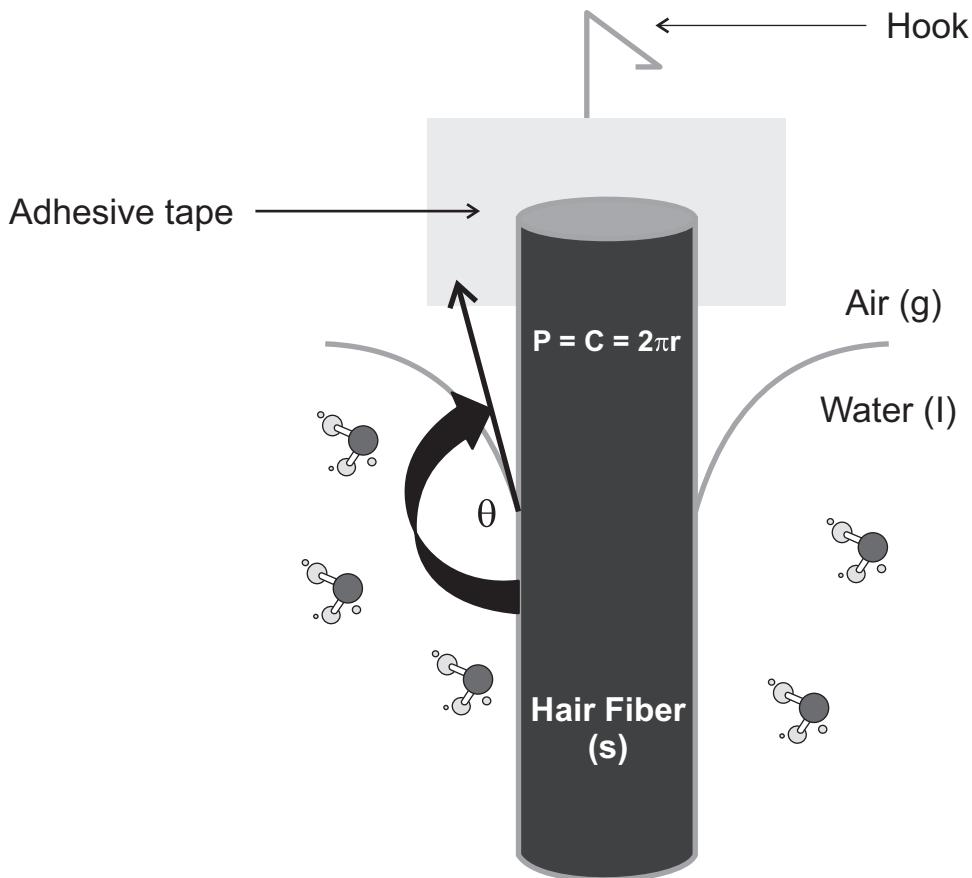
The advantage of the sessile drop method is that multiple measurements on the sample surface may be used to gauge surface energy heterogeneity. That is, if measurements are done carefully, the variation between contact angle measurements will be indicative of the wetting variability as well as the gradation 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 re-ceded 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,

$$F = \gamma P \cdot \cos\theta_{a,r} + W - \rho gyA, \quad (4)$$

where  $F$  is the maximum measured force,  $P$  is the wetted perimeter of the fiber,  $\theta_a$  is the advancing contact angle,  $\rho$  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* ( $\theta_r$ ) 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  $\cos \theta = F/\gamma P$ , where  $P$  is the wetted perimeter of the solid,  $\theta$  is the advancing or receding contact angle, and  $F$  is the wetting force corrected for buoyancy and fiber mass.  $P$  is approximately  $2\pi r$  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 ( $\theta_a$ )
1	1	72
2	2	73
3	3	67
4	4	99
5	5	101
6	6	103
<b>Root</b>		

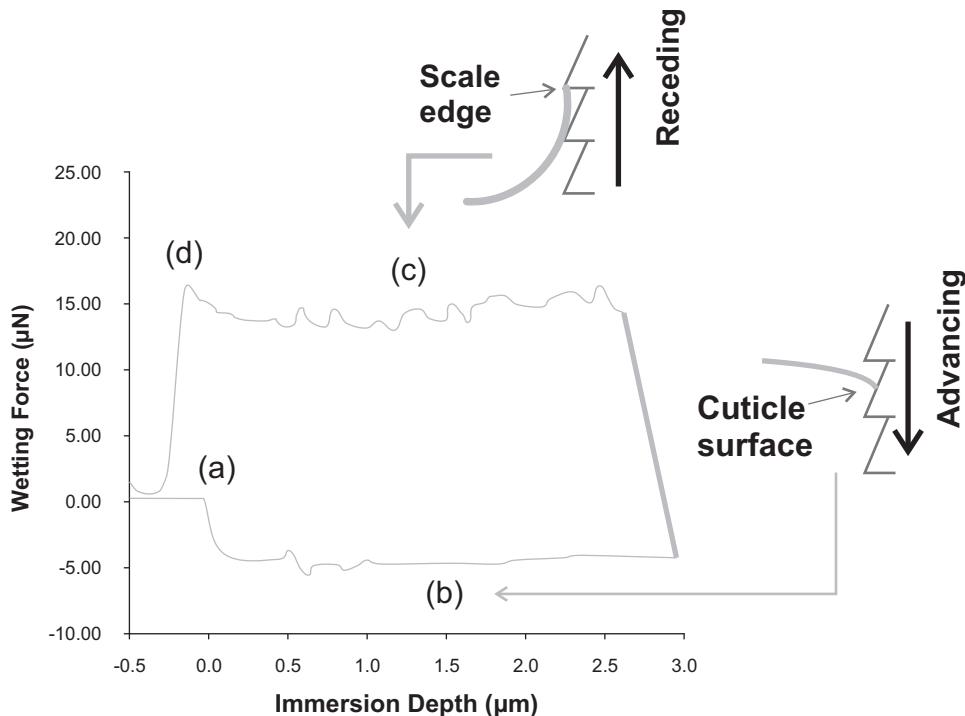
Using Wilhelmy techniques, Molina et al. [37] studied the impact of chemical strains on the physicochemical properties of the integument. They evaluated the application of alkaline solutions with and without 0.6% H<sub>2</sub>O<sub>2</sub> and compared to untreated hair. Because hydrogen peroxide is 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,

$$W_{SL} = W_{SL}^{LW} + W_{SL}^{AB}, \quad (5)$$

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],

$$W_{SL}^{AB} = \lambda_L (1 + \cos \theta) - 2(\lambda_L^{LW} \cdot \lambda_S^{LW})^{1/2}, \quad (6)$$

where  $\lambda_L$  is the surface tension of the dry solid,  $\lambda L_{LW}$  is the dispersive component of the surface tension for the liquid, and  $\lambda S_{LW}$  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.

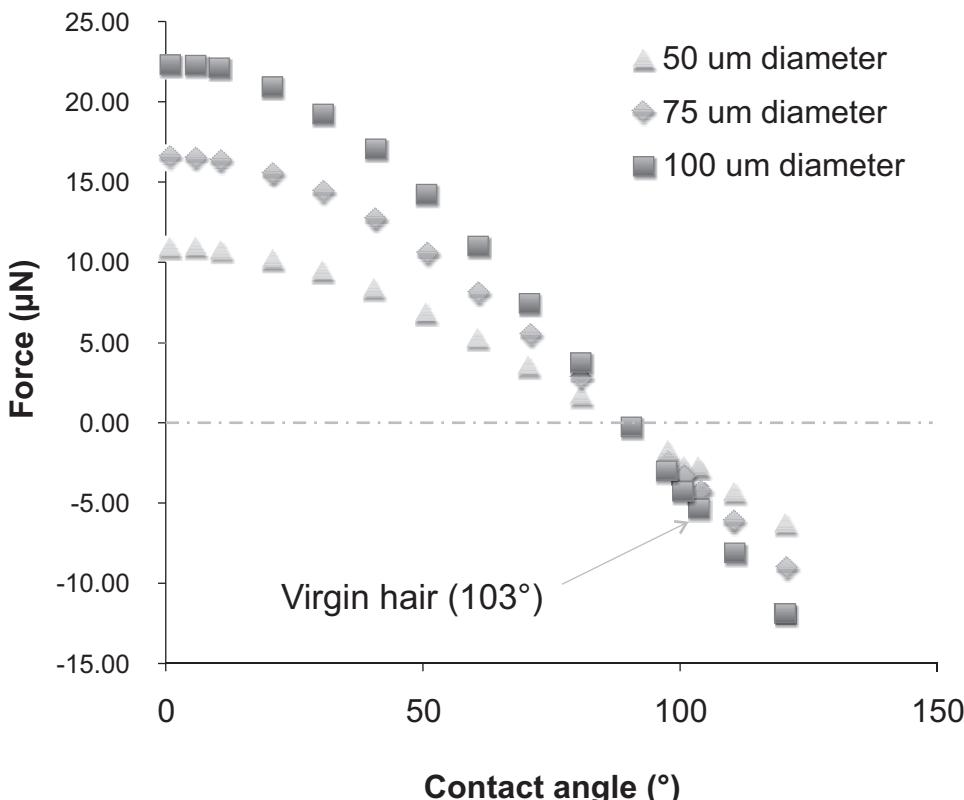


**Figure 10:** Wetting force as a function of immersion depth for a virgin hair fiber. The fibers are typically mounted in the against-scale direction. (a) Fiber penetrating the liquid interface; (b) fiber advancing into liquid surface; (c) fiber receding from interface; (d) meniscus breaks from fiber. The insets depict one reason for contact angle hysteresis ( $\theta_a - \theta_p$ ). 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* ( $\lambda_c$ ), where plotting  $\cos(\theta)$  versus  $\lambda_h$  with test liquids and extrapolating to  $\theta=0$  to find  $\lambda_c$ , Kamath [37] detailed the  $\lambda_c$  wetting argument into dispersive and polar terms,

$$\lambda_c = \lambda_s^d + \lambda_s^p, \quad (7)$$

where  $\lambda_c$  is the critical surface tension, and  $\lambda_{ds}=24$  mN/m and  $\lambda_{ps}=4$  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  $\lambda_c$  and  $\lambda_s^p$  – 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  $\theta_A$  is approximately  $103^\circ$  [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 ( $70\pm 7^\circ$ ) and with the application of conditioner ( $88\pm 9^\circ$ ) to virgin hair ( $103\pm 4^\circ$ ); however, application of commercial conditioner to permed hair ( $79\pm 1^\circ$ ) 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  $\theta_r$  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  $\lambda_c$  than the cuticle surface, and that the hydrophobic oils have  $\lambda$  less than the  $\lambda_c$  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.

### Incomplete wetting on surface



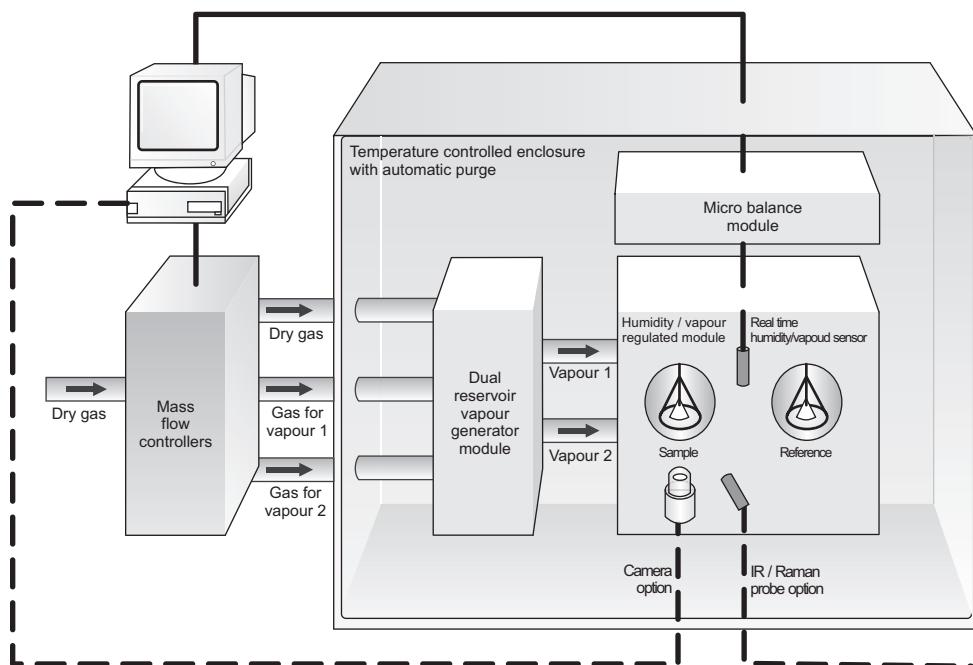
**Figure 12:** Preferential wetting of virgin hair by squalane. Squalane spreads uniformly across the relatively polar cuticle edge, whereas sparse wetting is visible on the hydrophobic cuticle surface.

### 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  $\alpha$ -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 (%)	$W_m$ (%)	$C_g$	$t_r$ (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

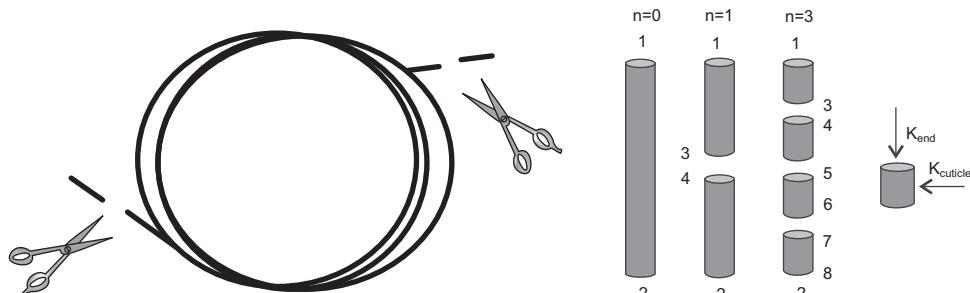
The authors rationalized the results in **Table 5** by noting that oxidative degradation mechanisms corrupt the surface and the way hair naturally intermingles with water; therefore, although perming significantly increased the maximum regain, bleaching is considered to have the most detrimental effects on hair by rendering the fibers more permeable to water. Bleaching lessens  $W_m$ , 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 ( $t_r$ ); however, neither treatment significantly affects  $C_g$ . 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  $D_A$ ,  $t_r$ , and  $W_m$  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:

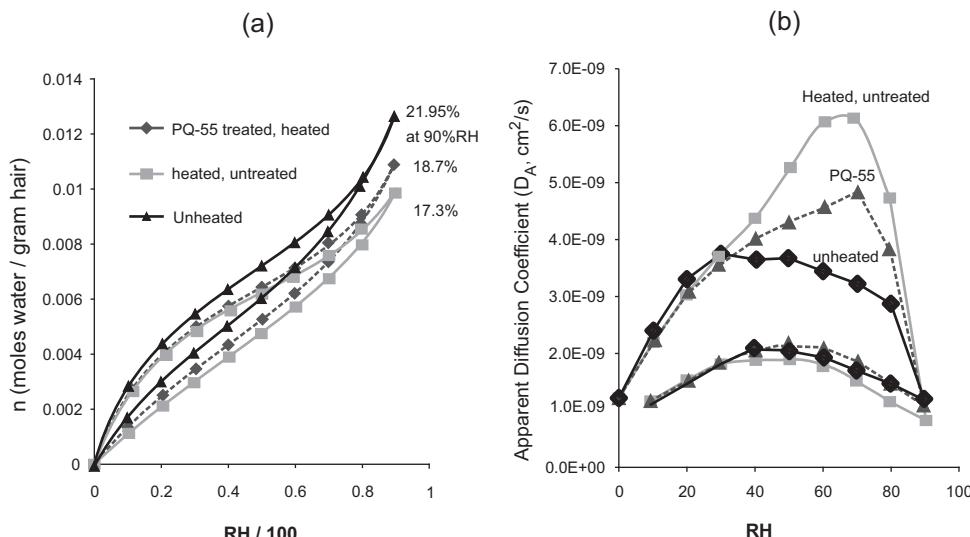
1. Preconditioning: Prior to drying of the loops, samples were exposed to 95% RH for 1 hour to minimize the variations in “humidity history” (56).
2. Initial drying: The loops were dried at 25°C and 0% RH for 12 hours.
3. Absorption curve: The humidity was ramped from 0% to 90% in 10% RH steps. Each 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.

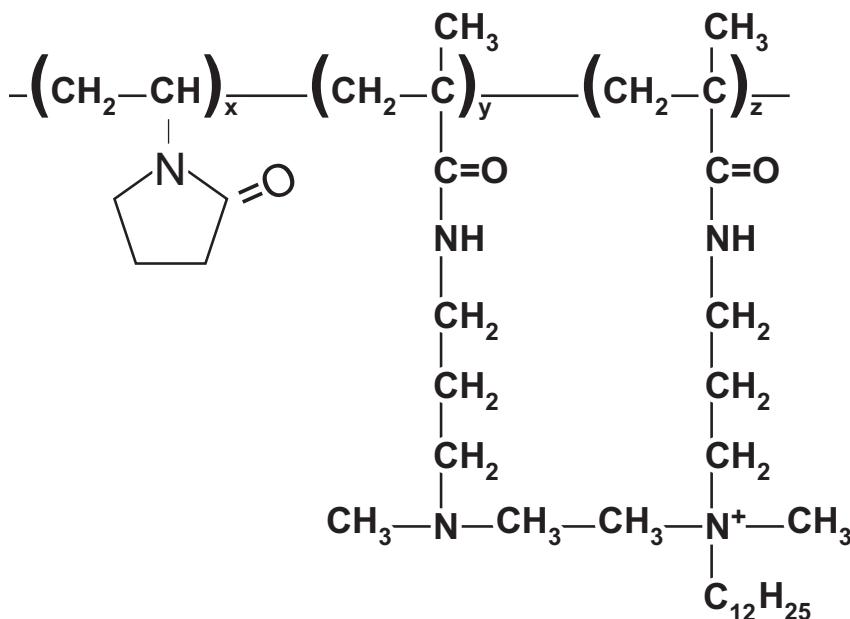
**Figure 15** shows the DVS isotherm and  $D_A$  overlays for the unheated, heated and untreated, and Polyquaternium-55 (PQ-55) treated and heated hair tresses (PQ-55, **Figure 16**). Note that the maximum water vapor regain trend is unheated > PQ-55 treated and heated > heated and untreated (Fig. 3a). 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  $\alpha$ -keratins denaturing to  $\beta$ - 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  $\alpha$ -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:

$$R_{\max} = M_v / M_D * 100 \quad (8)$$

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

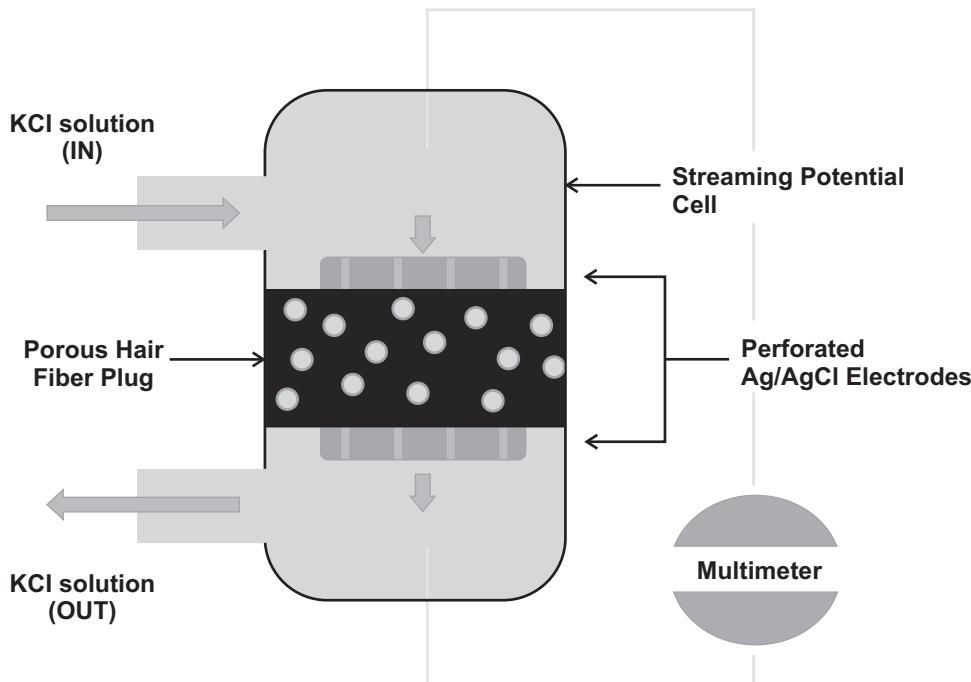
Streaming Potential, or *Dynamic Electrokinetic and Permeability Analysis* (DEPA), incorporates the measurement of electrochemical properties while a flowing test solution interacts with the surface of a planar or fibrous sample. It has been used to study the adsorption and desorption of various ingredients on hair [68, 69, 70]. The empirical data for a single DEPA experiment includes the streaming potential ( $E$ ), conductivity ( $\kappa$ ), pH, liquid flow rate, and temperature—all measured as a function of flow volume and time. The zeta potential,  $\zeta$ , 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),

$$\zeta = 4\pi\eta\kappa E / Pe, \quad (9)$$

where  $P$  is the drive pressure applied to the flowing liquid, and  $\eta$  and  $\varepsilon$  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 ( $5 \times 10^{-5}$  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  $E$ ,  $\kappa$ , flow rate, and  $\zeta$ . 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  $\zeta$  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,  $E$ ,  $\zeta$ , and  $\kappa$  data may be used to better design intelligent treatments to ameliorate fiber damage.



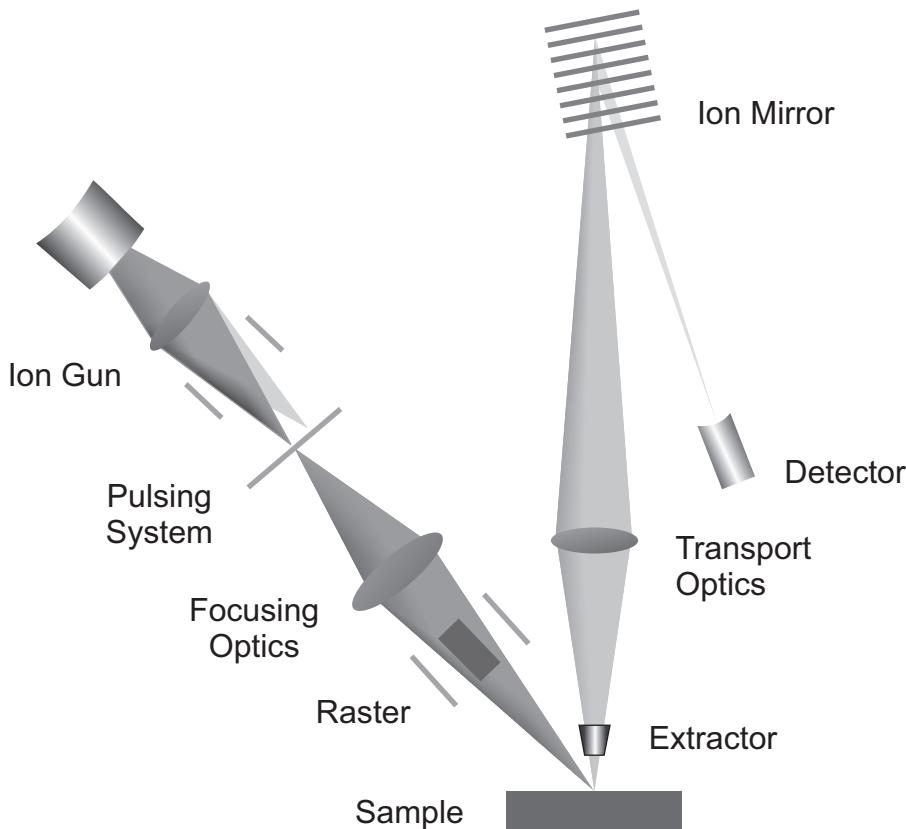
**Figure 17:** Schematic of Streaming Potential Cell showing perforated Ag/AgCl electrodes surrounding the compressed hair fiber plug (Adapted from Dynamic Electrokinetic Analyzer manual, Better Cosmetics LLC, US).

### 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**).

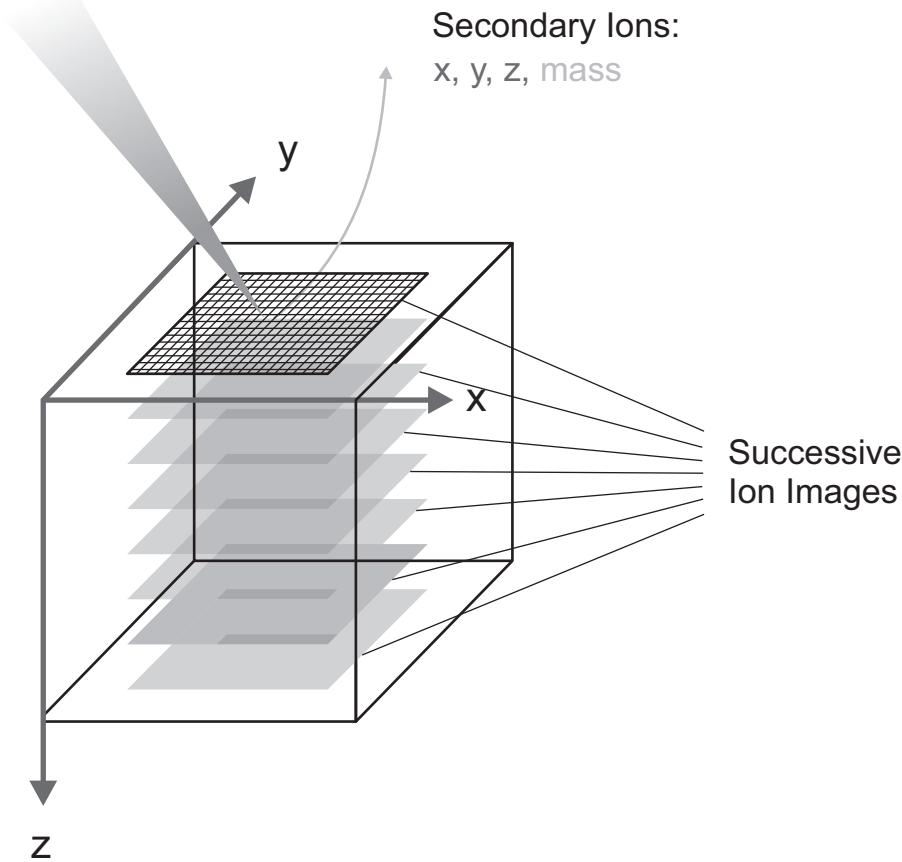
In its simplest mode, ToF-SIMS provides a mass spectrum of the region of interest. However, by rastering the primary ion beam across the surface, two-dimensional images are generated to view the spatial distribution of specific chemical moieties as a function of x-y space. For each pixel in the image, 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 matrices (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).

### Scanned Primary Ion Beam

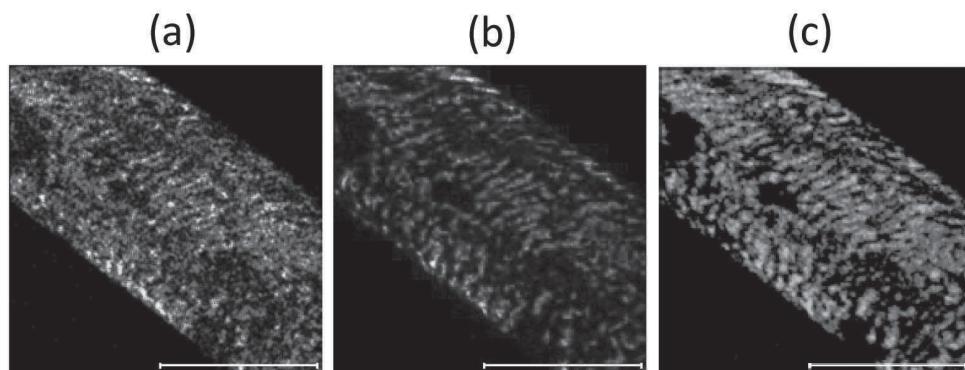


**Figure 19:** The primary ion beam may be rastered on a microarea of the sample to generate a 2-D image. By using a sputter beam to remove successive surface layers, a depth profile of the substrate (3-D) may be generated to view the layers of substrate below the surface monolayer (courtesy of ION-TOF 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  $\text{H}_2\text{O}_2$ , and others were treated with UV light ( $3500 \mu\text{W}/\text{cm}^2$ ) for various exposure times (48–480 hrs). For each studied fiber, a  $75 \times 75 \mu\text{m}$  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)  $\text{CNO}^-$  moiety, from surface protein, (b) ( $\text{lauryl sulfate}$ )<sup>-</sup> from shampoo residue, (c) and (18-MEA)<sup>-</sup> 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  $\sim 100^\circ$  to  $\sim 57^\circ$ . 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  $65^\circ$  to  $\sim 90^\circ$ ) 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 ( $\pm 0.1\text{--}1.0$  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  $10\ \mu\text{m}$ , 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.

$$\text{Binding energy} \approx \text{X-ray energy} - \text{Kinetic energy of emitted electron} \quad (10)$$

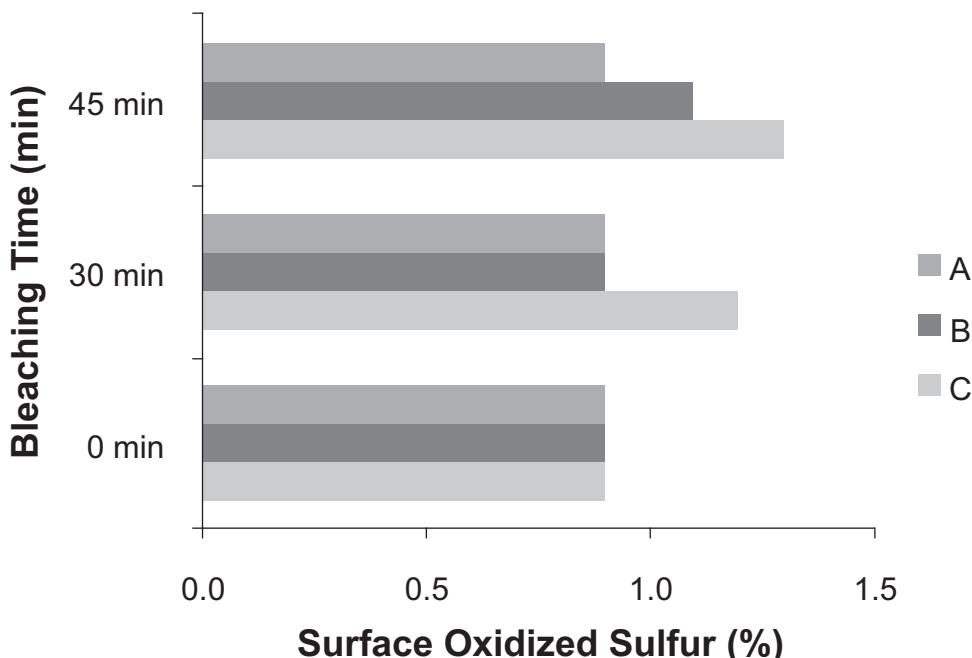
In combination with SEM, Beard et al. [84] studied the application of surface specific XPS to spectroscopically discern the effects of bleaching and subsequent conditioning on the surface chemistry of hair fibers. The impact of pH and the addition of surfactant on the efficacy of the bleaching process 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 ( $\text{Al } k_{\alpha}$  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  $\text{H}_2\text{O}_2$ .

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  $\text{Cl}^-$ . 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  $\text{Cl}^-$  counterion is absent because it is released when the  $\text{N}^+$  of the ester quat directly associates with the anionic sulfonic functionalities of the oxidized protein surface.



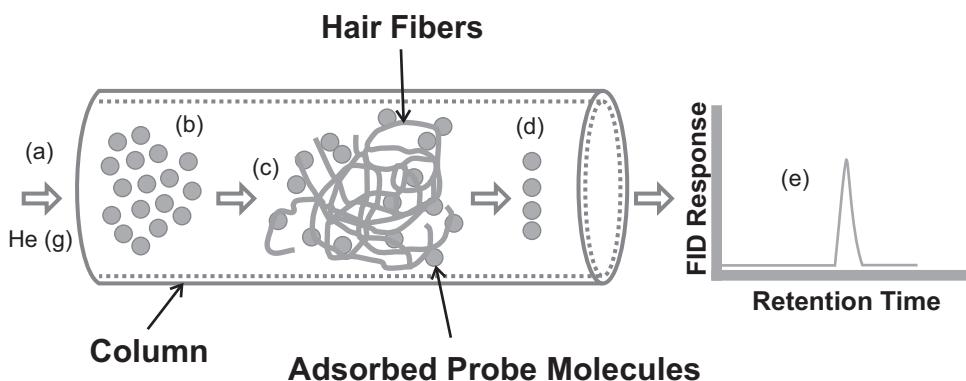
**Figure 21:** Total oxidized surface sulfur content (cysteic acid) by XPS for (A) bleached hair, (B) bleached hair, pH=9, (C) bleached hair, pH=9, SLS. Comparing B and C at 30 min., note that the addition of SLS eliminates the inherent induction period for surface oxidation (adapted from Beard et al. [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).

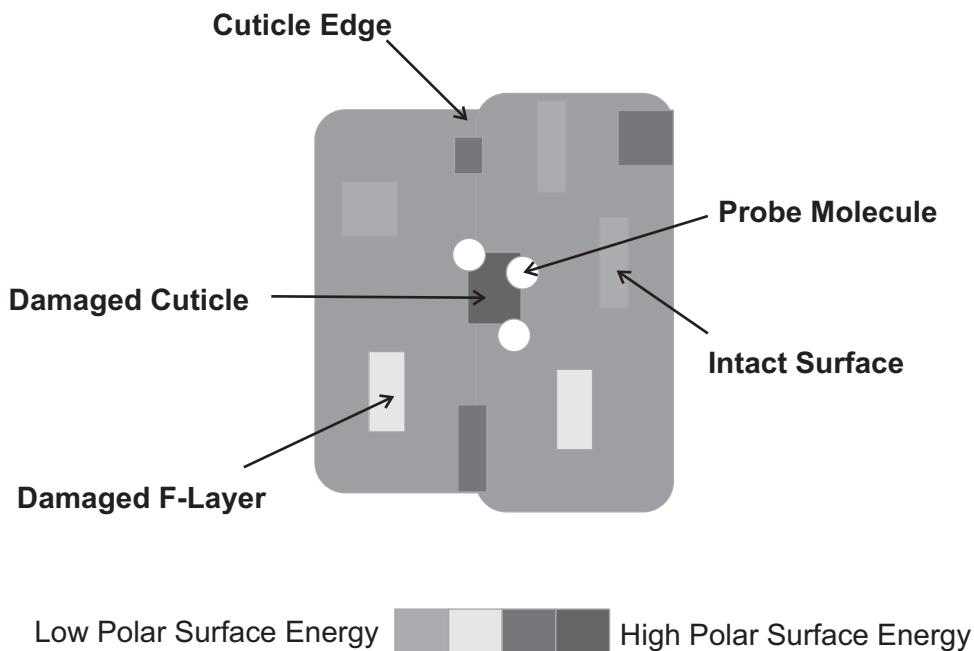


**Figure 22:** Diagram of an IGC experiment showing the interaction of probe vapor with intact hair fibers that have been packed into a custom-sized chromatography column. (a,b) Helium carrier gas and vapor pulse introduced to the column head; (c,d) retention time of the probe molecules delayed by fiber-probe 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  $\mu\text{m}$  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 ( $\gamma_s^{\text{Tot}}$ ), which is a sum of the dispersive (nonpolar,  $\gamma_s^D$ ) and acid-base (polar,  $\gamma_s^{AB}$ ) surface energy components, respectively (Eq. 11).

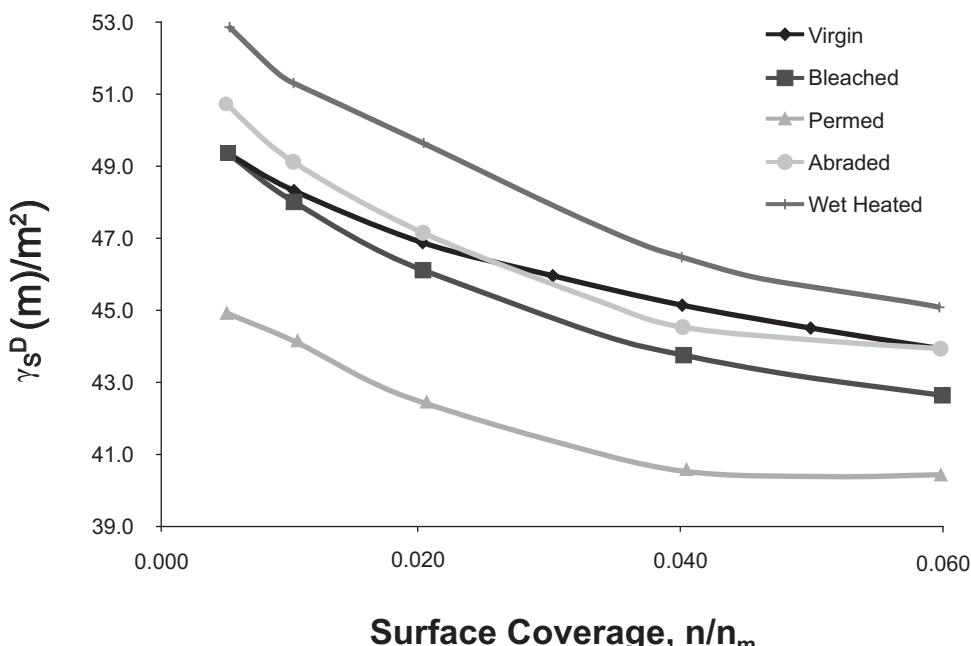
$$\gamma_s^{\text{Tot}} = \gamma_s^D + \gamma_s^{AB} \quad (11)$$

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.

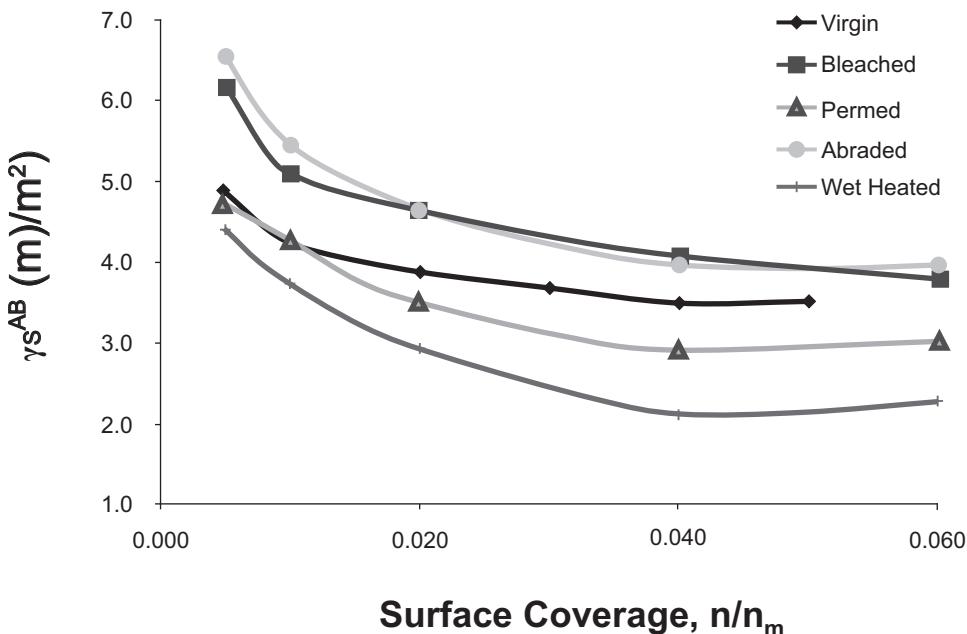
**Table 6:** Details of treated European Dark Brown hair tresses later examined by IGC

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.

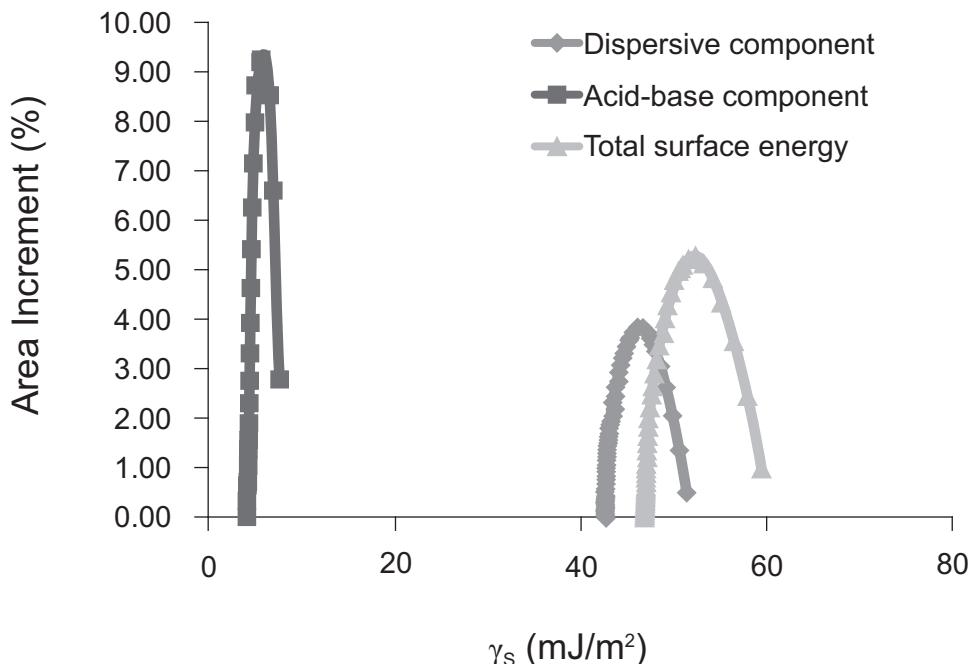


**Figure 24:** Dispersive surface energy profiles overlay for virgin, bleached, permed/conditioned, abraded, and flat-ironed wet hair fiber bundles. Pulsing discrete volumes of nonpolar probes into the column controls the fraction of probe-covered surface, where 100% coverage = 1.00 (i.e.,  $n = n_m$ ), 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).



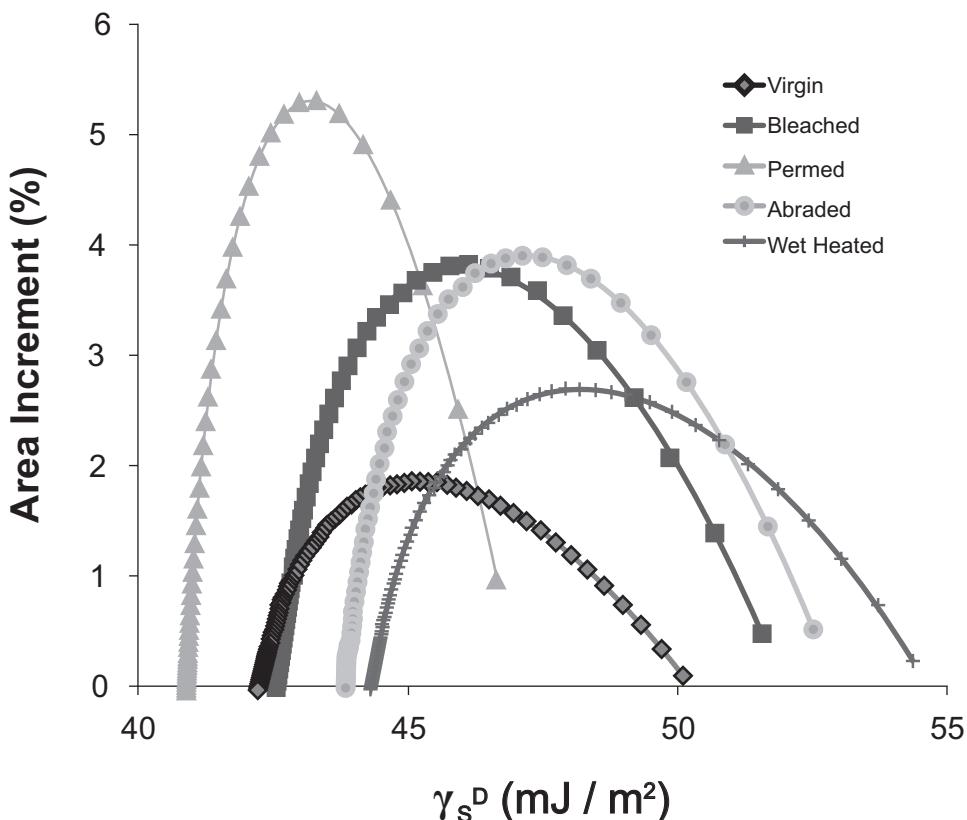
**Figure 25:** Specific (acid-base) surface energy profiles overlay for virgin, bleached, permed/conditioned, abraded, and flat-ironed wet hair fiber bundles. Pulsing discrete volumes of polar probes into the column controls the fraction of probe-covered surface, where 100% coverage = 1.00 (i.e.,  $n = n_m$ ), 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  $\gamma_S^{Tot}$  distribution more resembles  $\gamma_S^D$  rather than  $\gamma_S^{AB}$ . Hence, the contributions to  $\gamma_S^{Tot}$  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).

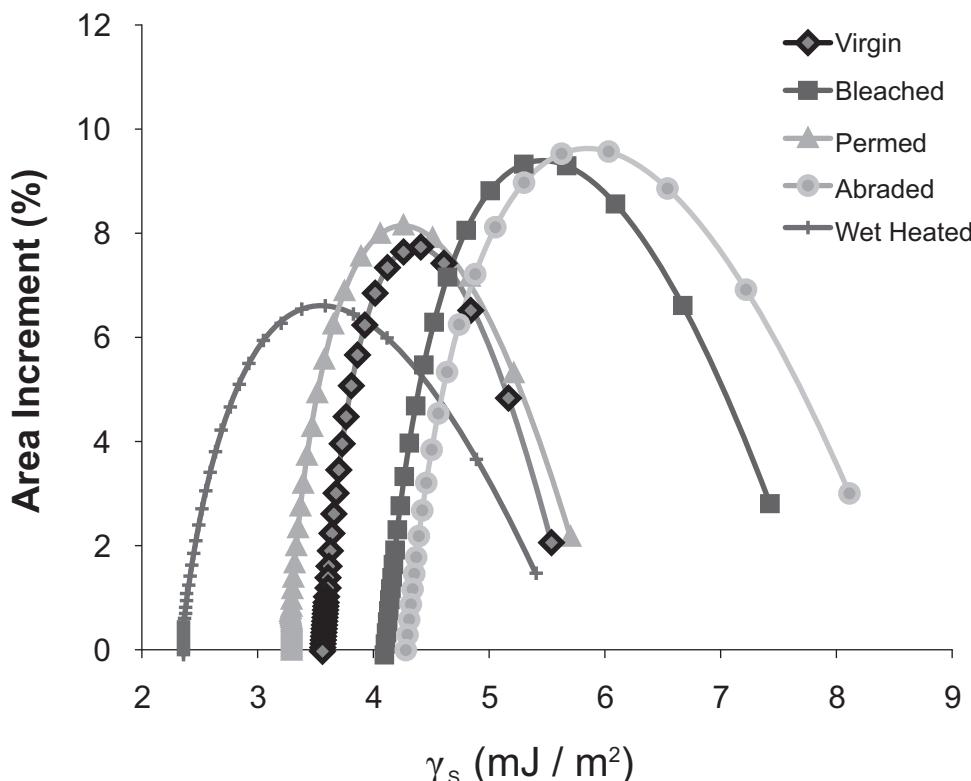
The trends in the dispersive and acid-base energy profile/distributions in Figures 24–28 clearly suggest that abrading, bleaching, perming/conditioning, thermal abuse, and associated grooming and SLS washing processes modify the accessible surface chemistry of the respective cuticles. Heavy abrading 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  $\gamma_s^D$  *surface heterogeneity*, which is gauged from the breadth (i.e., highest  $\gamma_s^D$  - lowest  $\gamma_s^D$ ) in each dispersive surface energy profile in the overlay (**Figure 24**), as follows: wet flat-ironed > abraded  $\approx$  **virgin** > bleached > permed. The  $\gamma_s^D$  *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<sup>2</sup>) > abraded (52.52 mJ/m<sup>2</sup>) > bleached (51.51 mJ/m<sup>2</sup>) > **virgin** (50.07 mJ/m<sup>2</sup>) > permed (46.68 mJ/m<sup>2</sup>). 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  $\gamma_s^D$  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

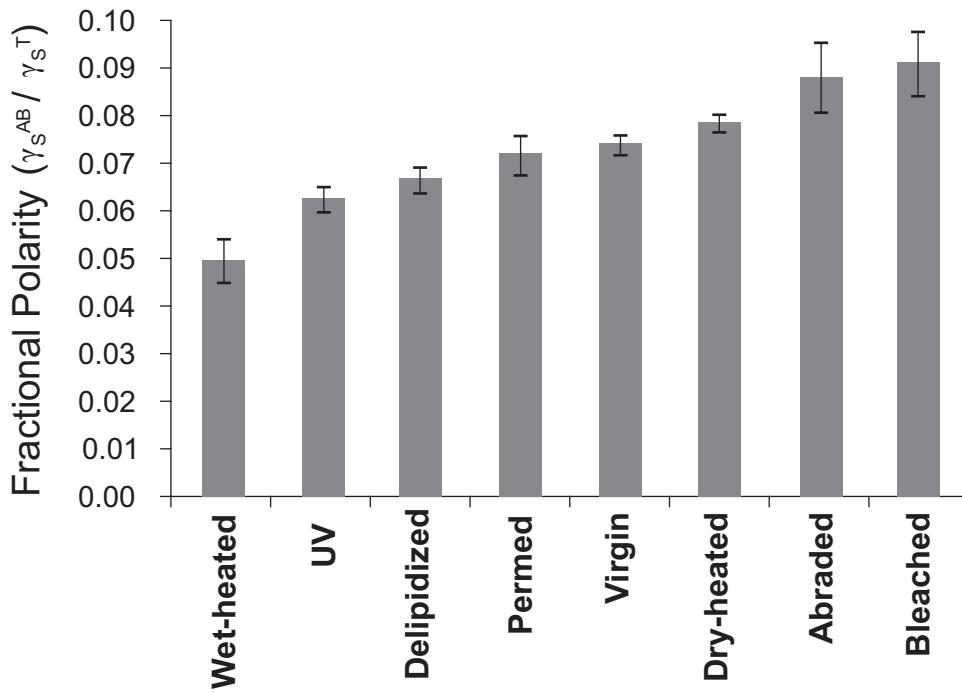
involved, treatments involving heat, abrasion, perming, and bleaching may add uniform submicron-level surface roughness to the cuticle surface; hence, it is likely that the number of active adsorption sites increased accordingly. Interestingly, bleaching and the lengthy perming process produced the narrowest  $\gamma_s^D$  heterogeneity and lowest  $\gamma_s^D$  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  $\gamma_s^D$  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  $\gamma_s^D$  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  $\gamma_S^{AB}$  heterogeneity ranked in the order: abraded  $\approx$  wet flat-ironed  $>$  permed  $>$  **virgin**  $\approx$  bleached; whereas the trend in  $\gamma_S^{AB}$  peak maxima positioned in the following way: abraded ( $8.10 \text{ mJ/m}^2$ )  $>$  bleached ( $7.42 \text{ mJ/m}^2$ )  $>$  permed ( $5.68 \text{ mJ/m}^2$ )  $\approx$  **virgin** ( $5.53 \text{ mJ/m}^2$ )  $\approx$  wet flat-ironed ( $5.39 \text{ mJ/m}^2$ ). The virgin and bleached samples had the narrowest  $\gamma_S^{AB}$  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  $\gamma_S^{AB}$  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  $\gamma_S^{AB}$  surface energy heterogeneity. The  $\gamma_S^{AB}$  maxima rankings imply that the abraded and bleached samples have elevated fractions of accessible higher energy  $\gamma_S^{AB}$  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  $\gamma_S^{AB}$  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  $\gamma_S^{AB}$  to  $\gamma_S^{Tot}$  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)

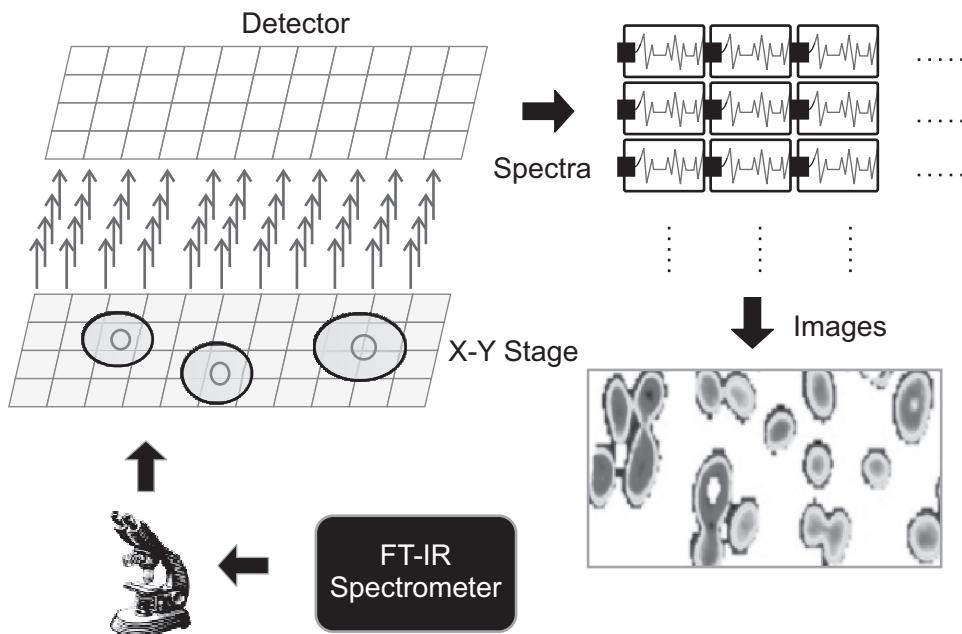
Infrared (IR) light is radiation that resides between the visible and microwave regions of the electromagnetic continuum. The IR spectrum is arbitrarily divided into the near, mid, and far infrared spectral regions. The wavelength ( $\lambda$ ) of near IR (0.8–3.0  $\mu\text{m}$ ) is closest to red visible light, whereas the wavelength range of far IR (30–300  $\mu\text{m}$ ) is nearest to the microwave region. Since mid IR (3.0–300  $\mu\text{m}$ ) 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 ( $\bar{v}$ ), which are in units of  $cm^{-1}$  (i.e.,  $\lambda^{-1}$ ). Note that the term “absorption frequency” is synonymously interchanged with “wavenumber” because the two magnitudes are both proportional to the imposed IR energy.

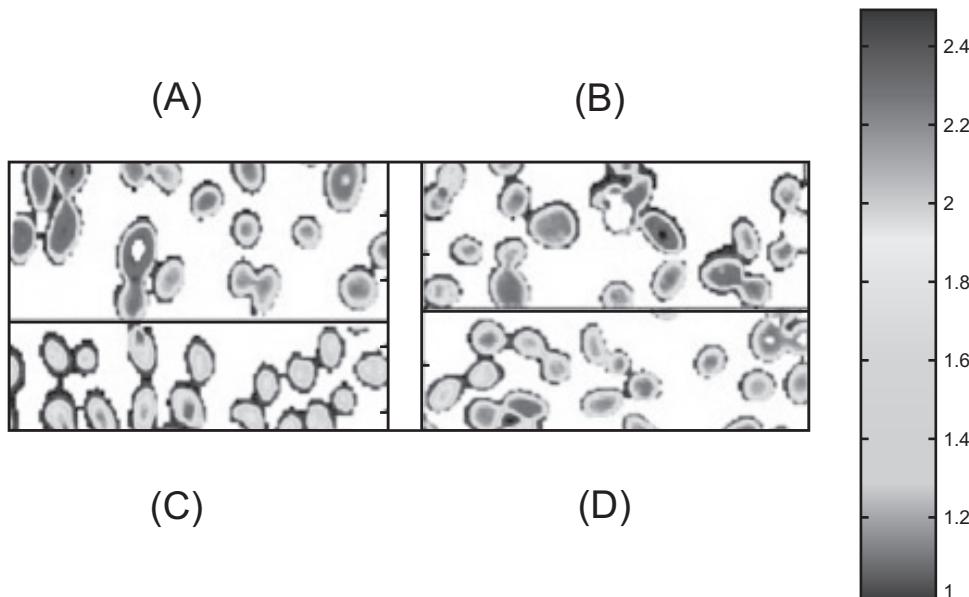
A modern FT-IR instrument uses an interferometer to produce a superimposed interference spectrum called an interferogram that contains a complete description of all of the IR frequencies produced by the IR source. The produced interferogram is then passed through the sample, which subsequently absorbs 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  $\mu\text{m}$  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  $\mu\text{m}$  thick cross-sections that were collected on CaF<sub>2</sub> 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:**  $-\text{CH}$  asymmetric stretching band ( $2960 \text{ cm}^{-1}$ ) from  $-\text{CH}_3$  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  $6.25 \mu\text{m} \times 6.25 \mu\text{m}$  x-y area coordinate on the sample surface. More specifically, the pixels correspond to the relative concentration of terminal  $\text{CH}_3$  residues in the fiber protein (with a minor contribution from the integral lipid), which absorb mid-IR energy at  $2960 \text{ cm}^{-1}$ . 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  $2960 \text{ cm}^{-1}$  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  $\alpha$ -helix cortical hydrogen bonding, which affected the quality and sustainability of the secondary structure (e.g., irreversible  $\alpha$ -helix conversion to  $\beta$ -sheet).

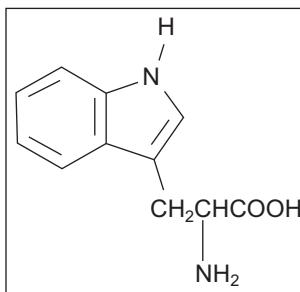
IR Imaging is an indispensable tool for viewing molecular changes to materials such as keratinous substrates. By coupling the technique with other analytical tools, such as DSC, DVS, and sensorial appeal, it may be possible to directly relate claims and performance attributes to trends and variations 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

$$\text{Ratio of tryptophan intensity} = \frac{\text{Emission intensity at } 336 \text{ nm of experimental}}{\text{Emission intensity at } 336 \text{ nm of reference}} \quad (12)$$

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:

$$\left( \frac{\text{ratio tryptophan intensity before treatment} - \text{ratio tryptophan intensity after treatment}}{\text{ratio tryptophan intensity before treatment}} \right) \times 100\% \quad (13)$$

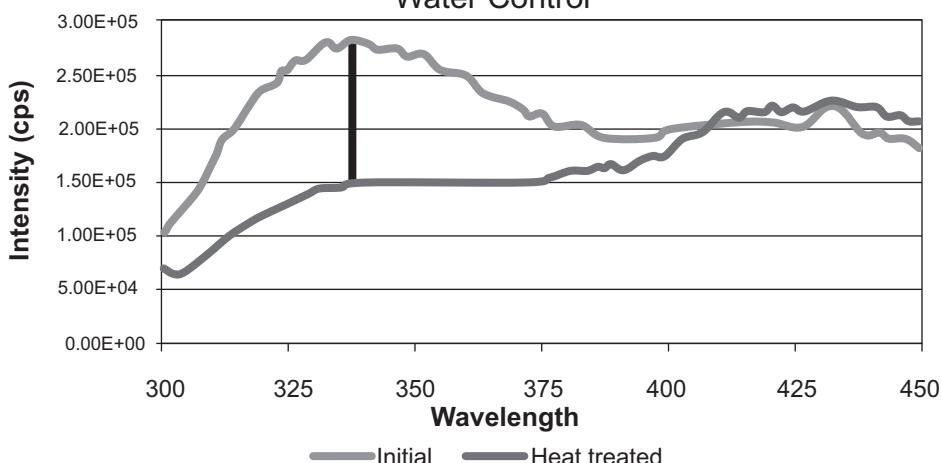
% Protection is calculated as:

$$\left( \frac{\% \text{ degradation of control} - \% \text{ degradation of experimental}}{\% \text{ degradation of control}} \right) \times 100\% \quad (14)$$

**Figures 33 and 34** provide examples of fluorescent spectra 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.

### Flourescence Analysis

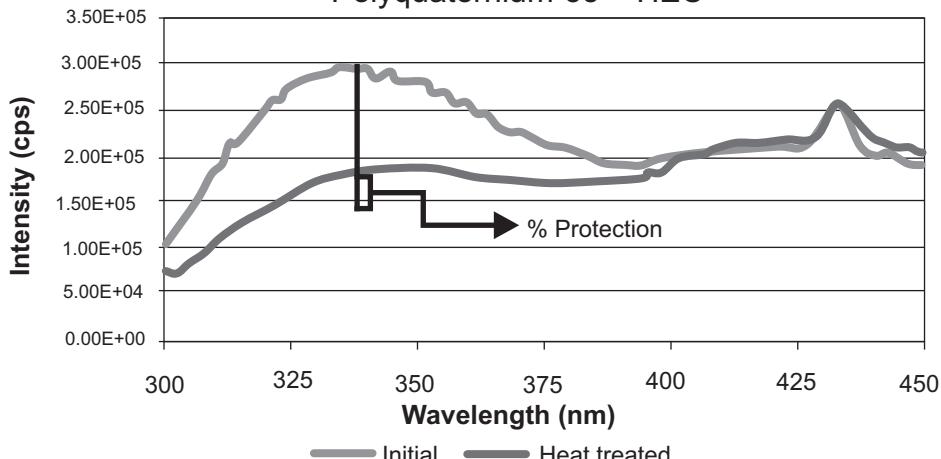
#### Water Control



**Figure 33:** Fluorescent spectrum of control tress before and after thermal exposure

### Flourescence Analysis

#### Polyquaternium-55 + HEC



**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 indispensable 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***

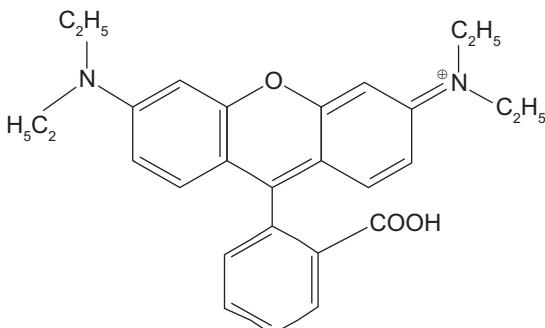
When a material absorbs and subsequently re-radiates light, fluorescence or phosphorescence occurs. Fluorescence, as was noted in the section on Spectrofluorimetry, occurs almost instantaneously (in the time frame of a microsecond), whereas phosphorescence consists of a longer lifetime emission after 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].

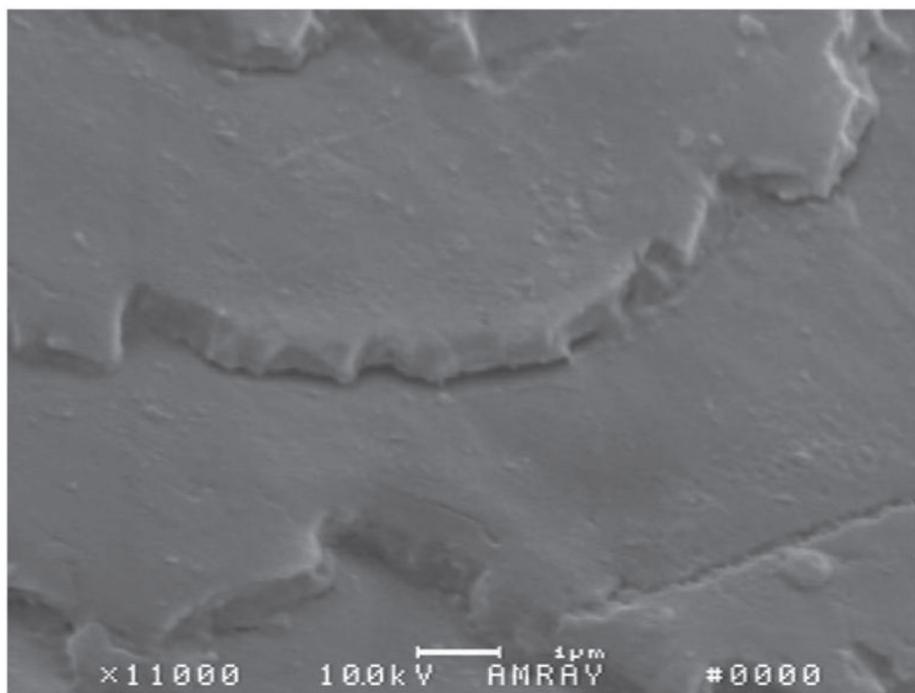
Polymer deposition was also characterized by fluorescein-labeled polyquaternium-10. Here the fluorophore was covalently linked to the reactive sites on the parent polymer. The synthetic pathway and initial deposition results are described by Goddard and Winnik [121]. As indicated by microspectrophotometry, 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)**

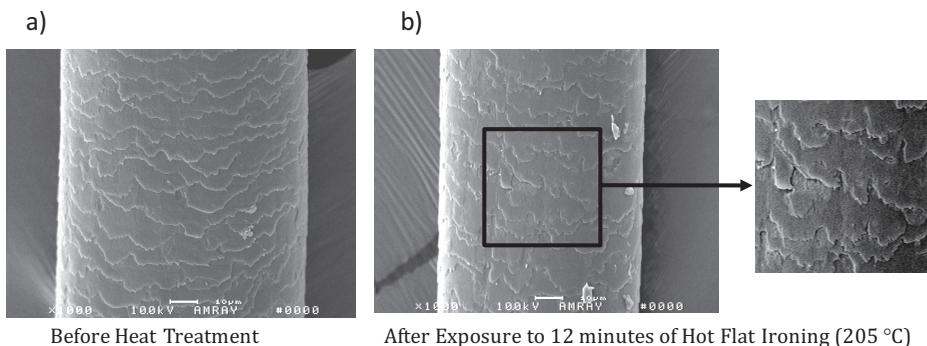
This technique has been utilized extensively in hair research. Technical articles abound with high-resolution SEM photomicrographs of the surface of hair. These have illustrated, for example, characteristics of surface morphology for forensic evidence, surface damage through the effects of chemical treatments, 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.

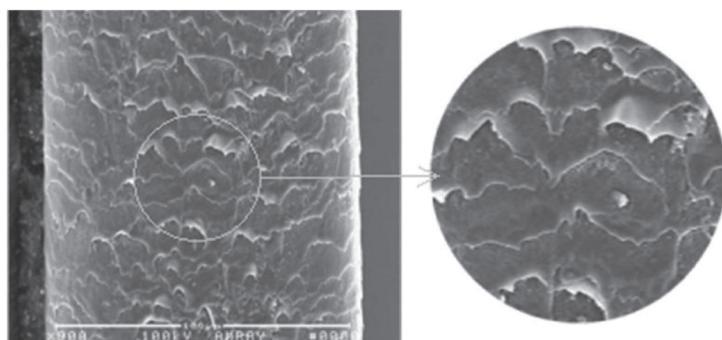


**Figure 36:** High magnification of the cuticle using SEM (B. Thompson, Ashland Analytical Dept.)

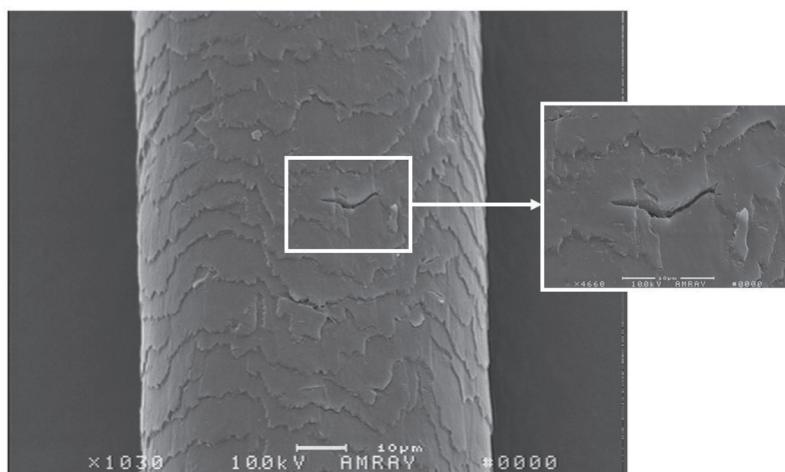
SEM has shown great utility in capturing various types of hair damage. Brown and Swift have used it as a diagnostic tool to show hair damage from various straining experiments (39). Examples of some of the different types of hair damage are portrayed in **Figures 37 through 40**. Protein denaturation 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.



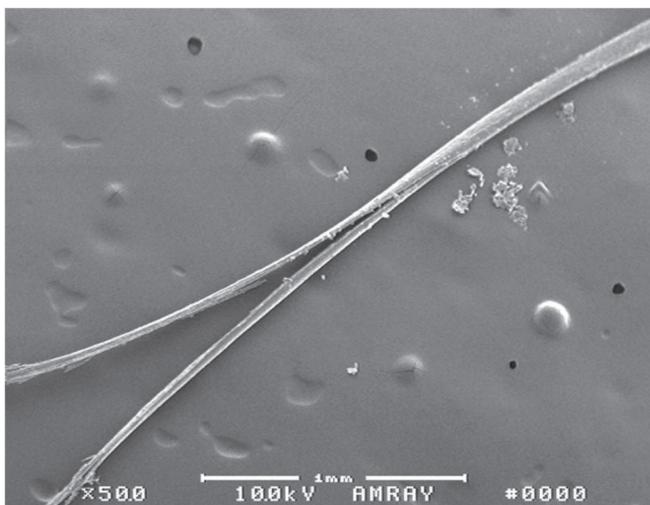
**Figure 37:** SEM of a) undamaged fiber; b) heat treated fiber (B. Thompson, Ashland Analytical Dept.)



**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

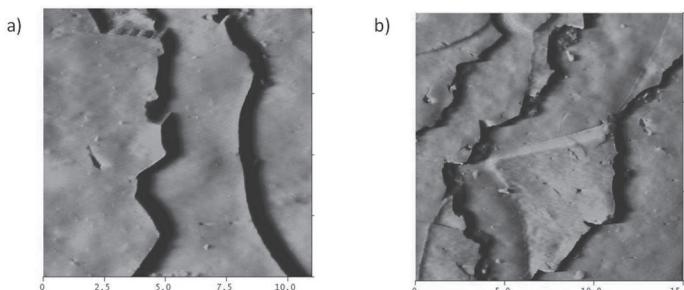
and its utility in the personal care industry has advanced over the last two decades and it is anticipated that this trend will not change.

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  $20 \times 20$  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 pyrithione (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.

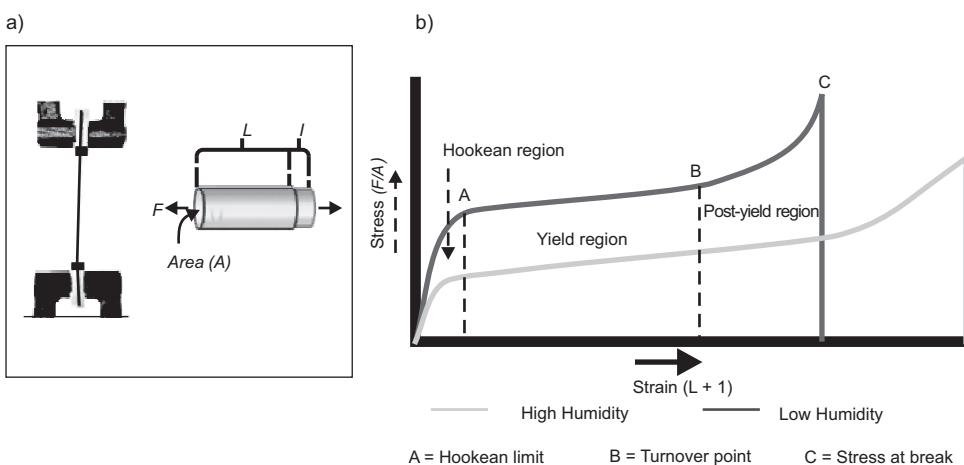
Reutsch found that multiple treatments of hair fibers with a cationic conditioning polymer, Polyquaternium-10, reduce the surface hardness of the cuticle face.

The reduced surface hardness was indicated by an increase in indentation from  $49.4 \pm 5.6$  nm to  $61.9 \pm 8.8$  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<sup>2</sup>) and requires two key measurements in its calculation. First, an average diameter of the fiber is measured so that cross-sectional area ( $\pi r^2$ ) 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 ( $\text{Stress}=\text{F/A}$ ).

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.

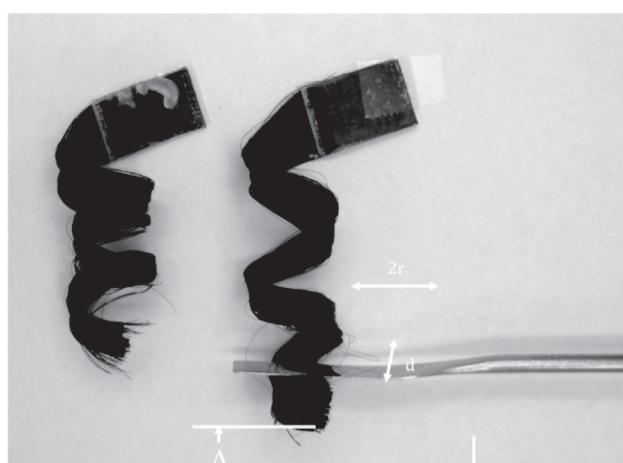
Using the Dia-stron CYC800 equipment, Evans treated his resultant fatigue data in a statistical manner to produce survival probability plots that could be used to predict the number of cycles to break a fiber based on a controlled and repeatable applied stress [144]. He first produced what he termed S-N curves, which are constructed by plotting applied stress ( $\text{g}/\text{um}^2$ ) 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.



$$\Delta = \frac{K F r^2 n}{G d^4} \quad (15)$$

where:

$\Delta$  = 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

$$G = \frac{128\pi Il}{T^2 d^4} \quad (16)$$

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.

$$S = \frac{2.3}{n} \log_{10} \frac{a_1}{a_n} \quad (17)$$

Where:

$S$  = logarithmic decrement

$a_1$  = displacement on the first cycle

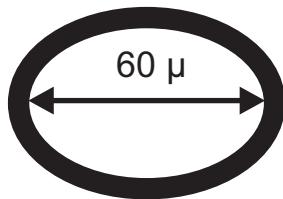
$a_n$  = displacement on the nth cycle

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].

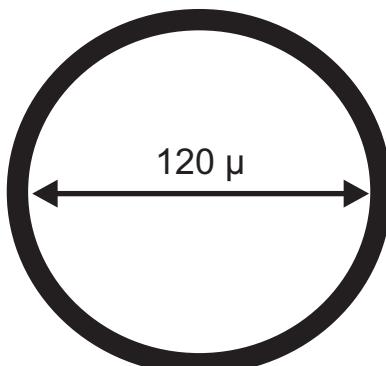
There are various morphological features of hair that affect the torsional modulus of the fiber. When a fiber is twisted one can visualize that the section of hair that suffers the most from the torsional deformation is the outer circumference of the fiber and that this stress progressively decreases 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.

Fine Caucasian Hair Fiber



Asian Hair Fiber



**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.

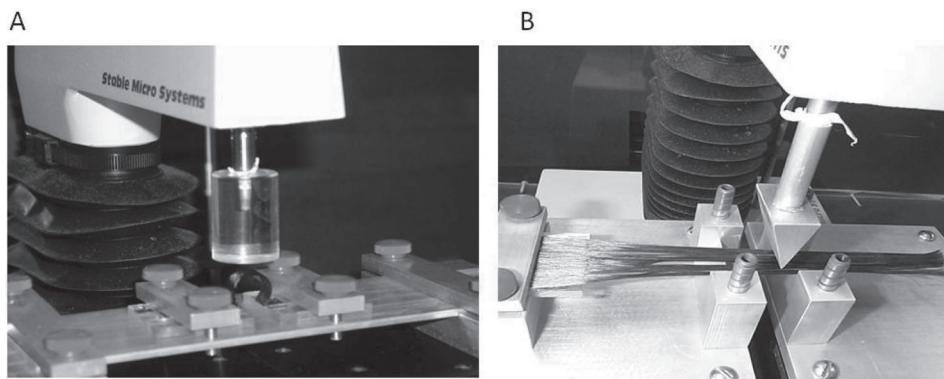
When measurements are taken with wet hair, the torsional modulus decreases compared to hair measured at 65% relative humidity [146]. Hair tends to absorb water, especially the lower cross-linked endocuticle layer of the cuticle. This tends to plasticize the cuticle and to reduce its torsional modulus. 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 microfibrillar region of the cortex, as well as other subcellular structures of the cortex and cuticula.

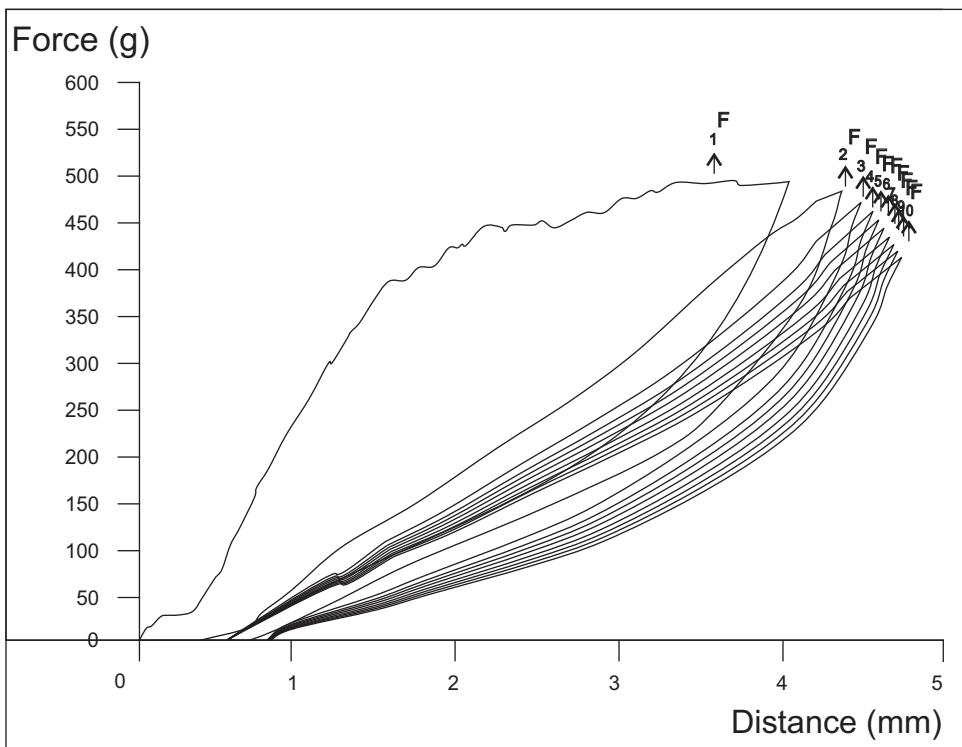
The texture analyzer is a force-measuring device. As with the tensile tester, it has a load cell that is designed to measure the force required to deform an object, such as a hair tress, and to measure the resultant forces during the compression cycle. As can be seen in **Figure 45**, a probe attached 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.



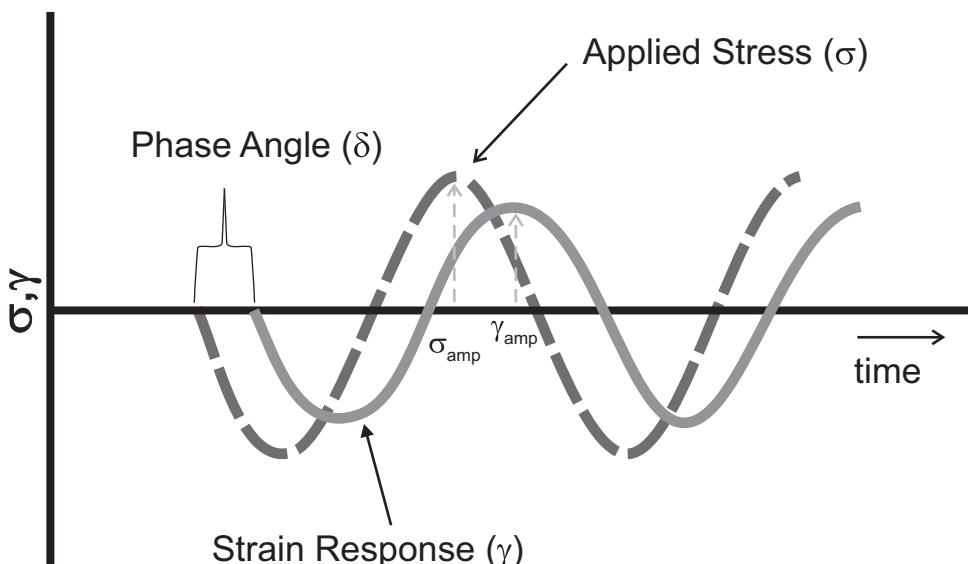
**Figure 46:** Typical stress-strain curve produced by a Texture Analyzer

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 pre-treatment 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 ( $\sigma$ ) oscillation by yielding, or straining ( $\gamma$ ), sinusoidally. The phase angle ( $\delta$ ) is the difference in phase between the dynamic stress and dynamic strain and relates to the level of elasticity in the sample; and  $\sigma_{amp}$ , and  $\gamma_{amp}$  refer to the applied stress and resultant strain amplitudes, respectively. The measurement response is a function of applied stress, time, frequency, temperature, and humidity.

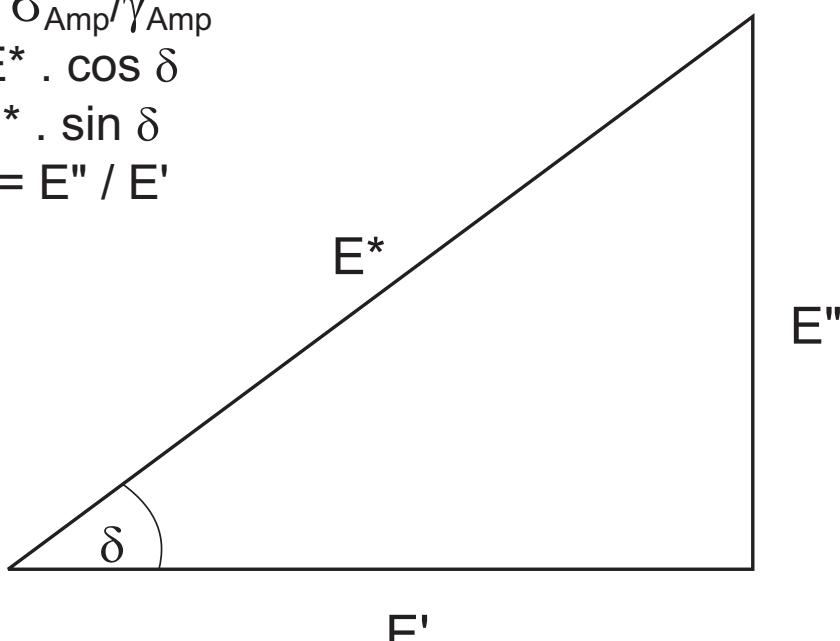
The stiffness is reported as the *elastic (or storage) modulus ( $E'$ )*, and the energy dissipated as heat is described by  $\tan \delta$ ; where  $\tan \delta$  is the ratio of the *viscous (or loss) modulus ( $E''$ )* to the elastic modulus (see **Figure 48**). A perfectly elastic response will have 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 ( $\delta = 90^\circ$ ). The phase lag for *viscoelastic* materials lies between the two extremes (i.e.,  $0^\circ < \delta < 90^\circ$ ). 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  $\tan \delta$ . 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  $\tan \delta$ . 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  $\tan \delta$  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.

$$|E^*| = \sigma_{Amp}/\gamma_{Amp}$$

$$E' = E^* \cdot \cos \delta$$

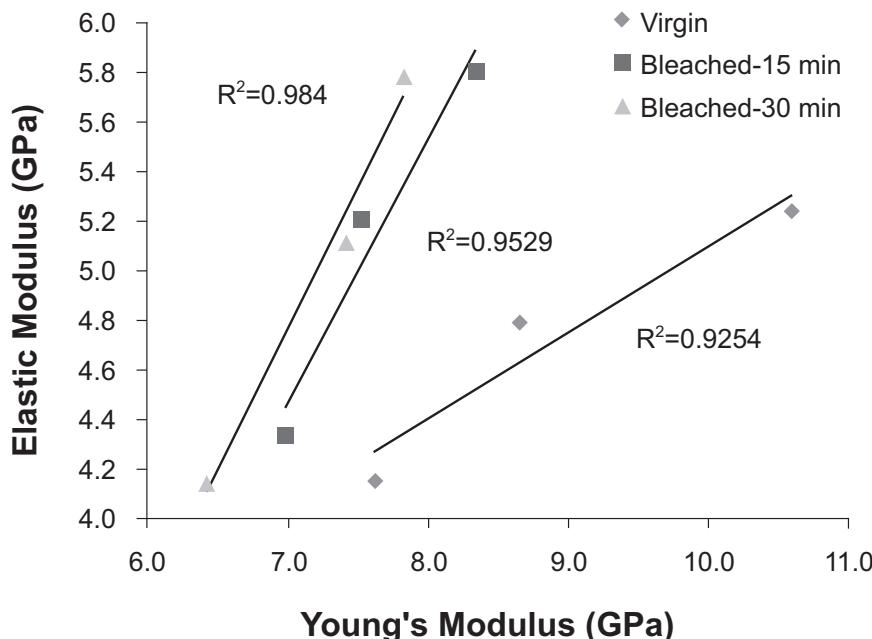
$$E'' = E^* \cdot \sin \delta$$

$$\tan \delta = E'' / E'$$

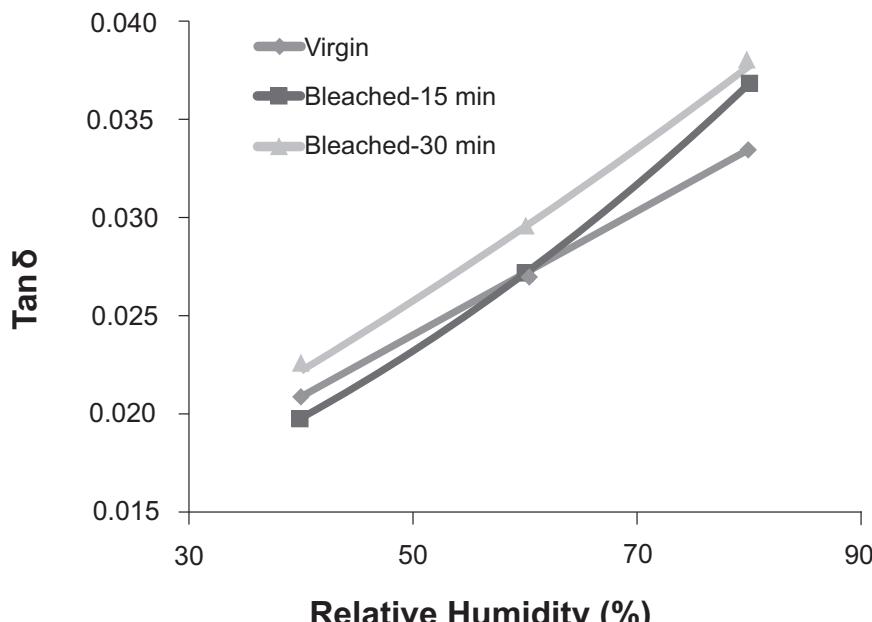


**Figure 48:** Argand diagram relating the real axis ( $E'$ ) to the imaginary axis ( $E''$ ). Hence, the complex modulus ( $E^*$ ) has both elastic and viscous components, meaning  $E^*$  is a mechanical blend of a Hookean spring and a viscous fluid.

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  $\tan \delta$  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 ( $\tan \delta$ ) 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.

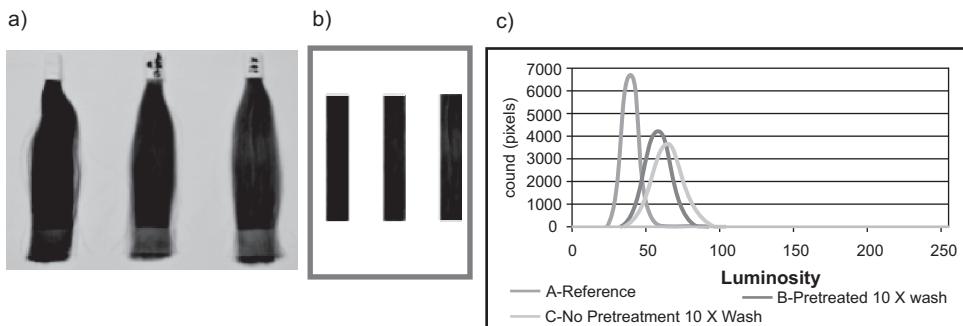
Quantification of the level of shine has been achieved through image analysis [159, 160]. In this method, hair is positioned on a semi-cylindrical barrel. A specular band of light is observed when a polarized beam of light is directed at the hair tress at a particular angle. A digital picture is then 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

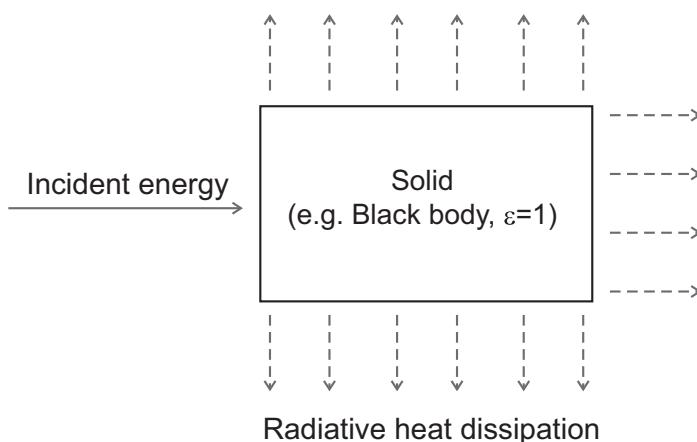
#### f. Infrared Thermography (IRT)

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 ( $\lambda=3-15 \mu\text{m}$ ) 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.

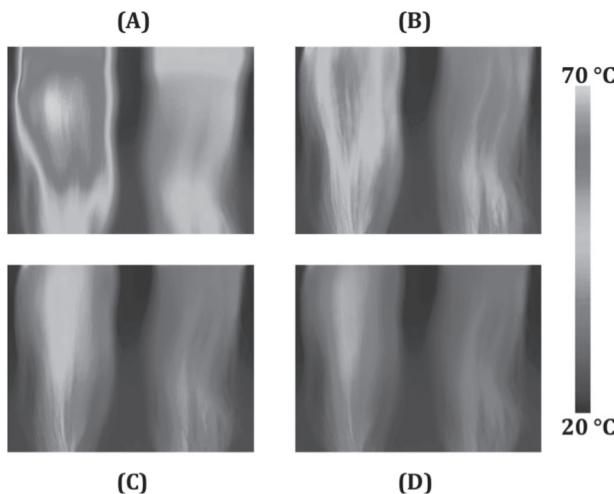
**Figure 52** invokes the Stefan-Boltzmann law, which is the mathematical connection between the maximum attainable emissive radiation across all wavelengths,  $Q$ , and the fourth power of the absolute surface temperature,  $T$ , of a material. For a solid black body radiator, which is a nonrealizable ideal,  $Q$  reaches its maximum because the *emissivity* ( $\varepsilon$ ) 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  $\varepsilon$  approaches unity and  $Q$  is nearly proportional to  $T^4$ ; for example, the  $\varepsilon$  of water and human skin are 0.96–0.98 [162], and the  $\varepsilon$  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.

$$Q = \varepsilon\sigma T^4$$



**Figure 52:** The Stefan-Boltzmann law describes the relationship between the observed radiation intensity ( $Q$ ) and the absolute temperature ( $T$ ), where  $\sigma$  is the proportionality, or Stefan-Boltzmann constant. Black body radiators have emissivity ( $\varepsilon$ ) 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 ( $\varepsilon=0.93$ , 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  $\Delta E$  and  $\Delta C$  are utilized to follow color changes. These are defined as follows (Eqs. 18 and 19):

$$\Delta E = ((L_t - L_o)^2 + (a_t - a_o)^2 + (b_t - b_o)^2)^{1/2} \quad (18)$$

$$\Delta C = ((a_t - a_o)^2 + (b_t - b_o)^2)^{1/2} \quad (19)$$

Where:

L = luminance

a = red to green shift

b = blue to yellow shift

$\Delta E$  = Total change in color including lightness

$\Delta C$  = 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.hunterlab.com](http://www.hunterlab.com).

Using the values of E and C in comparison to an appropriate control can be calculated from equation 20.

$$\% \text{ Color Retention} = \% \Delta E_{improvement} = \frac{\Delta E_{treatment} - \Delta E_{control}}{\Delta E_{control}} \times 100 \quad (20)$$

Attempts have been made to prevent color fading in hair by investigators working in the personal care industry. For example, by utilizing  $\Delta E$  and to a lesser extent,  $\Delta C$ , 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  $\Delta E$  and  $\Delta C$ , as well as percent protection. By considering the equations to determine  $\Delta E$  and  $\Delta C$ , one may take note that the color difference takes into consideration multiple color indices. If, for example,  $\Delta E$  and/or  $\Delta C$  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  $\Delta a$ ,  $\Delta b$ , or  $\Delta L$  individually. For example, Gao monitored  $\Delta a$ , which is a change in the saturation of red, for different red hair colors containing different levels of phosphate esters.

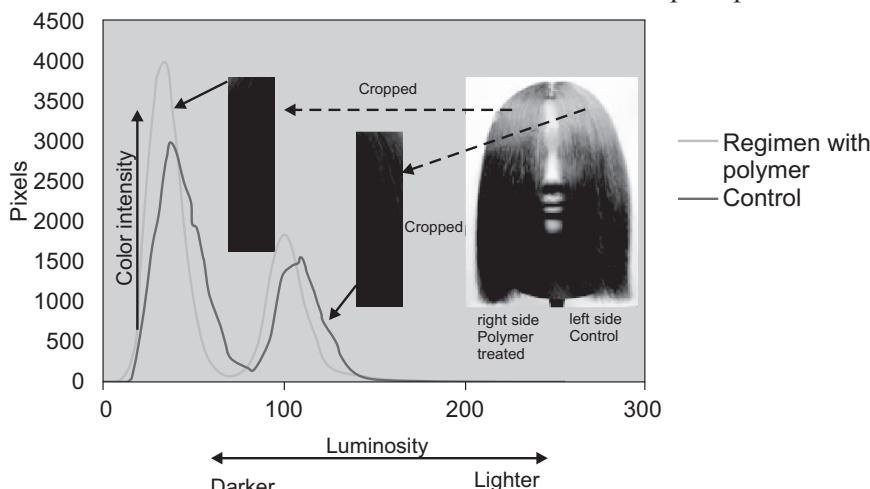
It should be mentioned that percent protection needs to be used with caution when claiming color protection. For example, a large percent protection can be calculated from very small changes of  $\Delta E$  of the experimental treatment versus the control, which may not be consumer perceivable.

## 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

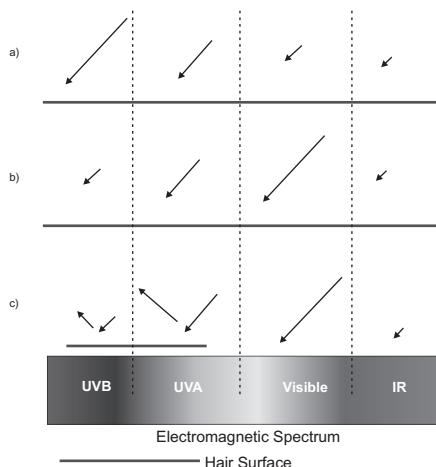
### b. Color Protection from UV-Induced Fading

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<sup>2</sup>. 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  $\Delta E$  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.<sup>125</sup> 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 <sup>14</sup>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.

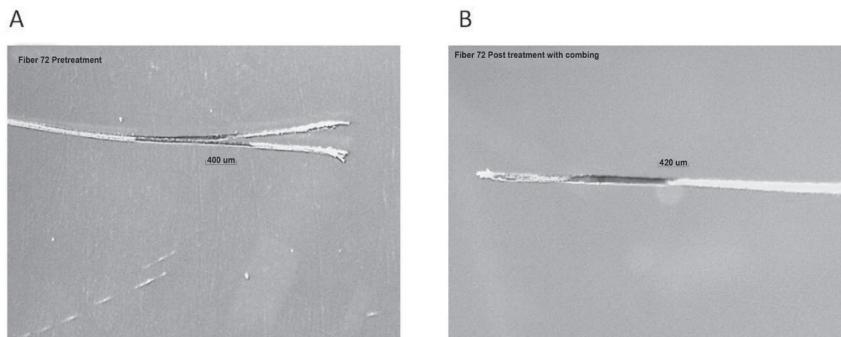
Gamez-Garcia studied this phenomenon of cuticle decentration further and found that cuticle lifting increased with the number of applied tensile cycles and was sensitive to moisture, whereas at lower humidity levels it took less strain and/or fewer cycles to initiate cuticle lifting [178]. The mechanistic 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 stearoxypropylidimethylamine (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.

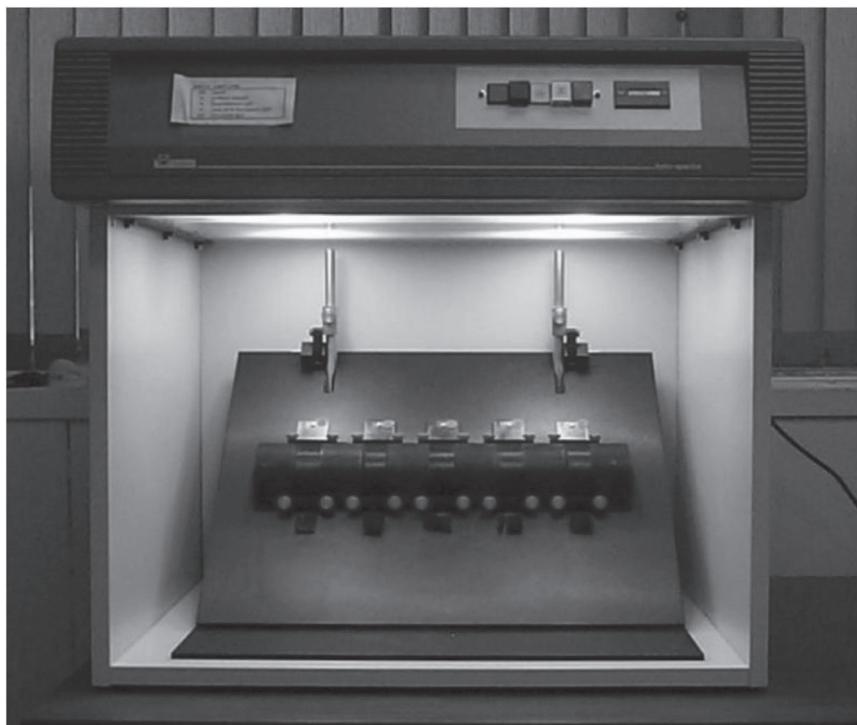
There are essentially two types of designs for panel tests. One is based on an absolute and another on a relative scoring system. In an absolute scoring system, the panelist judges a particular hair characteristic against set criteria. For example, if the hair attribute is ease of combing and if the 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

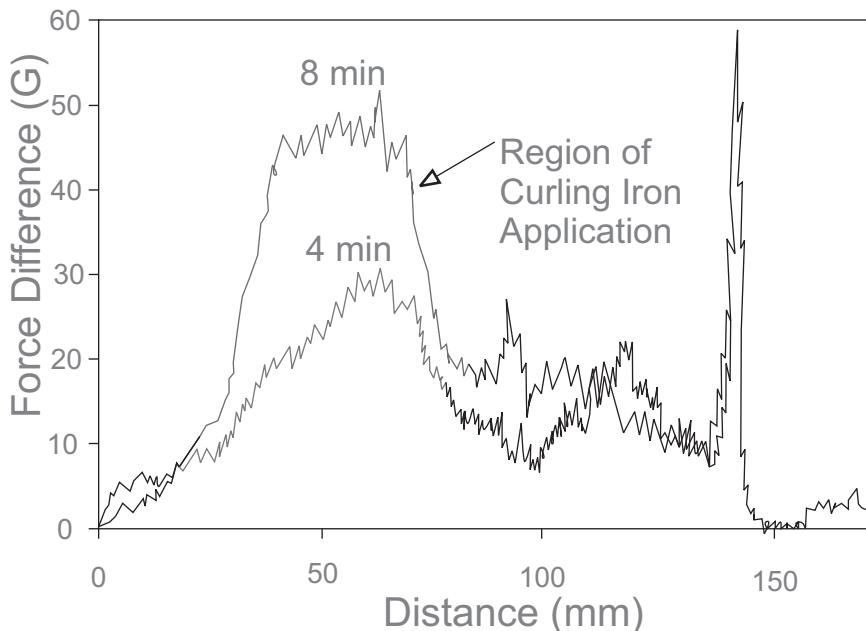
*Combing methods*—Breakage through combing imparts many mechanical stresses to hair. Test methods have been employed where hair is combed by hand in a controlled fashion or combed with a mechanical device and the number of fibers that have broken from the hair are quantified. The number of fibers, when 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 glyptic line structure. The replica samples have been imaged using many different angles of illumination. In some experiments we have used a narrow-angle ( $\sim 10^\circ$ ) 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 photoaging 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.

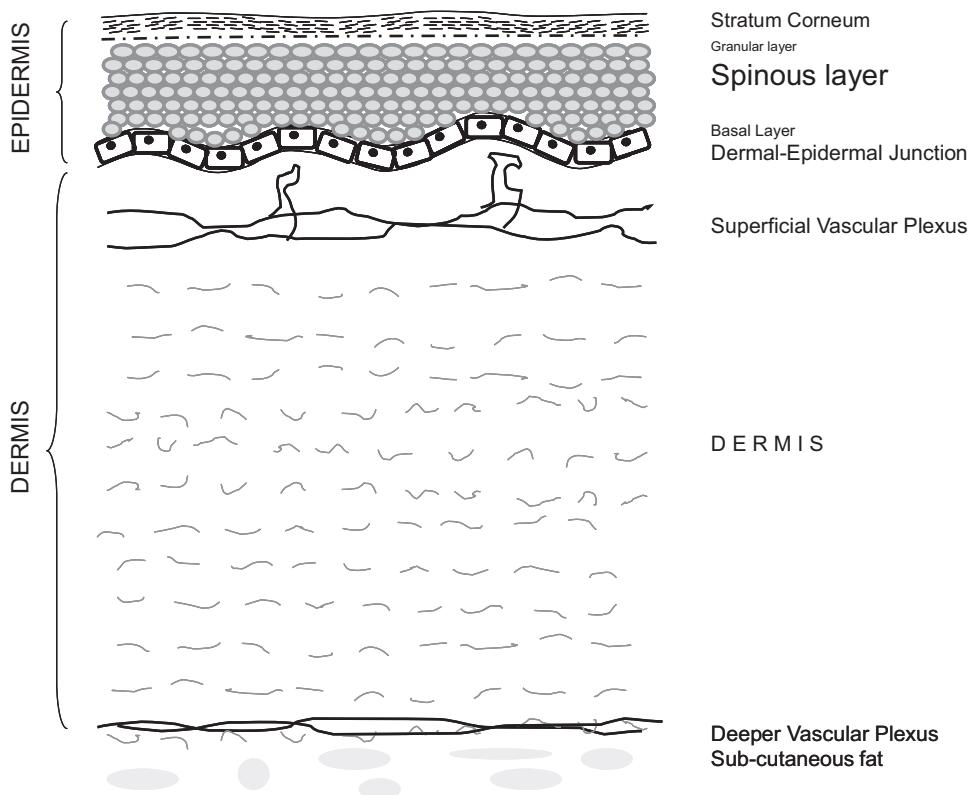
## 11.5.3 SKIN STRATA

Human skin covers the whole body, an approximate surface area of  $2\text{ m}^2$ , 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.



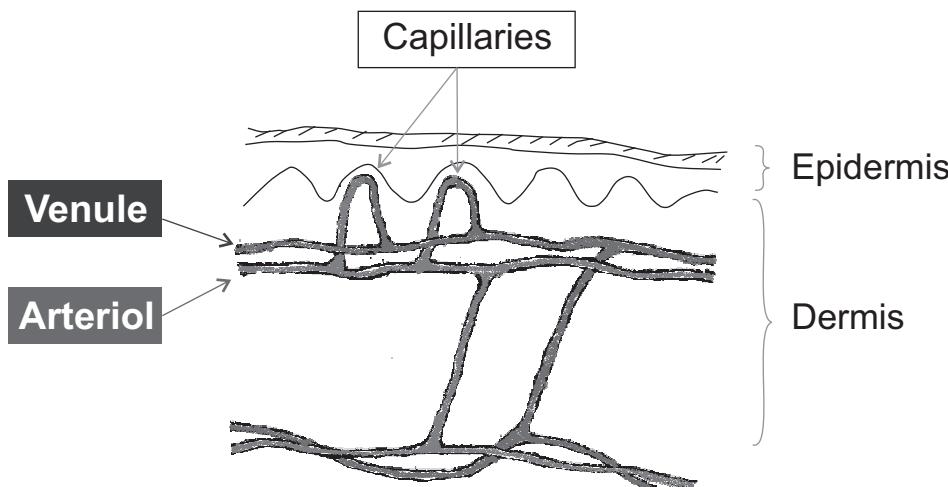
**Figure 1:** A general sketch showing the various strata of skin.

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 micro-vasculature.

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.

### MICRO-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 lamellar 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  $21^\circ \pm 1^\circ$  Celsius ( $70^\circ \pm 1^\circ$  Fahrenheit) and relative humidity of either  $40\% \pm 5\%$  or  $50\% \pm 5\%$  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.

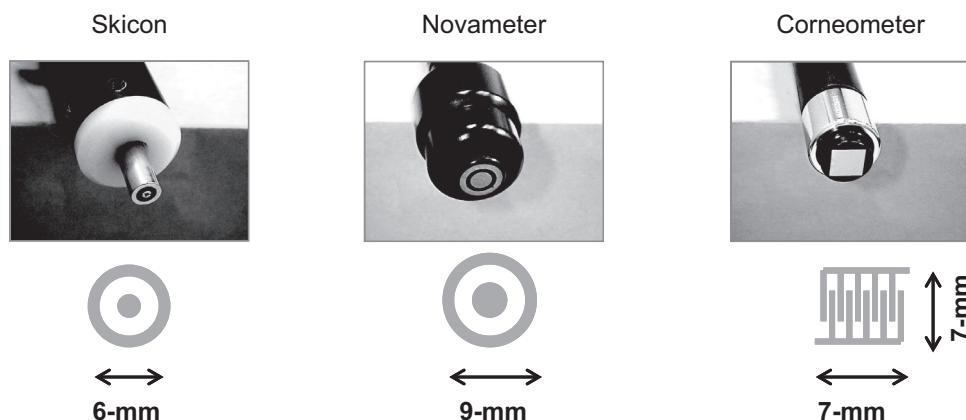
The temperature and humidity are continuously recorded on a chart recorder. The recommended time for acclimation of subjects is between 15 and 30 minutes, but could be longer for some experiments.

### 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.

#### Stratum Corneum Hydration



**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 ( $\mu\text{S}$ ) 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.

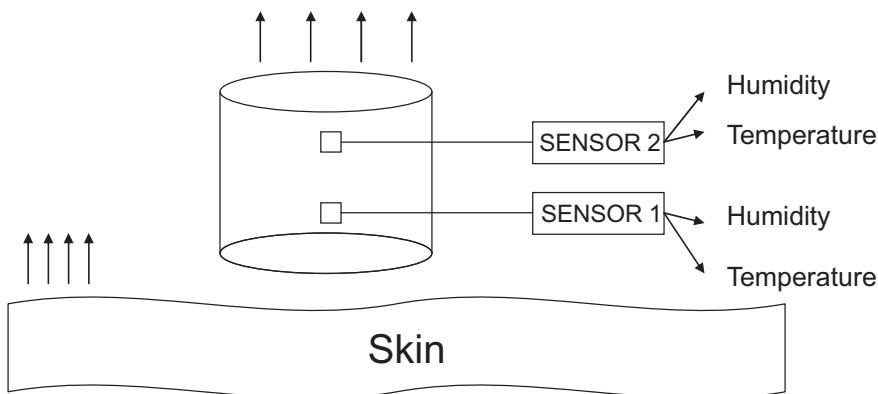
#### e. Moisture Accumulation Test

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  $\text{g}/\text{m}^2\text{h}$ . 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.

## Trans-Epidermal Water Loss (TEWL)



**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  $70^\circ \pm 0.5^\circ\text{F}$  and a relative humidity of  $40 \pm 5\%$ . 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  $\text{g}/\text{m}^2\text{h}$ .

### 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  $70^\circ \pm 0.5^\circ\text{F}$  and a relative humidity of  $40 \pm 5\%$ . 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  $\text{g}/\text{m}^2\text{h}$ .

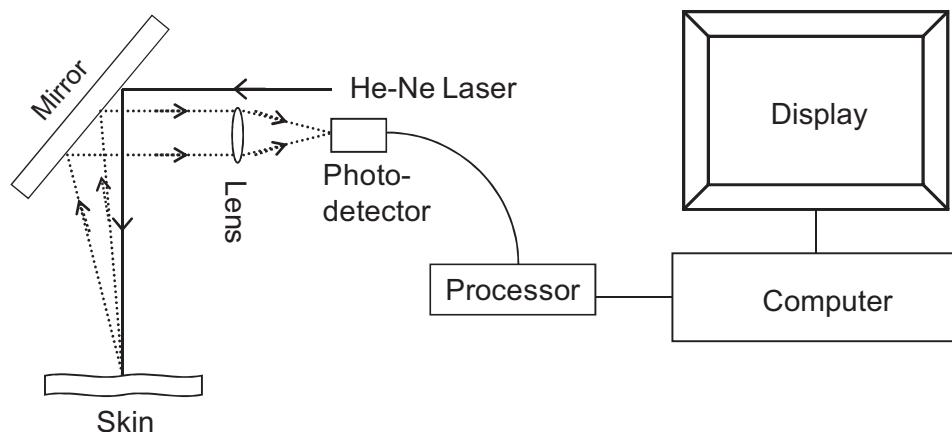
## 11.5.7 SKIN BLOOD FLOW, COLOR, ERYTHEMA

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  $250 \times 250$  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.



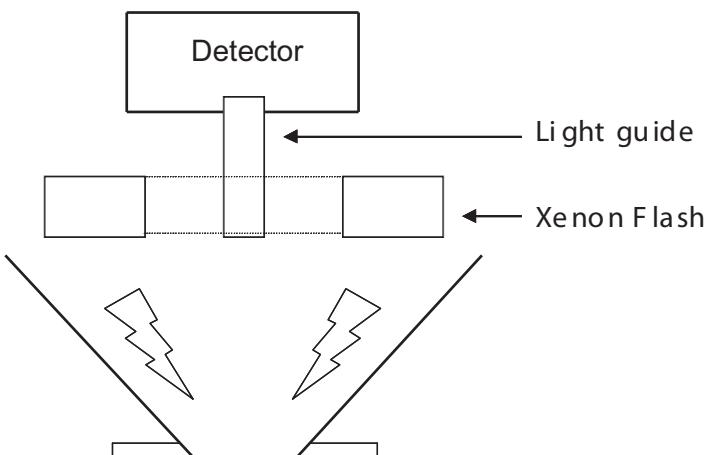
**Figure 5:** Schematics of the Laser Doppler Scanner.

### 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.

## Chromameter



**Figure 6:** The probe head of the chromameter.

### c. Reflectance Spectrophotometry

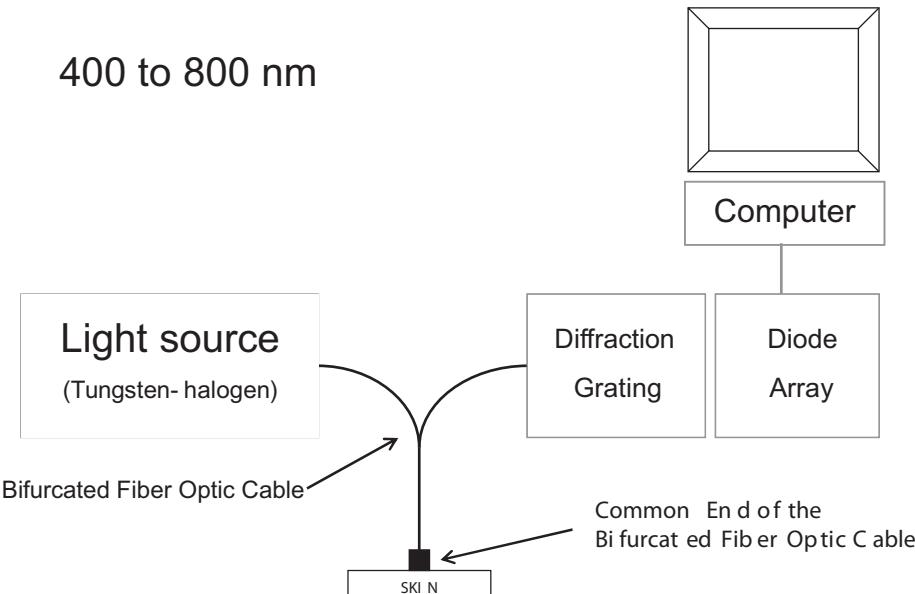
The spectrophotometric measurement of erythema is based on assessing the hemoglobin content of skin by measuring the specific absorbance peaks. Oxy-hemoglobin have absorbance peaks at 415, 542, and 577 nm, and deoxy-hemoglobin have peaks at 430 and 555 nm. The common end of a bifurcated fiber optic cable 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:

$$A(\lambda) = \log_{10} \{ W(\lambda) / S(\lambda) \}$$

Where  $A(\lambda)$  is the apparent absorbance,  $W(\lambda)$  is the white reference spectra, and  $S(\lambda)$  is the spectra from skin.

## Reflectance Spectrophotometry



**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:

$$A(\lambda) = \log_{10} \{ [W(\lambda) - D(\lambda)] / [S(\lambda) - D(\lambda)] \}$$

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  $-45^\circ$ ,  $0^\circ$ ,  $+45^\circ$  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).

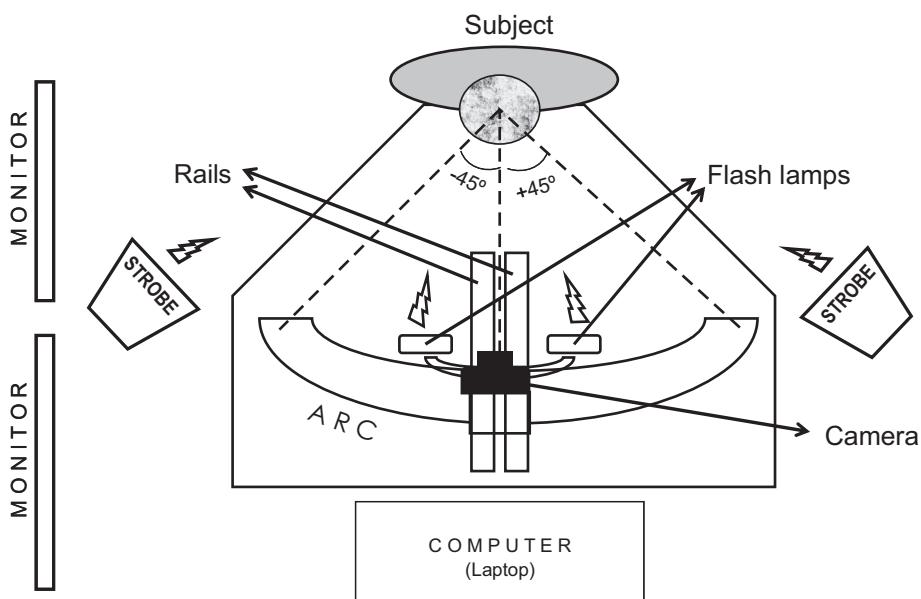
#### 1. Face Photography

The photos of the left, front, and right sides of the face are obtained using a computerized photography system with a 21.1-Megapixel ( $5616 \times 3744$  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:  $0^\circ$  (Front face),  $-45^\circ$  (Right face), and  $+45^\circ$  (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 ( $0^\circ$ ) is obtained. The photos of the right and left sides of the face are obtained sequentially after moving the camera  $-45^\circ$  (Right face) and  $+45^\circ$  (Left face).

### FACIAL PHOTOGRAPHY SYSTEM



**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***

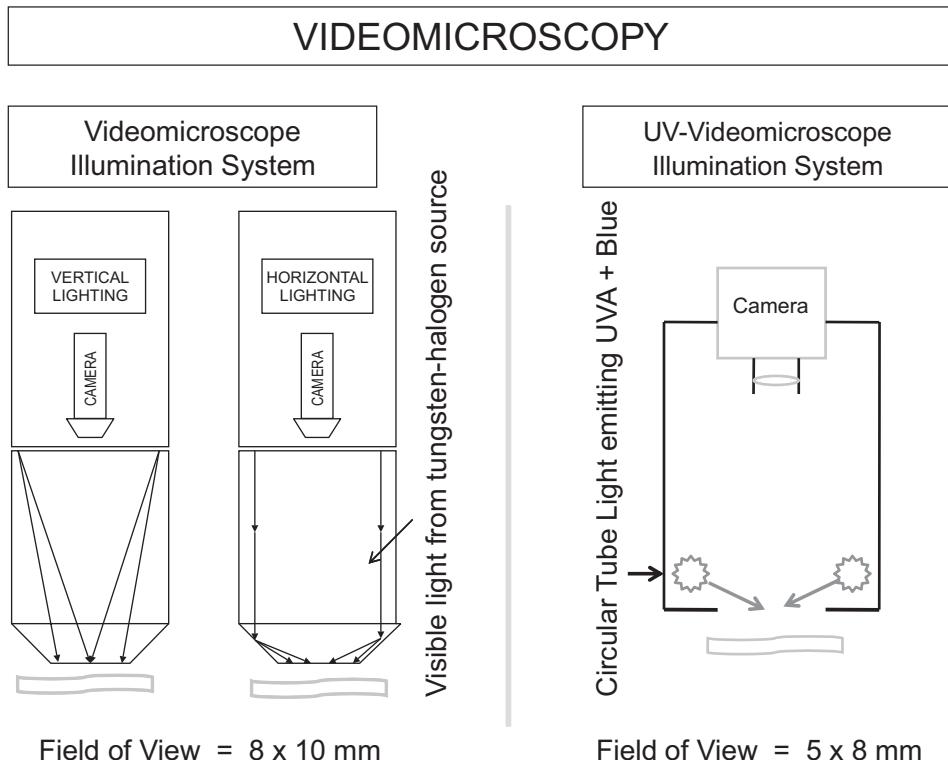
For enhanced visualization of pigmented lesions and melanin content of skin, photography with light in the ultraviolet wavelength range (320 to 400 nm) is used. A 35-mm film camera with a 60 mm lens and a 400 W twin-flash lamp is used. Optical filters, passing Ultraviolet-A light (UVA, peak at 365 nm) 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 (Hi-scope, 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.

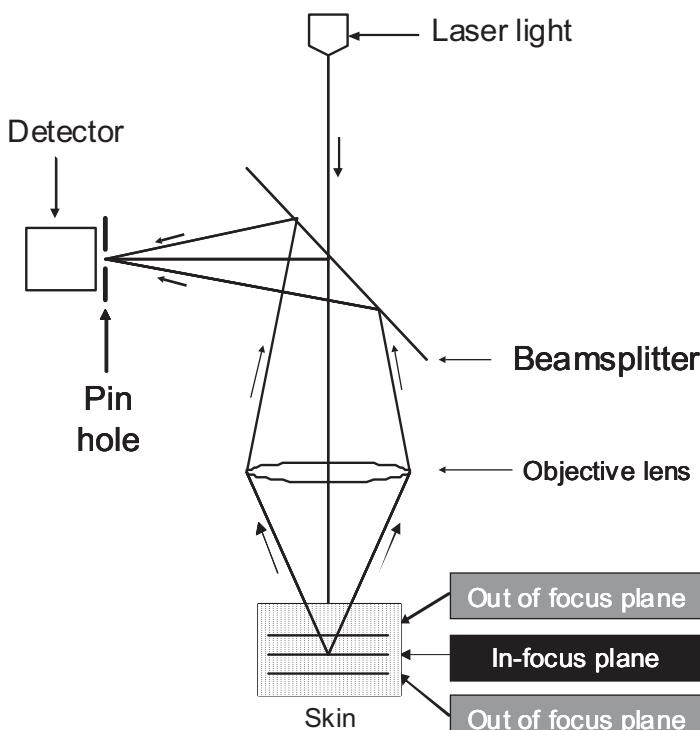
Using the videomicroscope with an appropriate illuminating system, one may follow various dermatoses and treatment regimens over a period of time, recording images along the way on the computer disk. In many cases the skin lesions can be located reasonably accurately by comparing current image with the 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.

## CONFOCAL MICROSCOPY



**Figure 10:** The Optics of the Confocal Microscope.

The confocal scanning laser microscope Vivascope1000 (Lucid, Inc. Rochester, NY) with an objective lens of  $30\times$  and 0.9 NA, scanning a field of  $155 \times 155 \mu\text{m}$  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  $100 \mu\text{m}$ . 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  $3.6 \mu\text{m}$  steps. A horizontal resolution of  $1.3 \mu\text{m}$  and a vertical resolution of  $5 \mu\text{m}$  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.

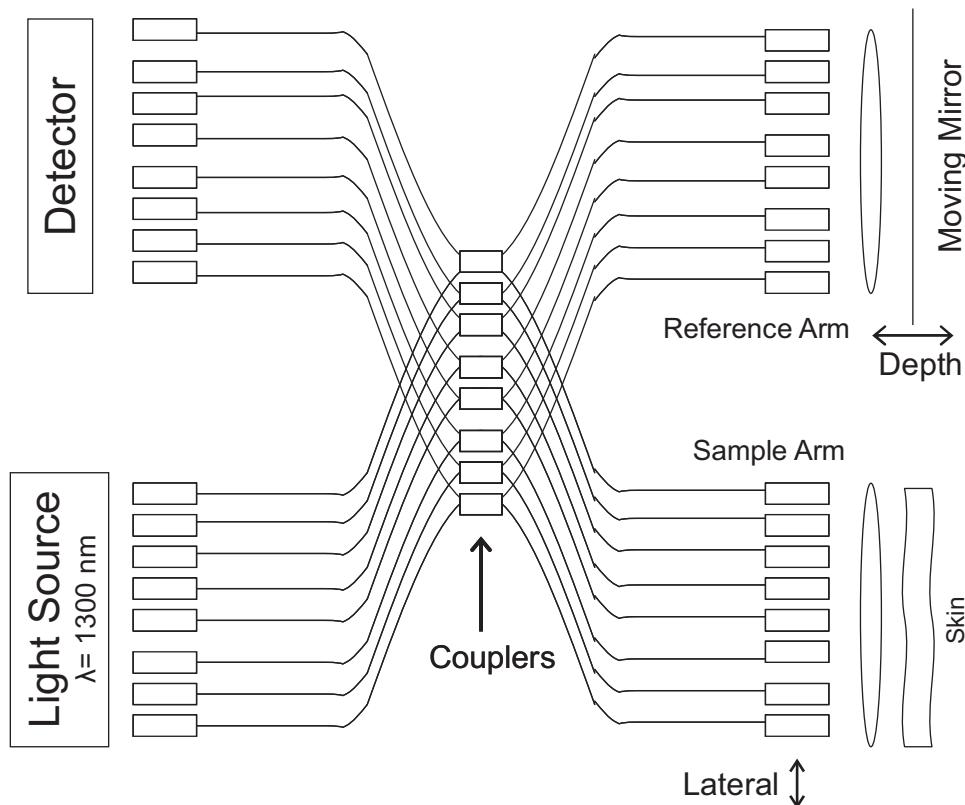
A more advanced confocal microscope, Vivascope 1500 (Lucid, Inc. Rochester, NY, now called caliber I.D.), was later introduced with Z-steps of 1.6 microns, but with somewhat lower magnification. The new software can capture multiple images, as a block by x-y mapping as well as a stack in depth by z-mapping. 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  $500 \times 500 \mu\text{m}$ . 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 lesions 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.

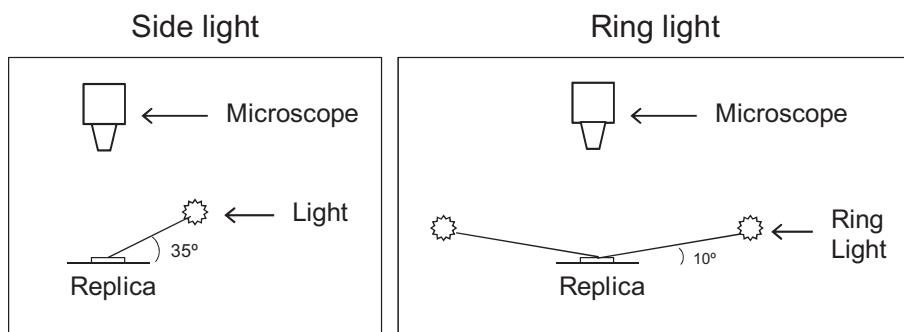
### 11.5.9 SKIN TOPOGRAPHY

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.

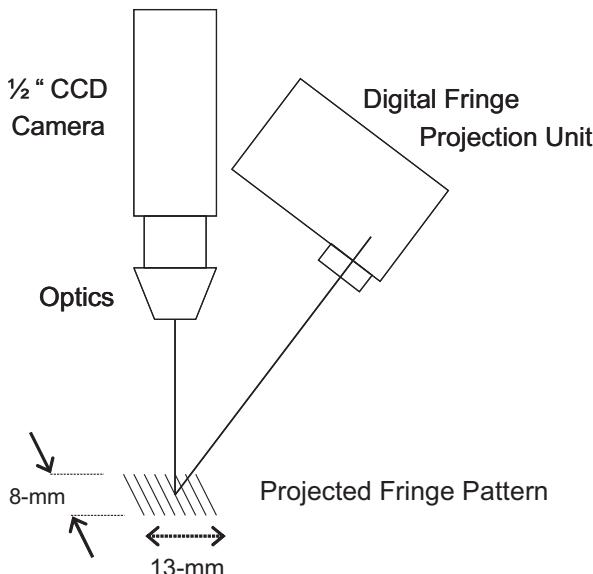


**Figure 12:** Imaging of Replica by Stereomicroscope with a side-light (left) and a ring-light (right).

### 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  $\text{Cos}^2$  type in a rectangular area of  $13 \text{ mm} \times 18 \text{ mm}$  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.

## Topography by Fringe Projection Device



**Figure 13:** Schematic showing the basic design of the Fringe Projection Device.

### c. Calculation of Roughness Values

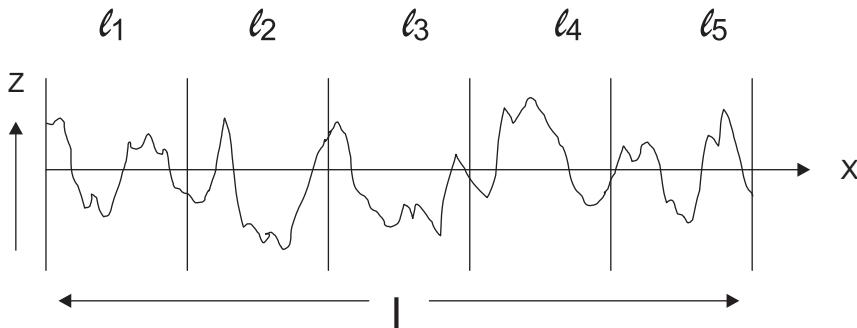
First the hairs from the image are removed by a mathematical filter, then a fifth-order polynomial ( $n=5$ ) is applied to the 3-D image to remove the gross contours of the skin surface. This flattening of the surface is done using the following equation:

$$\sum_{i=0}^n \sum_{j=0}^n \left( X - \frac{X_{\max}}{2} \right)^i \left( Y - \frac{Y_{\max}}{2} \right)^j$$

$$Z(x, y) = a_{ij} \cdot X.$$

$$i = 0 \quad j = 0$$

On this processed image, 16 radial lines are marked inside a circle, each consecutive line subtending an angle of  $22.5^\circ$ . 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:



$$Rz = \frac{1}{n} z \sum_{i=1}^{i=n} Z_{\max}(i) - Z_m \quad \dots \quad [n = 1, 2, \dots 5]$$

$$Ra = \frac{1}{\ell} \int_0^{\ell} (x).dx$$

#### 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<sup>2</sup>. Due to rough topography of the skin surface, the actual area would always be larger than 234 mm<sup>2</sup>. The rougher the surface, the larger the area. Therefore, change in the surface area would indirectly indicate change in surface roughness.

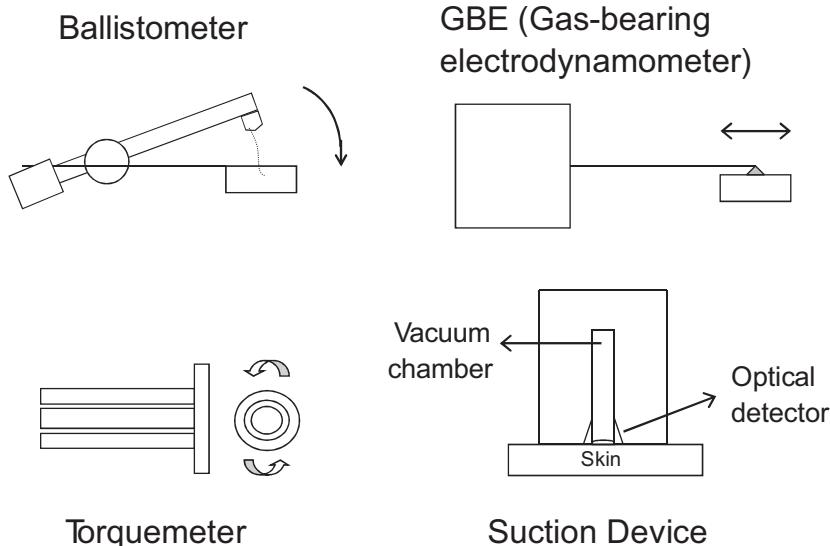
### 11.5.10 VISCOELASTIC MEASUREMENTS

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).

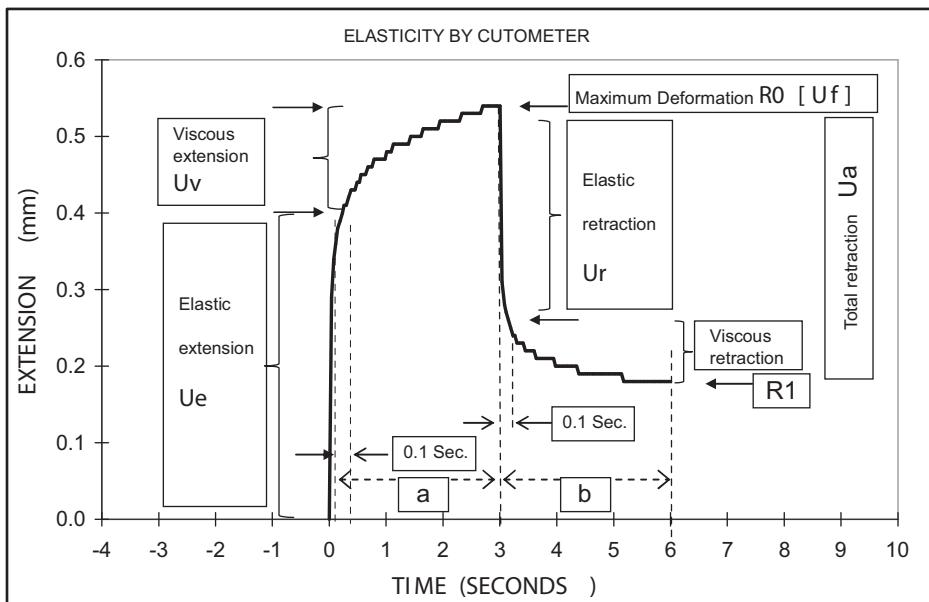
#### a. Suction Device

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.  $R_0 = e(a) = [U_f]$  Maximum Amplitude of the first cycle (Maximum Deformation). Value of extension “e” at the end of the first suction period “a.”
2.  $R_1 = e(a+b)$  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.  $R_2 = (R_0 - R_1)/R_0 = [U_a/U_f]$  Gross Elasticity: Ratio of return to maximum extension in the first cycle.
4.  $R_5 = [U_r / U_e]$  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.  $R_6 = [U_v / U_e]$  Viscoelastic Ratio: Ratio of the viscous extension to the elastic extension.

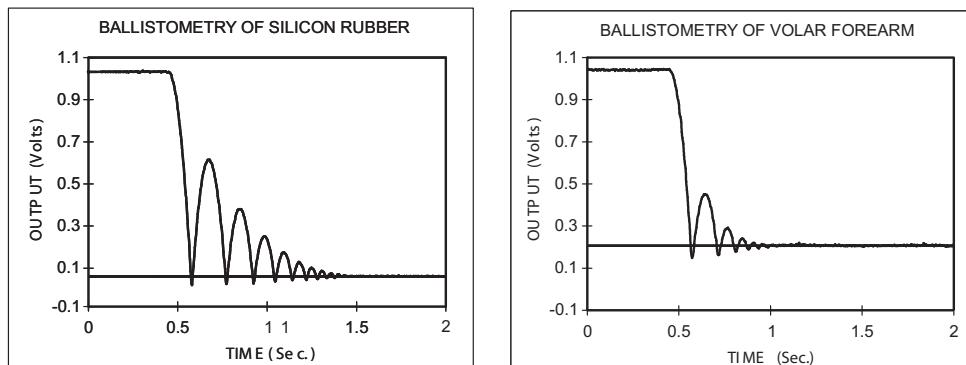
There are many other ratios calculated, particularly when multiple cycles are used. Generally, three cycles of suction and release are recorded and some parameters comparing the first cycle to the third cycle are evaluated. In some experiments large number of cycles are used, e.g., 20 or 30 cycles, to 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.

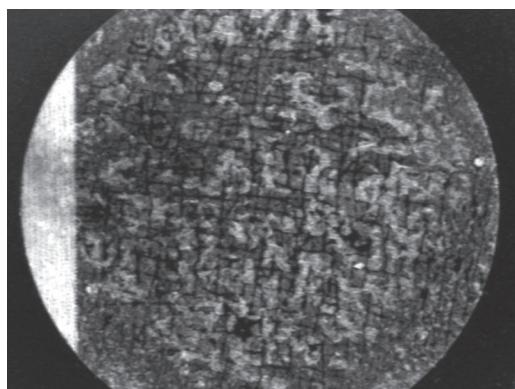


**Figure 16:** Graphs of diminishing peaks recorded for silicon rubber pad (left) and volar forearm of a volunteer (right).

### 11.5.11 SOME EX VIVO TECHNIQUES

#### a. Desquamation 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.

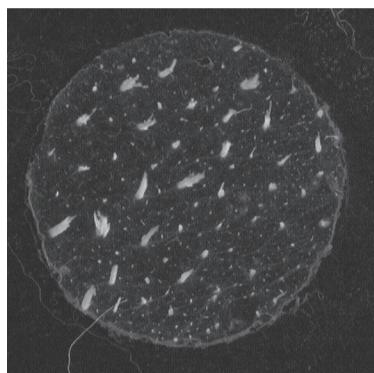


**Figure 17:** D-Squame sample showing scales removed from the skin.

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  $1\text{ cm}^2$  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.

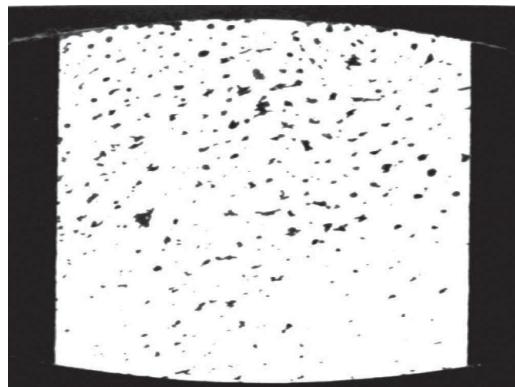


**Figure 18:** Cyanoacrylate surface biopsy from face showing fluorescent follicular casts.

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 ( $\text{mm}^2$ ) are calculated.

### c. Sebum Collection Assay

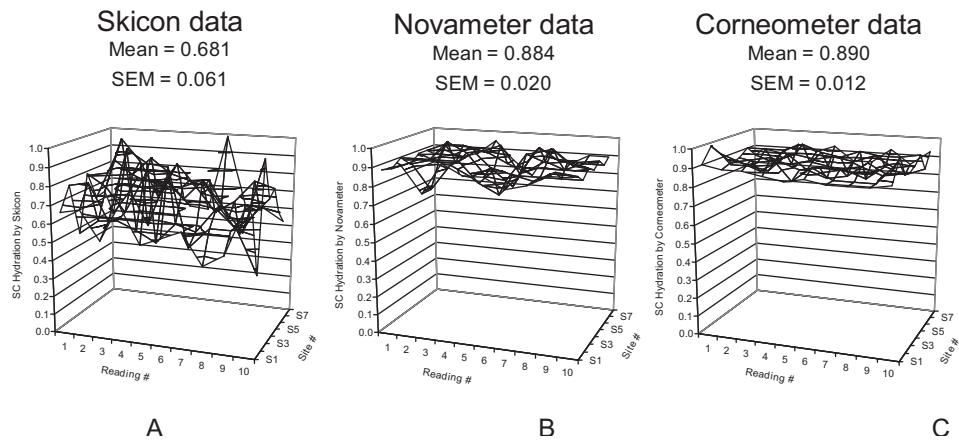
A porous translucent plastic film, Sebutape (CuDerm Corp., Dallas, TX), which when placed on skin collects sebum, which turns the film transparent. When the film is placed on a black card the collected sebum appears as black spots (see Figure 19). The sebum output is usually assessed on the cheeks and 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).

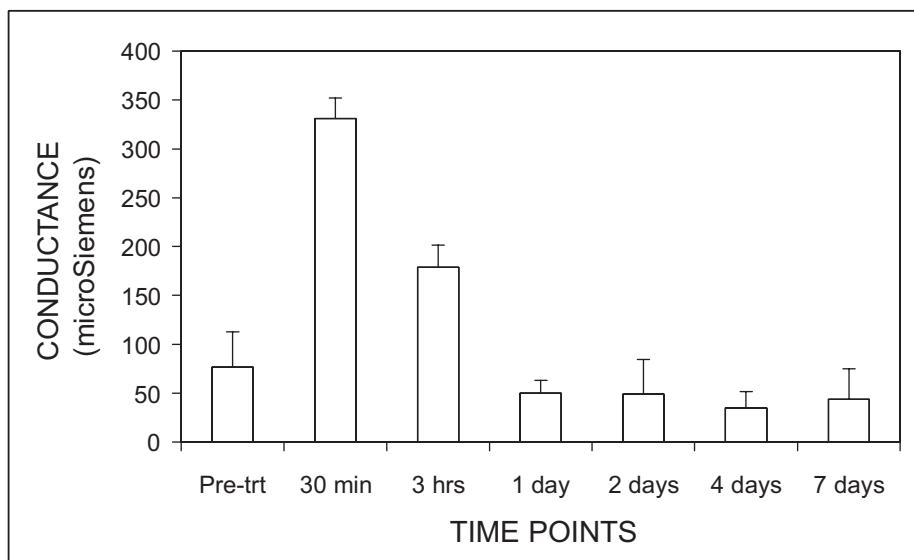
The Standard Error of the Mean (SEM) was also calculated. Table 1 shows the data with Mean, Standard deviation (SD), and SEM.

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.

### Hydration Post Lactic Acid [Skicon]



**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**

Reading →	SKICON										MEAN	SD	SEM	
	1	2	3	4	5	6	7	8	9	10				
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	

Reading →	NOVAMETER										MEAN	SD	SEM	
	1	2	3	4	5	6	7	8	9	10				
S1	132	136	116	140	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

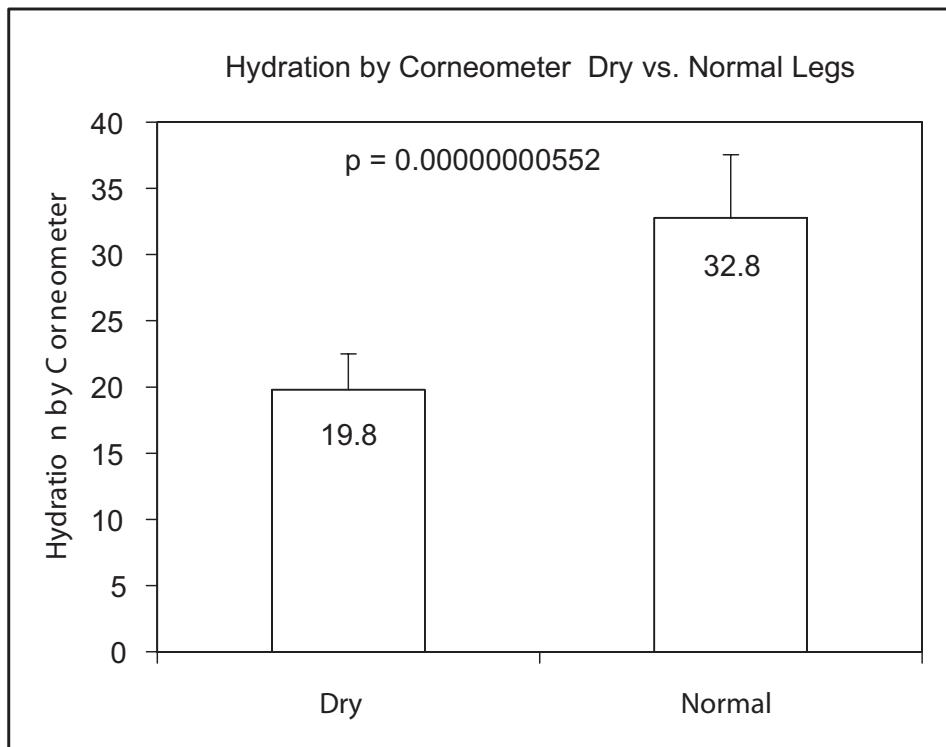
<b>S5</b>	134	128	128	138	124	112	140	144	132	130	>	131.00	9.055	0.022
<b>S6</b>	134	140	140	136	142	120	142	132	132	134	>	135.20	6.613	0.015
<b>S7</b>	128	128	136	132	124	118	128	122	128	128	>	127.20	5.007	0.012
<b>S8</b>	130	124	128	140	124	112	136	124	132	132	>	128.20	7.800	0.019

Max> 148 Min> 112 MEAN (All)> 130.85 7.312 0.018

### CORNEOMETER

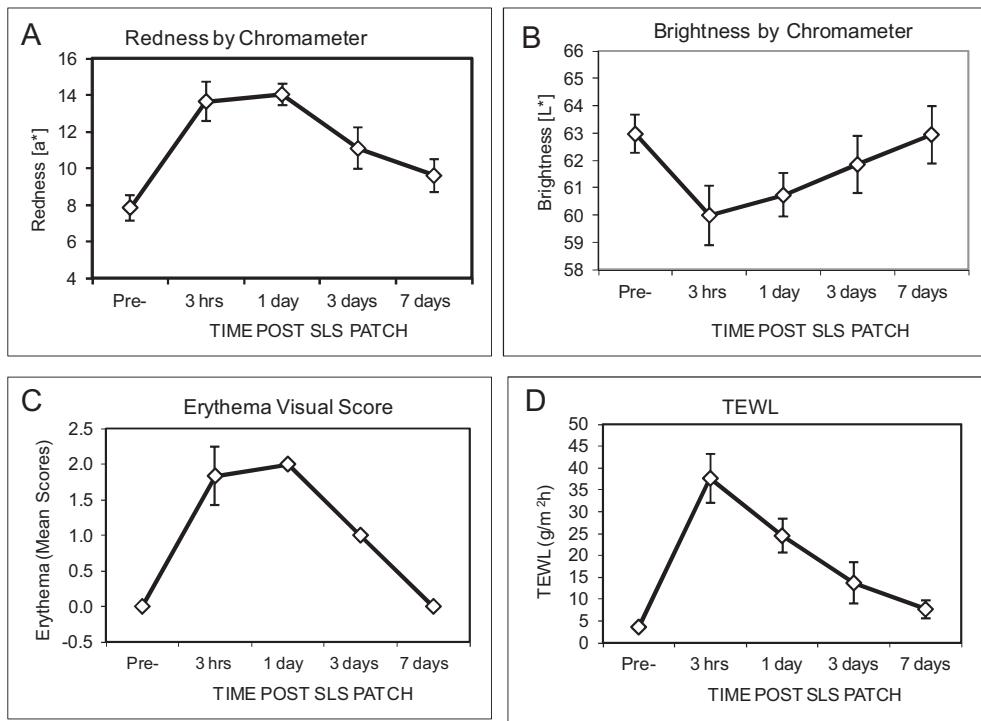
Reading →	SITE ↓	CORNEOMETER										MEAN	SD	SEM
		1	2	3	4	5	6	7	8	9	10			
<b>S1</b>	48	47	47	46	45	46	45	45	45	46	>	46.00	1.054	0.007
<b>S2</b>	52	48	49	50	44	48	45	45	45	51	>	47.70	2.830	0.019
<b>S3</b>	49	47	45	48	46	47	47	48	46	47	>	47.00	1.155	0.008
<b>S4</b>	45	45	44	49	45	43	44	48	45	46	>	45.40	1.838	0.013
<b>S5</b>	46	45	46	48	47	48	47	49	46	47	>	46.90	1.197	0.008
<b>S6</b>	48	48	48	50	48	47	49	48	48	49	>	48.30	0.823	0.005
<b>S7</b>	45	43	44	43	42	40	44	42	44	44	>	43.00	1.414	0.010
<b>S8</b>	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.

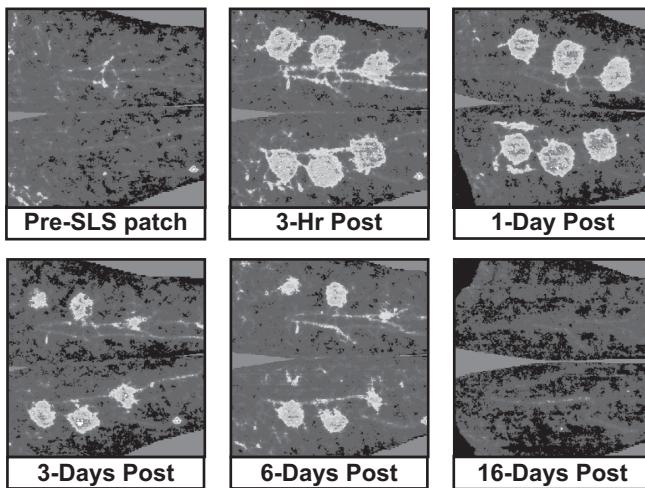
Experimental irritation was induced by applying sodium lauryl sulphate (SLS) 0.5% aqueous for 24 hours. The effects were evaluated at 3 hours, 1 day, 3 days, and 7 days post-SLS patch removal by measuring Redness ( $a^*$ ) and Brightness ( $L^*$ ) by Chromameter and TEWL by Dermalab as well as visually 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.

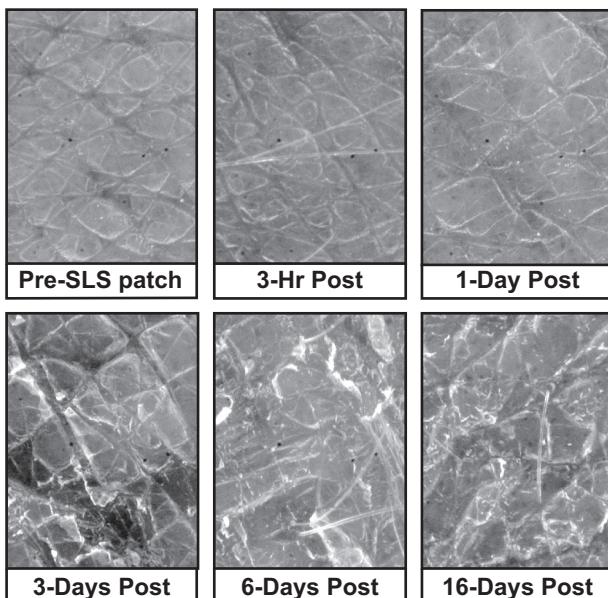
In another “Irritation by SLS” experiment, six SLS 0.5% patches were applied to the volar forearms, three on the left and three on the right, for 24 hours. Laser Doppler Imaging, side-lighted videomicroscopy, and UV videomicroscopy were done before SLS patch application and then at 3 hours, 1 day, 3 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.



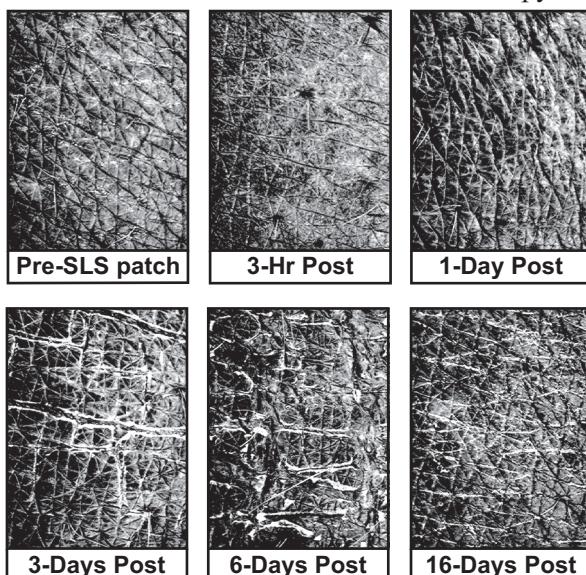
**Figure 24:** Laser Doppler Images of the volar forearms, pre-SLS patch applications and then 3 hours, 1 day, 3 days, 6 days, and 16 days post-SLS patch removal.

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.



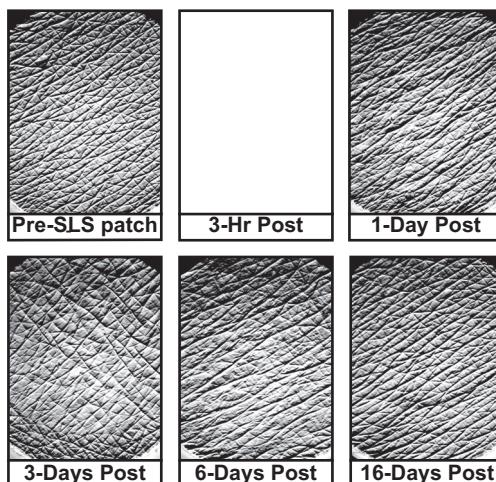
**Figure 25:** Side-lighted videomicroscope images of the volar forearms, pre-SLS patch applications and then 3 hours, 1 day, 3 days, 6 days, and 16 days post-SLS patch removal. Strong scaliness appears on days 3 and 6. Scaliness starts to reduce by day 16.

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.

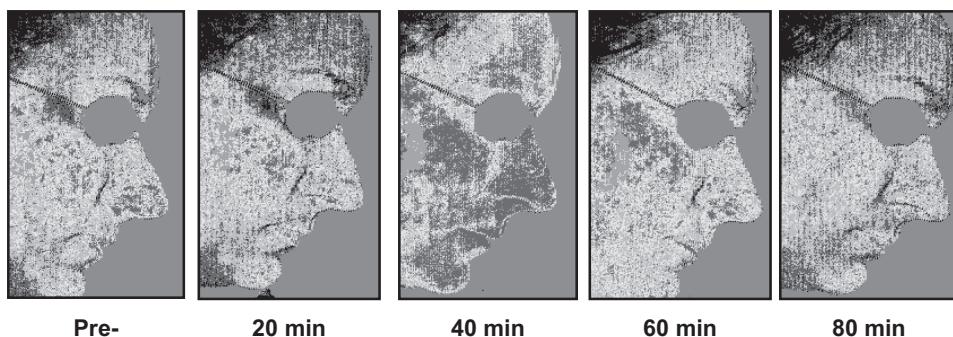
Silicon rubber replicas of skin show the distortion of glyptic pattern post-SLS patch on days 3 and 6, and then recovery by day 16. Figure 27 shows the series of replica images. Replica was not obtained at “3-Hr Post” time point because replicas don’t form very well if the skin surface is somewhat moist.



**Figure 27:** Series of replica images showing the distortion and recovery of glyptic pattern post-SLS patch over a period of 16 days.

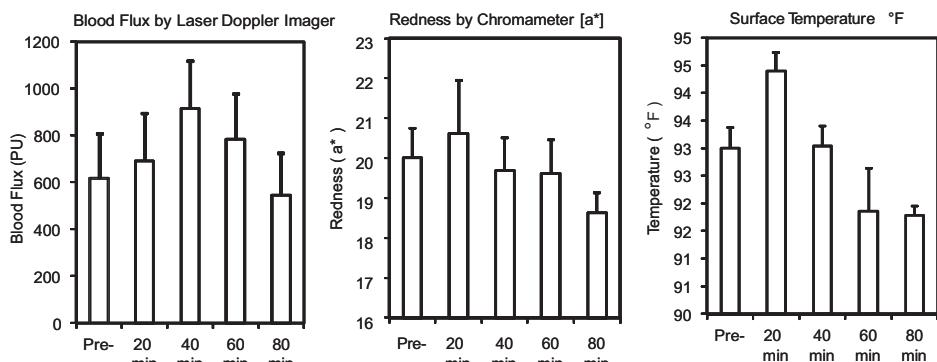
To show the changes in the degree of erythema and blood flow, oral niacin (250 mg) was used by a healthy volunteer and Laser Doppler Blood Flow Imaging of the cheek was performed at several time points. The blood flow values in Perfusion Units were calculated. Skin redness values were also evaluated 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.



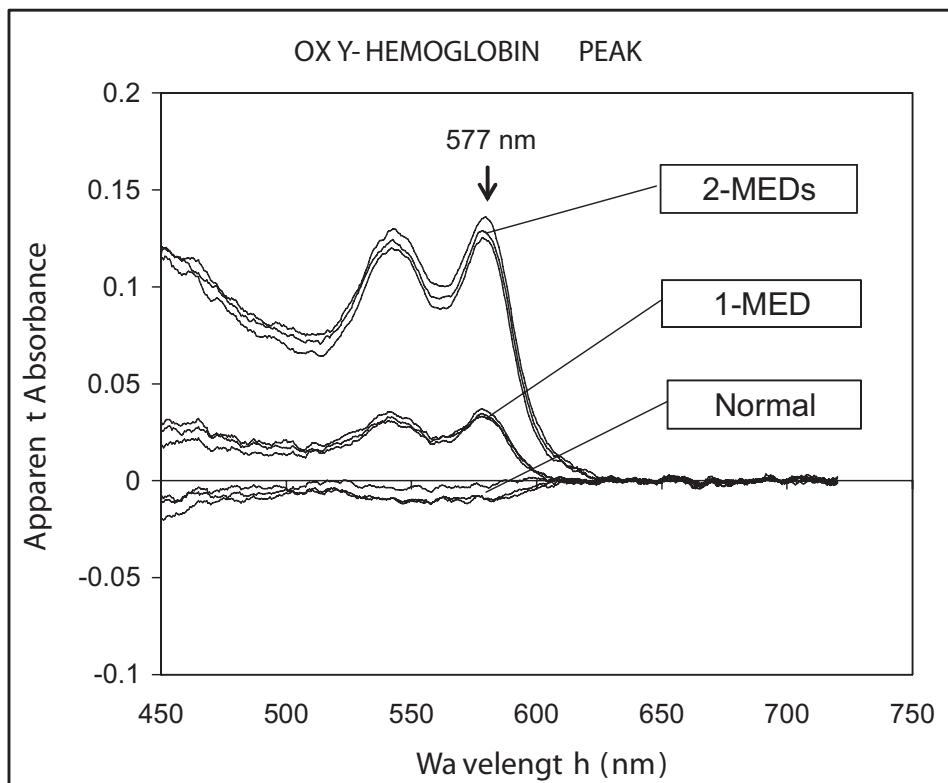
**Figure 28:** Laser Doppler Images show increased blood flow after orally taking niacin 250 mg. Facial blood flow peaked at 40 minutes, then reduced somewhat at 60 minutes and finally subsided at 80 minutes.

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 Bifurcated 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.

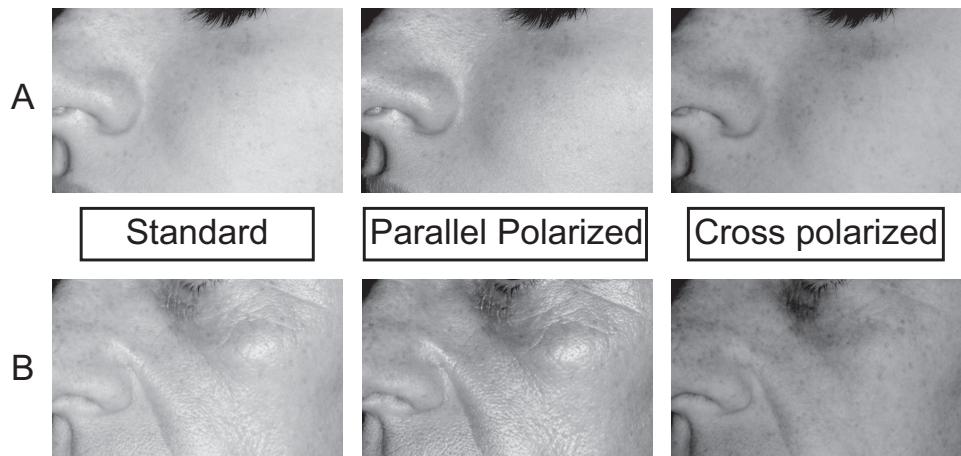
Decades of cumulative sun exposure of skin results in the gradual structural degradation and the appearance of an assortment of unwelcome signs of

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.

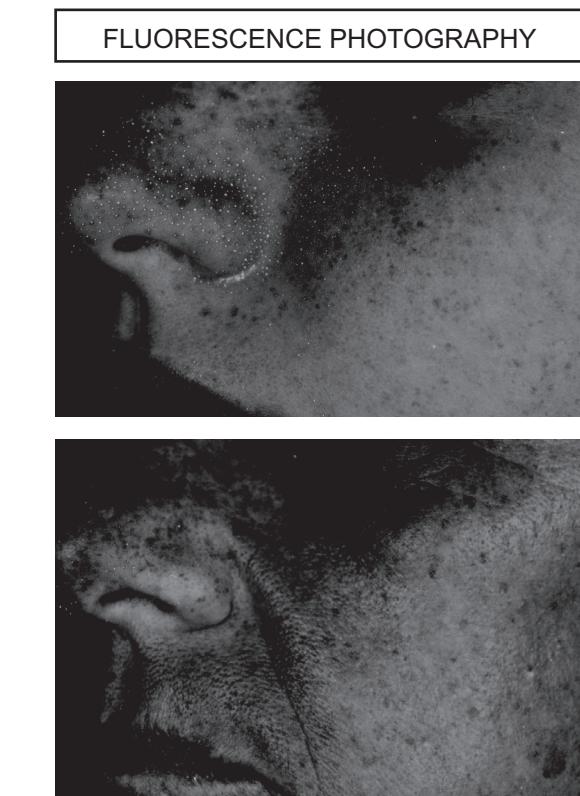
The older photo-damaged face (B) shows more wrinkles, roughness, prominent naso-labial fold and dyspigmentation compared to the young face (A). The wrinkle and roughness features are enhanced in the parallel polarized photos while the cross-polarized photos show the dyspigmentation more prominently.

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.

### DIGITAL PHOTOGRAPHY



**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.



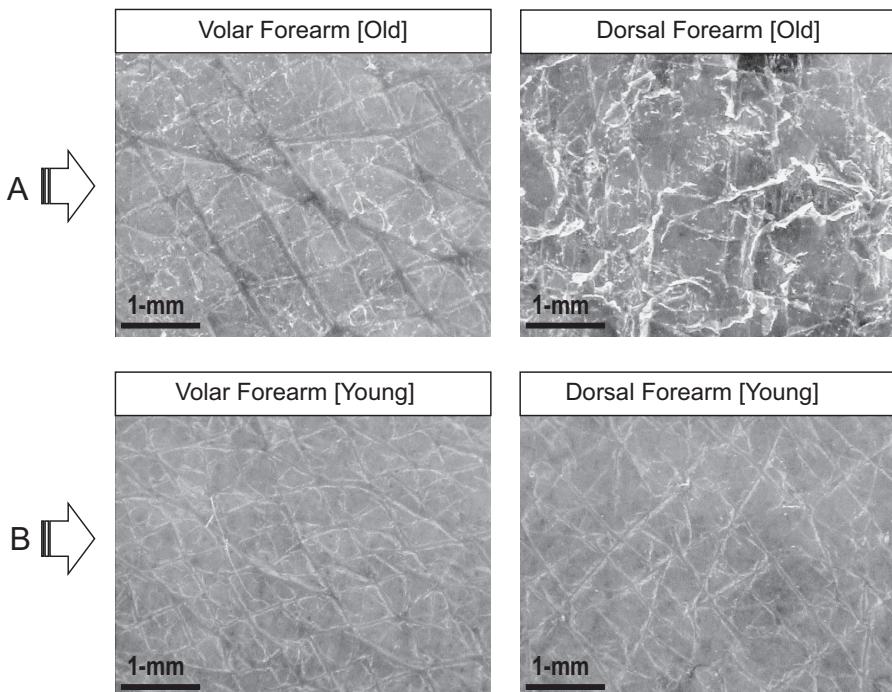
**Figure 32:** Fluorescence photos with the excitation wavelength centered at 417 nm show enhanced dyspigmentation, more on the older face (B) compared to the younger face (A). The young face also shows fluorescing follicular cast (yellowish spots) on the nose and surrounding area, some of them showing 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 $\times$  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 glyptic 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 glyptic pattern and barely any difference in pigmentation.

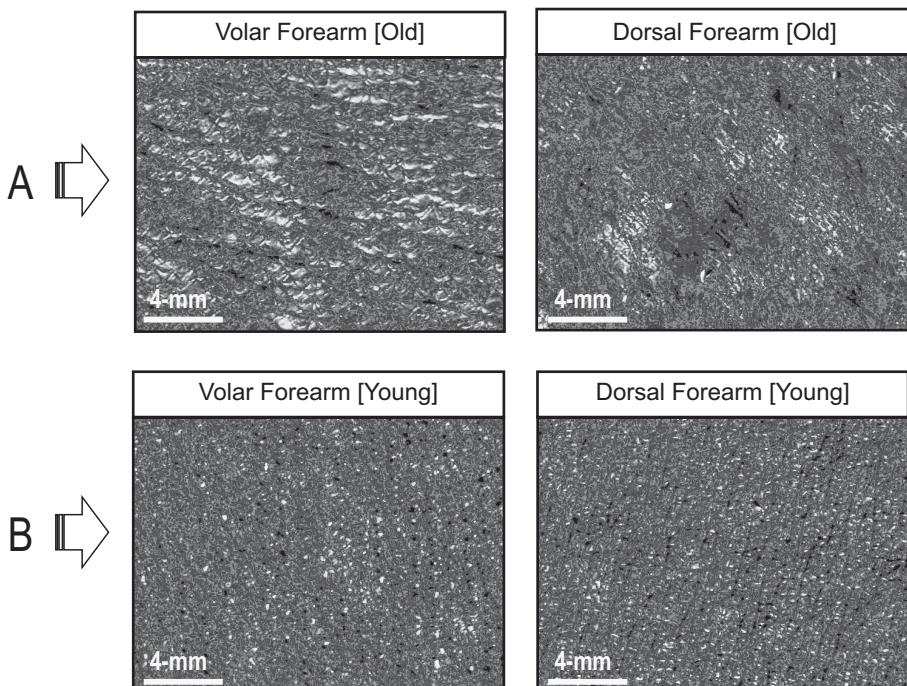


**Figure 33:** Videomicroscopy at 60 $\times$  reveals the differences in the relative photo-damage between the volar and dorsal forearms. The younger subject (B) shows slight difference in the glyptic pattern while the older subject (A) shows gross differences. The glyptic lines are practically obliterated, 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-dimensional image, shows differences in photo-damaged skin of the dorsal forearm compared to the volar forearm. Figure 34 shows the 3-D images.

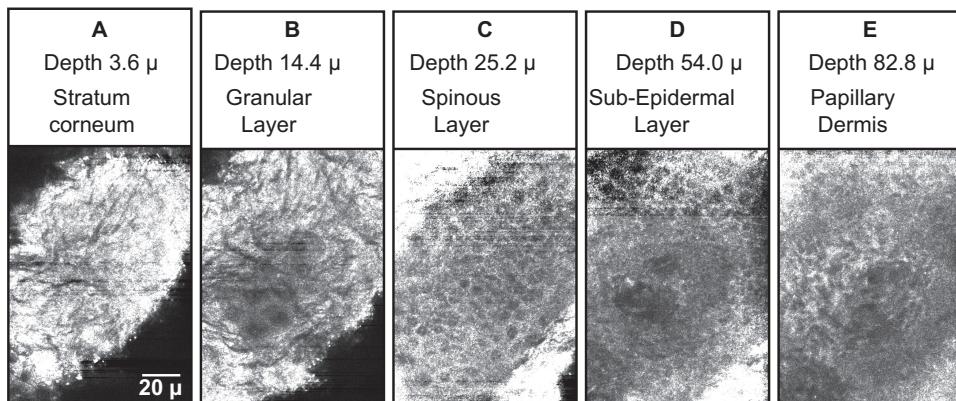
A mathematical construct of the primary image, after flattening the gross contour of the skin surface but leaving the microtopography intact, is presented. The color-coded image of the surface for heights show gross differences in the volar vs. dorsal forearm of older subject (A) while the younger subject (B) show slight

difference. The photo-damaged dorsal forearm of the older subject (A) shows gross distortion of the microtopography, loss of glyptic 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.

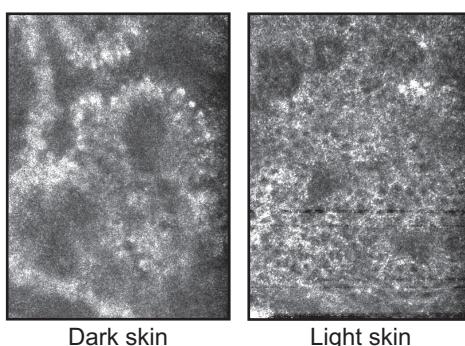
The confocal scanning laser microscope (CSLM) images horizontal sections of skin at various depths showing the structures of tissue and cells. Figure 35 shows confocal images of normal skin (volar forearm) at various depths. In normal skin the confocal images of SC appears as a bright structure due to strong reflection. The wrinkle lines and surface structure along with some glyptic lines can be observed. The *stratum corneum* at a depth of 3.6  $\mu\text{m}$  is shown in picture A. The cellular arrangement of *stratum granulosum* is shown in picture B at a depth of approximately 14.4  $\mu\text{m}$ . 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  $\mu\text{m}$ . Picture D shows the dermal-epidermal junction, with some capillary loops shown in the sub-epidermal layer, at a depth of 54.0  $\mu\text{m}$ . The dermis is shown in picture E at a depth of 82.8  $\mu\text{m}$ .



**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.



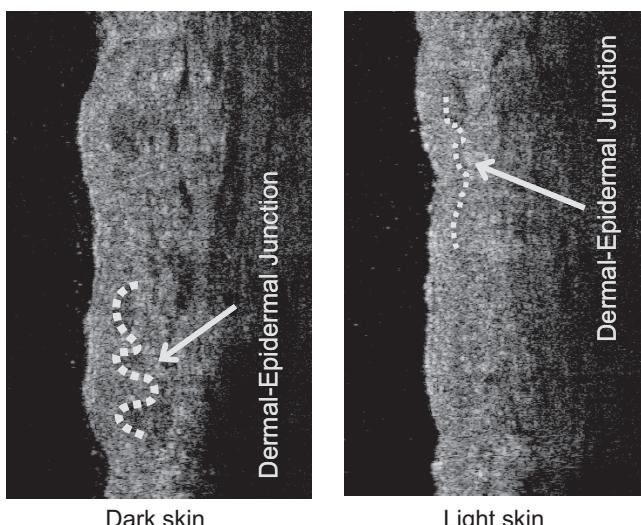
**Figure 36:** Confocal microscope section at the dermal-epidermal junction of a dark-skinned individual (left) compared to a light-skinned individual (right). Melanin near the basal layer is observed as bright spots as the horizontal section slices the dermal-epidermal junction.

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 lesions such as comedones and seborrheic keratosis can be visualized.

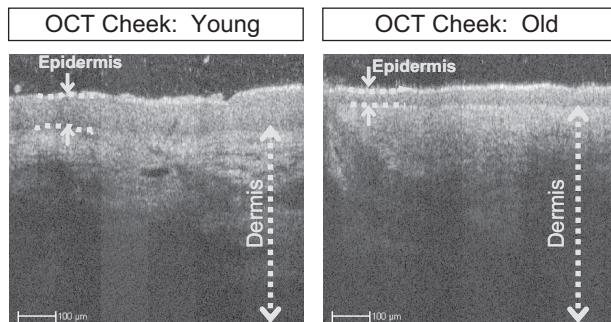
As it was shown by the Confocal Microscopy, the difference between dark and light skin can also be shown by the OCT at the dermal-epidermal junction. Figure 37 shows an example of an OCT image of dorsal forearm of a dark-skinned individual compared to image of a light-skinned individual.

The dark skin shows wavier dermal-epidermal junction compared to the light skin.



**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.

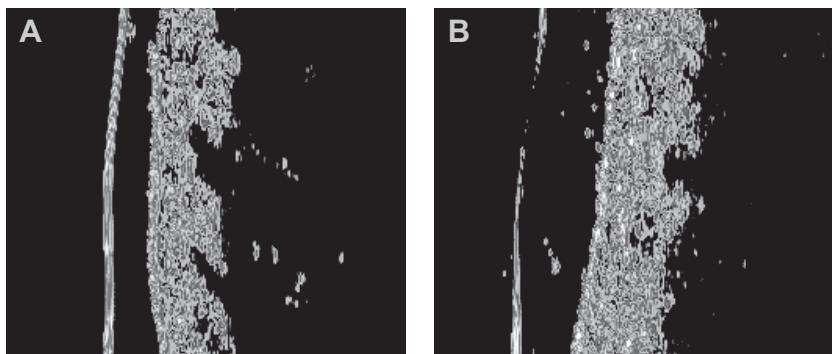


**Figure 38:** OCT image of the photo-damaged cheek (right) show thinning of epidermis and flattening of the papillary junction. The younger, relatively less photo-damaged skin (left) shows much thicker epidermis.

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.



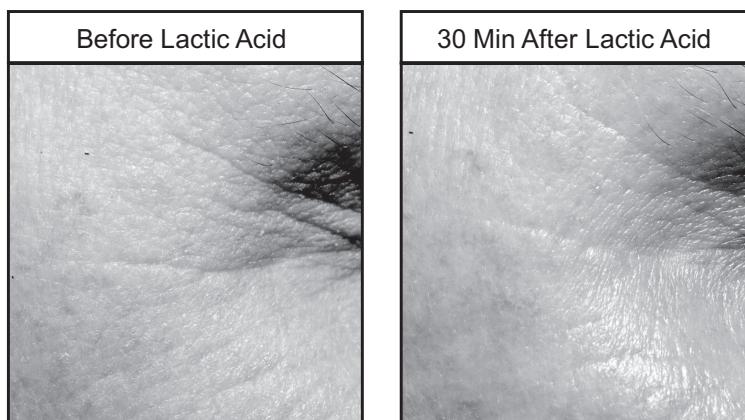
**Figure 39:** The effect of massaging on cellulite as revealed by ultrasound. The ultrasound images were obtained from the region of thigh showing cellulite, before treatment (A) and then after one month of daily massaging (B).

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.

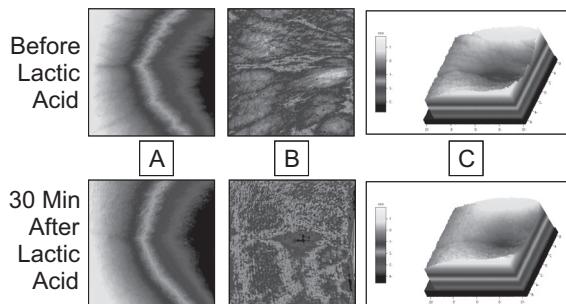
The digital photos obtained before and after the lactic acid treatment show the reduction of crow’s-feet wrinkles clearly (see Figure 40). The 3-D imaging by the fringe projection device also shows this difference (see Figure 41).

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.

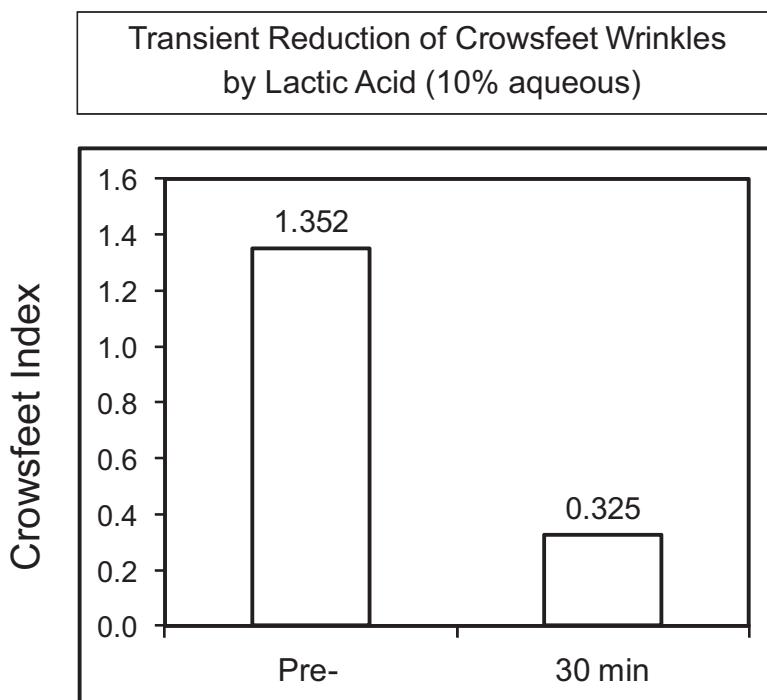


**Figure 40:** Digital photos of the crow’s-feet wrinkle area, before lactic acid treatment (left) and 30 minutes post-lactic acid treatment (right).

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.



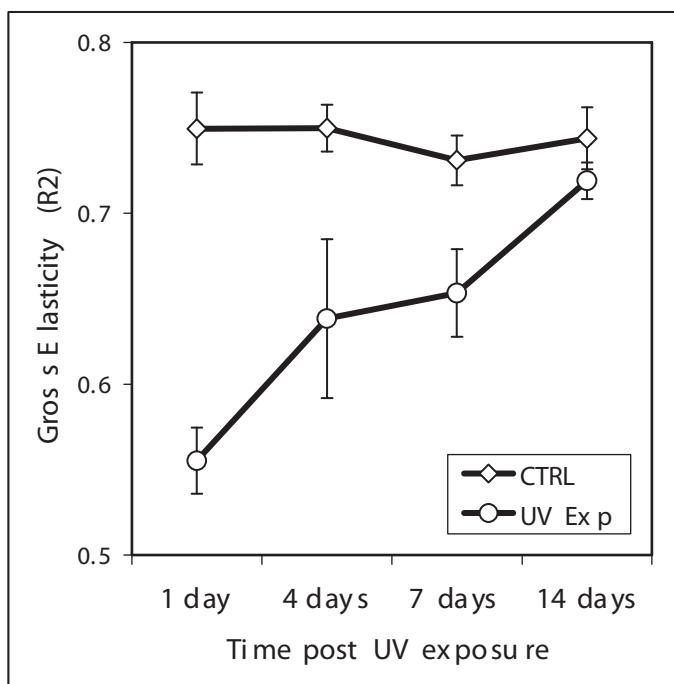
**Figure 41:** Fringe projection images before (top) and after (bottom) lactic acid treatment. The images are color-coded for heights. The original images (A) were processed to remove the gross contours to show flat images but with the wrinkles intact (B). The geometric 3-D images are shown in (C).



**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.

Apparent Loss of Elasticity after UV Exposure



**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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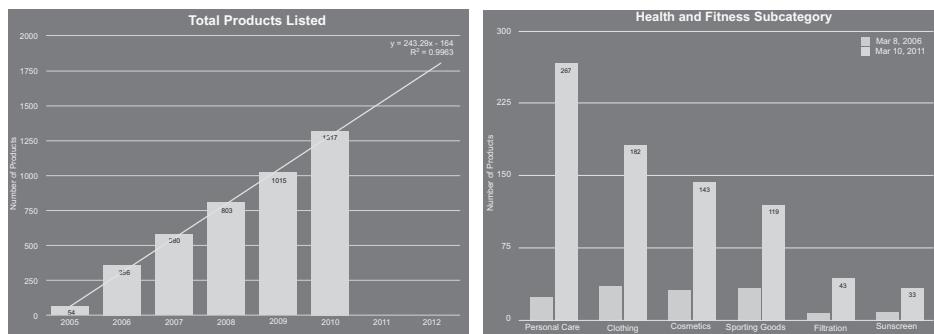
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### 11.6.1 INTRODUCTION

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  $\text{TiO}_2$  and  $\text{ZnO}$ , provide broad UV-protection and do not cause adverse cutaneous health effects.<sup>2</sup>

Silver nanoparticles are well known for their antibacterial properties<sup>3,4</sup> and are widely used throughout many industries.<sup>5</sup> 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 anti-bacterial 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.<sup>6</sup>

**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.*” 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.”<sup>7</sup> 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.

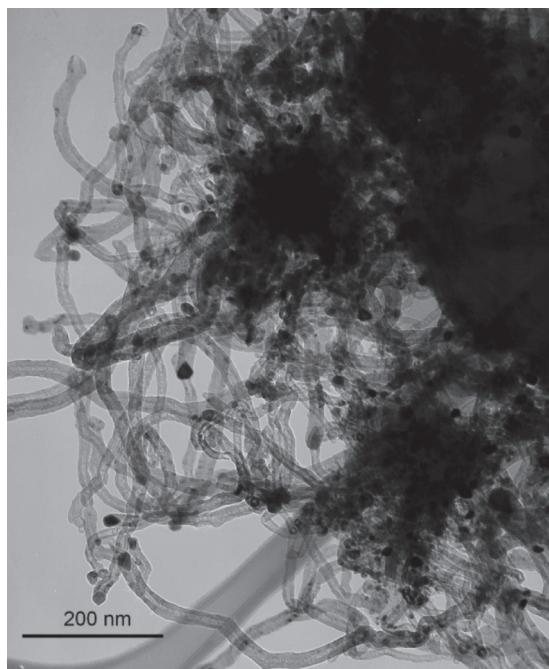
Linsinger recently produced a very comprehensive summary of the different analytical techniques available for the characterization of nanoparticles, and their relative strengths and weaknesses.<sup>8</sup> It must be noted that no one technique will provide a full description of the particle size and 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.<sup>9,10</sup> 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.

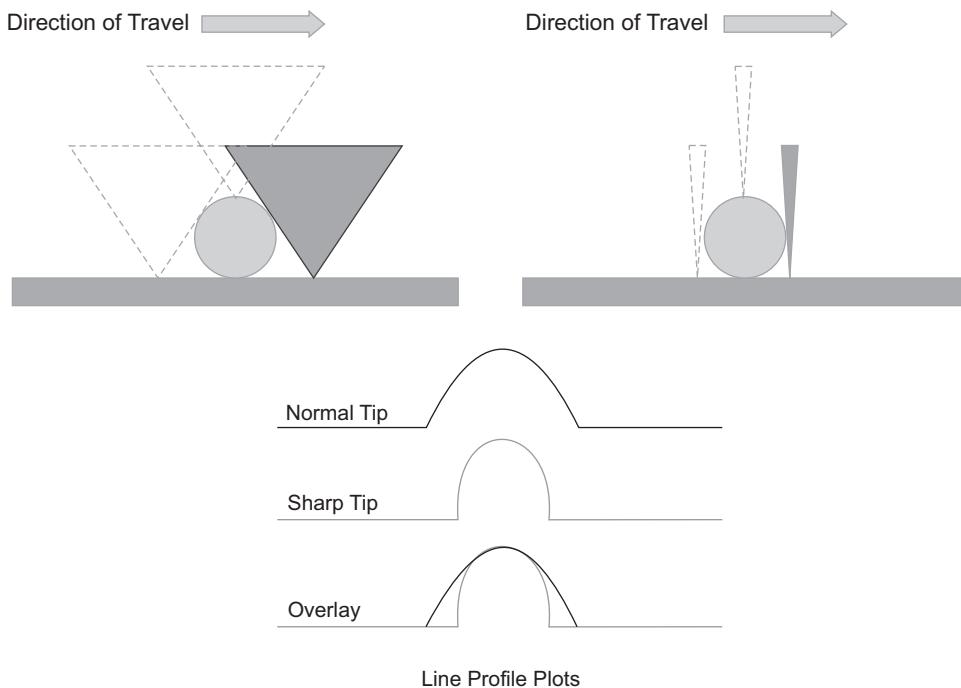
SEM or TEM coupled with energy-dispersive X-ray Spectroscopy (EDS) allows elemental information to be gained for individual particles. Variations in chemical composition may also be studied or mapped, even within individual particles. Again, it is important to note that almost all other techniques will 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<sup>11</sup> [(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),<sup>12</sup> scanning surface potential microscopy (SSPM)<sup>13</sup> and conductive probe atomic force microscopy (CP-AFM)<sup>14,15</sup> have proven useful for the study of the nanoscale electrical properties of various conducting and insulating materials including metal-insulator composites,<sup>16</sup> semiconductors,<sup>17</sup> and nanomaterials.<sup>18</sup>

For both STM and AFM,<sup>11</sup> 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.<sup>19</sup> 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.<sup>20, 21, 22</sup> 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.

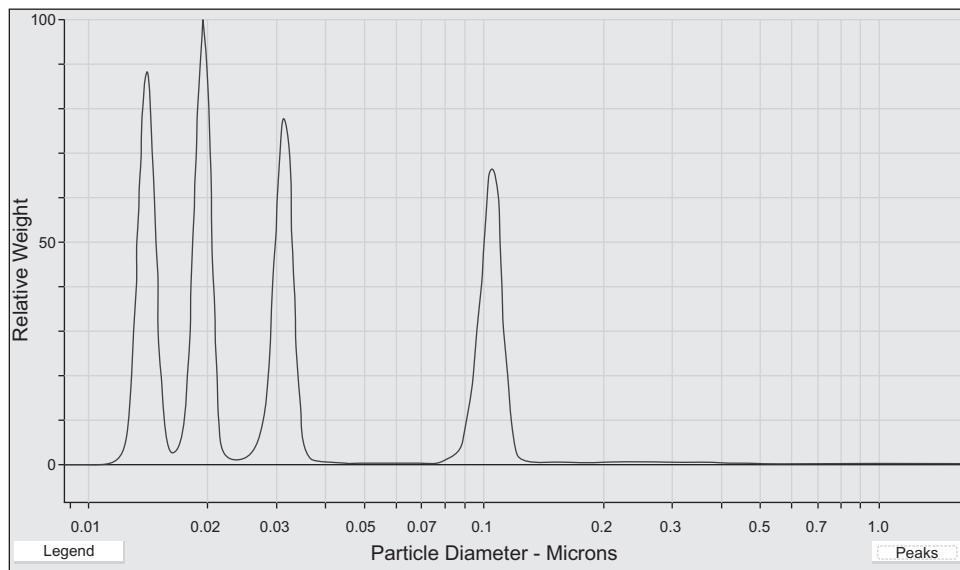
A significant advantage of AFM over many other techniques is that a nanomaterial can be imaged in either air or fluid environments. This allows for development of an improved understanding of how the individual particles, fibers, or tubes behave in various media. Of course, as with all probe-based techniques, 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.<sup>23</sup> 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.<sup>24</sup> 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.<sup>25</sup> 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.<sup>26</sup> 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.<sup>27</sup> 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 photoelectron 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).<sup>26</sup>

Because of optics limitations, XPS lacks the ability to focus the incident radiation beam to practical sizes smaller than  $\sim 10$  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.<sup>28, 29</sup> 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.<sup>30</sup>

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.<sup>31</sup> SIMS is performed by bombarding a surface with a focused primary ion beam. Numerous ion beam sources are available, including  $\text{Ga}^+$ ,  $\text{Cs}^+$ ,  $\text{Bi}_1^+$ ,  $\text{Bi}_3^+$ ,  $\text{C}_{60}^+$ , and other cluster sources, each of which affords advantages for

specific applications.<sup>32, 33</sup> 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,<sup>34</sup> 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 ( $\rho_s$ ), porosity, total pore volume (TOPV), pore size distribution, as well as activity and metal dispersion in the case of catalysts.

### a. BET Surface Area

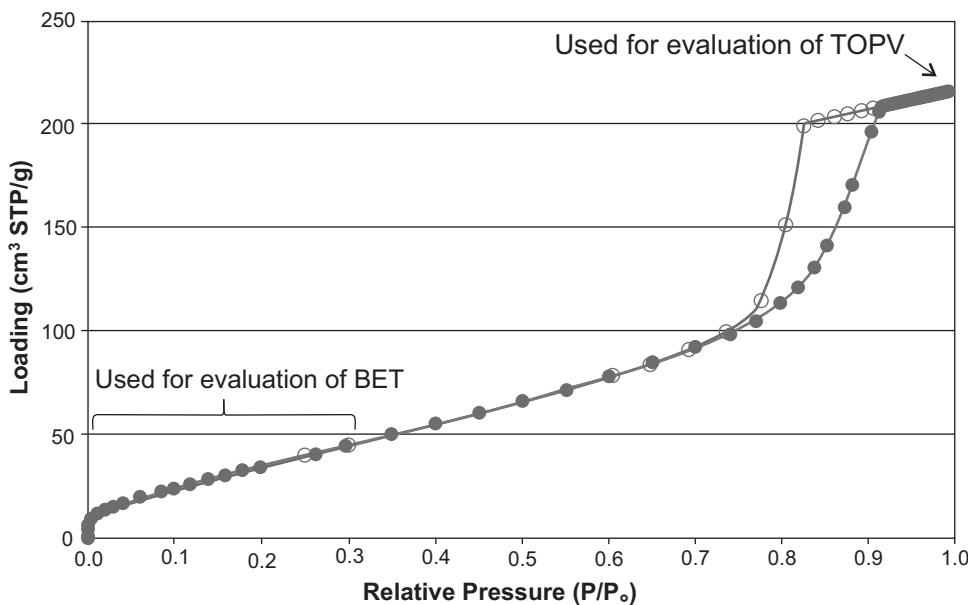
Although the underlying theory is known to have some weakness,<sup>35</sup> the BET technique remains one of the most popular methods for determining the surface area of powders and porous materials and is likely the most common sorption technique. Nitrogen gas is generally employed as the probe molecule 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<sup>2</sup>/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).<sup>36</sup> 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.<sup>37</sup> 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<sub>2</sub> at 77 K ( $\approx$ 230 torr). At relative pressures above the point at which a N<sub>2</sub> 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<sub>2</sub> 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.<sup>38</sup> 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<sub>2</sub> 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<sub>2</sub>, 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  $\sim 20\text{ \AA}$  to below  $\sim 1500\text{ \AA}$  ( $0.15\text{ }\mu\text{m}$ ). For materials containing larger pores, mercury porosimetry is the preferred experimental technique and spans the pore range from  $\sim 35\text{ \AA}$  to  $\sim 200\text{ }\mu\text{m}$ .



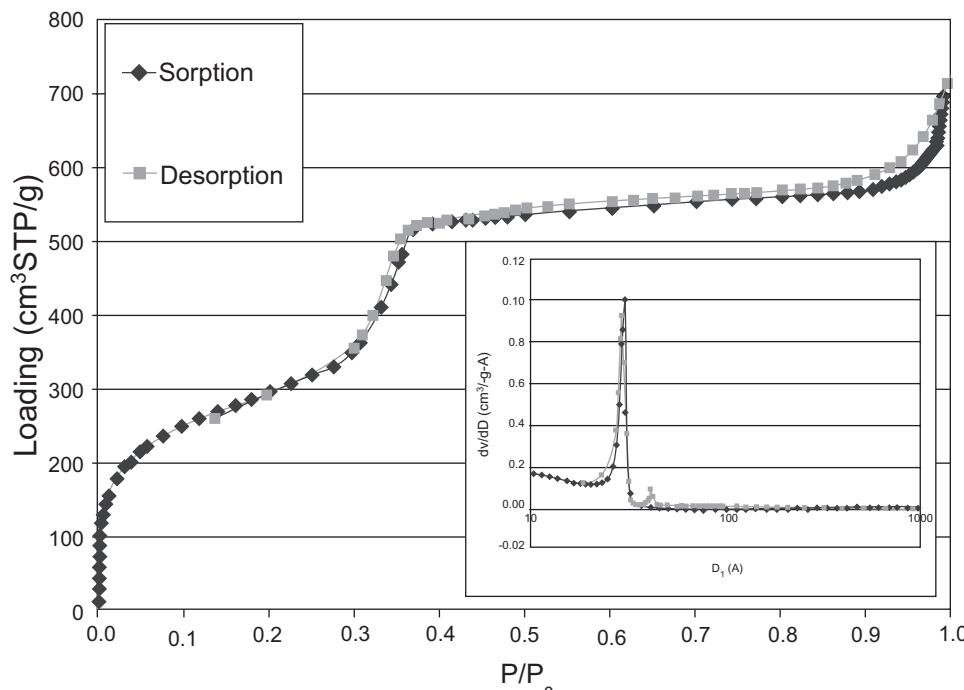
**Figure 3.1.** Typical  $N_2$  adsorption/desorption isotherm measured at 77 K. Closed circles represent adsorption while open circles represent desorption.

### b. Porosity

Since  $N_2$  condenses in the pores of the solid, TOPV has units of  $\text{cm}^3$  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  $\text{cm}^3$  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  $\rho_s$  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,<sup>36</sup> 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  $\text{N}_2$  isotherm with a well-defined pore structure. The sharp rise in the loading at low values of  $P/P_0$  indicates a high surface area (see Figure 3.1 for comparison), the abrupt loading transition within the  $P/P_0$  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  $P/P_0$ . The inset to Figure 3.2 shows the pore size distribution from a modified Kelvin analysis using a BJH thickness curve.<sup>36</sup>



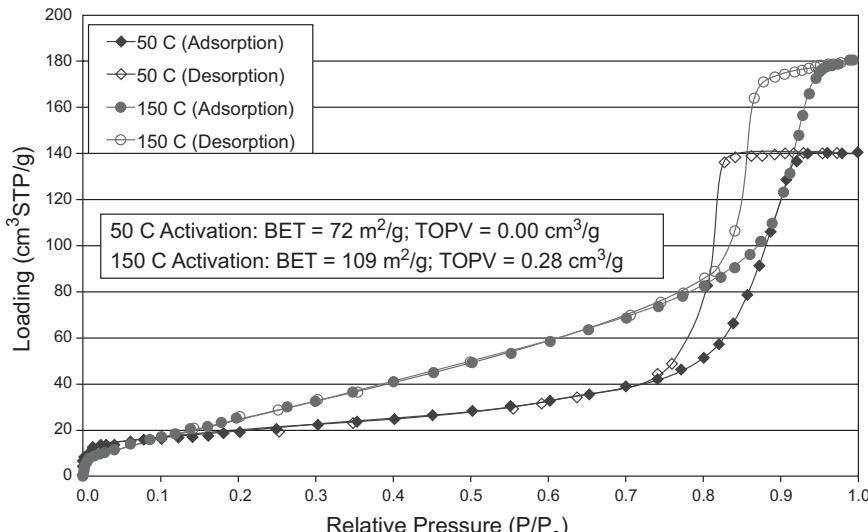
**Figure 3.2.** Typical  $\text{N}_2$  adsorption/desorption isotherm measured at 77 K. Inset is the pore size distribution evaluated from analysis of the isotherm.

### c. Sample Preparation

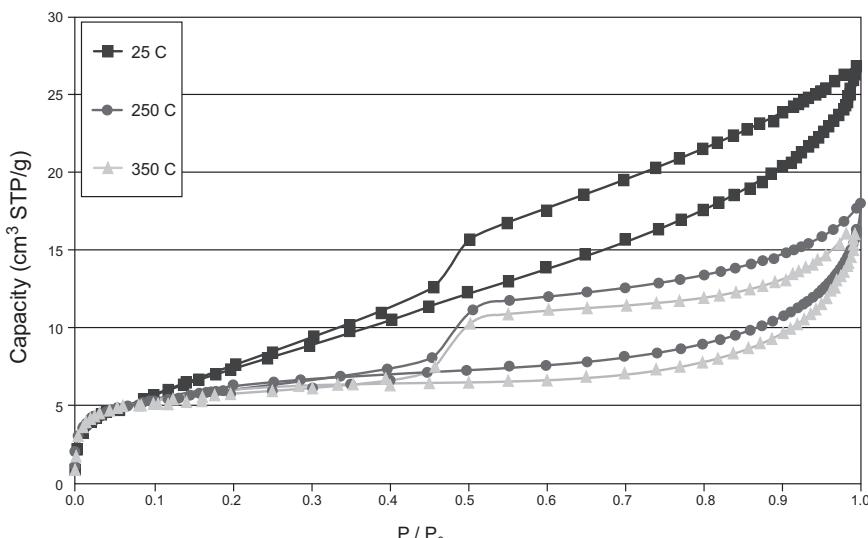
Lastly, the consideration given to sample preparation (or activation) prior to sorption analysis is a key aspect of material characterization. To perform a meaningful analysis the material should be in a state that is representative of how it will be used and pretreated accordingly. Often materials are 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

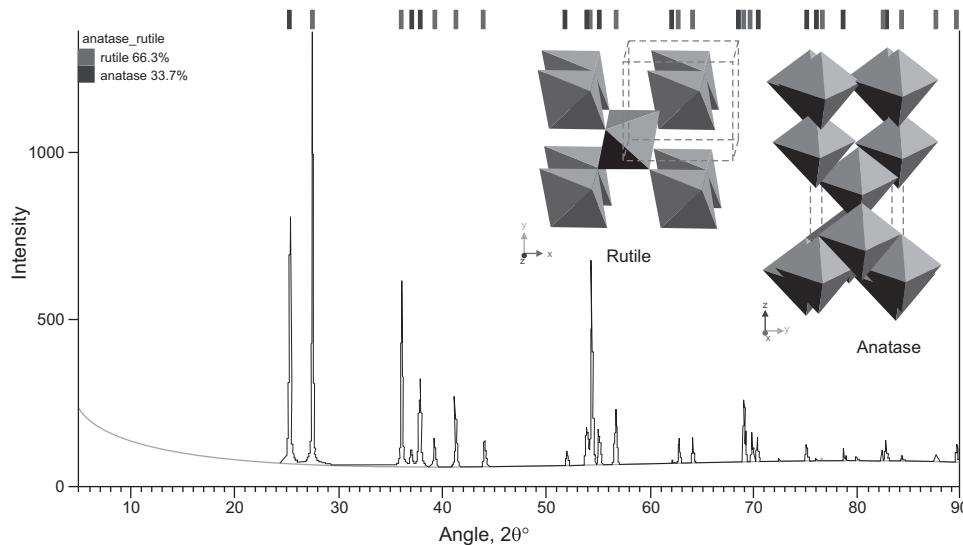
X-ray diffraction (XRD) is a nondestructive technique used to identify and quantify crystalline phases in a material.<sup>39</sup> Samples are typically analyzed as dry powders. However, crystalline phases in suspensions, gels, and creams can also be analyzed. XRD utilizes the elastic, coherent scattering 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<sub>2</sub> 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.<sup>39</sup> 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<sub>2</sub> when used as a UV protectant.<sup>40</sup> However, XRD can be used to identify and quantify polymorphs like rutile and

anatase in  $\text{TiO}_2$  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.<sup>41</sup> 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.

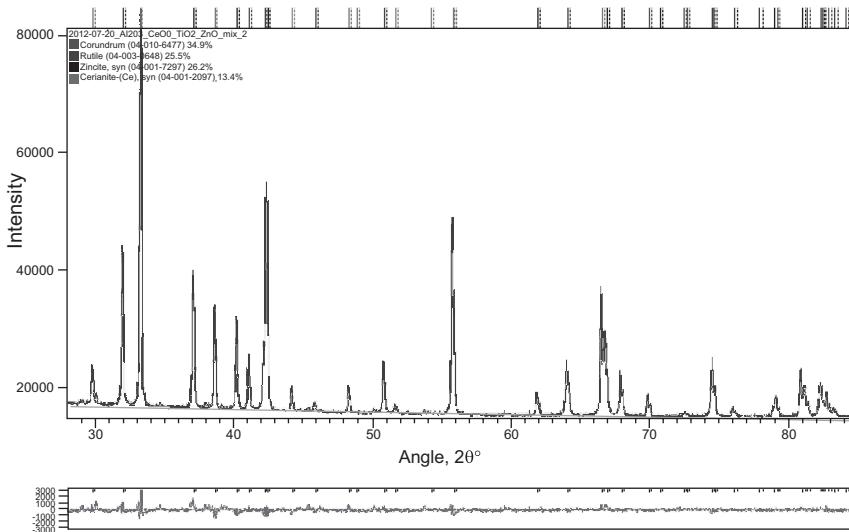


**Figure 4.1:** Comparison of XRD patterns for rutile and anatase simulated from crystal structures. The inset shows the difference in structure between the two polymorphs.

### b. Phase Composition using XRD

Calculation of the concentrations of crystalline phases in a sample is most accurately performed using the Rietveld Method.<sup>42</sup> 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.<sup>42</sup> Figure 4.2 shows a fit of calculated intensities to observed data from a sample mixture of Al<sub>2</sub>O<sub>3</sub> (corundum), CeO<sub>2</sub>, TiO<sub>2</sub> (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.<sup>42</sup> 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.

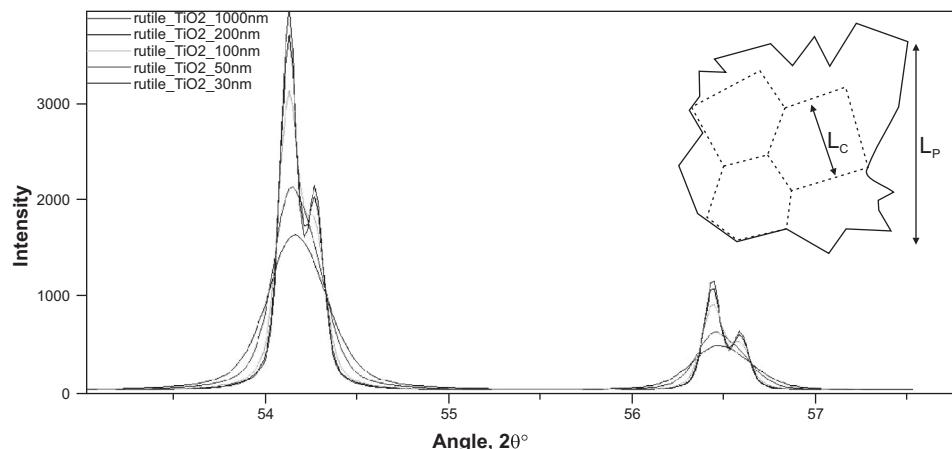


**Figure 4.2:** Fit of calculated intensities from refined sample model to observed XRD data.

### 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  $\text{TiO}_2$  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<sup>39</sup> 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<sup>43</sup>) or by calculating size and strain from peak profile parameters refined using the Reitveld method.<sup>44</sup> 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  $\text{TiO}_2$ . The inset illustrates the difference between crystallite size and particle size,  $L_p$ .

### 11.6.5 BULK METALS ANALYSIS

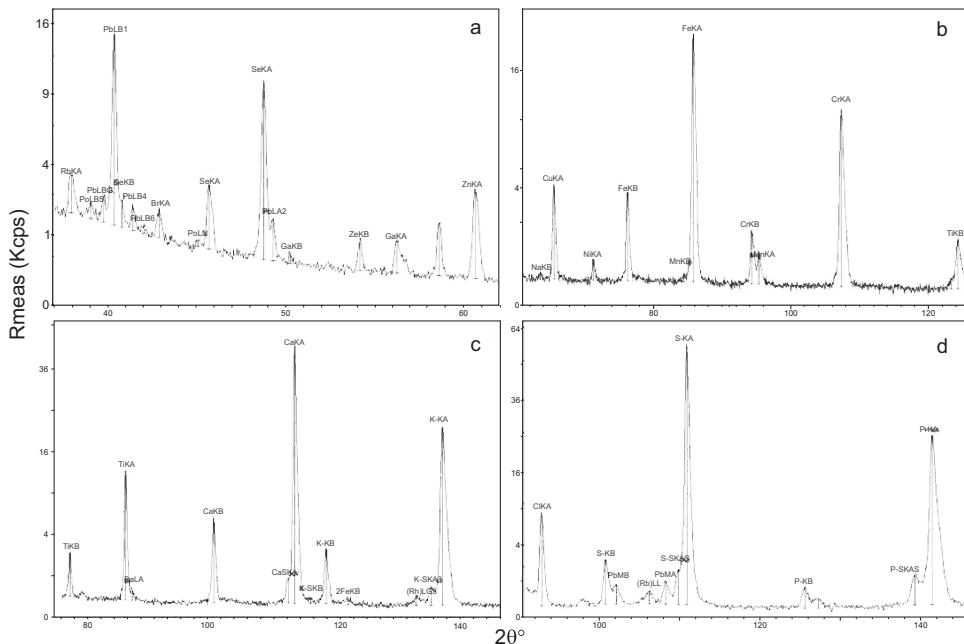
The two primary functions of elemental analysis in the cosmetics industry are to qualify raw materials and to quantify concentrations of trace elements that are toxic to the environment and personal health. The qualification of dry, raw materials, such as metal oxide powders or minerals, can be done using X-ray fluorescence

(XRF) spectroscopy.<sup>45</sup> The quantitation of trace and ultra-trace elements can be performed using flame atomic absorption spectroscopy (FAAS)<sup>46</sup> or inductively coupled plasma (ICP) techniques such as optical emission spectroscopy (OES)<sup>47</sup> or mass spectrometry (MS).<sup>48</sup> 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  $\text{TiO}_2$ ) are often mixed with zincite ( $\text{ZnO}$ ) to produce a mixture with superior protection across the spectrum of UV light compared to the individual components.<sup>40</sup> The attenuation of UV over a range of wavelengths is dependent upon the relative concentrations of  $\text{TiO}_2$  to  $\text{ZnO}$ . 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.<sup>49</sup> 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  $\text{N}_2$  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.<sup>50</sup>

Of particular concern are metals like lead and mercury, which exhibit toxicity to numerous organs and systems in the body.<sup>50</sup> 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.<sup>51</sup> Mercury compounds can significantly affect the kidneys, central nervous system, liver, and other systems after both acute and chronic exposure.<sup>52</sup> Recent cases involving

exposure to high levels of mercury in skin-lightening creams have illustrated the importance of metals testing to cosmetic products.<sup>53</sup>

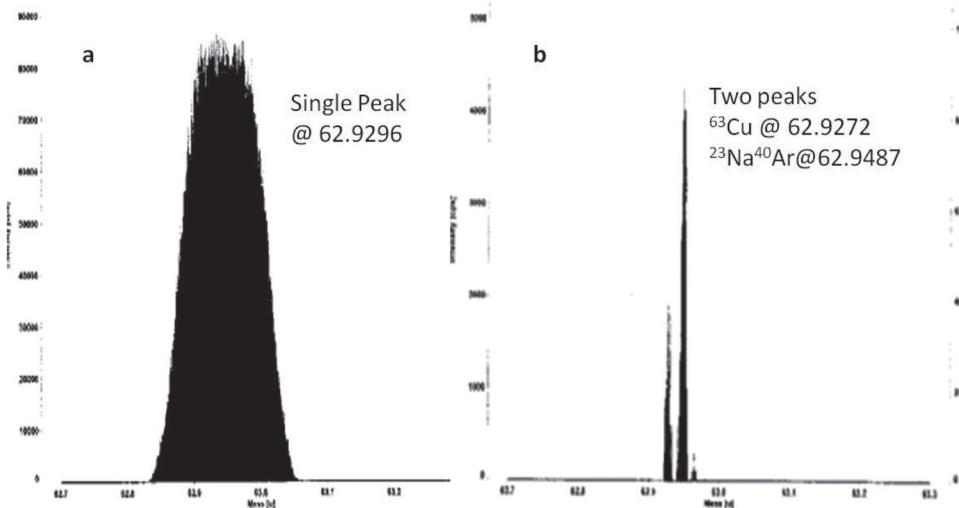
At such low concentration levels, analytical methods such as FAAS, ICP-OES, and ICP-MS are required to provide accurate quantitation. All of these methods depend upon the ability to dissolve the sample into an aqueous solution while retaining the elements of interest throughout the sample preparation 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  $\text{AuCl}_3$  to stabilize  $\text{Hg}^{2+}$  in an  $\text{HNO}_3$  solution.<sup>54</sup>

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  $^{63}\text{Cu}$  with  $^{23}\text{Na}^{40}\text{Ar}$  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  $^{65}\text{Cu}$  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  $^{63}\text{Cu}$  with  $^{23}\text{Na}^{40}\text{Ar}$  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

<b>TEM</b>	Transmission electron microscopy
<b>SEM</b>	Scanning electron microscopy
<b>STM</b>	Scanning tunneling microscopy
<b>AFM</b>	Atomic force microscopy
<b>EFM</b>	Electrostatic force microscopy
<b>SSPM</b>	Scanning surface potential microscopy
<b>CP-AFM</b>	Conductive probe atomic force microscopy
<b>DLS</b>	Dynamic light scattering
<b>AES</b>	Auger electron spectroscopy
<b>XPS</b>	X-ray photoelectron spectroscopy
<b>DSIMS</b>	Dynamic secondary ion mass spectrometry
<b>TOF-SIMS</b>	Time-of-flight secondary ion mass spectrometry
<b>TOPV</b>	Total pore volume
<b>BET</b>	Brunauer, Emmett, and Teller theory
<b>DFT</b>	Density functional theory
<b>PSD</b>	Pore size distribution
<b>XRD</b>	X-ray diffraction
<b>ICP-OES</b>	Inductively coupled plasma optical emission spectroscopy
<b>ICP-MS</b>	Inductively coupled plasma mass spectrometry
<b>FAAS</b>	Flame atomic absorption spectroscopy
<b>MDL</b>	Method detection limit
<b>LOQ</b>	Limit of quantitation

## EQUATIONS

*Equation 2.1*

$$E_k = h\nu - E_B - \phi_{sp}$$

Where  $E_k$  is the kinetic energy of the emitted electron,  $h\nu$  is the energy of the photon beam,  $E_B$  is the binding energy of the electron, and  $\phi_{sp}$  is the work function of the spectrometer.

*Equation 3.1*

$$\text{BET} \left[ \frac{\text{m}^2}{\text{g}} \right] = \frac{4\pi R^2}{\frac{4}{3}\pi R^3 \rho_s} = \frac{3}{\rho_s R} = \frac{6}{\rho_s D}$$

Where  $R$  is the pore radius,  $D$  is the pore diameter, and  $\rho_s$  is the skeletal density.

*Equation 3.2*

$$\begin{aligned} \text{TOPV} \left[ \frac{\text{cm}^3}{\text{g}} \right] &= \text{Max Loading} \left[ \frac{\text{cm}^3 \text{STP}}{\text{g}} \right] \times \\ &\left[ \frac{1 \text{ mole}}{22,414 \text{ cm}^3 \text{STP}} \right] \times \text{MW} \left[ \frac{\text{g}}{\text{mole}} \right] \times \frac{1}{\rho_L} \left[ \frac{\text{cm}^3}{\text{g}} \right] \end{aligned}$$

Where MW and  $\rho_L$  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<sup>3</sup>, respectively.

*Equation 3.3*

$$\text{Porosity} = \frac{\text{cm}^3 \text{void}}{\text{cm}^3 \text{solid} + \text{cm}^3 \text{void}} = \frac{\text{TOPV}}{\frac{1}{\rho_s} + \text{TOPV}}$$

Where TOPV is the total pore volume found by Equation 3.2, and  $\rho_s$  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 ubiquitylation 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

<b>Agent</b>	<b>Effect on DNA Methylation</b>	<b>Mechanism of Action</b>	<b>Reference</b>
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 ubiquitylation, 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  $\text{H}_2\text{O}_2$  (Forneris et al. 2005). Since there are readily available commercial probes that react with  $\text{H}_2\text{O}_2$  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-Deazaneplanocin 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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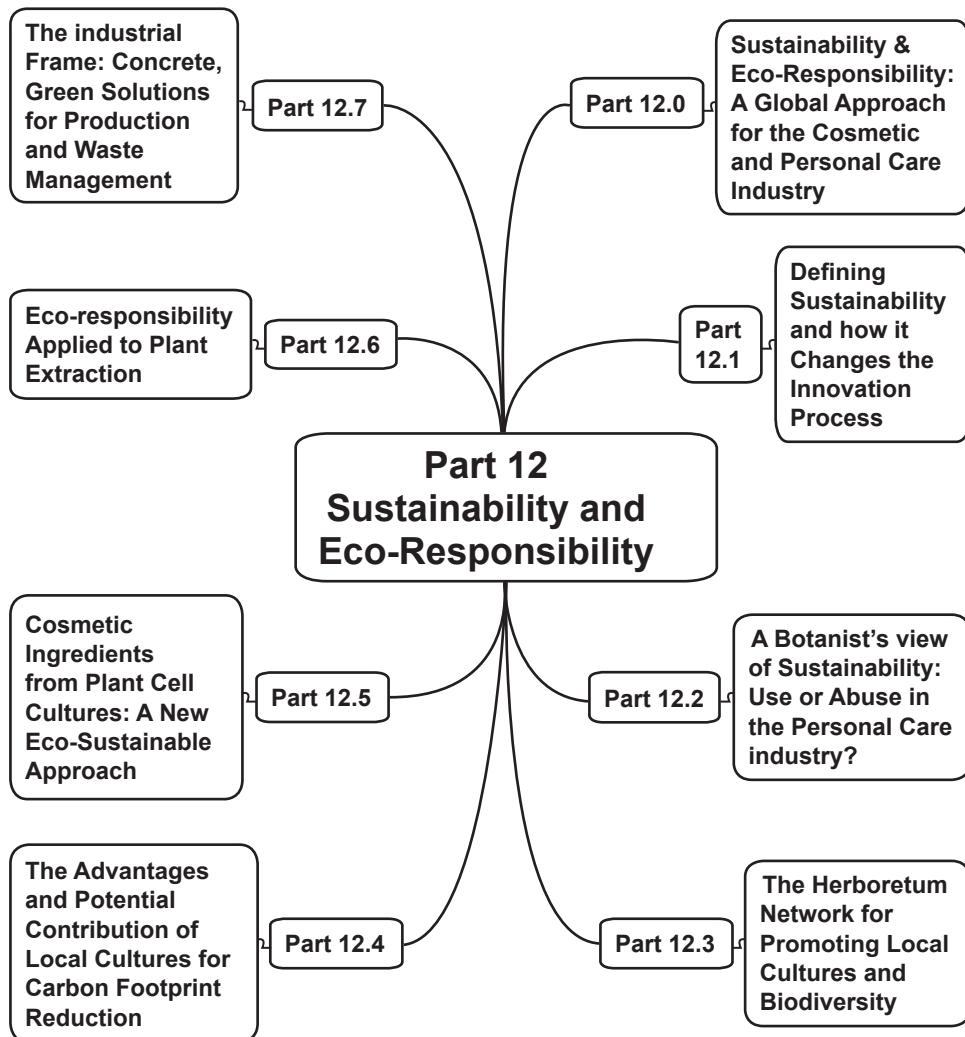
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### SUSTAINABILITY & ECO-RESPONSIBILITY



## 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**

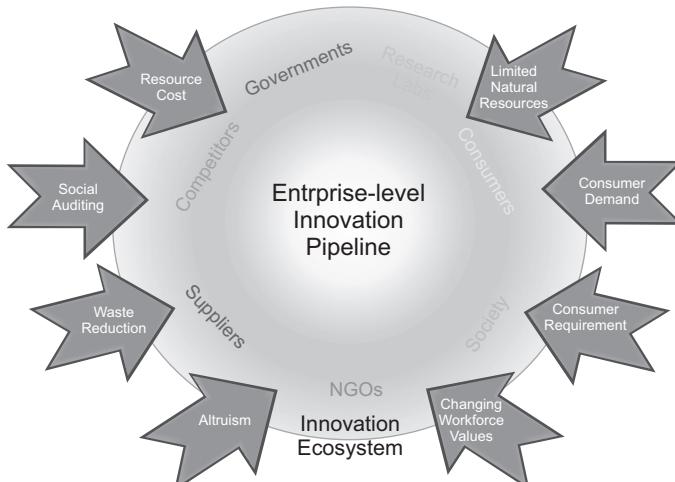
The complexity of sustainability and the many parts that are required to truly follow a green path necessitate that a company must collaborate. No company can source all its ingredients, packaging, or manufacturing equipment eternally. Each of these components plays a significant role in the environmental 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**

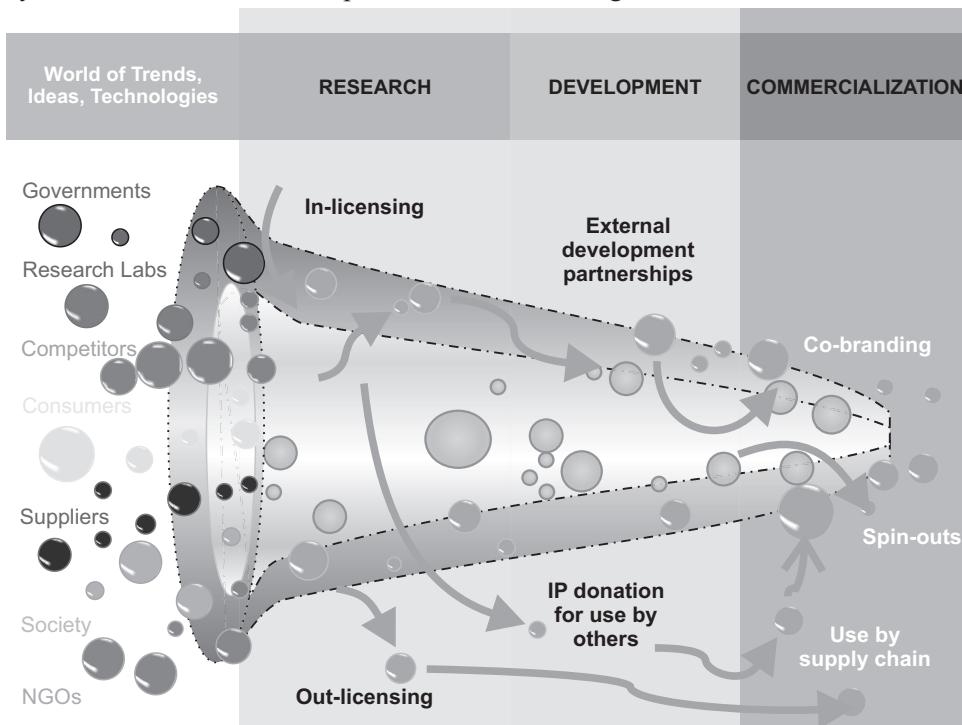
- In the process of becoming more sustainable, companies typically become more “open”—for example, they work with their suppliers, customers, and NGOs to understand sources of environmental or social impact along their supply chains. The relationships established in the process of opening up can then 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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<b>12.2.2 What happens once you find a species of interest?</b>	<b>2011</b>
1. Accurate identification of botanicals	2011
2. Understanding why the plant is used in the product, and what part or form will give the best result to the consumer	2011
3. Truthful representation of the local uses of the plant in marketing efforts	2011
4. Making sure the environment is not degraded as a result of harvesting botanicals	2012
5. Ensuring that local communities are not negatively impacted by the harvest of the plant	2012
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	2012
<b>12.2.3 Sustainable production of wild-harvested products</b>	<b>2012</b>
<b>Acknowledgments</b>	<b>2016</b>
<b>References</b>	<b>2016</b>

### 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 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<sup>1</sup>**

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 (*Bertholetia 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](http://pdf.usaid.gov/pdf_docs/PNABT501.pdf).

An alternate, less technical, but useful analogy for 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.<sup>2</sup> 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 mind-sets 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 (fox-glove, 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 cortège 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 competitiveness.

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

### Author

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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:

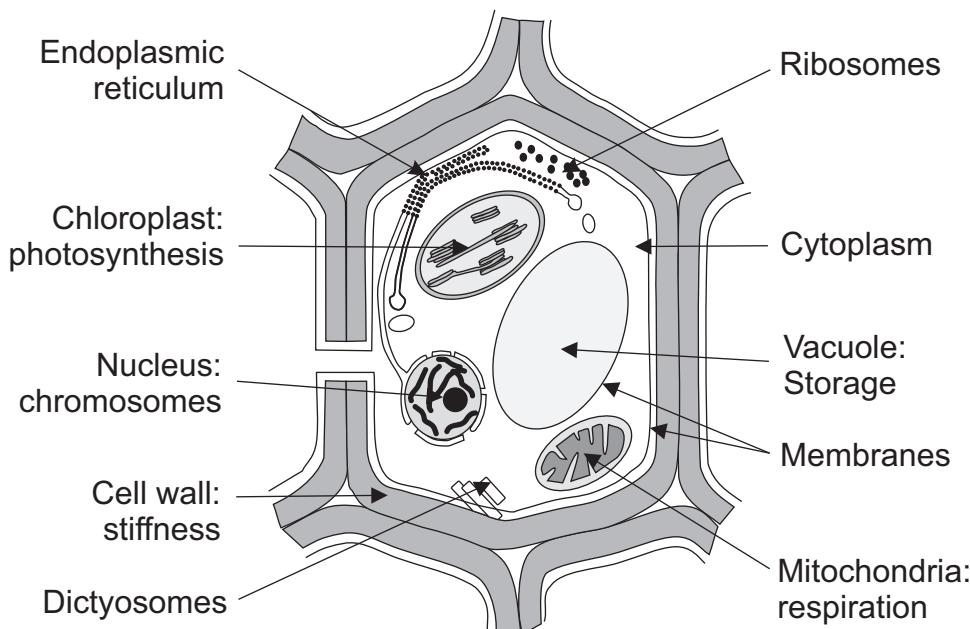
- Cosmetics-sector abandonment of ingredients derived from animals in the wake of Bovine Spongiform Encephalopathy (BSE);
- Campaigns warning against excessive consumption of medications in so-called developed countries;
- A collective realization of the richness of nature and its biodiversity;
- Prestigious 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 re-planting 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.

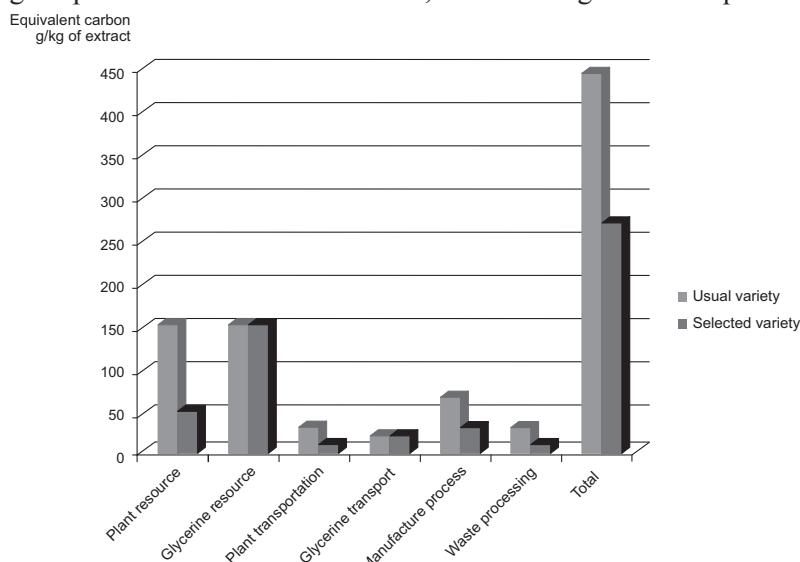
The example of cosmetic extracts obtained from the flower of a selected variety of German chamomile (*Matricaria recutita* L.) shows that it is possible to obtain good-quality extracts equivalent to the concentration of active ingredient by using three times less plant mass. Also, the risk of undesirable contaminants is mathematically reduced by three.

**Table 4.2**

Composition	German chamomile Flower <i>Matricaria recutita</i>		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 recutita* 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 micro-organisms 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

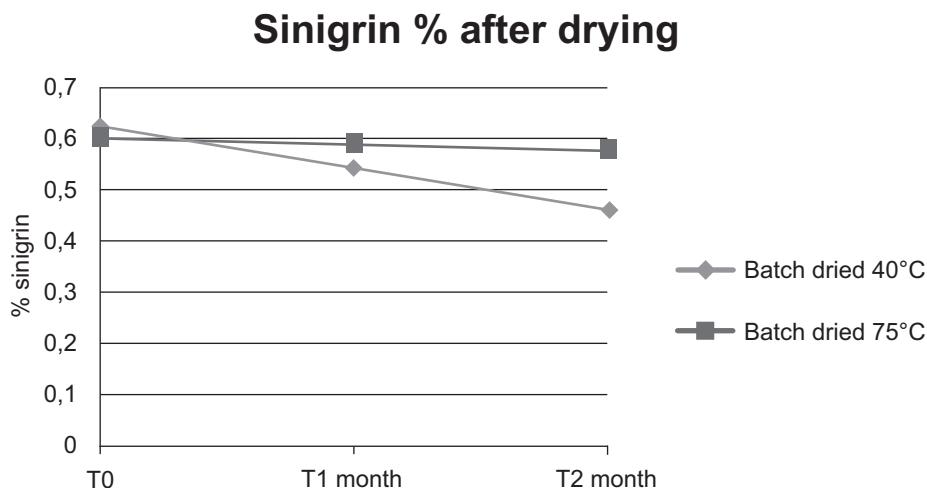
The drying technique is the most commonly used to preserve plants. However, this must also be perfectly handled. If it is carried out badly or in the wrong conditions it can be dangerous to quality and safety.

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 *Brassicaceae* 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 repellent or antimicrobial role.

However, for the cosmetic use of *erisimum* 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 *erisimum* 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  $\mu\text{g}/\text{kg}$ ; 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.*

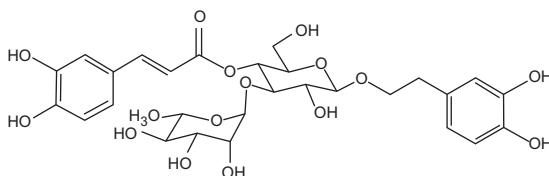
#### **12.5.4 ADVANTAGES OF PLANT CELL CULTURES: THE NEW ALTERNATIVE**

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<sup>2</sup> 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<sup>2</sup> 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 <sup>2</sup> )	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 peroxy 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- $\kappa$ B (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- $\kappa$ B 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](http://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 12<sup>th</sup> 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 (propane-diol)
- 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-drying 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 zeolites (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 epidemic, 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  $-20^{\circ}\text{C}$ , 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 ( $\leq 40^\circ\text{C}$ ) 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 m<sup>2</sup> 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, ranunculus, 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**

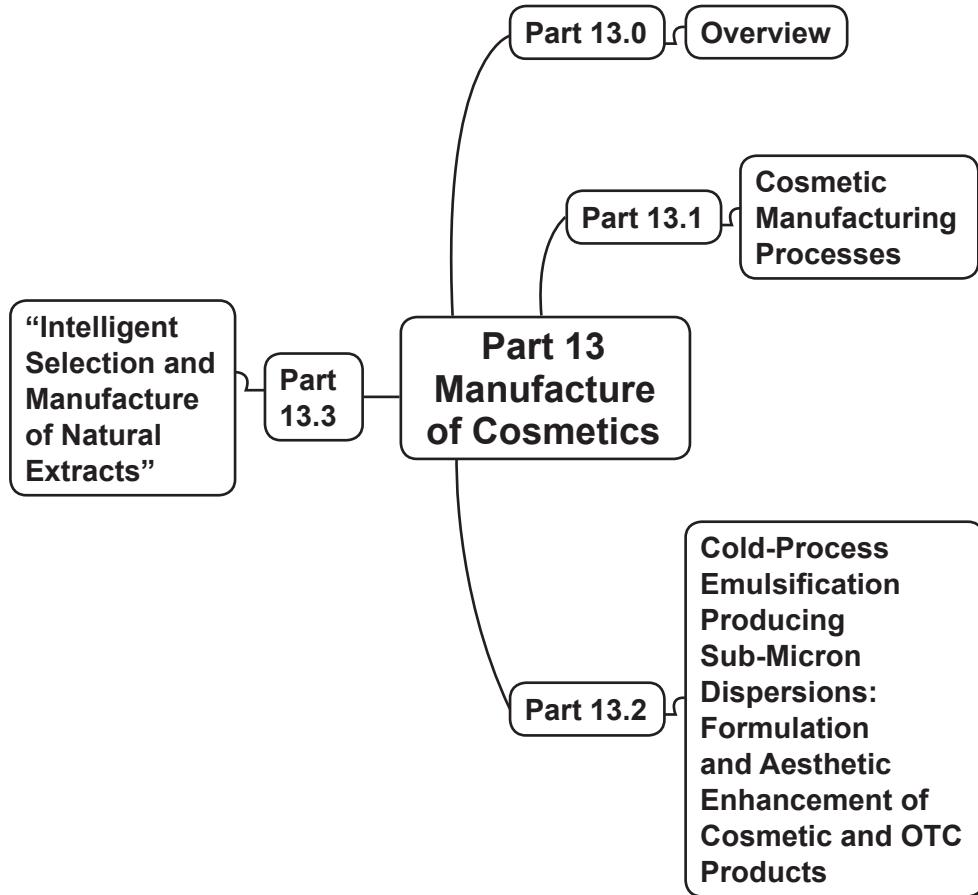
By re-creating the wetlands, our water gardens have witnessed a spontaneous return of flora and small animals to the recovered habitats. The new riverbanks have encouraged the return of amphibians (frogs, tree frogs, newts, etc.), the most endangered species because of their disappearing biotope. Insects, 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 Cosmetology*.

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

## COSMETIC MANUFACTURING PROCESSES

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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) Degaeration 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) Degaeration 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 non-uniformities 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.

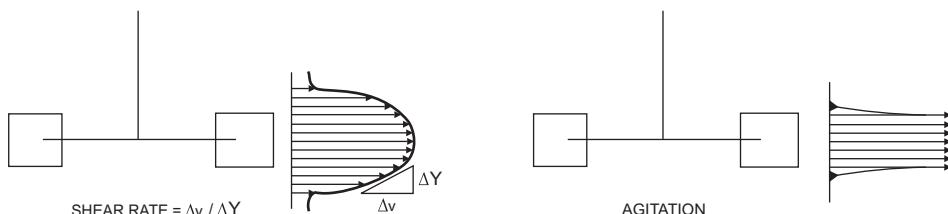
## **2. Mixing Rheology**

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

$\rho$  = Density Of Liquid In Grams Per Cubic Centimeter

$\eta$  = Viscosity Of Liquid In Centipoise

$C$  = Conversion Constant Of  $1.67 \times 10^{-4}$

Equation 13.1 Reynolds Number

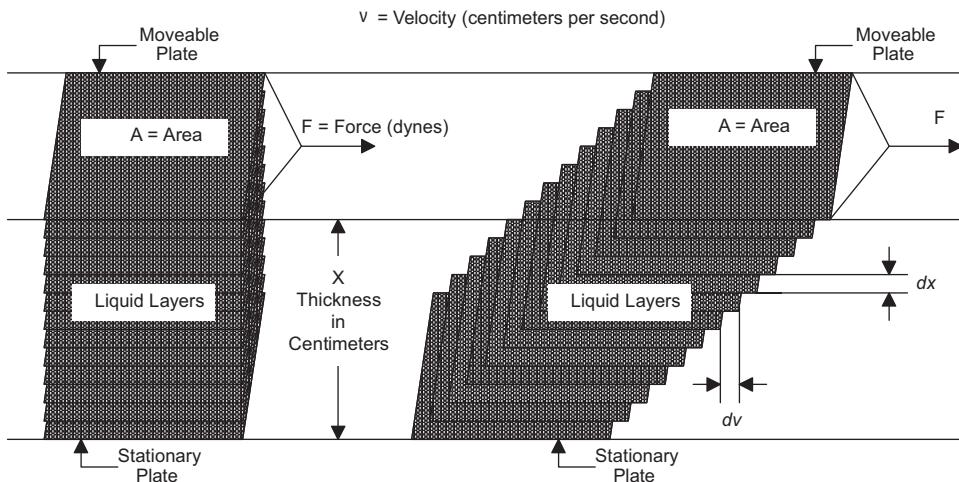
Experience has shown that the onset of turbulence occurs at Reynolds Numbers above 2,000. For fully developed turbulence, Reynolds Numbers greater than 10,000 are required and these higher values are found in many cosmetic mixing processes. As shown in Equation 13.1, it becomes more difficult to achieve turbulence as the viscosity ( $\eta$ ) 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 ( $v$ ) 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 ( $v$ ) 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.

$$F = \eta \times A \times v$$

Where  $F$  = Force in gram-centimeters per second squared

$\eta$  = Coefficient of Viscosity of Liquid in gram per centimeter-second squared

$A$  = Cross-sectional Area of the liquid in square centimeters

$v$  = 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.

$$F = (\eta_{app})^n \times A \times v$$

Where: F = Force in gram-centimeters per second squared

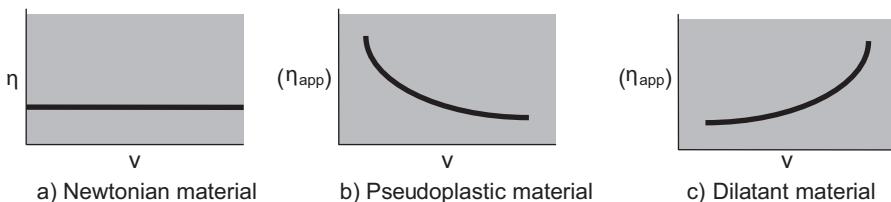
$\eta_{app}$  = Apparent Viscosity of Liquid in gram per centimeter-second squared  
n = exponent

A = Cross-sectional Area of the liquid in square centimeters

v = 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  $\mu\text{m}$  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 ( $v$ ) plotted against viscosity ( $\eta$ ) or apparent viscosity ( $\eta_{app}$ )

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 ( $\Delta T_{LM}$ ) 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

$\Delta T_{LM}$  = Log Mean Temperature gradient between the product and the heat transfer fluid (difference) in °C

#### Equation 13.4 Heat transfer

$\Delta T_{LM}$  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  $\Delta t$  (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

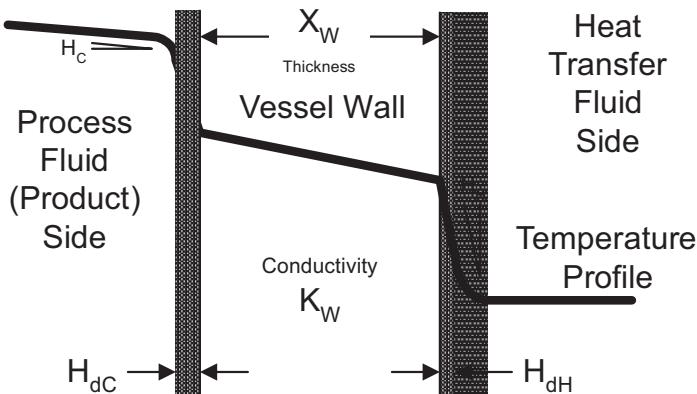
$X_w$  = Thickness of vessel wall in meters

$K_w$  = 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  $\Delta t$  (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,  $\Delta T$ . The driving force for mass transfer is  $\Delta C$ , 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**

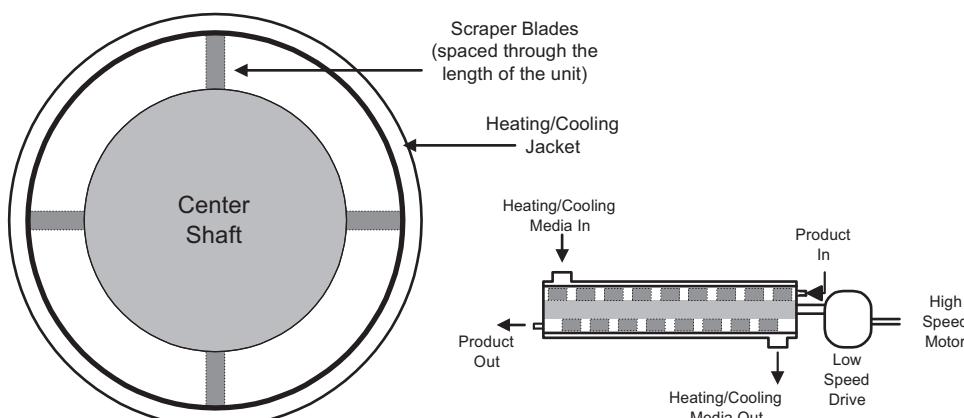
The most common heat transfer method used in the cosmetic industry is jacketing of a vessel/pipe. The jacket may be used for either heating or cooling with steam, water, or other heat transfer medium (specially designed oils and glycols). Most liquid products can be made using this type of cooling regardless 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:** Scraper-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)

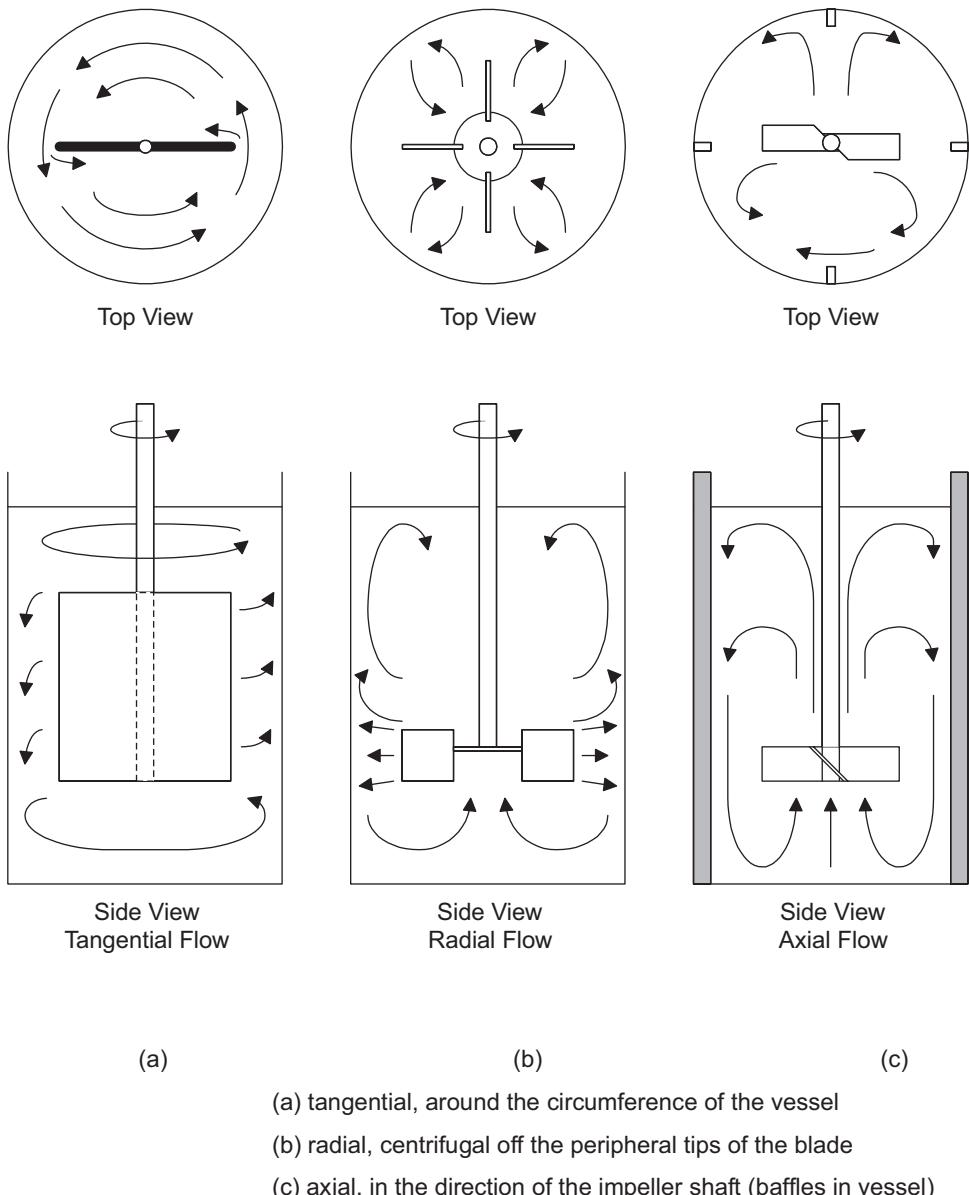
Flow patterns in agitated vessels can be resolved into three principal types: tangential flow, radial flow, and axial flow.

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.



**Figure 13.6:** Flow patterns with different agitators

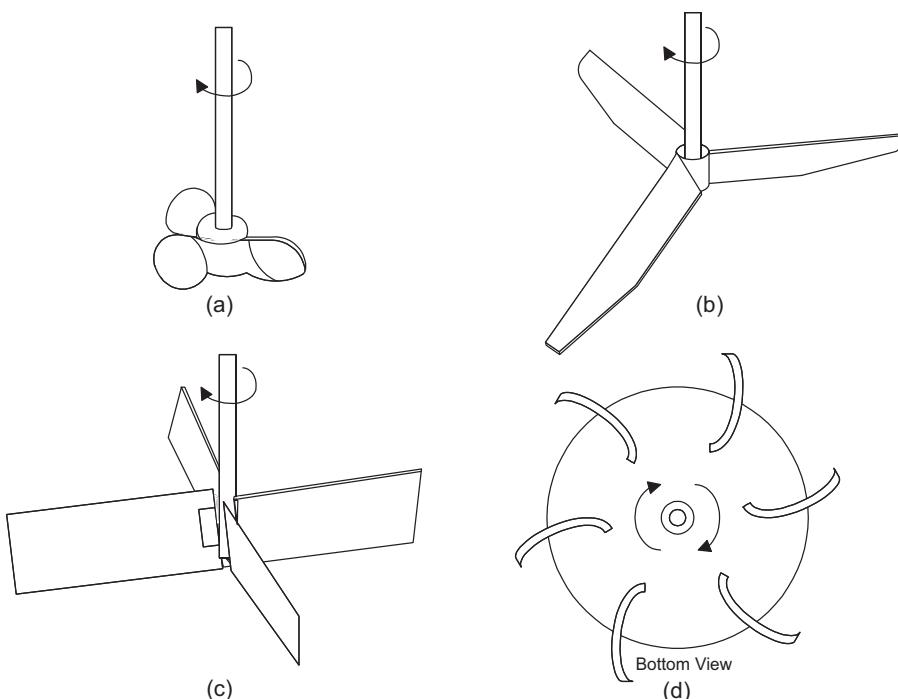
Viscous liquids showing shear-thinning characteristics present considerable problems to the cosmetics processor. The fluid close to the rotating impeller of a mixer is sheared at a high rate and so becomes relatively mobile. But as this is pumped away from the impeller, regions of less intense flow and 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

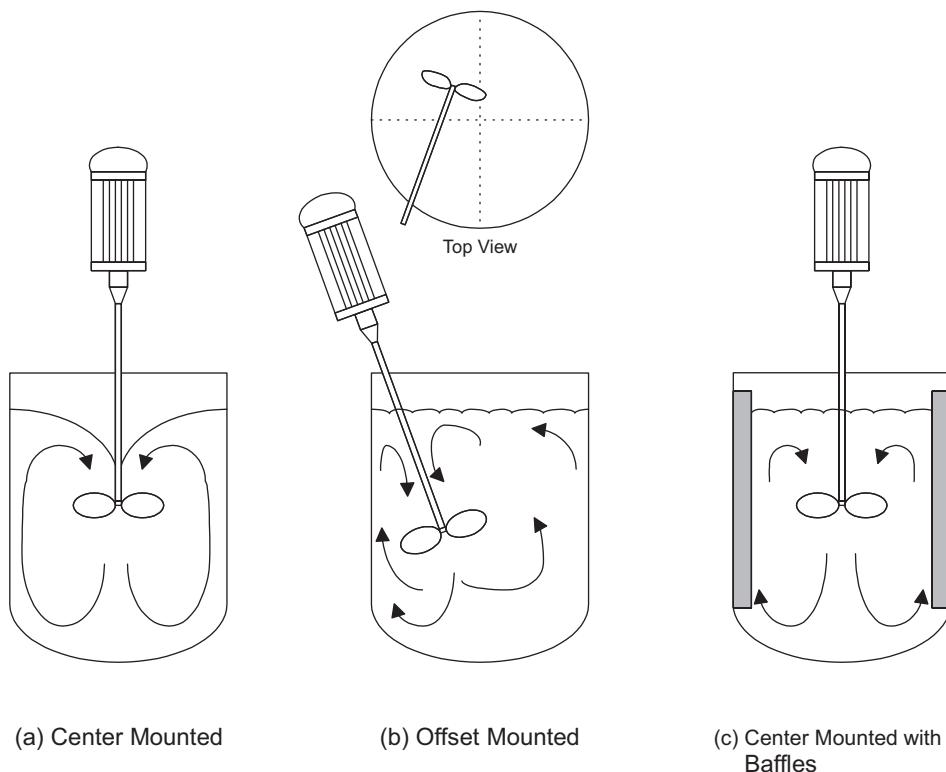
**Paddle mixers** produce mainly tangential flow and are usually mounted centrally because of their large diameter compared with that of the tank. For viscous liquids, the paddle is often of an anchor design equipped with scraper blades that remove product from the walls of the vessel.

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  $0.33 \pm 0.05$ , 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-entrainment. 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

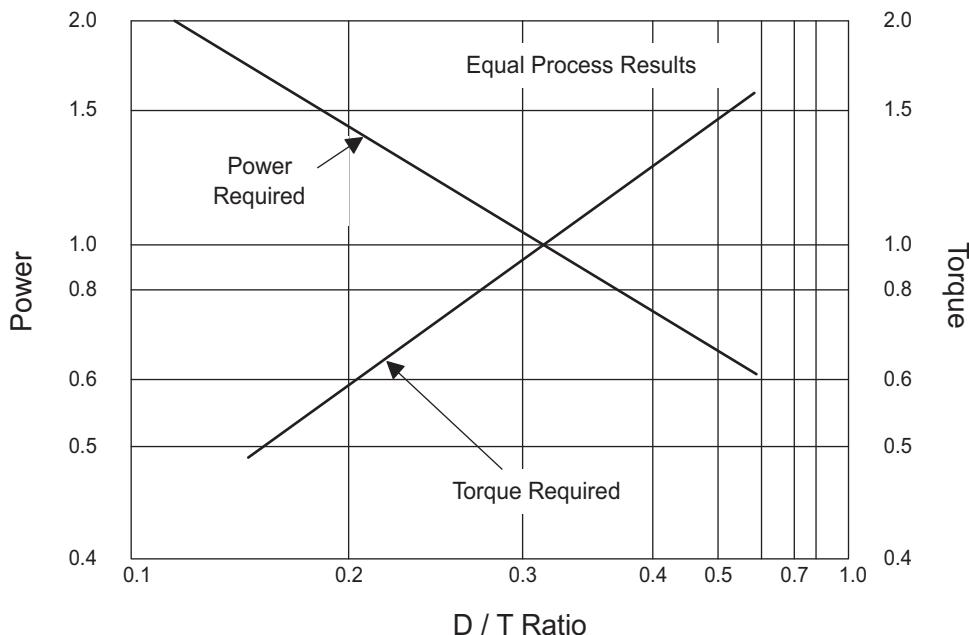
$H$  = Velocity Head in meters

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.



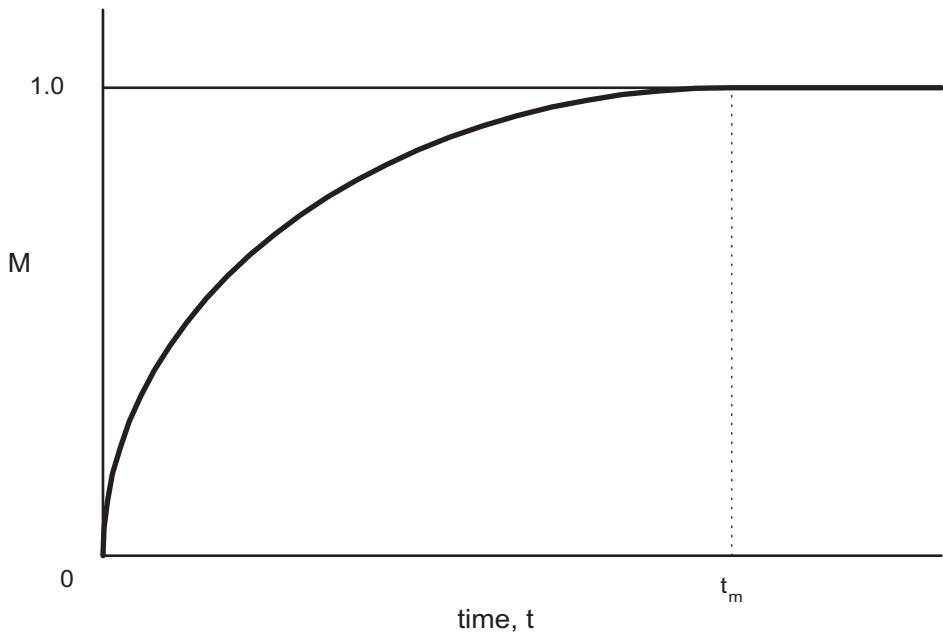
**Figure 13.9** Power and torque relationship for equal process results [9]

### e. Mixing Time

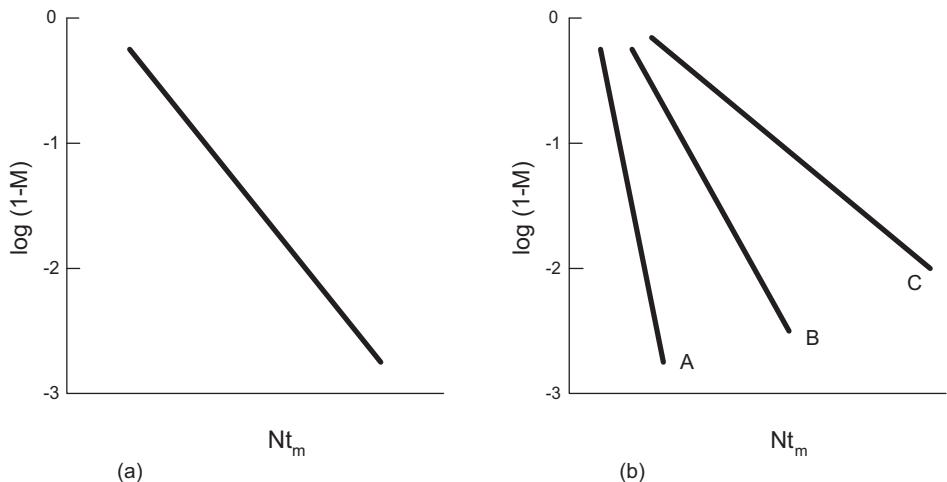
Another important measurable parameter is mixing time,  $t_m$ . This is the time taken to achieve the desired degree of homogeneity in the mixture. There are many methods by which this characteristic may be measured, but perhaps the most obvious is the time taken for a soluble dye to become uniformly 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,  $Nt_m$

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

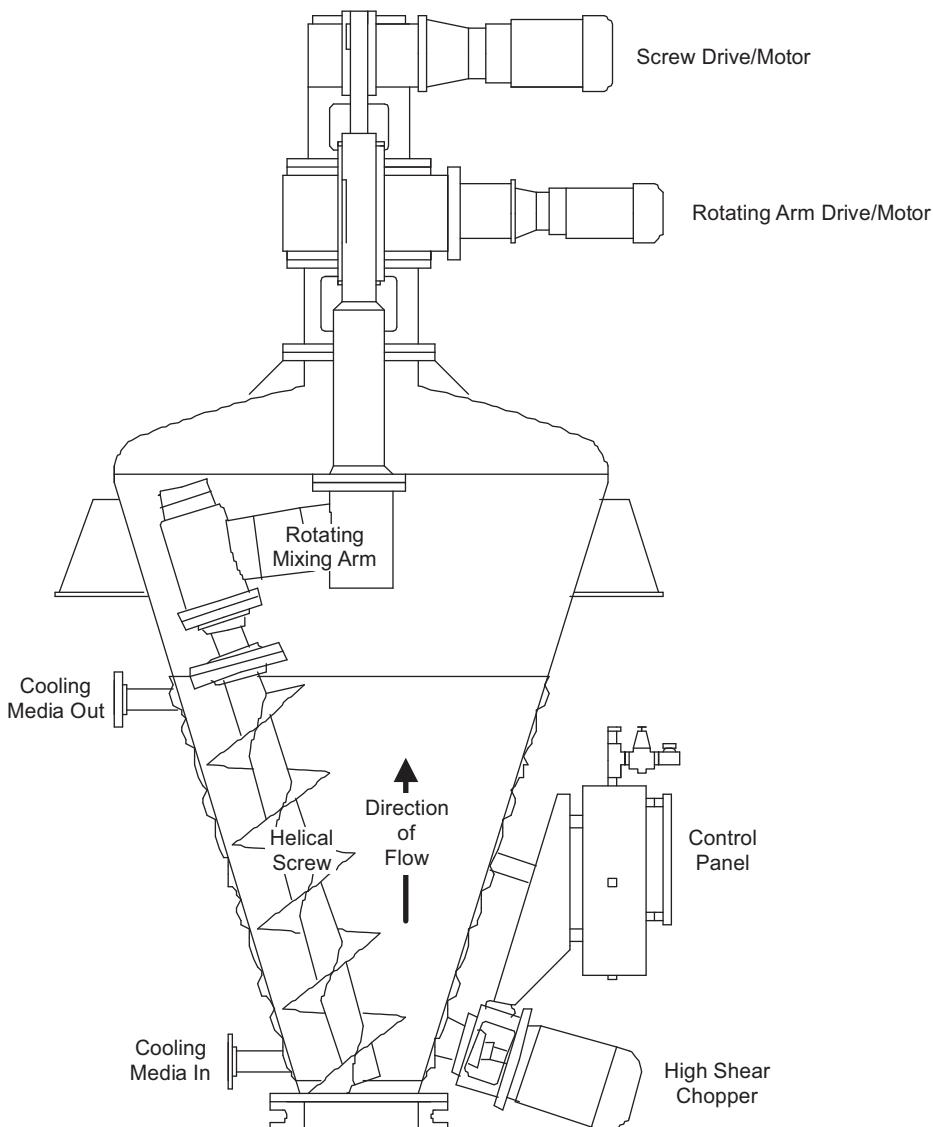
actives, preservatives, and perfumes. The relatively high-energy input required while mixing viscous materials also influences the mechanical construction of the mixing machinery and the method by which mixing is achieved.

### **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.

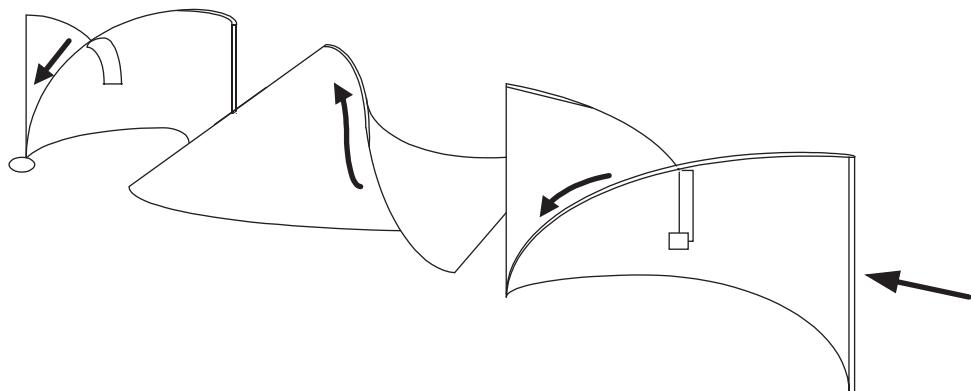


**Figure 13.12:** High solids blender [11]

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.

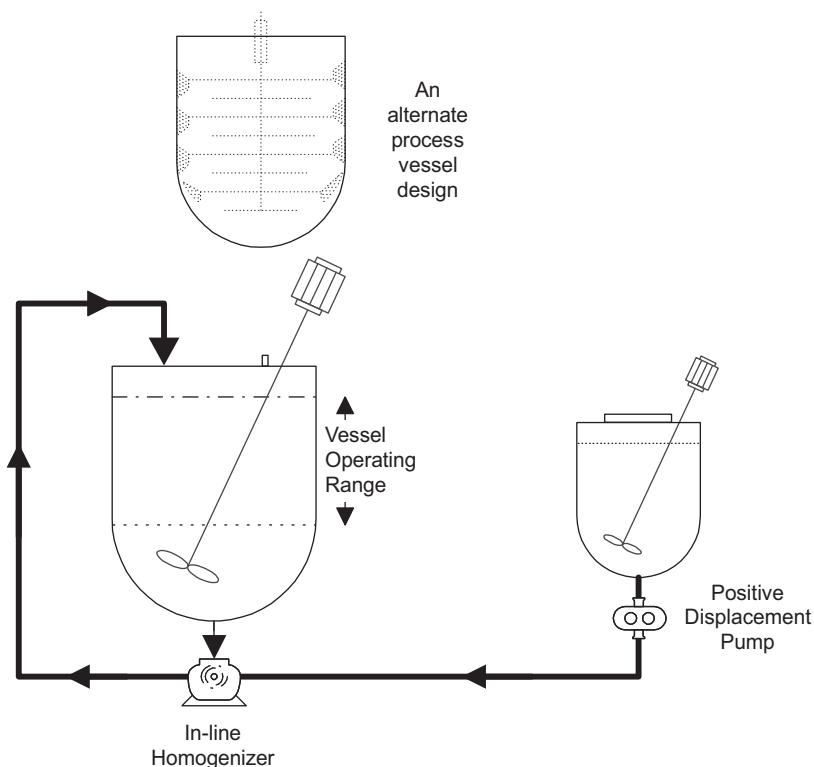
A less-used method is one in which both the water and oil phases are added alternately to the emulsifying agent. Usually, the small improvement in product quality obtained by the use of this method does not warrant the complication it causes in the manufacturing procedure.

### 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

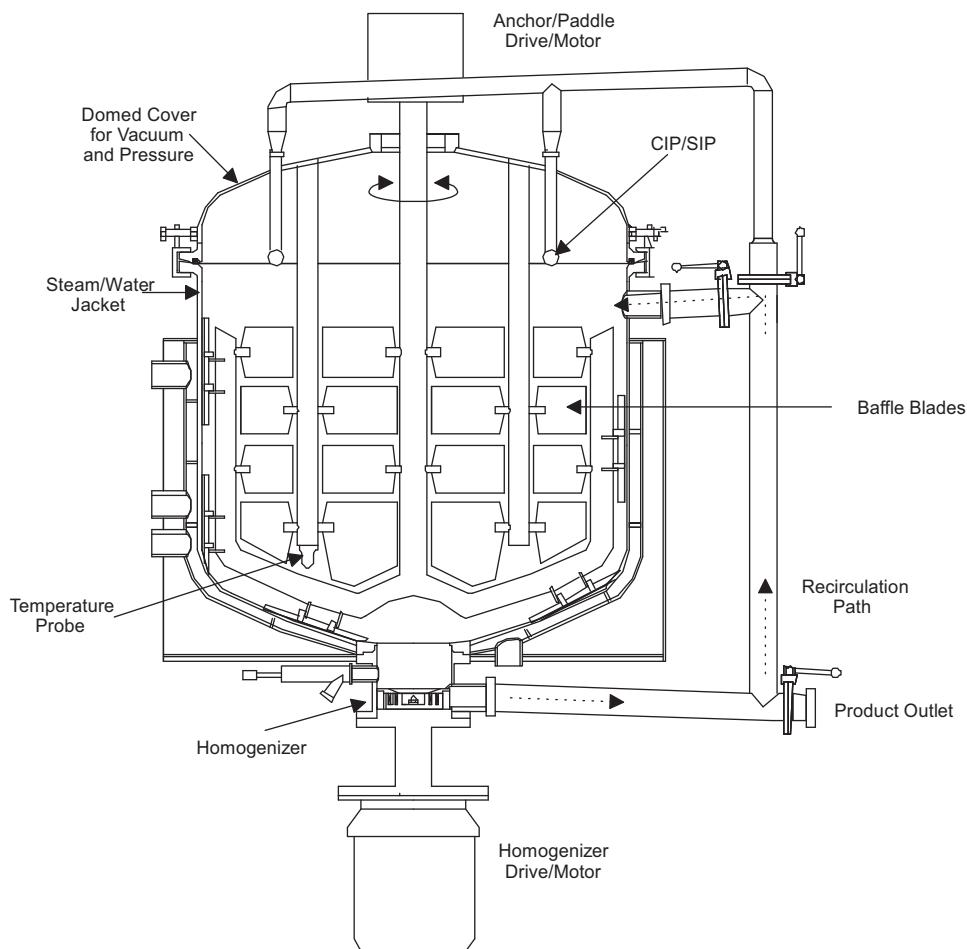
It is evident from the discussion so far that there are two important elements of emulsion processing: shear for emulsification and particle size reduction and flow for circulation of vessel contents through the region of maximum shear. Flow is also important in the heating and cooling of the emulsion. 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:

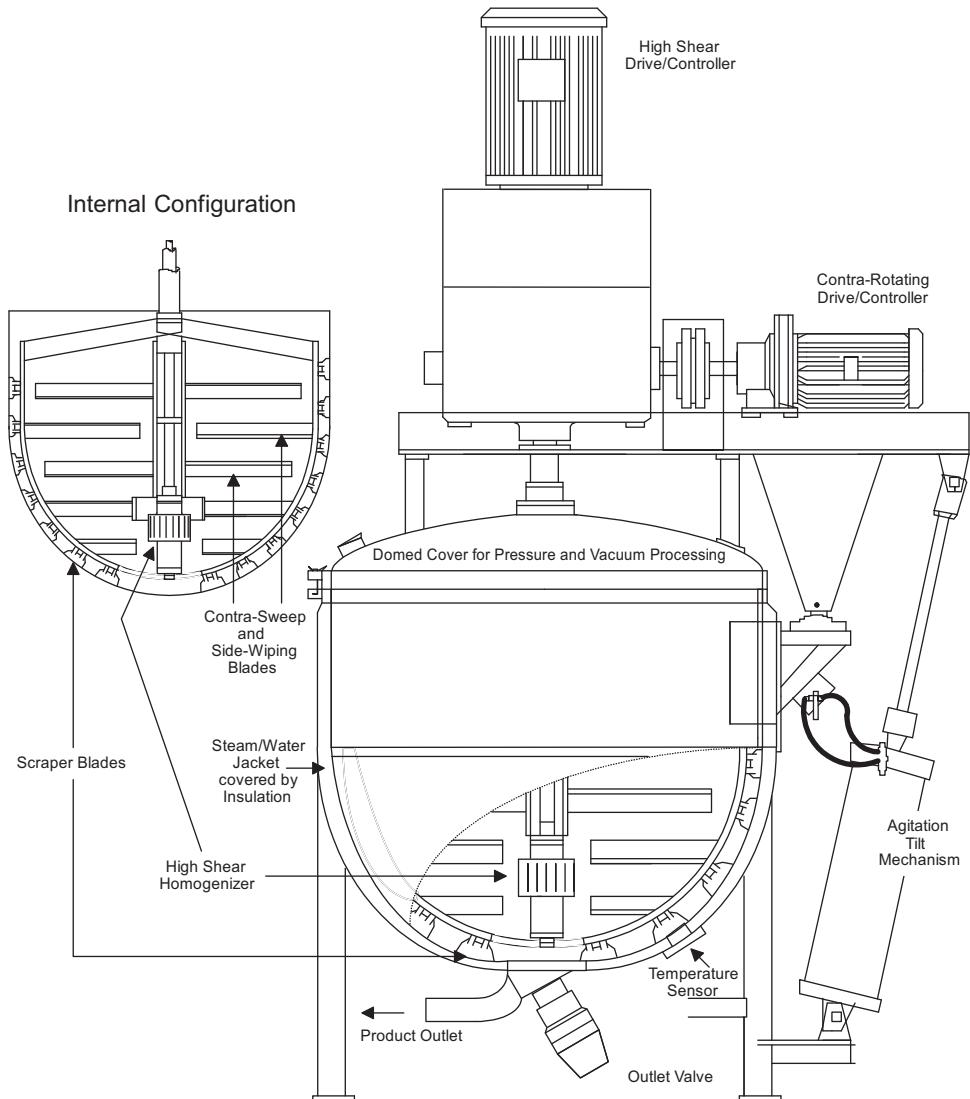
- Universal in application. It is capable of handling the entire viscosity range from light lotions to heavy ointments.
- Efficient presentation of the batch to the homogenizer for all batches. Efficient mixing ensures that all parts of the batch will see the same quantity of energy.
- Vacuum capable 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.



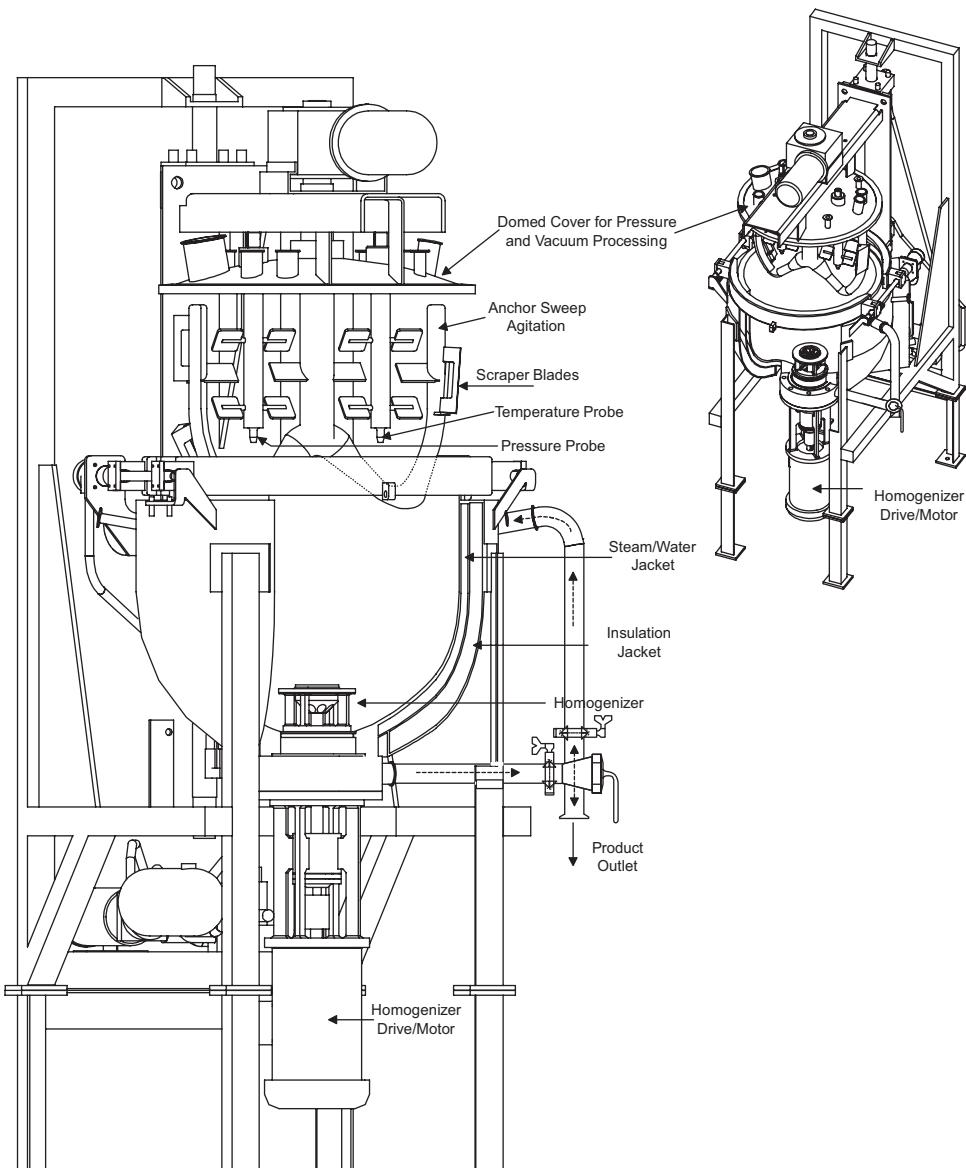
**Figure 13.15:** Batch emulsion processing vessel—SYMEX® Style - homogenizer in the down position [13];

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]

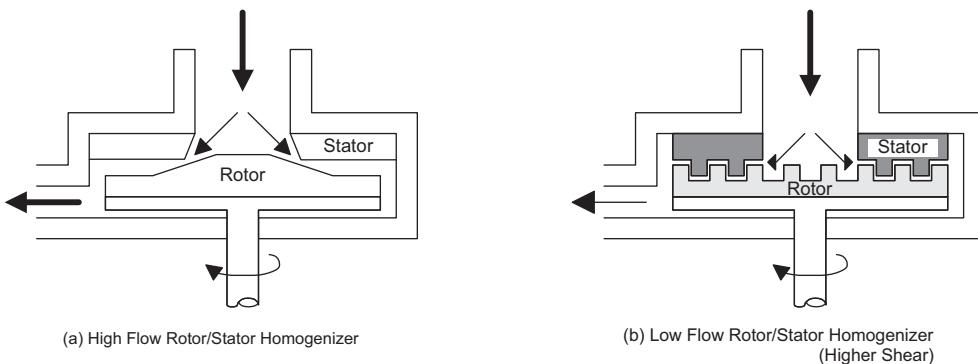
Figure Courtesy of Chemineer®-Greerco®, North Andover, Massachusetts.

### 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.

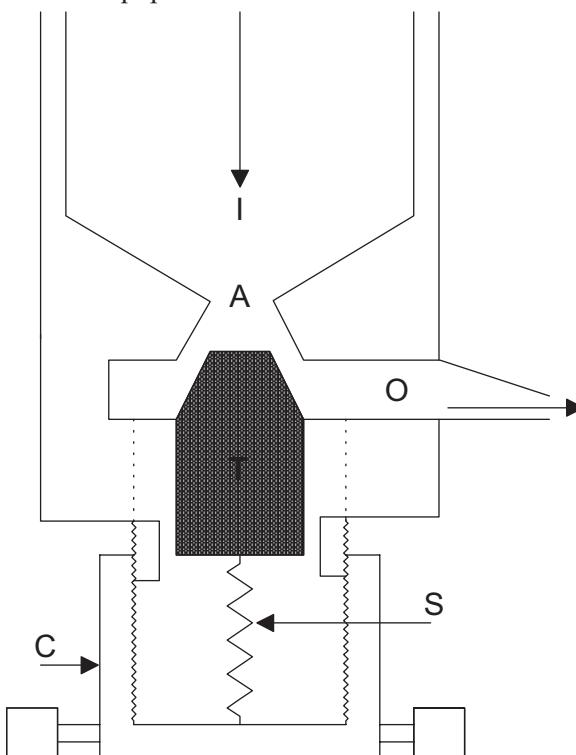
A disadvantage of the top-entering homogenizer is the tendency of the mixer to cause aeration due to vortex formation—particularly when the homogenizer head is not completely covered. For this reason, high-shear devices are often incorporated into the bottom of the processing vessels. Additional advantages 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.

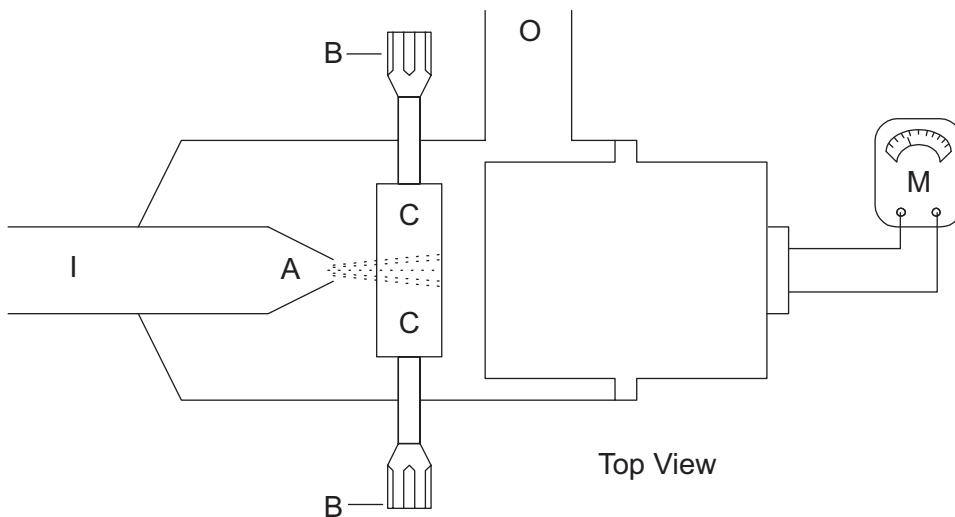
Perhaps the highest shear stress is generated by the valve homogenizer, which is still extensively used in the production of emulsions with very fine internal phase droplets. A valve homogenizer, Figure 13.19, consists of a high-pressure pump, which forces product through a small orifice at pressures 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.

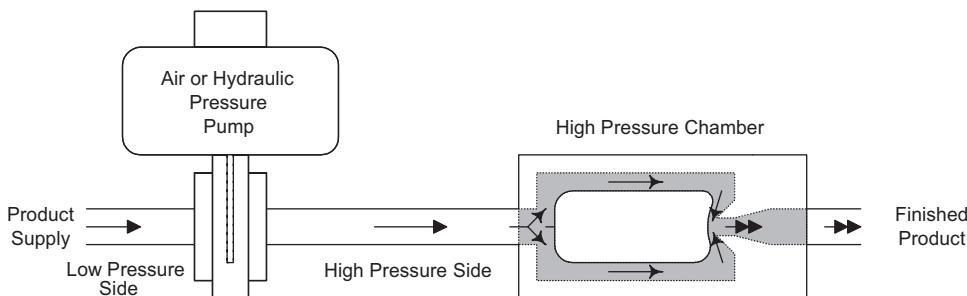


The roughly premixed product enters at I. It passes through orifice A and is subjected to intense ultrasonic energy by vibrating blade C. The treated product leaves via O. The meter, M, and “tuning” devices at B combine to allow the operator to maximize the settings for each product

**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).

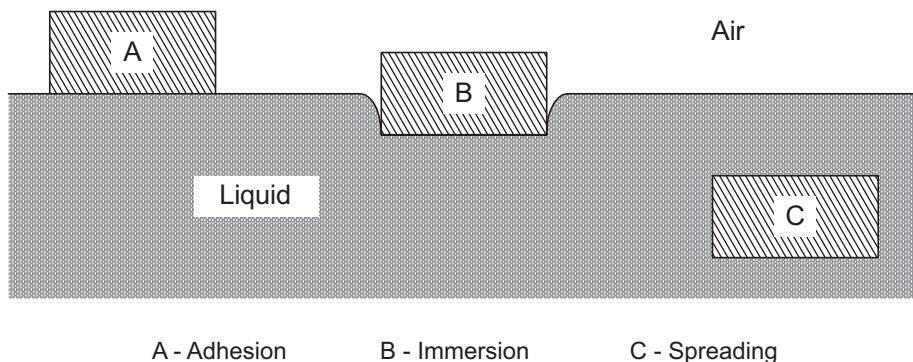
### 13.1.3.3 WET SYSTEMS—LIQUID–SOLID SYSTEMS

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.

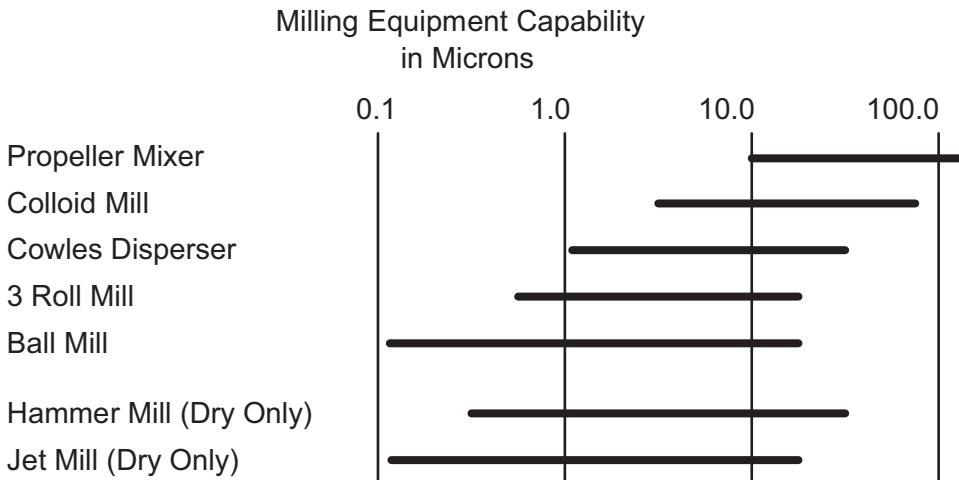
Homogeneous suspension exists when the particle concentration and (for a range of sizes) the size distribution are the same throughout the tank. The homogeneous suspension is always more difficult to achieve and to measure than the complete suspension. Nevertheless, homogeneous suspension is very desirable 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.

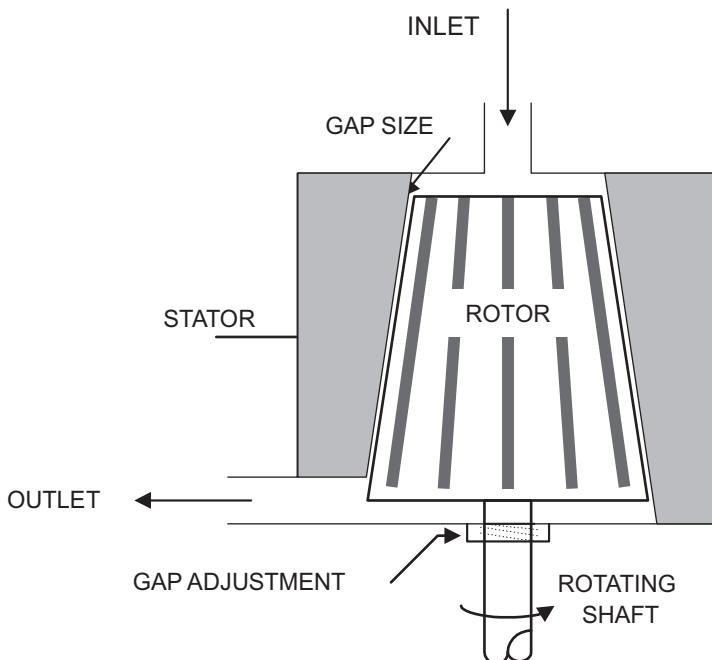


**Figure 13.23:** Particle-size comparison for milling equipment  
(some processing may require multiple passes)

### 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.

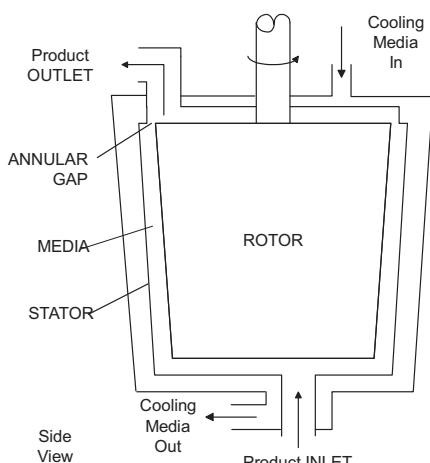
Retention time is the amount of time the product is exposed to the milling energy. The longer the product is in the mill, the smaller the particle size will be. Retention time is dependent on the flow rate, rotor speed, backpressure, and the gap size. The flow rate is easily measurable and should be 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.

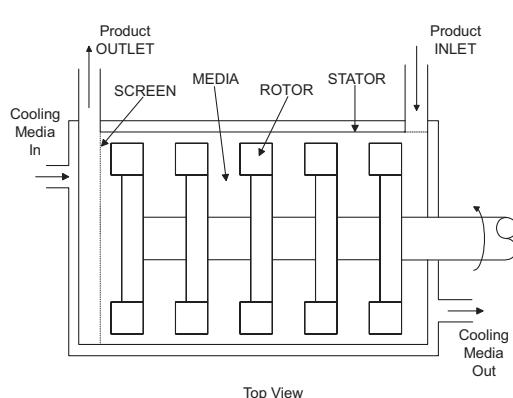
#### d. Ball Mills

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:

$$t = V_v / Q \quad \text{and} \quad V_v = V_c - V_m$$

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

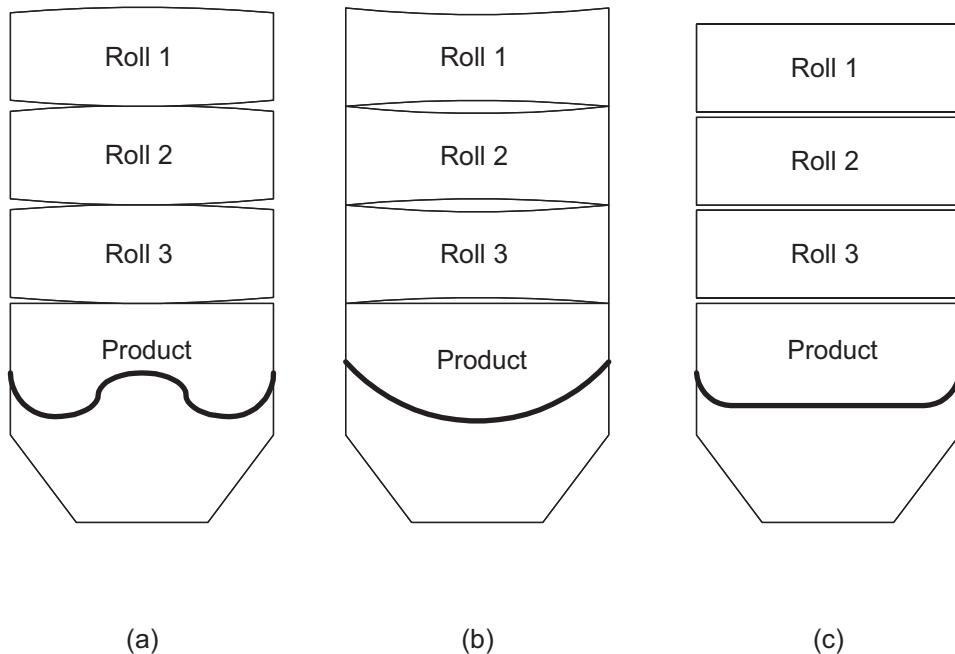
**Three-roll (roller) mills** are typically used as side batching or in-process devices. Like the ball mill, they are not normally used on the finished product, just on the pigmented sub-phase. Unlike the ball mill, particles are ground down to reduce their agglomerate size. Roller mills are used extensively 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

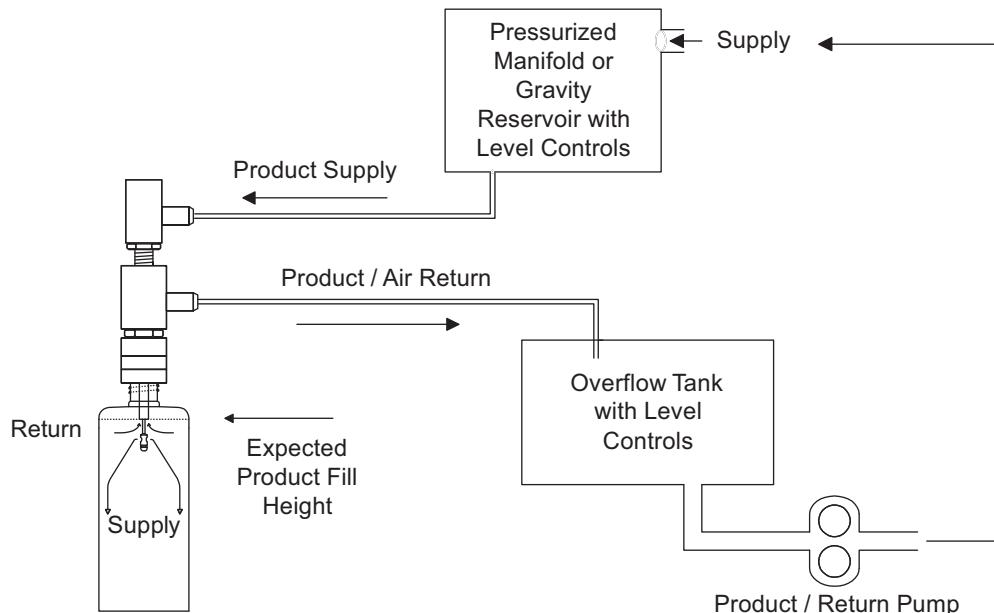
not filling. Both the excess fill and the bypassed product are returned to the filling hopper. The product is then available to be reused during the filling run. A level sensing system may use a slight air pressure flow that is delivered through a control line attached to the tip of the filling nozzle. 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.

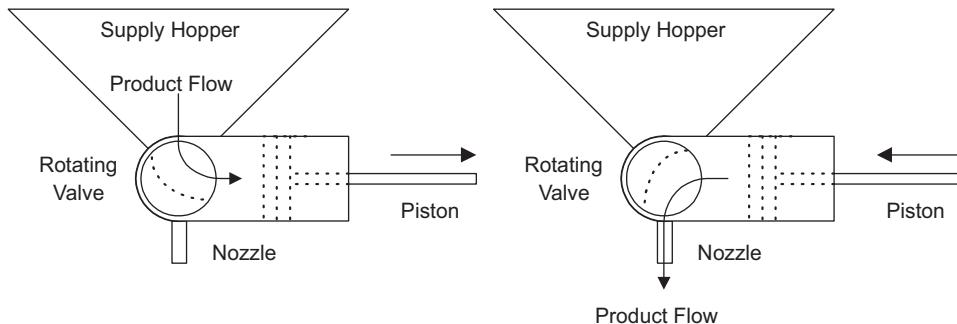


**Figure 13.28:** Liquid fill-to-level system

#### 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.

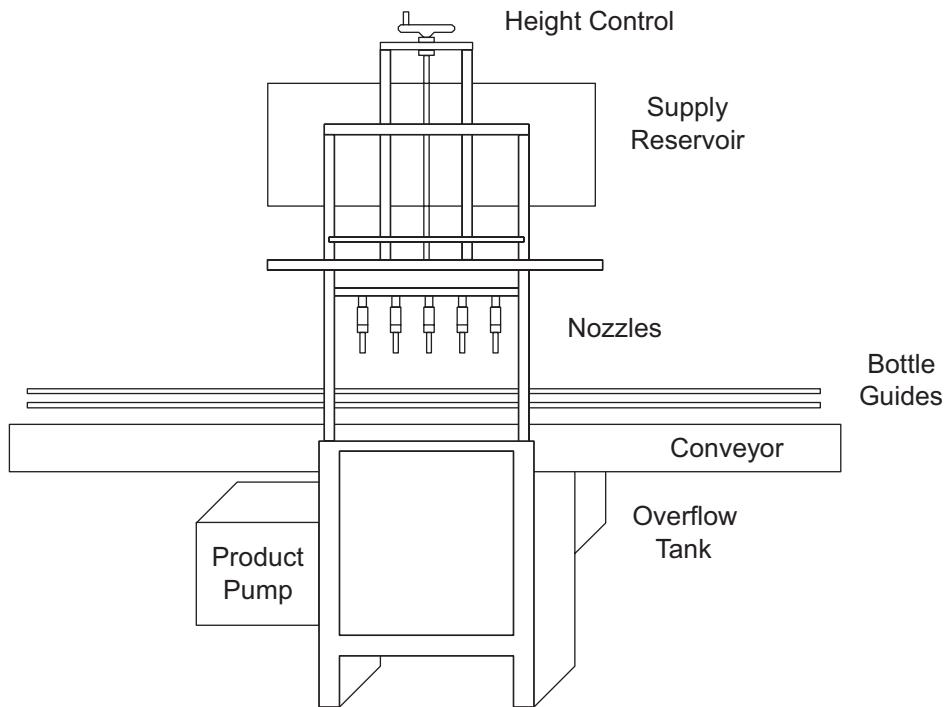
The filling speed is critical in filling these types of products. Dispensing speed must be carefully adjusted as it may create unnecessary air or bubbles at the point of dispensing.

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.

## **1. 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  $\pm 1^{\circ}\text{C}$ , heat-traced lines and filler assemblies, pre-heating 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.<sup>8</sup>

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

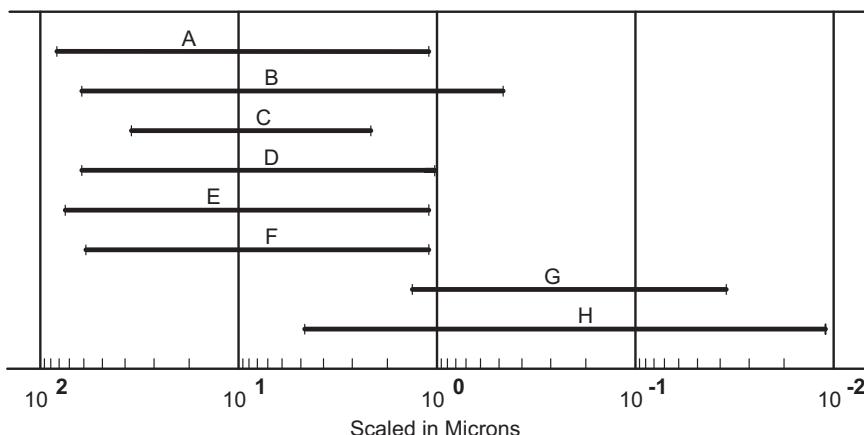
transfer and heat/cooling transfer rate should always be considered carefully during formulation and scale-up.

#### 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.<sup>23-26</sup> 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.

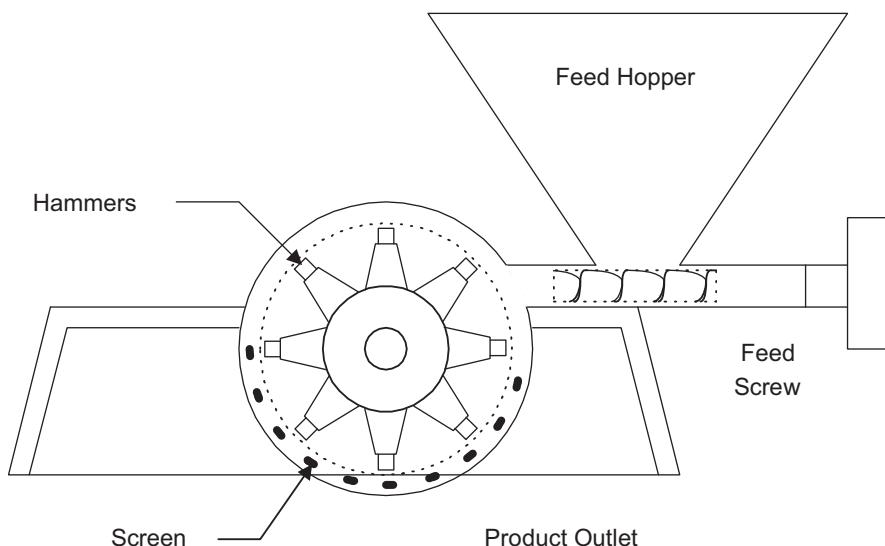
The use of the liquid binder to assist in the development of color is a technique to be used only under special circumstances. The liquid binder will assist the high-shear equipment to disperse the pigments onto the chosen substrate. However, the pigments will be much more temperature sensitive to color 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 ( $60\text{--}80 \text{ m s}^{-1}$ ) 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  $\mu\text{m}$  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 ( $\geq 0.125"$ ). (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.

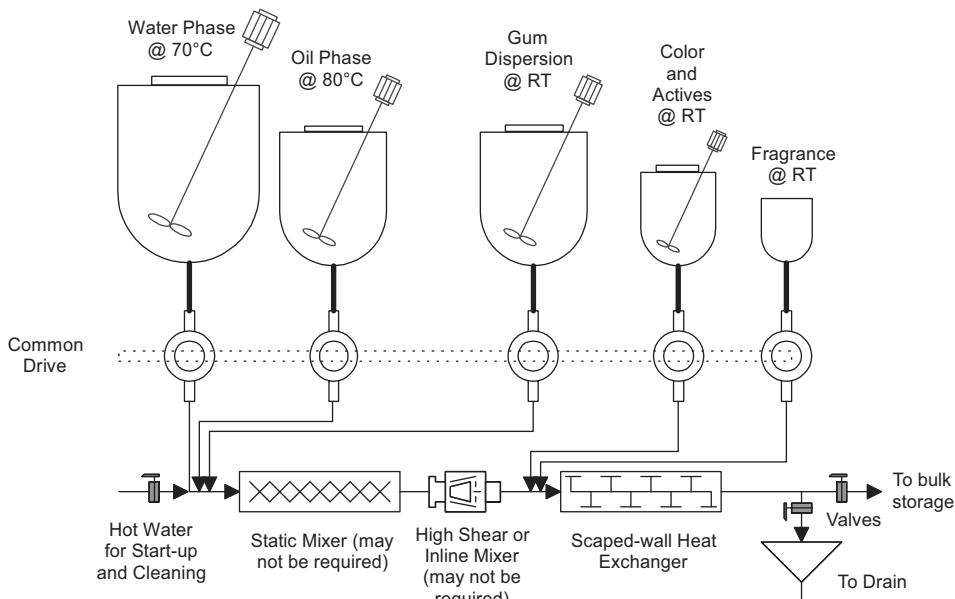
### 13.1.7.1 WET CONTINUOUS PROCESS

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 often-times 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  $\pm 0.5^{\circ}\text{C}$  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 upgradable 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.

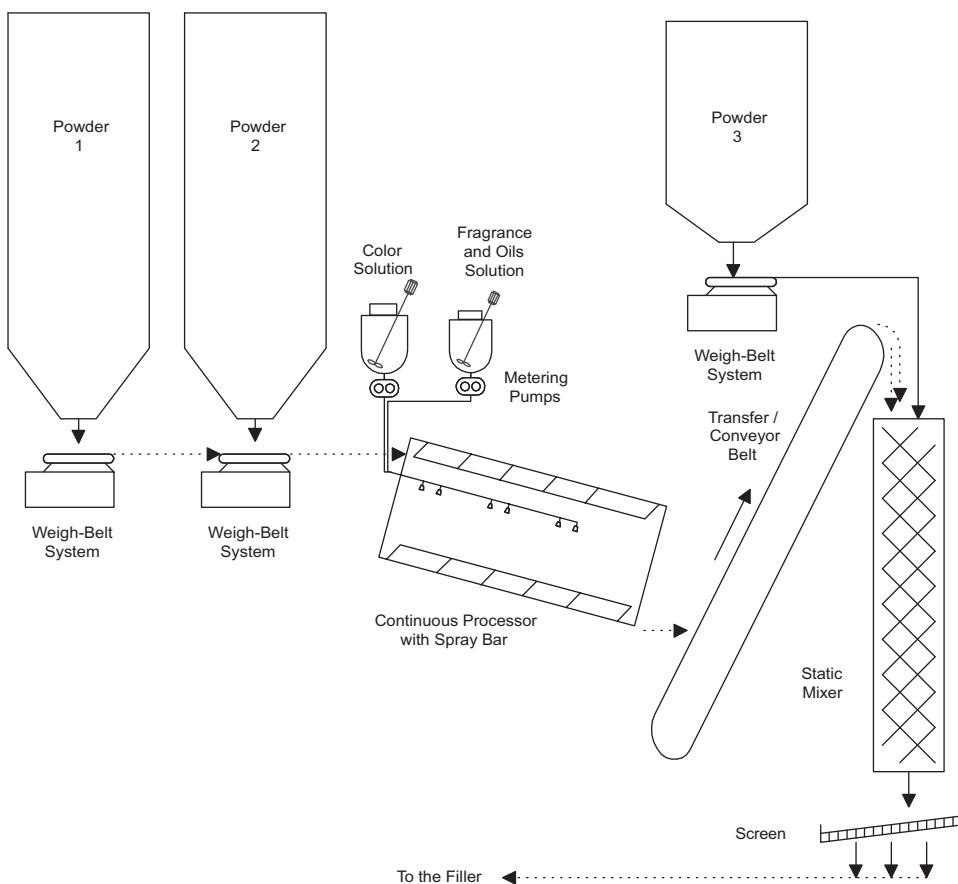
The system is shut down in reverse order as it was started up. Simultaneously the pumps are diverted into their recycle position; hot water is flushed through the pipeline, mixers, and heat exchangers and routed to the drain. This flushes the system in preparation for cleaning and sanitization for the 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

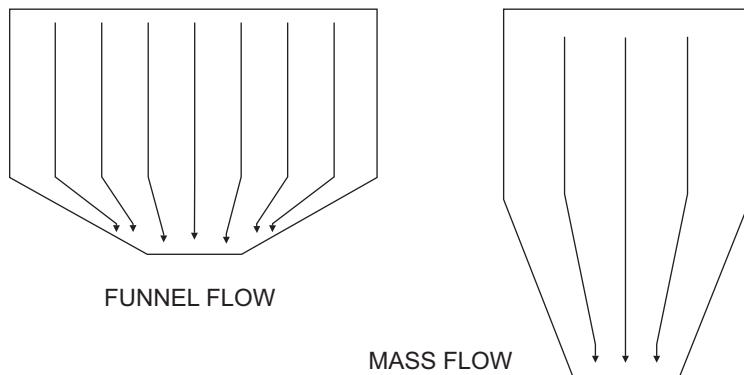
### a. Bulk Powder Storage

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 deflation (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 ( $\Omega$ ), 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.

**Table 1:** Delivery Systems Most Commonly Used in Personal Care

Classification	Description	Diameter	Materials Carried
Liposomes	Phospholipid-based unilamellar or multilamellar bilayer vesicles.	100-500 nm	Hydrophilic and hydrophobic actives
Nanodispersions	Phospholipid-based micellar 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/ Clathrate	Clathrate 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 emulsifier, 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.  
$$V = (2gr^2)(d_1-d_2)/9\mu$$
- $V$  = velocity of fall ( $\text{cm sec}^{-1}$ ),  $g$  = acceleration of gravity ( $\text{cm sec}^{-2}$ ),  $r$  = “equivalent” radius of particle (cm),  $d_1$  = density of particle ( $\text{g cm}^{-3}$ ),  $d_2$  = density of medium ( $\text{g cm}^{-3}$ ), and  $\mu$  = viscosity of medium (dyne sec  $\text{cm}^{-2}$ ).
- **200 nm particle is 680 times more stable than 5  $\mu$  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-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 ( $6.227 \times 10^9$ ) 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 micellar 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 undesired 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.

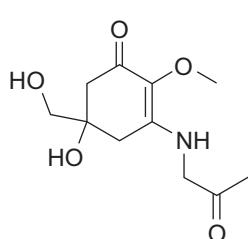
### b. Microorganisms

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 sphingolipids have been produced by fermentation of *Pseudomonas aeruginosa* and *Candida bomicola*, respectively. [10]

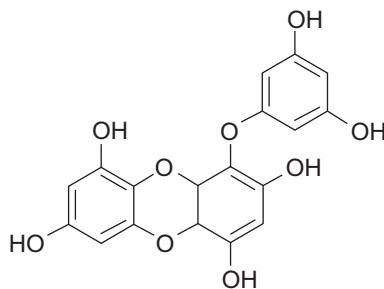
Choosing an appropriate microbial strain that can produce high yields of the desired molecule is crucial for production using fermentation. Subsequent downstream processing, to separate the actives from the broth, may involve cell separation and concentration of the extract followed by precipitation or drying of the broth on a carrier or extraction by using an organic solvent.

### c. Algae

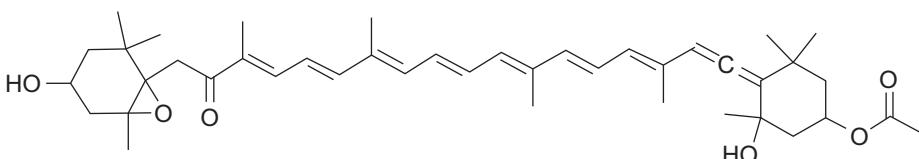
In the past decade, interest in algae as a source of novel natural compounds has been growing rapidly. Algae of many types floating on the oceans are common in various regions of the world. Some of the most common ingredients extracted from algae are alginates, carrageenan, and agar, which are used for 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]



Mycosporine glycine



Eckol



Fucoxanthin

**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 de-watering, 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  $4.9 \times 10^9$  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 ( $\tan \delta$ ):

$$\tan \delta = \epsilon' \epsilon /$$

where  $\epsilon'$  is dielectric loss, which is the efficiency of converting microwave energy into heat and  $\epsilon$  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 ( $\tan \delta$ ) 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.

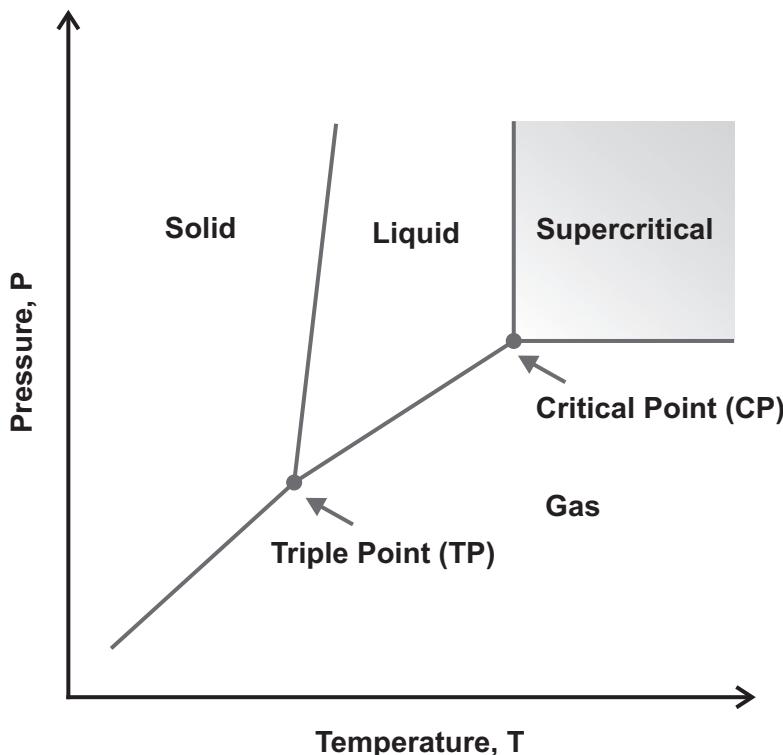
UAE has found application for extraction of essential proteins and bioactives from a variety of bio-sources. [34] UAE improved the extraction of carnosic acid from rosemary using ethanol as the extracting solvent. The extracting yields were comparable to that obtained from the use of ethyl acetate and 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

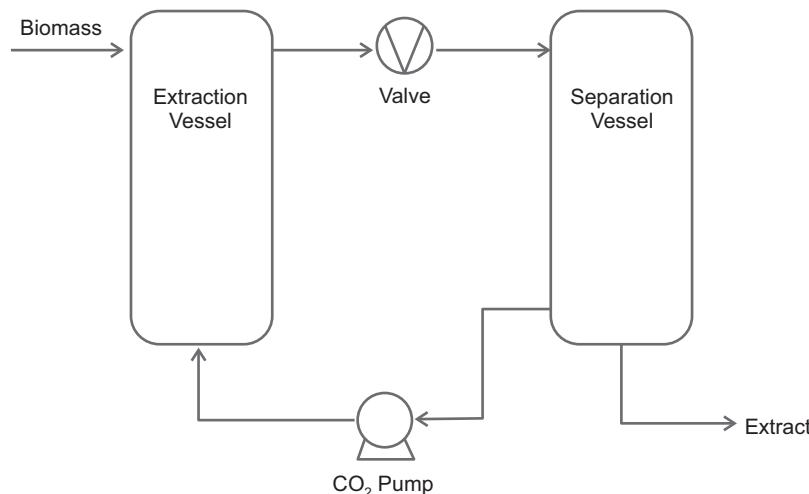
**Table 2.** Critical properties of a few common solvents

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<sub>2</sub> results in a solvent-free extract since CO<sub>2</sub> is a gas under ambient conditions. Also, the critical temperature of 31.2°C for CO<sub>2</sub> makes it an attractive candidate for extraction of thermo-labile compounds. The efficiency of extraction of actives using CO<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub> 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<sub>2</sub>.



**Figure 3.** A schematic of SCFE – CO<sub>2</sub> process.

During extraction phase, the solid or liquid biomass is charged into the extraction vessel, where the biomass is then exposed to CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> has limited ability to dissolve highly polar molecules. A co-solvent is often used to improve the polarity of SC-CO<sub>2</sub> 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 SFCFE. [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 SFCFE has led to it being used in many applications with a wide variety of bio-sources. For example, SFCFE 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. SFCFE has been used for successful recovery of variety of essential oils, without the loss in activity. [47] SFCFE has also been used for extraction of natural pigments and dyes, and turmeric extract rich in curcuminoids was extracted using SFCFE 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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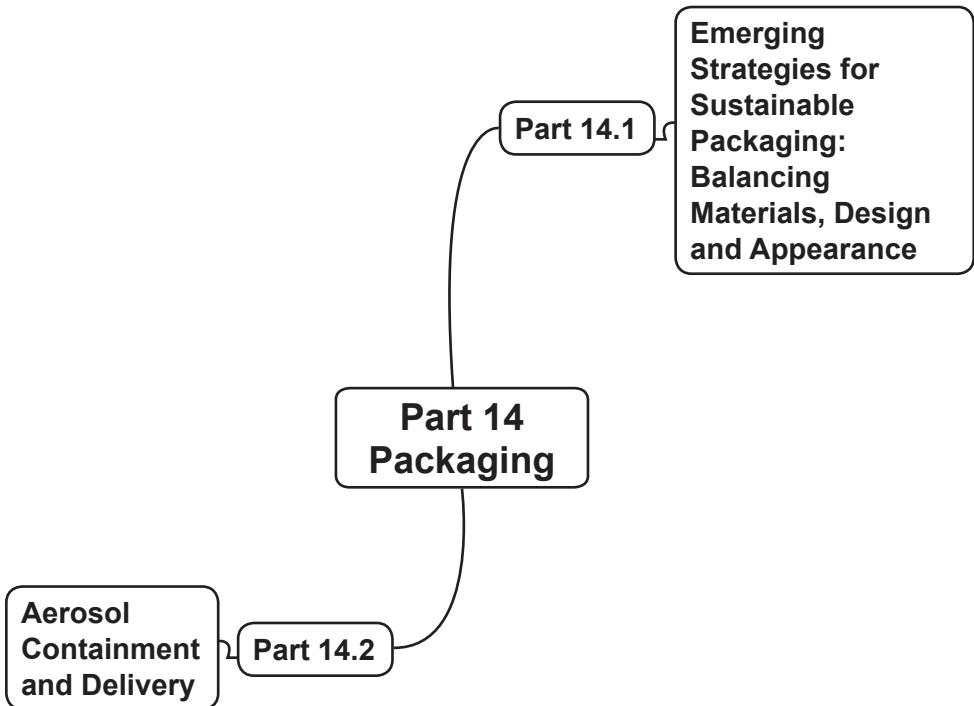
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## PACKAGING



## 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.<sup>1</sup> 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 bio-resins 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.<sup>2</sup>

#### 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),<sup>3</sup> 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
<b>Total</b>	<b>18.4</b>

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!*<sup>4</sup> *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<sup>5</sup> 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.”<sup>7</sup>

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 Lilenfeld, 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](http://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](http://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  $50\mu$  and usually is less than  $10\mu$  *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.

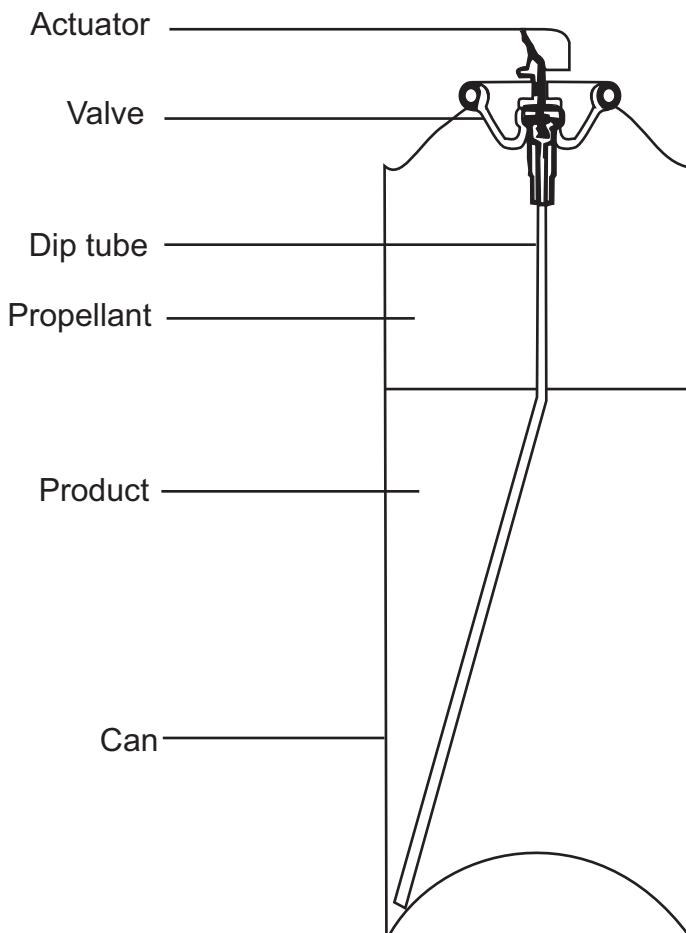
#### c. Barrier pack systems

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 8<sup>th</sup> 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](http://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.

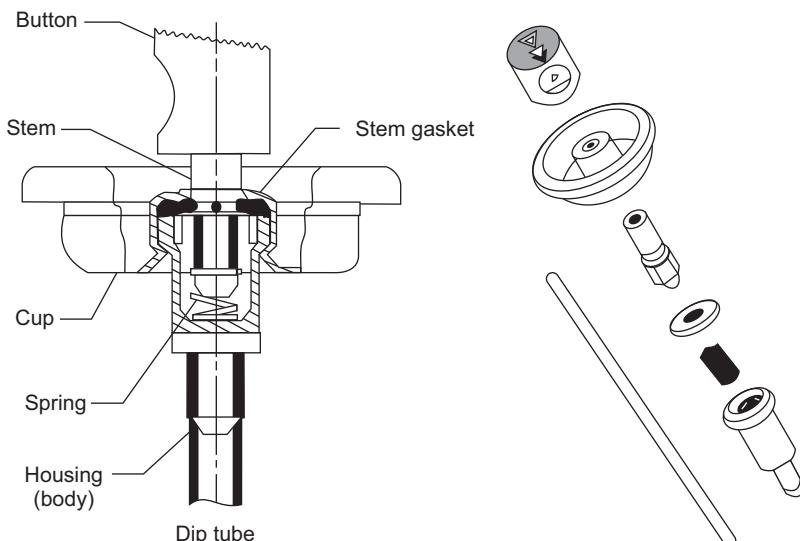
### 14.2.6 VALVE

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

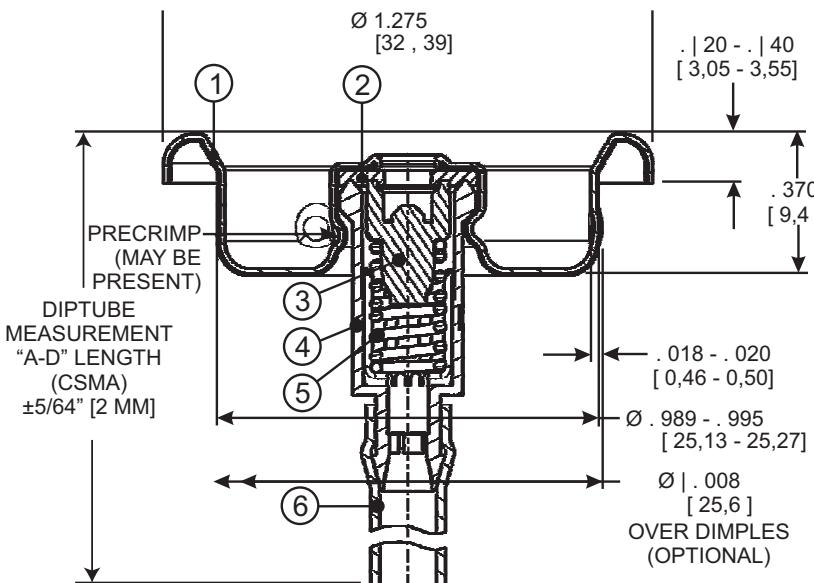
seven spirals. The housing may be provided with a vapor tap that allows dispensing in a position in which the dip tube does not contact the product. The dip tube is commonly prepared from polyethylene or polypropylene and is firmly attached to the housing. Finally, the mounting cup, which is crimped 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 8<sup>th</sup> 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 CANØ 1.065-1.075 [27,05-27,31]  
HEIGHT .180-.190 [4,57-4,83]1 DIMENSIONS IN BRACKETS ARE IN  
MILLIMETERS2 TOLERANCE FOR ORIFICES LESS THAN .060"  
[1.52 MM] DIA: ±.002" [0.05 MM]3 ALL OTHER TOLERANCE ±.005" [0.13 MM]  
UNLESS OTHERWISE SPECIFIEDPOSSESSION OF THIS DRAWING IS AN ACKNOWLEDGEMENT THAT IT AND  
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ANY FORM WHATSOEVER WITHOUT WRITTEN PERMISSION FROM SEAQUIST.Title AR-74 VALVE ASSEMBLY,  
STANDARD HEIGHT TAPERSEAL

Drawn By RJS Date 02-Dec-91 Chkd By TFE Date 10-May-10

Material - Scale 3:1

**SeaquistPerfect**  
DISPENSING

Approved	Date
Oral Mgl	CAC 02/02/07
R & D	RLC 13/04/09
Lab Ngr	MER 17/23/07

Rev Ltr B

**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  $\pm 0.13\text{mm}$

Cup Style	3-Piece tin plate can		Aluminum can outside curl		Aluminum can inside curl	
	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  $\text{CH}_3\text{CHF}_2$  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<sub>2</sub>, N<sub>2</sub>O, and N<sub>2</sub>, 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<sub>2</sub> 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<sub>2</sub>. Using a saturator, CO<sub>2</sub> 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).

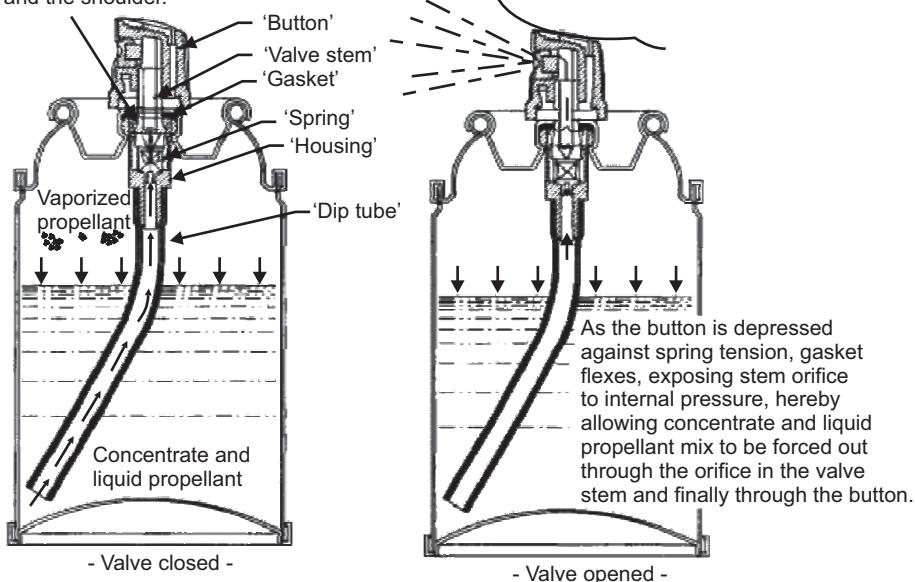
The volume of the fill is then easily computed. Some minor correction is made for the space occupied by the valve. A level of about 20% of the overflow capacity

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.

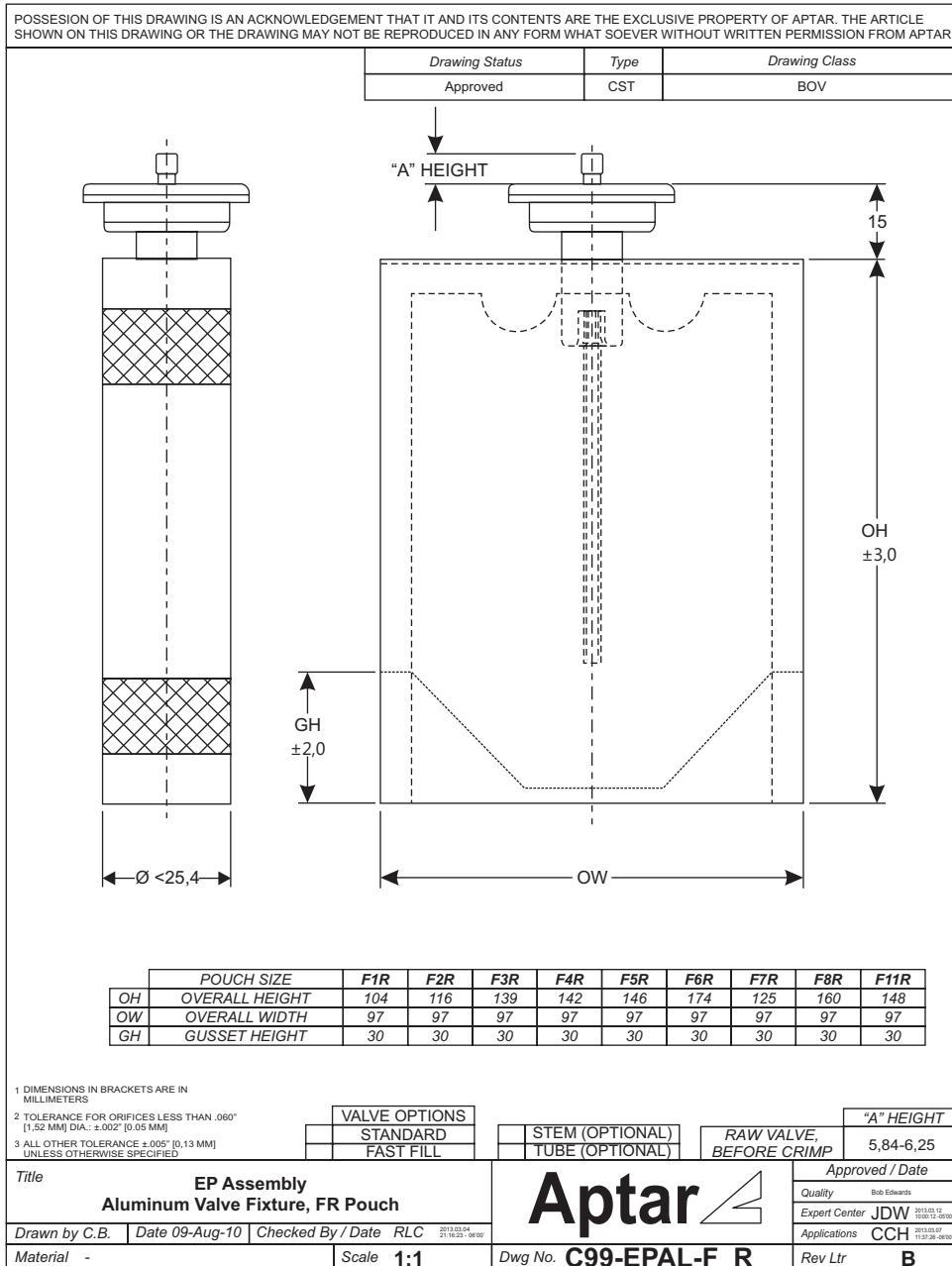
The gasket prevents the flow of concentrate and liquid propellant mix (under pressure) by sealing the valve stem at the orifice and the shoulder.



**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 Cosmetiology 8<sup>th</sup> 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.



**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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