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GREEN ANALYTICAL CHEMISTRY

MIGUEL DE LA GUARDIA AND SERGIO ARMENTA

GREEN ANALYTICAL CHEMISTRY:
THEORY & PRACTICE

COMPREHENSIVE ANALYTICAL CHEMISTRY

VOLUME *57*

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Dedication:

To my sons, Alberto and Miguel, my daughters Julia, Irene, and Sofia, and my wife Amparo.

Miguel de la Guardia.

To the memory of my parents and to my wife Ana.

Sergio Armenta.

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SERIES EDITORS PREFACE

As Series Editor, I have great pleasure in introducing this new book on green analytical chemistry, the first on this particular topic in the Comprehensive Analytical Chemistry series.

Green Analytical Chemistry, Theory and Practice contains ten chapters starting with the Origins of Green Analytical Chemistry that includes the state-of-the-art of this practical concept created to avoid the adverse side effects of chemistry in the analysis of samples, by using, for instance, fewer solvents in the laboratory. The second chapter covers the basis for a greener analytical chemistry and the third is an evaluation of existing analytical methods from a green perspective. Avoiding sample treatments is an interesting chapter that shows different ways of avoiding chemicals that are familiar to us. Perhaps we never thought that some of the techniques we use could be considered "green," like tele-detection, direct analysis on solid or liquid samples without sample damage by, for instance, vibrational spectroscopy (MIR, NIR, and Raman), and others like laser ablation of solids. An alternative to avoiding sample treatment is the so-called greening of sample treatments by techniques like supercritical fluid extraction of analytes and superheated water extraction SPE, SPME as well as other extraction strategies, for instance, those based on membrane techniques.

A further group of chapters deals with analytical methods, encouraging the use of multianalyte determinations versus one-at-a-time methodologies, mainly based on chromatography–mass spectrometry techniques or downsizing the methods by miniaturization—the so-called lab on a chip or the use of biosensors and/or nano techniques.

The last three chapters describe the practicalities of green analytical chemistry like how to move to cleaner wastes, ideas for bringing about a change of mentality and practices, and ending with the practical consequences of a green analytical chemistry. The final message of the book is clear and goes directly to the societal benefits to convince the reader to use greener analytical chemistry methods for a better, more sustainable way of living.

One of my tasks as editor of CAC was to convince the authors to edit or perhaps write a book. My sincere thanks are due to Miguel de la Guardia and Sergio Armenta. I was positively surprised when they told me that they were even willing to write the complete book and not to prepare an edited work.

The analytical chemistry community hopefully will appreciate the tremendous amount of work that is involved in writing a book with only two authors.

Finally, I would recommend the book to a wide audience—lecturers, instructors, students, and technicians in general chemistry and related disciplines like biology, environmental science, and food science. It is suitable material for courses not only at the university level but also for industrial, non-profit organizations and governmental courses.

D. Barceló
Barcelona, July 2010

PREFACE

Green Chemistry can be considered the conceptual framework that allows us to avoid the undesirable effects of Chemistry. It has evolved from the pollution prevention approach developed within the USA Environmental Protection Agency. However, if we consider the dates of publication of classical texts by Paul Anastas, we see that prior to the formulation of the 12 principles of Green Chemistry, many efforts were made in the field of Analytical Chemistry to avoid the side effects of the analytical methodologies and to reduce the costs of analysis. The development of clean and environment-friendly methods of analysis was based on the conscientious work of analysts in fields like automation and miniaturization as an ethical compromise with society and operators and, at the same time, as an effort to find and benefit from economic opportunities.

Nowadays, both Green Chemistry (specially green synthesis) and Green Analytical Chemistry can be integrated in a common effort to modify the social perception of Chemistry as an important cause of pollution and its related problems to one that is based on the fact that Chemistry is absolutely necessary for both remediation and pollution prevention. It was this idea that motivated us in 2008 to publish a review article in *Trends in Analytical Chemistry* devoted to the Green Analytical Chemistry, and from the impact of the aforementioned study in the analytical literature, Elsevier decided to incorporate this hot topic into the prestigious Wilson & Wilson Comprehensive Analytical Chemistry Series.

This book aims to provide a basic coverage of the fundamentals and principles of the so-called Green Analytical Chemistry and to revise the main strategies for greening the analytical methods. At the same time, we have reviewed the contributions of many authors working in this field. This text has been an intellectual journey that the authors have made in close cooperation and we hope that you both enjoy and benefit from it. However, it is clear that Green Analytical Chemistry is an area in constant development and this book can only claim to be a starting point from which to survey it. It is our hope that it will provide many fertile ideas to the readers.

M. de la Guardia and S. Armenta
Valencia, May 2010

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ABBREVIATIONS

μTAS	Micro total analytical system
2D GC	Two dimensional gas chromatography
2D LC	Two dimensional liquid chromatography
ACS	American Chemical Society
AES	Auger electron spectroscopy
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
APPI	Atmospheric pressure photoionization
ASE	Accelerated solvent extraction
ASTM	American Society for Testing and Materials
ATR	Attenuated total reflectance
BGE	Background electrolyte
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BTEX	Benzene, toluene, ethylbenzene, and xylene
CAS	Chemical Abstracts Service
CCD	Contactless conductivity detection
CCDs	Charge coupled detectors
CE	Capillary electrophoresis
CFME	Continuous-flow microextraction
CI	Chemical ionization
cITP	Capillary isotachophoresis
CLAs	Colloidal liquid aphrons
CMC	Critical micellar concentration
CPE	Cloud point extraction
CV-AFS	Cold vapor-atomic fluorescence spectroscopy
DART	Direct analysis in real time
DEUF	Dendrimer enhanced ultrafiltration
DI-SDME	Direct immersion single drop microextraction
EI	Electron ionization
EOF	Electroosmotic flow
EPA	Environmental Protection Agency
ESI	Electrospray ionization
ETAAS	Electrothermal atomic absorption spectroscopy
FAAS	Flame atomic absorption spectroscopy

FASS	Field-amplified sample stacking
FDA	Food and Drug Administration
FFF	Field-flow fractionation
FIA	Flow injection analysis
FIR	Far-infrared
FPA	Focal plan array
FTIR	Fourier transform infrared
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionization detector
GD	Glow discharge
GDMS	Glow discharge mass spectrometry
GDOES	Glow discharge optical emission spectrometry
GLP	Good laboratory practice
GPC	Gel permeation chromatography
HDC	Hydrodynamic chromatography
HPHTSE	High-pressure, high temperature solvent extraction
HPSE	High-pressure solvent extraction
HPLC	High performance liquid chromatography
HS-SDME	Headspace single drop microextraction
HSSE	Headspace sorptive extraction
HS-SPDE	Headspace-solid phase dynamic extraction
HS-SPME	Headspace-solid phase microextraction
HTWE	High temperature water extraction
HWE	Hot water extraction
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectroscopy
IMS	Ion mobility spectrometry
INCAT	Inside needle capillary adsorption trap
IPC	Ion-pairing chromatography
IR	Infrared
ISI	Institute for Scientific Information
ISO	International Organization for Standardization
ISS	Ion scattering spectroscopy
LC	Liquid chromatography
LC-API–MS	Liquid chromatography-atmospheric pressure ionization–mass spectrometry
LCW	Liquid-core waveguide

LD_{50}	Medium lethal doses
LDPE	Low-density polyethylene
LED	Light-emitting diode
LIBS	Laser-induced breakdown spectrometry
LIF	Laser-induced fluorescence
LLLME	Liquid–liquid–liquid microextraction
LODs	Limits of detection
LOQs	Limits of quantification
LOV	Lab-on-valve
LP-GC	Low-pressure gas chromatography
LPME	Liquid phase microextraction
LVSS	Large volume sample stacking
MAE	Microwave-assisted extraction
MASE	Membrane-assisted solvent extraction
MEEKC	Microemulsion electrokinetic chromatography
MEKC	Micellar electrokinetic chromatography
MEUF	Micellar enhanced ultrafiltration
MIMS	Membrane introduction mass spectrometry
MIR	Mid-infrared
MISPE	Molecularly imprinted solid-phase extraction
MLC	Micellar liquid chromatography
MS	Mass spectrometry
MSDS	Material safety data sheet
MWCO	Molecular-weight cut-off
NEMI	National Environmental Methods Index
NIOSH	National Institute for Occupational Health and Safety
NIR	Near infrared
NMR	Nuclear magnetic resonance
NP-LC	Normal phase liquid chromatography
NSM	Normal stacking mode
OLEDs	Organic light-emitting diodes
PAHs	Polycyclic aromatic hydrocarbons
PBT	Persistent, bioaccumulative, and toxic
PCBs	Polychlorinated biphenyls
PCs	Priority chemicals
PDMS	Polydimethylsiloxane
PEEK	Polyether ether ketone
PeEUF	Polyelectrolyte enhanced ultrafiltration

PEG	Polyethylenglycole
PET	Polyethylene terephthalate
PEUF	Polymer enhanced ultrafiltration
PHSE	Pressurized hot solvent extraction
PHWE	Pressurized hot water extraction
PLE	Pressurized liquid extraction
PME	Polymeric membrane extraction
PSE	Pressurized solvent extraction
PTR-MS	Proton transfer reaction-mass spectrometry
RCRA	Resource Conservation and Recovery Act
REACH	Register, Evaluation, Authorization and Restriction of Chemicals
RP-LC	Reversed phase liquid chromatography
RSC	Royal Society of Chemistry
RSD	Relative standard deviation
RTILs	Room temperature ionic liquids
SBQ	Sociedade Brasileira de Quimica
SBSE	Stir bar sorptive extraction
SCI	Science Citation Index
SDME	Single drop microextraction
SDS	Sodium dodecyl sulphate
SEC	Size exclusion chromatography
SERS	Surface enhanced Raman scattering
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
SHWE	Superheated water extraction
SIA	Sequential injection analysis
SIMS	Secondary-ion mass spectroscopy
SORS	Spatially offset Raman spectroscopy
SPDE	Solid phase dynamic extraction
SPE	Solid phase extraction
SPMDs	Semipermeable membrane devices
SPME	Solid phase microextraction
SPS	Solid phase spectrophotometry
SSE	Subcritical solvent extraction
SWE	Subcritical water extraction
TDLs	Tunable diode lasers
TLV	Threshold limit value

TLV–STEL	Threshold limit value–short term exposure limit
TLV–TWA	Threshold limit value–time weighted average
TOF	Time of flight
TRI	Toxic release inventory
uHTS	Ultra-high throughput screening
UPLC	Ultra performance liquid chromatography
UPLC	Ultra pressure liquid chromatography
UV	Ultraviolet
UV-DOAS	Ultraviolet differential optical absorption spectrometry
VLCE	Vesicular liquid coacervate extraction
WHO	World Health Organization
XPS	X-ray photoelectron spectroscopy
XRF	X-ray fluorescence
XRPD	X-ray powder diffraction

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CHAPTER 1

Origins of Green Analytical Chemistry

Contents

Green Chemistry is nowadays a well established topic which is clearly identified as the efforts to improve chemistry-related activities by avoiding their undesirable side effects. So, it is easy to imagine a green industrial process, a green synthesis, and also a green analytical chemistry. However, in the literature we see that before the popularization of the concept of Green Chemistry there was a consciousness among analytical chemists of the need to develop sustainable methodologies, in order to save reagents and solvents, and to replace the most toxic solvents by other innocuous or less toxic ones. In fact, before the pioneering theoretical contributions and clean analytical methods proposed in the literature, it is evident that people working in the analytical field were aware of the challenges offered by automation, miniaturization, and direct analysis. These challenges allowed analytical chemists to avoid the use of reagents or solvents, thereby enhancing the safety of their work and becoming more environmentally friendly.

In this chapter, we will introduce the scenarios and facts which made possible the tremendous interest in Green Analytical Chemistry that exists today and the origin of many of the terms currently used, also highlighting the close relationship between Analytical Chemistry today and the ecological mentality.

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1.1. THE ECOLOGICAL PARADIGM

Professor H. Malissa, from the Technical University of Vienna, presented a talk at the Euroanalysis VI conference in September 1987, under the title "Changes of paradigms in Analytical Chemistry" [1]. This suggestive, rather unconventional, presentation followed the recommendations made by Professor Laitinen in 1973 when he said that "the usual approach to predicting the future is to project past trends" [2] and he showed that scientific activities must be adapted to societal needs in order to surmount any crises by a correct projection of the past to the future, making adequate behavioral changes to avoid errors made in the past and to incorporate new ideas into old practices.

Malissa used the term paradigm as a pattern and a syntax-like scheme but "also as a guide for new friends in our academic society, giving them new tools for solving new problems and a new platform for discussion and communication". In fact, what he proposed was not to describe the nucleus of the Analytical Chemistry as a discipline strongly linked to atomic and molecular theory and the crystalline state theory, which ultimately explains the fundamentals of all the analytical methods used to detect and quantify atoms, molecules, or structures in any material. Rather, in evaluating the relationship between Analytical Chemistry and social activities, he identified six successive paradigms in the evolution of Analytical Chemistry—from Archeochemistry to Alchemy, Iatrochemistry, Chemiology and Chemiurgy and finally to Ecological Chemistry.

In this presentation, the reasons for the revolutionary changes of paradigms were clear. The original archeological practices were integrated into the Alchemy paradigm, which was impregnated with religious and mystical ideas. This first theoretical structure crashed due to factors introduced by the Renaissance and the organization of the Chemistry and analytical knowledge around the search for natural compounds suitable to preserve or restore human health. However, the crisis of Stahl's phlogiston theory and its transition to the oxidation theory together with the rationalist empirical systematic study of chemical reactions made by Lavoisier in 1789 moved the old Iatrochemistry paradigm to a logical chemistry, the Chemiology paradigm, which provided the fundamentals of Chemistry as a scientific discipline. This led to the creation of chemistry-based industries and the Chemiurgy paradigm was a combination of the exploitation of synthesis processes and new products. However, some of the excesses of this latter period created controversy between industrial development

and ecology and forced the people engaged in Chemistry to think about their activities, thus allowing a new change of paradigm.

In the scenario depicted by Malissa, the conclusion is that Analytical Chemistry today must be closely related to environmental protection and that all analytical activities must consider the different aspects related to the preservation of our ecosystem. At this point, we would like to recall the letter that the great chief of Seattle sent in 1855 to the president of United States in which he said "And with all your strength, with all your might and with all your heart, preserve it [the land] for your children, and love it" [3]. Thus, we must be conscious that in our professional activity we need to take care of both the safety of our operators and the preservation of the environment.

As can be seen in Figure 1.1 there is no clear transition between the archeological period and the different paradigms but nowadays the ecological mentality must inform our work and our relationship with society. So, when thinking about the analytical process, we must not only pay attention to the problems, samples, and data to be obtained, but also to the amounts and nature of the reagents to be used, the energy requirements,

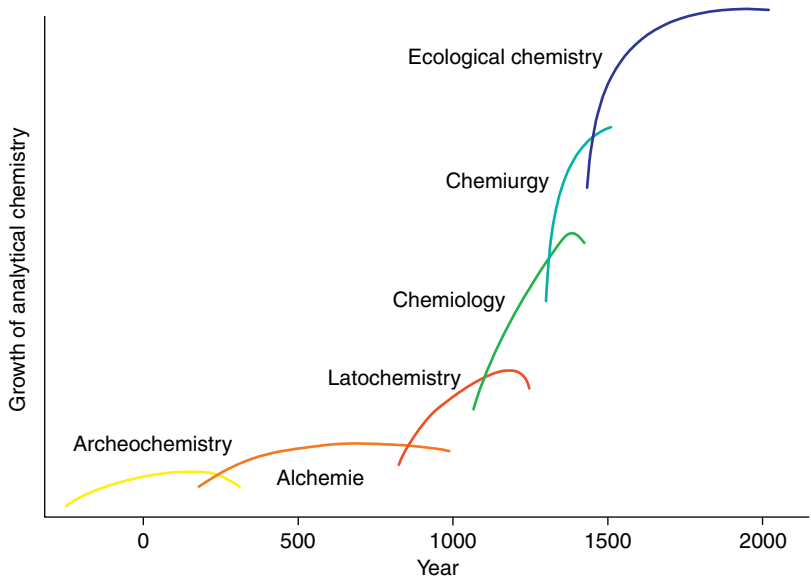

Figure 1.1 The Malissa thesis about the changes of paradigms in Analytical Chemistry (*adapted from Reference* [1]).

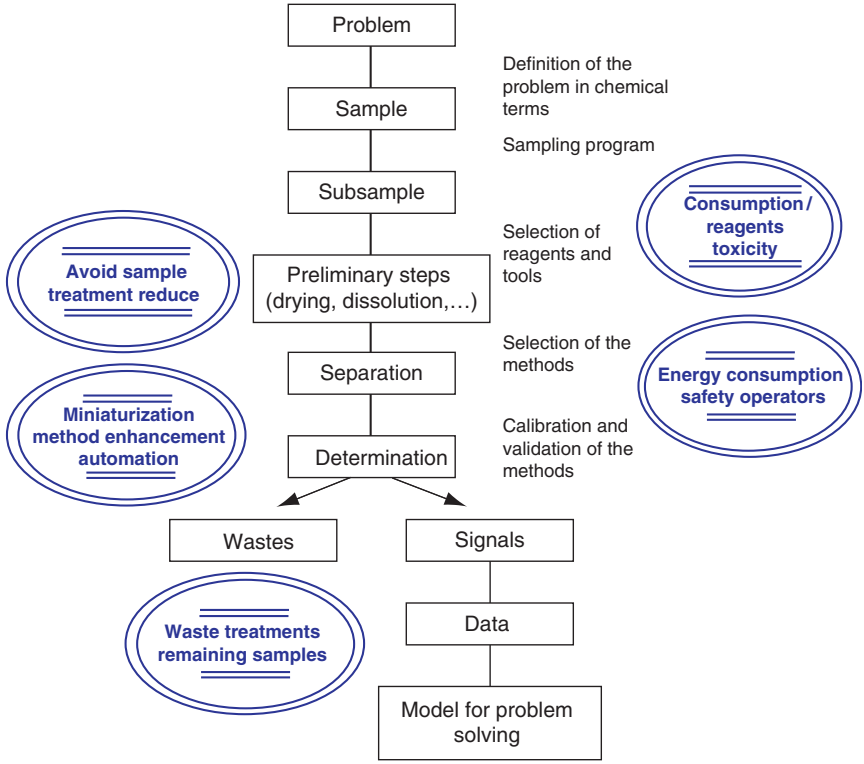

Figure 1.2 Steps of the analytical process to be considered in the frame of the Ecological Paradigm.

the emissions and wastes generated during the whole process and the risks to operators and the environment.

Figure 1.2 shows a modified proposal of the traditional schemes of the steps of analytical process and, as can be seen, the ecological mentality extends the aspects to be considered by incorporating new questions and parameters involved in method development and the comparison of methods.

1.2. THE ENVIRONMENTAL OPPORTUNITIES FOR ANALYTICAL CHEMISTRY

George Pimentel, who developed a vast research and teaching activity in Chemistry from 1943 till 1989, which included some basic studies on free radicals, infrared photochemistry, and chemical lasers, was deputy director of the National Science Foundation from 1977 to 1980 and president of the American Chemical Society (ACS) in 1986. He organized and

presented a report to the National Academy of Sciences entitled "Opportunities in Chemistry" [4] which was published in 1985 and had an important influence in the teaching of Chemistry in American high schools.

The Pimentel report evaluated the state-of-the-art of Chemistry at the middle of the 1980s and the main aspects of the discipline, including its instrumentation. Although there is no specific mention of Analytical Chemistry, it is clear that in the remark by Jeanette Graselli that "we know only what we can measure" there is a tribute to analytical chemists [5,6]. In this context, of special interest are the proposals to the Environmental Protection Agency (EPA) in the form of four recommendations including: (i) the increase in the percentage of research and development funding devoted to exploratory research, particularly on environmental problems for the future, (ii) improvement of fundamental research on reaction pathways for substances of environmental interest, (iii) the detection of potentially undesirable environmental constituents at levels below their expected toxicity, and (iv) the EPA support of Analytical Chemistry in a prominent way. The Pimentel report opened special opportunities for Analytical Chemistry and improved the measurements of toxic substances in the environment.

The increase of the analytical activity on environmental samples and on undesirable environmental constituents had the knock-on effect of increasing the amount of analytical wastes and provided notable changes in the mentality of laboratories about the impact of their residues.

In Figure 1.3, we summarize the evolution of the ecological mentality within analytical laboratories from the deep evaluation of the analytical figures of merit of new methods to the environmentally friendly Green Chemistry as a consequence of the bad conscience about the side effects of the increase of reagent consumption and waste generation.

So, finally we have evidence that the new opportunities offered by Chemistry at the end of the last century were closely related to the ecological mentality and, as will be discussed in the following sections, Green Analytical Chemistry offered excellent opportunities from both the academic and business points of view.

1.3. THE BAD CONSCIENCE OF CONSUMERS OF REAGENTS AND WASTE GENERATION

The increase of analytical activities from both basic research and applications also increased the amounts of wastes and reagents consumed, thus creating new problems in the acquisition and management of reagents.

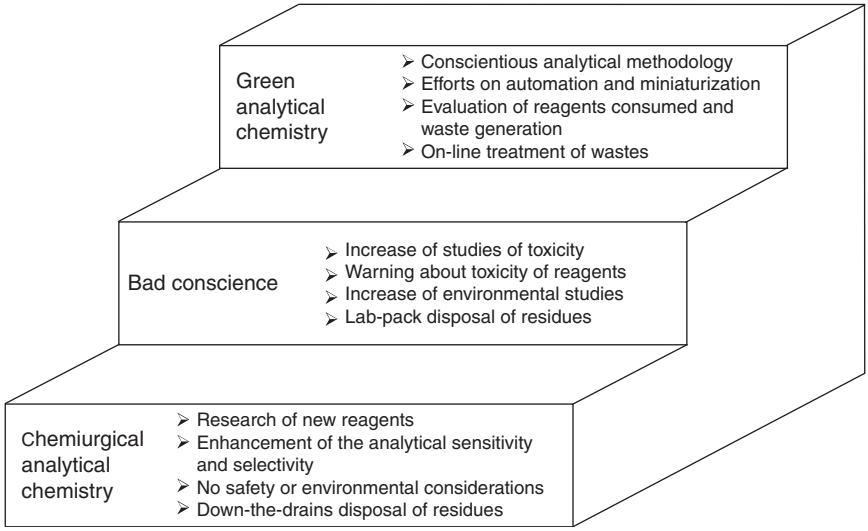

Figure 1.3 Steps in the evolution of the ecological mentality of the analytical laboratories.

At the same time, this also provided new challenges that focused on the practical characteristics of methods related to the time of analysis, costs, safety considerations, and side effects of environmental problems as a result of the analytical activities, in addition to those concerning their main features. So, laboratories started to evaluate the scale of their analysis, scaling down the volume of reagents and solutions prepared to do the measurements, and trying to minimize the amounts of solvents and reagents consumed. It was originally a matter of economy but it then moved to safety and toxicity aspects.

In the beginning of the 1990s, research and teaching laboratories around the world began to pay attention to their responsibilities regarding the health risks to operators and the environmental damage of their activities, with organizations creating special archives cataloging the toxic characteristics of chemicals, adding comments about the risks of the use of reagents and solvents and ensuring a better organization of the transport and storage of chemicals.

One of the consequences of the bad conscience of the analytical laboratories about the undesirable side effects of their activities was linked to the increase of environmental studies and the problems related to the disposal of polluted samples together with that of laboratory wastes. All the aforementioned problems facilitated a change of mentality of teachers and

researchers and, within this framework, the Chemiurgy paradigm was replaced with the Ecological paradigm.

On the other hand, the new challenges once again offered new opportunities together with new problems. The increase of information about toxic characteristics of reagents led to the replacement of widely used traditional solvents and chemicals for new innocuous or, at least, less toxic ones than those employed before and the downscaling of methods offered cheaper alternatives. Thus automation and miniaturization offered excellent alternatives to solve problems related to the management of reagents and to increase the productivity of the laboratories faced with an increased demand for measurements and data.

Some of the most innovative advances in sample preparation, measurement, and data handling related to flow injection analysis (FIA), microwave-assisted sample digestion and extraction (MAE) and chemometrics, which were introduced in the middle of the 1970s. They found in the paradigm of ecological chemistry new arguments for their use in method development and, as indicated in Figure 1.4, the methodological milestones required to make analytical chemistry greener were mainly achieved before the concepts of Green Analytical Chemistry were formulated.

The starting practices involved the generalized use of lab-pack disposal of residues and the recovery of expensive reagents and solvents. However, off-line practices increased the magnitude of the problems and the accumulation of residues in the labs also, thereby increasing their risks. So, it was clear that moving from "cleaning" methodologies to "clean" ones could be a real alternative for both practical and economic reasons. Additional efforts were thus made to avoid the increase of the magnitude of environmental problems and waste disposal by improving on-line treatments.

1.4. CLEAN ANALYTICAL METHODS

During the XXVIII Colloquium Spectroscopicum Internationale in York, UK, 1993, a discussion between Miguel de la Guardia and Harpal Minhas, who at that time was the editor of *The Analyst*, the journal of the Royal Society of Chemistry (RSC), evolved some ideas regarding the possibility offered by the combination of photo-assisted degradation processes linked to the FIA manifolds employed in spectrometric determination of phenolic compounds. One idea was to replace the word waste by a more convenient term—such as clean waste—offering an alternative method which

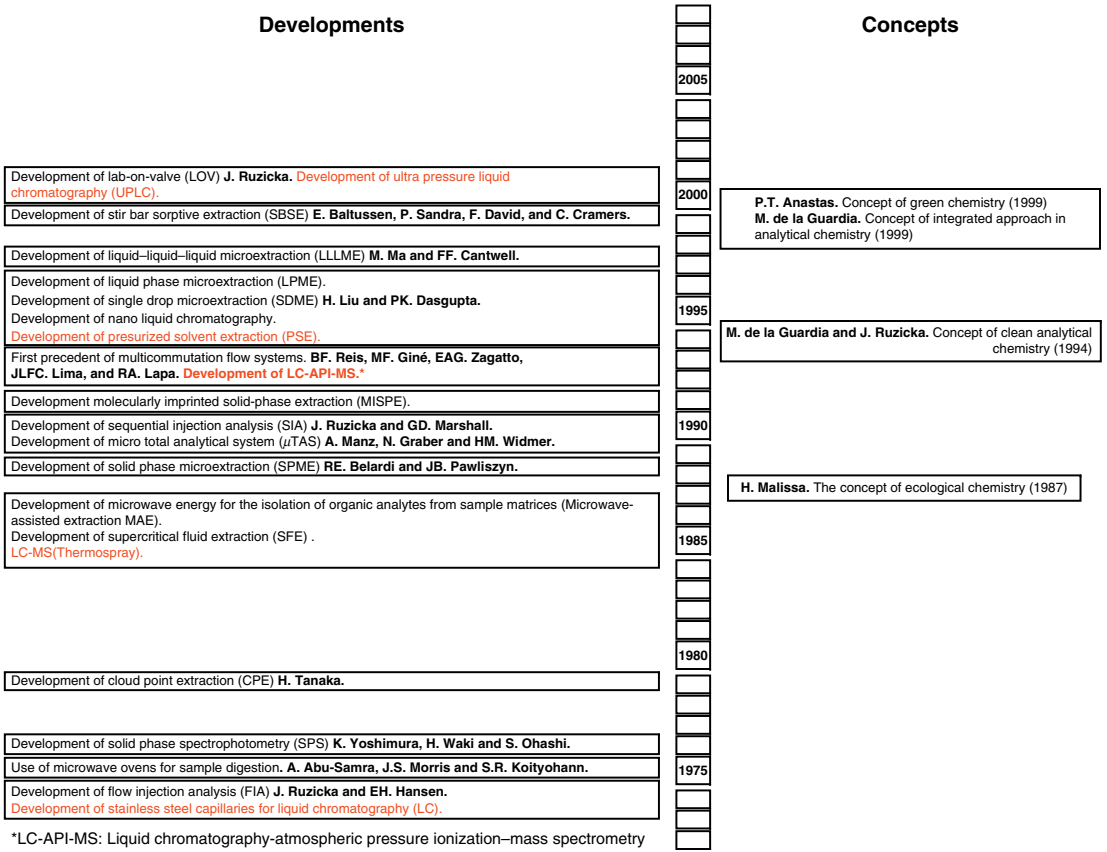

Figure 1.4 Milestones of Green Analytical Chemistry; concepts and methodologies (*adapted from Reference* [7].)

incorporated an additional chemical effort to reduce the environmental impact of FIA determinations. The result of that meeting was the edition of a special issue of *The Analyst* devoted to Clean Analytical methods.

In fact, in the framework of Flow Analysis VI, Toledo, Spain, in June 1994, the poster "A clean analytical method for the determination of propoxur" won the prize for the most innovative poster. The aspect of the on-line degradation of wastes was an important contribution of the aforementioned study which was previously proposed for the detoxification of wastes generated through the use of an additional reactor, located at the end of the flow line, after the determination step. In such a reactor, the wastes are merged with a TiO_2 slurry and irradiated with an UV lamp at 254 nm in order to guarantee the on-line complete mineralization of reagents. Additionally, the catalyst was recovered by filtration and used again [7].

One year later, in 1995, the special issue of *The Analyst* confirmed the possibilities offered by the contribution of degradation processes and FIA to enhance analytical methods. The title of the invited editorial "Towards environmentally conscientious Analytical Chemistry through miniaturization containment and reagent replacement" is the first declaration of the principles of what is today called Green Analytical Chemistry [8]. In this editorial, the replacement of toxic chemicals by harmless ones was fixed as an ideal goal to be achieved. However, it was advanced as a realistic approach: to start by reducing by a factor of 10 the use of dangerous chemicals in order to contribute to pollution prevention as conceptualized in the USA by the EPA. To do it, many ways were suggested, such as the use of (i) robotics, (ii) miniaturized chromatographs, (iii) flow techniques like FIA and sequential injection analysis (SIA), and (iv) advances in sensor technology. So, it was the first approach to establish a theoretical support to search for the minimization of the impact of the millions of assays carried out daily and also to create a new mentality for the education of future generations.

However, it should be emphasized that the special issue of *The Analyst* was not appealing enough for the scientific community as it only included five contributions, covering topics like photocatalytic treatment of laboratory wastes, supercritical fluid extraction of pollutants, micellar spectrophotometry, chemometric determination of gasoline properties, and a clean analytical method including the on-line photocatalytic degradation of wastes. So, the impact of this first approach was practically minimal until the publication of the paper of Paul. T. Anastas in 1999 concerning "Green Chemistry and the role of analytical methodologies development" [9].

In 1995, David W. Green et al. presented a report entitled "Waste minimization in Analytical Methods" at the DOE Pollution Prevention Conference XI, held in Knoxville, Tennessee, which is considered to be one of the precursor works of Green Analytical Chemistry. The purpose of that paper was to add "waste minimization" to the list of characteristics, such as accuracy, precision, limits of detection, cost, and interferences among others, to be considered during the selection of appropriate analytical methods. In that report, modifications of existing analytical methods for waste characterization were evaluated for both reduction of the cost of analysis and volume of generated wastes. Although the term Green Analytical Chemistry was not used, the green idea was inherently present. Green et al. compared new analytical methods with existing ones in terms of accuracy, precision, and waste volume reduction, paying special attention to three research areas: sample injection for inorganic analysis, dissolution of waste samples for radiochemical analysis, and sample preparation for analysis of organic constituents.

1.5. GREEN CHEMISTRY

Between 1994 and 1998, Paul Anastas edited a series of texts devoted to Green Chemistry in the ACS symposium series: "Benign by design: alternatives synthetic designs for pollution prevention" [10], and "Green Chemistry: designing chemistry for the environment" [11] together with two books published by Oxford University Press entitled *Green Chemistry: theory and practices* [12] and *Green Chemistry: frontiers in benign chemical synthesis and processes* [13]. As a consequence of his experience in the field Anastas devoted a critical review to the role of Analytical Chemistry in the frame of Green Chemistry. In fact, the review of Anastas fixed the term Green Analytical Chemistry as the most appropriate to describe what were previously known as clean or environmentally friendly methodologies.

Green Chemistry was defined as "the movement toward pursuing chemistry with the knowledge that the consequences of synthesis and the use of reagents do not stop with the properties of the target molecule or the efficacy of a particular reagent. The impacts of the chemistry designed by chemists are felt by the people that come in contact with the substances that they make and use and by the environment". It was clear for Anastas that there is an ethical responsibility of chemists to develop new chemistries that must be more benign than those employed to date but, additionally, the industrial and societal costs of actions to

improve environmental protection could be reduced in this way. In fact, one of the merits of Green Chemistry is to integrate efficacy, efficiency, and economic criteria, highlighting the relationship between chemists and society. In this way, Green Chemistry could contribute, by making an additional chemical effort, to avoid or, at least, reduce the environmental impact of the chemical activities.

The ideas considered by Anastas on evaluating the relationship between Analytical Chemistry and the environment are of particular interest, first at all considering that during the process of measuring the environmental problems the analytical chemistry methodologies used could contribute themselves to further environmental problems and also on evaluating process analytical chemistry to prevent pollution at the source and the use of real-time in-process sensors, which can be of a great value to detect the formation of hazardous substances.

On considering the different aspects of Green Chemistry in the laboratory, Anastas evaluated some of the new approaches that could contribute to greener methods such as: (i) field analysis, (ii) screening, (iii) extraction, (iv) dilution, (v) digestion methods, and (vi) the use of alternative mobile phases. All those aspects considered the use of reduced amounts of reagents and solvents and the generation of as small amounts of wastes as possible, but, as indicated in previous sections, additional efforts like on-line recycling, passivation, or degradation of toxic wastes together with the use of remote sensing or chemometric treatment of data obtained for untreated samples could contribute to reduce simultaneously the cost and environmental impact of analytical methods.

The tremendous editorial activity by Anastas in the last decade has been of a great benefit to bring about the change of mentality in the chemistry community. Thus the term Green Chemistry is nowadays universally accepted and therefore Green Analytical Chemistry seems the best alternative title to define the efforts made in the Analytical Chemistry community to avoid side effects of pollution and to improve the sustainability of the new alternative methods.

As evidence of the appropriateness of the term, Table 1.1 provides an updated list of books and journals devoted to Green Chemistry. So, we need to move away from the old terms such as clean technologies or clean production which were in the title of journals, like the classical *Journal of Cleaner Production* edited by Elsevier from 1993 or the modern *Clean Technologies and Environmental Policy* edited by Springer from 2008 and adopt the use of green terms.

Table 1.1 Books and journals devoted to Green Chemistry

Title of the book or journal	Authors book/Web site journal	Editor	Year
Green Chemistry: designing chemistry for the environment	P.T. Anastas and T.C. Williamson	American Chemical Society	1996
Green Chemistry: Theory and Practice	P.T. Anastas and J.C. Warner	Oxford University Press	1998
Green Chemistry: Frontiers in Benign Chemical Synthesis and Processes	P.T. Anastas and T.C. Williamson	Oxford University Press	1998
Real-world cases in Green Chemistry	M.C. Cann and M.E. Connelly	American Chemical Society	2000
Green Chemistry: An Introductory Text	M. Lancaster	Royal Society of Chemistry	2002
Introduction to Green Chemistry: Instructional Activities for Introductory Chemistry	American Chemical Society	American Chemical Society	2002
Green Chemistry: Environment Friendly Alternatives	R. Sanghi and M. M. Srivastava	Alpha Science International	2003
New Trends in Green Chemistry	V. K. Ahluwalia and M. Kidwai	Kluwer Academic publisher	2004
Green Chemistry and Catalysis	R.A. Sheldon, I. Arends, and U. Hanefeld	Wiley-VCH	2007
Green Chemistry	http://www.rsc.org/greenchem	Royal Society of Chemistry	1999
Green Chemistry Letters and Reviews	http://www.tandf.co.uk/journal/titles/17518253.asp	Taylor and Francis	2007

Many research sites focusing on Green Chemistry have been created around the world, as can be seen in Table 1.2. The main activities of the aforementioned research teams concern catalysis, organic synthesis, or clean manufacturing technologies but there are also groups concerning general Green Chemistry and environmental chemistry.

1.6. THE INTEGRATED APPROACH TO ANALYTICAL CHEMISTRY

In 1999, a review article was published in the *Journal of the Brazilian Chemical Society* summarizing the conference presented during the annual meeting of the Sociedade Brasileira de Quimica (SBQ) in Caldas de Rei, Brazil [14]. In this study, the general process of Analytical Chemistry was presented as a whole tool for problem solving and the different steps, from sampling to sample storage, sample preparation, determination and data and waste treatment, were evaluated in the frame of the ecological paradigm in order to provide accurate, economic, fast, and safe analytical methodologies.

In this review, 10 subjects were identified as the main keywords of Analytical Chemistry at the end of the last century such as: (i) traceability, (ii) chemometrics, (iii) flow analysis and robotics, (iv) in-field sampling, (v) microwave-assisted treatment, (vi) hyphenation, (vii) speciation, (viii) sensors, (ix) screening methodologies, and (x) decontamination of wastes. It is remarkable that seven of these keywords clearly focus on the principles of Green Analytical Chemistry.

However, the main contribution of the so called integrated approach was in developing a consciousness of the inputs and outputs of the analytical methods and the identification of the responsibility of the laboratory for the wastes produced during the analysis of samples and the importance that waste treatments have on the total cost of the analytical operations. The aforementioned approach offered excellent possibilities for both basic research and applied analysis, because the main proposal was to maintain the required analytical properties of methods such as accuracy, sensitivity, and selectivity, while also looking for a drastic reduction of costs, sample handling and size of assays, and to demonstrate an improvement in the safety and comfort of operations, in the repeatability of assays and in laboratory productivity. So, by thinking about all of the analytical steps in an integrated way it was seen that the whole process could be improved from

Table 1.2 Research internet sites regarding Green Chemistry

Country	Institution	Web site
Serbia	Mediterranean Countries Green Chemistry Network	http://www.incaweb.org/megrec/
UK	Centre for Clean Technology, York	http://www.york.ac.uk/depts/chem/staff/Clark-home.html
	Green Chemistry Network	http://www.chemsoc.org/gcn
	Institute for Applied Catalysis	http://www.iac.org.uk
	Centre for Alternative Technology (CAT)	http://www.cat.org.uk
	Questor	http://questor.qub.ac.uk/
	Queens University Ionic Liquids Laboratory	http://quill.qub.ac.uk/
	University of Nottingham—Clean Technology Research Group	http://www.nottingham.ac.uk/~pczsp/clnthome.html
	University of Reading—The Reading Centre for Surface Science and Catalysis	http://www.chem.rdg.ac.uk/dept/catrg/catrg.html
	Cardiff School of Biosciences—Clean Technology Group	http://www.cf.ac.uk/uwc/biosi/associates/ctgroup/
	University of Leeds—Leeds Cleaner Synthesis Group	http://www.chm.leeds.ac.uk/people/CMR
	University of Leicester—Leicester Green Chemistry Group	http://www.le.ac.uk/chemistry/greenchem
	University of York—Green Chemistry Group	http://www.york.ac.uk/res/gcg/GCG
Denmark	Technical University of Denmark—Center for Sustainable and Green Chemistry	http://www.csg.dtu.dk

Italy	Interuniversity Consortium of Chemistry for the Environment	http://www.unive.it/inca.html
	Italian Group of Catalysis	http://www.fci.unibo.it/gic/
Netherlands	Netherland Institute of Catalysis Research	http://www.chem.tue.nl/niok/
Australia	Murdoch University, Perth	http://wwwscience.murdoch.edu.au/teaching/m234/recycle32.htm
	Monash University, Clayton	http://www.monash.edu.au/
USA	Centre for Clean Technology (University of California)	http://cct.seas.ucla.edu
	National Center for Environmental Research and Quality Assurance (NCERQA)	http://es.epa.gov/ncerqa/
	National Center for Clean Industrial and Treatment Technologies (CenCITT)	http://es.epa.gov/ncerqa/cencitt/cencitt.html
	Environmental Protection Agency (U.S. EPA)	http://www.epa.gov/greenchemistry/
	Centre for Green Manufacturing, University of Alabama	http://bama.ua.edu/~cgm/
	University of North Carolina—The NSF Science and Technology Center for Environment	http://www.nsfstc.unc.edu
	Carnegie Mellon University—Institute for Green Science	http://www.chem.cmu.edu/groups/Collins
	Carnegie Mellon University—Institute for Green Science	http://www.chem.cmu.edu/groups/Collins

both the economical and scientific aspects and at the same time providing environmentally friendly alternatives to the old practices.

Figure 1.5 provides a scheme of the basic ideas of the integrated environmentally friendly approach of Analytical Chemistry. As can be seen, the size of laboratory samples can be reduced through the incorporation of in-field sampling strategies, carefully selected to avoid conservation problems of large volumes of samples and providing a good way for their

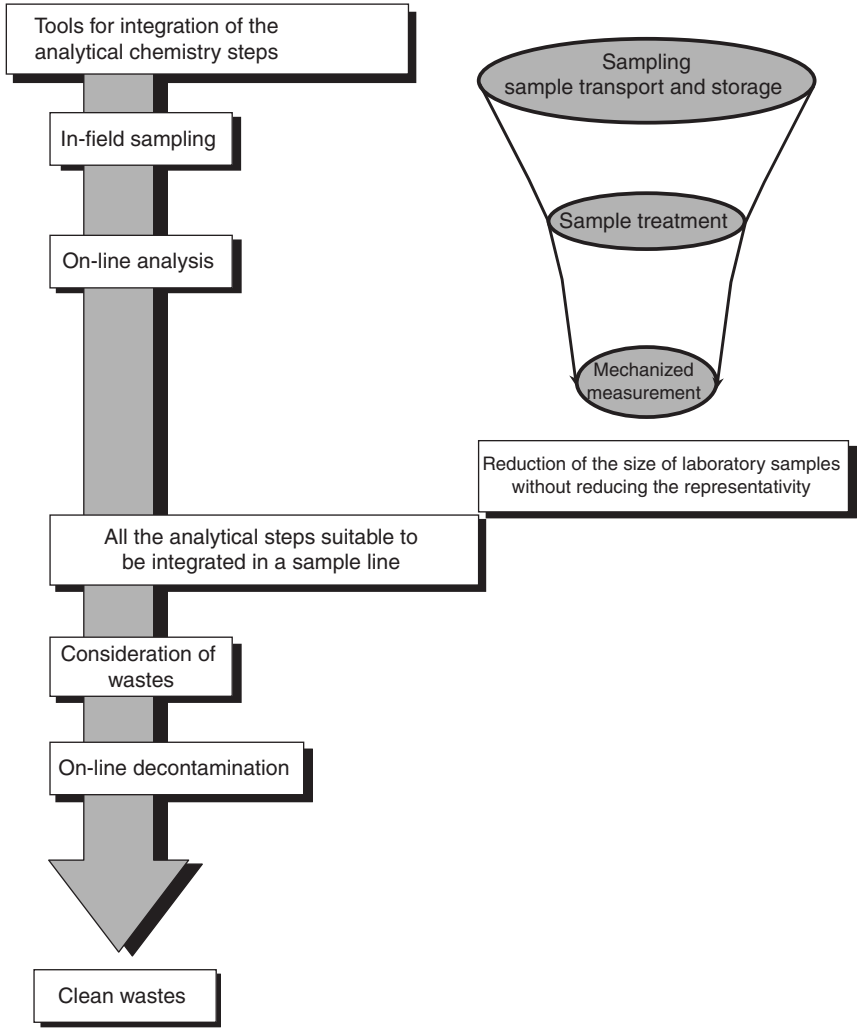

Figure 1.5 Basic ideas for an integrated environmentally friendly approach of Analytical Chemistry.

incorporation into analytical setups. Adsorbed samples can be on-line eluted and analyzed in a flow process which drastically reduces the amount of employed reagents and samples, also reducing the requirements for cleaning the laboratory material and thus involving a drastic reduction of generated wastes. However, from a green approach it is difficult to accept the generation of waste and, because of that, an additional effort concerning waste decontamination or waste passivation is required to be environmentally friendly. An additional chemical effort is therefore necessary and could be incorporated on-line to the analytical process.

1.7. THE STATE-OF-THE-ART OF GREEN ANALYTICAL CHEMISTRY

In a scientometric study recently made [15], it was observed that it is difficult to identify the analytical studies concerning green alternatives because there is not a wide and generalized use of a common term which can group the efforts to prevent wastes or to avoid the use of potentially toxic reagents or solvents together with those involving the decontamination of wastes.

As can be seen in Figure 1.6, the evolution of the literature covering green analytical methods, including the terms clean, sustainable, and environmentally friendly, compiled in the Chemical Abstracts Service (CAS), the U.S. National Library of Medicine and the Science Citation

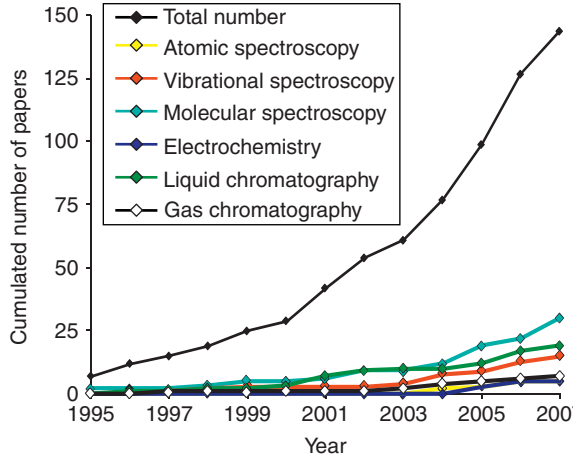

Figure 1.6 Evolution, as a function of time, of the literature covering clean, sustainable, or green analytical methods.

Table 1.3 Reviews published on Green Analytical Chemistry

Topic	Title	Number of References	Year	Reference
General	Waste minimization in analytical methods	13	1995	[16]
	An integrated approach of Analytical Chemistry	28	1999	[14]
	Green Chemistry and the role of analytical methodology development		1999	[9]
	Green Analytical Chemistry—some remarks	11	2001	[17]
	Green Analytical Chemistry—a new approach to analysis		2001	[18]
	Trends in environmental analytics and monitoring	10	2002	[19]
	Applications of the principles of Green Chemistry in Analytical Chemistry	52	2006	[20]
	Green Analytical Methodologies	110	2007	[21]
	Green Analytical Chemistry: Innovations, applications, and education		2008	[22]
	Green Analytical Chemistry	85	2008	[23]
Miniaturization	Electrochemical detection for microscale analytical systems: a review	23	2002	[24]

Continued

Table 1.3 Reviews published on Green Analytical Chemistry—cont'd

Topic	Title	Number of References	Year	Reference
	Electrochemical detection for capillary electrophoresis microchips: a review	48	2005	[25]
	Challenges of analytical microsystems	83	2006	[26]
Sensor devices	Novel sensor devices and monitoring strategies for green and sustainable chemistry processes		2007	[27]
Sample preparation techniques	Minimization of solvent consumption in pesticide residue analysis	41	1996	[28]
	Passive sampling	125	2002	[29]
	Analytical applications of membrane extraction for biomedical and environmental liquid simple preparation	161	2005	[30]
	Modern techniques of sample preparation for determination of organic analytes by gas chromatography	132	2007	[31]
	Sample preparation techniques for the determination of trace residues and contaminants in foods	204	2007	[32]
	Trends in solventless sample preparation techniques for environmental analysis	94	2007	[33]

Continued

Table 1.3 Reviews published on Green Analytical Chemistry—cont'd

Topic	Title	Number of References	Year	Reference
Flow analysis	Flow analysis strategies to greener Analytical Chemistry: an overview	44	2001	[34]
	Multi-pumping flow systems: the potential of simplicity	24	2007	[35]
Ionic liquids	Uses of ionic liquids in Analytical Chemistry	31	2003	[36]
	Ionic liquids in Analytical Chemistry	71	2006	[37]
	Application of Ionic Liquids in Liquid Chromatography	62	2007	[38]
Gas chromatography	Some remarks on gas chromatography challenges in the context of Green Analytical Chemistry	7	2002	[39]
Plasma-based techniques	Plasma chemistry as a tool for Green Chemistry, environmental analysis and waste management	62	2000	[40]
Spectroscopy	Spectroscopy: the best way toward Green Analytical Chemistry?	68	2007	[41]
Electrochemistry	Real-time electrochemical monitoring: toward Green Analytical Chemistry	35	2002	[42]

Index (SCI) database of the Institute for Scientific Information (ISI) in Philadelphia, USA, provided a relatively small number of less than 200 published papers, from 1995 till 2007, with a clear predominance of molecular spectroscopic methods and liquid chromatography. It can be concluded that the lack of a wide and generalized use of the term Green Analytical Chemistry reduces the real representativeness of studies on the evolution of this field. This makes it difficult to provide a good description of its real state-of-the-art and thus provides just an indication of the impact of the ideas of Green Analytical Chemistry in the research work of many teams.

Table 1.3 summarizes the review articles published from 1995 till 2008 concerning the general topic of Green Analytical Chemistry but also those concerning miniaturization, sensor devices, sample preparation, automation through flow analysis, and specific areas regarding green spectroscopy, green electrochemistry, green chromatography, or green plasma techniques. A special case is the importance of the research done in the first years of this century on the use of ionic liquids as a green alternative to the use of solvents.

In short, the evolution of the literature references including the use of the terms sustainable, clean, or green, together with the increase of the review articles concerning the general aspects of Green Analytical Chemistry or its application in different fields inform us about the evolution of the interest in this subject. It also permits us to hope for an advance in the search for a safe and nonpolluting Analytical Chemistry that can provide scientific alternatives to the available methods and which are also less expensive than the previous ones. Additionally, it is clear that the main goal of Green Analytical Chemistry is to avoid or reduce the undesirable environmental side effects of its practice, preserving the main features of the methods like accuracy, sensitivity, selectivity, precision, sample throughput, comfort, and cost.

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CHAPTER 2

The Basis of a Greener Analytical Chemistry

Contents

The 12 principles developed by P. Anastas and J.C. Warner for Green Chemistry involved [1]:

1. Prevent waste in order to avoid the need of cleaning or decontamination procedures.
2. Design safer chemicals and products to avoid their risks or toxic effects.
3. Design less hazardous chemical synthesis for both humans and environment.
4. Use renewable feedstock to replace depleting feedstock media for fossil fuel.
5. Use catalysts, not stoichiometric reagents to reduce the amounts of reagents used through the world.
6. Avoid chemical derivatizations to reduce once again the amounts of reagents to be used.
7. Maximize atom economy in order to reduce wastes and to improve the synthesis yield.
8. Use safer solvents and reaction conditions to improve the use of water or eco-friendly solvents that do not contribute to smog formation or ozone layer depletion.
9. Increase energy efficiency working, as possible at room temperature.

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10. Design chemicals and products to degrade after use, in order to avoid reagents accumulation in the environment and to assure that employed chemicals degrade to innocuous final products.
11. Analyze in real time to prevent pollution thus involving in-field analysis and real time monitoring of processes.
12. Minimize the potential for accidents like explosions, fires, and releases to the environment.

One of the aforementioned principles includes the development of fast analytical methods as a condition for a safe chemistry and many of these principles concern the reduction of reagents (including solvents) consumed and the replacement of toxic reagents by innocuous ones. On the other hand, the reduction of wastes and energy consumption has also been considered.

The aforementioned 12 principles of Green Chemistry have been adapted by Jacek Namiesnik [2] to four top priorities.

1. Elimination, or at least significant reduction, of consumption of reagents and organic solvents for analytical procedures.
2. Reduction of emission of vapors and gases, as well as solid wastes generation in analytical laboratories.
3. Elimination of reagents displaying high toxicity and/or ecotoxicity from analytical procedures.
4. Reduction of labor and energy consumption of analytical procedures.

Figure 2.1 summarizes the Green Chemistry principles and the four priorities of Green Analytical Chemistry together with the basics for greener Analytical Chemistry.

Taking into consideration all the aforementioned principles and priorities, an ideal methodology should be a reagentless procedure, nondestructive, with a reduced energy consumption, fast and capable of determining as many analytes and/or parameters as possible in a single run. So, the basic strategies for greening the analytical methods involve:

(i) The in-field direct analysis of untreated samples, (ii) the use of alternative, less energy-consuming, and less reagent-consuming sample treatments together with (iii) the miniaturization and automation of the methodologies. These three strategies contribute to the elimination or reduction of reagents consumed in analytical practice and also reduces energy requirements. (iv) The search for alternative reagents and solvents is mandatory. Moreover, when there is no other possibility, (v) the on-line decontamination of wastes could be of great value to reduce emissions and

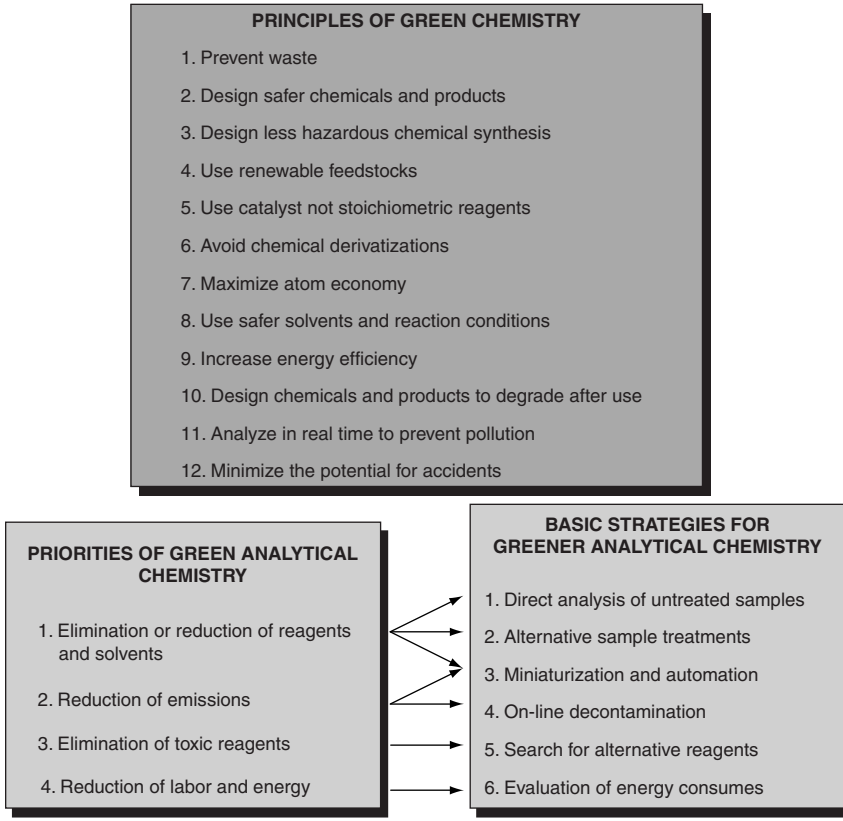

Figure 2.1 Basic principles, priorities, and strategies for greener Analytical Chemistry, based on the principles of Green Chemistry and the priority tasks of Green Analytical Chemistry defined in Anastas and Namiesnik studies [1,2].

to eliminate their ecotoxicity together with the advantages offered by miniaturization and automation to reduce the volumes of waste. (vi) The evaluation of energy consumption together with automation to assess the reduction of labor and energy consumption of analytical procedures.

The aforementioned basic strategies must focus on the deep evaluation of: (1) the side effects of reagents and solvents, (2) the energy cost of methods, and (3) the evaluation of the amounts and toxicity of analytical wastes in order to combine the strategies to provide the most environmentally friendly alternative procedure without sacrificing the basic characteristics of the analytical methods.

So, in the following sections we will evaluate those aspects of analytical methods which clearly affect their sustainability.

2.1. THE SIDE EFFECTS OF REAGENTS AND SOLVENTS

Toxicological information, in addition to the physico-chemical data and information on the risks of reagents and solvents required for the analytical measurement must be available for potential users. The Register, Evaluation, Authorization, and Restriction of Chemicals (REACH) norm established by the European parliament in 2006 [3] concerning the REACH is one of the recent examples of the need for a limited use, production, and commercialization of chemical substances that are potentially toxic. One of the key factors to a correct survey of the risks and exposures to the chemicals is correct information from both authorities and users.

We now need to establish policies concerning the restriction of the use of highly toxic reagents and solvents and to make mandatory the information in all documentation, including scientific publications, about the practical risks associated with all the reagents required for the analytical measurements. Information is the preliminary step to knowing the correct way toward greener chemistries.

In a recent review article, Keith et al. [4] have reported the greenness criteria elaborated by the ACS Green Chemistry Institute to evaluate the environmental methods in order to identify those procedures which use safer chemicals or minimize wastes. The aforementioned criteria have been applied to the National Environmental Methods Index (NEMI) which contains information regarding methods, summaries, metadata, and additional information about more than 800 methods [5].

An important contribution of the NEMI is the incorporation of greenness profiles to evaluate the analytical methods. The greenness criteria can be summarized by four key terms "persistent, bioaccumulative, and toxic reagents (PBT)," hazardous, corrosive, and waste, which concern the use of PBT as defined by the Environmental Protection Agency (EPA) and included in the toxic release inventory (TRI) [6]. Regarding hazardous, it indicates that the method uses a hazardous chemical of those listed on the TRI [6] or on the Resource Conservation and Recovery Act (RCRA) as classified D, F, P, or U hazardous wastes [7]. The term corrosive indicates the use of a pH during the analysis lower than 2 or higher than 12 and wastes indicate that the total amount of waste generated is higher than 50 g.

The aforementioned terms are reflected in a greenness profile symbol (see Figure 2.2) which is a circle with four quadrants labeled as hazardous, waste, corrosive, and PBT, filled in green when the method does not

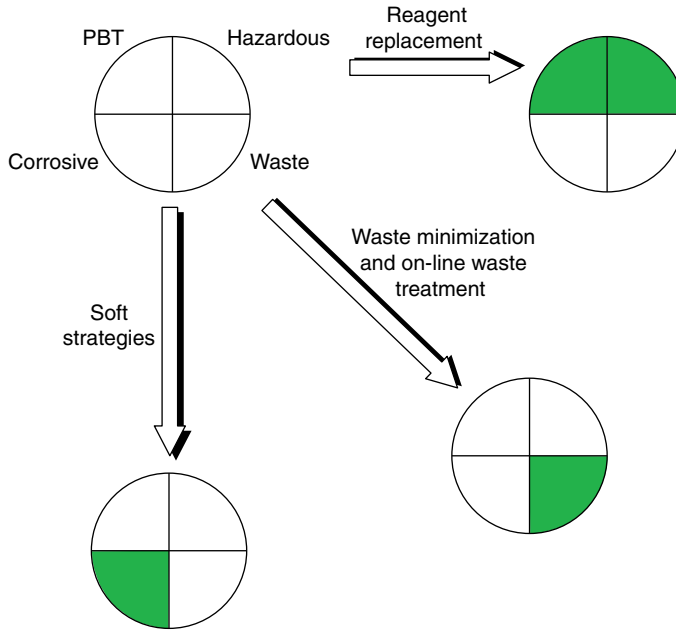

Figure 2.2 The greenness symbols of NEMI and the alternatives for greening the methods.

involve each one of the greenness criteria and left open when the method present a toxicity, corrosive, or waste problem.

So, it is of great interest that prestigious organizations incorporate green criteria for the information of users about the different available methodologies and editors of analytical journals should encourage the authors of papers to incorporate this kind of symbol in their publications. On the other hand, taking advantage of the facilities offered by the electronic editions of scientific journals, we also recommended adding a link to the material safety data sheets (MSDS) of all required chemicals to be employed in the proposed methods.

In short, both method developers and users must pay attention to the toxic and safety properties of chemicals in order to avoid the use of persistent, bioaccumulative, toxic, and hazardous products.

Obviously, in some cases, it is not evident that there are alternative reagents or solvents to do specific determinations. In such cases, it must be attempted to make the available procedures greener through the minimization of reagents consumed, the reduction of contact between

operators and reagents, and also to incorporate on-line waste treatments to avoid the disposal of chemical wastes into the environment.

2.2. THE ENERGY COSTS OF ANALYTICAL CHEMISTRY

Power supply is the key factor of the energy consumption of analytical instruments and apparatus. The use of high temperature sample preparation steps and long digestion or extraction procedures on heating conditions involves a high demand of electricity and contribute to the cost of analysis and the environmental impact of the analytical steps. Moreover, laboratory furniture, such as safety cabinets, refrigerators, and freezers that maintain the integrity of samples before their analysis, and ovens, that assure a constant temperature during the measurement, and which work continuously during long periods of time, requires elevated energy consumption. In 2007, an energy consumption study was conducted in order to compare different equipment commercial brands: freezers, safety cabinets, and centrifuges were considered to be the biggest energy drain in laboratories today. The results of this study demonstrated consumption of approximately 15,000 KWh $year^{-1}$ in a research laboratory.

Sample digestion and analyte extraction at high temperatures during several hours are another of the steps of the analytical procedures which have environmental side effects from the energetic point of view. They are time and energy consuming and involve a relatively high risk for operators. So, the change of mentality in today's analytical laboratories, moving away from the total destruction of samples to be analyzed to the use of softer or no sample pretreatments, as discussed in Chapters 4 and 5, involves a drastic reduction of both reagents employed and energy consumed.

On the other hand, the simultaneous treatment of several samples together, the increase of the speed of sample measurement processes through automation, the development of multianalyte determinations in a single run, and the use of portable instrumentation provide a strong reduction of the energy consumed, thus contributing to the sustainability of the method, and also involving a reduction of costs. This shows that Green Analytical Chemistry could be cheaper than the traditional one.

However, it is clear that for method evaluation and implementation the green mentality requires the evaluation of energy consumption and, as it is indicated in Figure 2.3, the consideration of the starting power supply and energy requirements of apparatus and instruments to be used

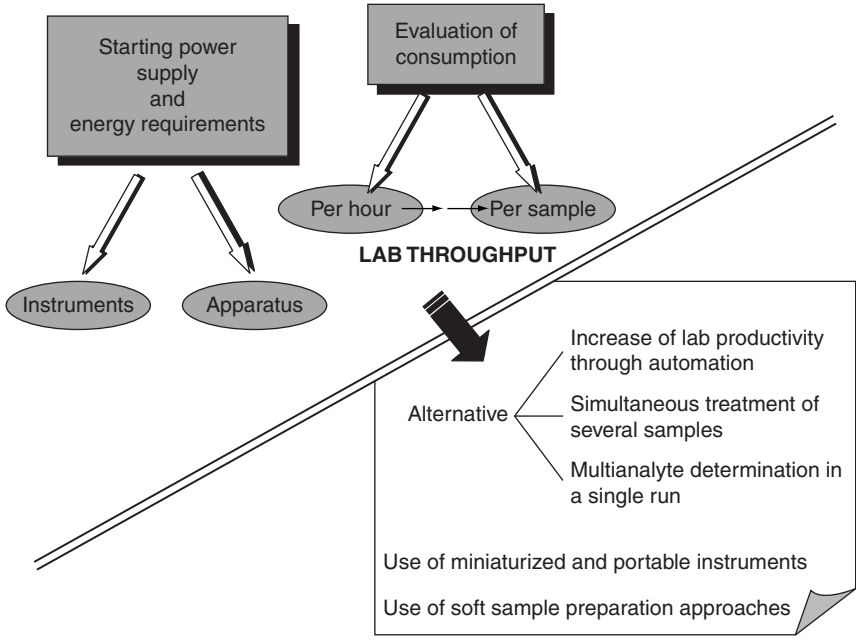

Figure 2.3 Energy costs of analytical methods.

during the analytical procedure. For a correct evaluation of the energy consumption, the watts consumed per hour or, even better than that, per sample, must be employed. In this regard, it is clear that the increase of productivity through automation of methods, the simultaneous treatment of several samples or multianalyte determinations, all contribute to reduce the energy consumption per analysis.

On the other hand, the replacement of the old sample pretreatments based on convective heating used in wet and dry ashing of samples by microwave-assisted ones, ultrasound treatments, or pressurized solvent extraction strongly reduces the energy consumption and increases operator safety, as indicated in Figure 2.4 [8].

Furthermore, the replacement of the traditional instrumentation by miniaturized apparatus [9,10], the use of sensors [11,12], and the evolution to portable instrumentation [13] reduces drastically the energy requirements, not only considering the maximum power employed but also by reducing the input voltage as it is summarized in Figure 2.5.

So, in our opinion method developers must provide to the potential users values about the energy requirements and energy consumption

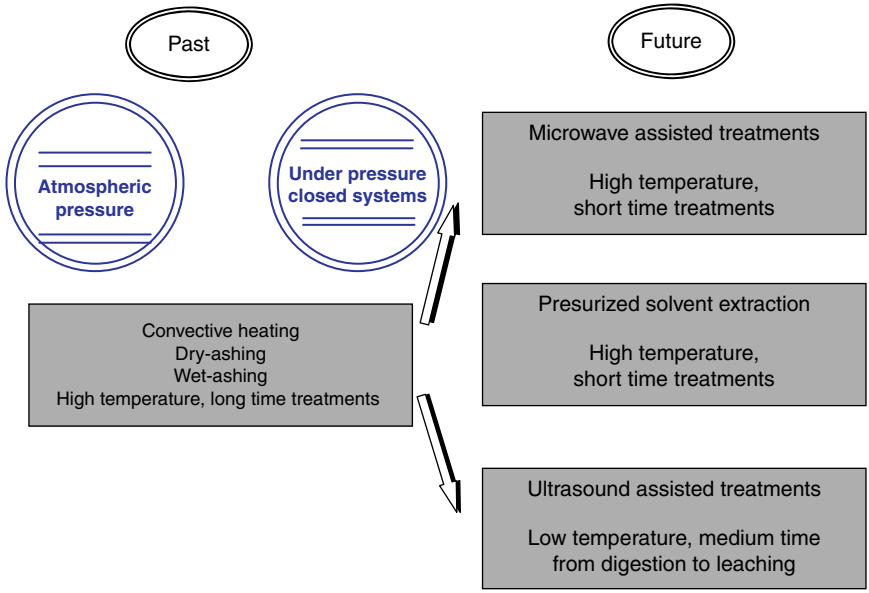

Figure 2.4 Greening the analytical methods through reduction of the energetic costs in sample preparation.

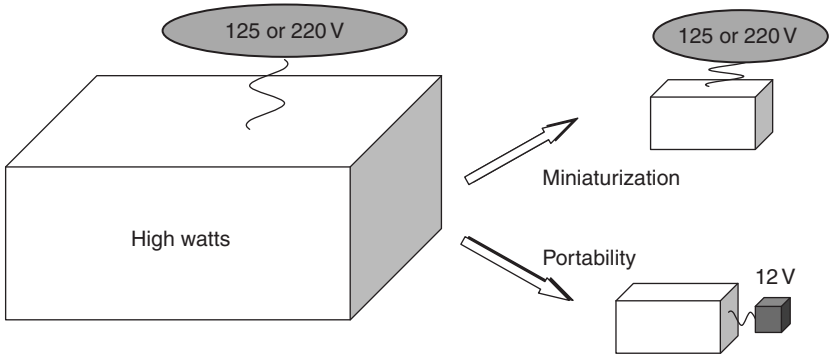

Figure 2.5 Greening the analytical methods through miniaturization and portability to save energy consumption and energy requirements.

involved in the whole procedure, especially when new methods are compared with reference ones. This kind of information will be of a great importance to evaluate the costs of analysis and could contribute to expand the green mentality among analytical chemists.

2.3. WASTE GENERATION AND ITS ASSOCIATED RISKS

Generally, analytical methods provide wastes together with data as final products of the measurement steps. It is evident that analytical methodologies devoted to in-field analysis, also called on-site, are the greenest ones, reducing reagent and energy consumption to the minimum while avoiding or reducing waste generation and the risks related to transport and storage of samples. In-field analysis is continuing to progress as practical and green methodologies for dealing with different analytical problems such as environmental incidents, site remediation, and terrorist actions and provides several important advantages. However, there are of course several disadvantages to using in-field procedures, the most important being, together with the reliability of such measurements, their nonacceptance as these are usually not in compliance with standards set by the International Organization for Standards (ISO), the EPA, the National Institute for Occupational Health and Safety (NIOSH), and the American Society for Testing and Materials (ASTM). This means that only "standard" procedures would stand up in court. Thus, it would be really interesting to see the full acceptability of in-field procedures as standard methods [14].

In the case of nondestructive direct determinations, the residue is minimized to the portion of the sample transported to the laboratory. For this reason it is interesting to not handle large amounts of samples and to try to provide a representative sampling in the field to save costs and risks in sample transport and sample processing in environmental conditions different to the original ones.

However, waste problems arise when the analytical procedures to be used involve sample dissolution or extraction steps followed by chemical derivatization, separation of components, and determination. The traditional use of batch methods involved the handling of amounts of samples of the order of few grams together with the preparation of volumes of reagents at the liter or hundred milliliters scale and thus the generation of large volumes of prepared samples mixed together with reagents. Moreover, the volumes of cleaning solutions contaminated with surfactants, acids, and solvents must be added to the residues of analytes, samples, and reagents employed. Fortunately, the downscaling of laboratory treatments together with the efforts made in method mechanization and automation [15] have reduced the waste generation from several liters per day to a few milliliters.

Method automation provides the best way for a drastic reduction of analytical wastes, because first of all, it allows the mixing on-line of sample and reagents, thus avoiding the preparation of volumes of treated samples higher than those required for analytical measurements.

One of the reasons for the continuous progress in flow analysis-based strategies is the need to minimize reagent consumption and waste generation. We can thus understand the evolution from classical flow injection analysis (FIA) to sequential injection analysis (SIA) and modern multicommutation as a search for the reduction of wastes and reagent consumption together with an enhancement of laboratory productivity [16] without sacrificing the main analytical figures of merit.

Table 2.1 summarizes, as an example, the amount of wastes and sampling throughput in the spectrophotometric determination of carbaryl, a persistent carbamate, using *p*-aminophenol as chromogenic reagent together with NaOH and KIO_4 and, as can be seen, depending on the automation strategy used, the total waste could be reduced from 960 to 27 mL h^{-1} with a sampling throughput varying from 20 to 90 h^{-1} which in short implies wastes from 10.7 to 1.44 or 1.35 mL per sample using FIA or multicommutation.

Figure 2.6 indicates schemes of the four different manifolds employed in the aforementioned comparison study. It must also be indicated that the micropump approach was based on the use of a light-emitting diode (LED) instrument which allowed the portability of the whole system [18], thus providing additional advantages in saving energy together with the reduction of reagents consumed and waste generated.

Moreover, the possibility to add on-line and fully mechanize clean-up or preconcentration steps based on the use of solid phase extraction units

Table 2.1 Waste generation and sample frequency obtained for the determination of carbaryl with *p*-aminophenol using different mechanized procedures

Methodology	Sampling (h^{-1})	Total waste (mL h^{-1})	Waste (mL analysis^{-1})	References
Classical FIA	90	960	10.7	[16]
SIA	20	27	1.35	[16]
Solenoid valves multicommutation	70	120	1.71	[16]
Micropumps multicommutation	72	104	1.44	[17]

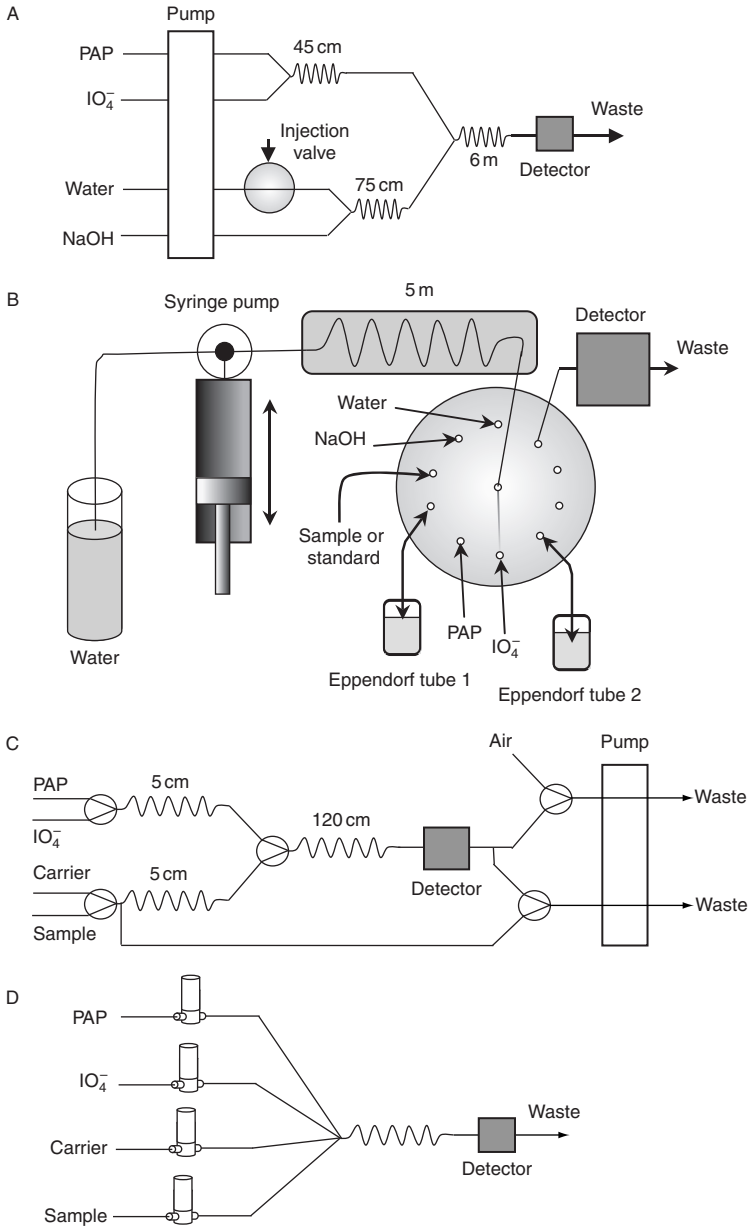

Figure 2.6 Comparison of the manifolds employed for the spectrophotometric determination of carbaryl with *p*-aminophenol using (A) classical FIA, (B) SIA, (C) solenoid valve multicommutation, and (D) micropump multicommutation in this latter system the detection was a portable LED photometer.

[19], capillary microextraction [20], or the on-line retention of reagents in the so called solid phase spectrophotometry [21,22], substantially reduces the solvent and reagent consumption. Furthermore, the self cleaning characteristic of the mechanized or automated system strongly reduces the use of glassware and the high volumes of cleaning solutions required to clean and decontaminate the material to be used in the analytical methods, this being an additional green advantage.

Finally, in considering waste generation, it must be remembered that wastes from the analysis of toxic products like metals or pesticides also become toxic and harmful and thus analytical wastes cannot be disposed of in the environment and must be treated. From an economic point of view, waste treatment is an expensive task which contributes to an increase of analytical costs, and the accumulation of residues in the laboratory before their transport to the treatment plants also contributes to an increase in the risks to operators and the environment due to an increase of their volume. Therefore, a great advantage provided by the use of mechanized methods is the possibility to incorporate on-line decontamination processes, also called end-pipe treatments, and this aspect will be considered in detail in Chapter 8.

2.4. STRATEGIES FOR GREENING ANALYTICAL CHEMISTRY

As indicated in Figure 2.1, the 12 principles of Green Chemistry and the four priorities of Green Analytical Chemistry can be manifested in six basic strategies for greening Analytical Chemistry and we encourage analysts to search for the best environmental alternative when carrying out a specific analysis. Figure 2.7 offers a chronology of decisions to be made on looking for the most sustainable method to solve an analytical problem and the advantages derived from each of these strategies. As can be seen, the in-field direct analysis of untreated samples could offer the best alternative because this kind of determination implies no reagents and generates no wastes, thus avoiding risks both to operators and the environment. However, in many cases, it is not possible to solve the analytical problem through the use of remote sensing analysis nor by direct measurements made on the untreated samples and thus it becomes necessary to dissolve the samples or extract the analytes and to carry out chemical reactions. In such cases, the first priority must be to use nontoxic, nonhazardous reagents in order to avoid risks and the generation of toxic wastes.

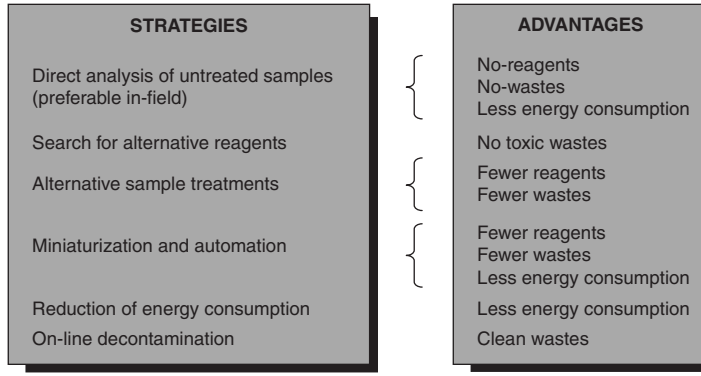

Figure 2.7 Chronology and advantages of the strategies proposed for greener Analytical Chemistry.

Alternative sample treatments could be of great value to assure the quantitative recovery of the analytes, without modifying the chemical form present originally in the samples and also to minimize matrix decomposition and solubilization, which could cause interferences in further analytical steps. Nowadays, the tendency in sample treatments has moved from hard to soft, from long and aggressive treatments to leaching ones. This contributes to the strategy of saving chemicals and energy and to reduce emissions and wastes.

In many cases, sample treatment and sample measurement processes can be greened through miniaturization and automation. These strategies allow us to reduce drastically the amount of reagents consumed and the generation of waste and also help reduce energy consumption. However, this latter aspect must be considered separately in order to make the whole analytical process compatible with the lowest energy consumption per sample while also incorporating the simultaneous treatment of several samples and the hyphenation of techniques to obtain as much information as possible from a single sample treatment.

Finally, if the method of choice involves the use of hazardous chemicals, additional efforts must be made to decontaminate on-line the analytical wastes or to reduce the amount and toxicity of residues, thus providing on-line recycling or recovery of solvents and reagents, the mineralization of organic compounds and the precipitation and passivation of metals. In the following chapters, the aforementioned strategies will be highlighted from the consideration of green alternatives proposed in the literature for sample treatment and measurement, and examples will be presented for greening our methods.

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CHAPTER 3

A Green Evaluation of Existing Analytical Methods

Contents

As mentioned in Chapter 2, the starting point for greening analytical methods is the correct evaluation of costs, energy consumption, risks to the operator, and environmental effects of reagents and wastes generated both by the procedure and its practice. Information is the key factor. In this chapter, we will discuss the basis for an evaluation of the existing methods of analysis, paying special attention to the green parameters to clearly identify the risks and weaknesses of methods but also looking for maintenance of the classical analytical parameters such as accuracy, sensitivity, selectivity, and precision.

3.1. TOXICOLOGICAL DATA OF REAGENTS

Chemical compounds are on many occasions hazardous due to their flammability or combustible characteristics which can create problems in their storage and transport or be poisonous to operators and biota. So, correct information about the safety of chemical handling and storage is of great importance to prevent accidental risks and to evaluate the specific conditions required for their use and disposal [1].

Toxicity to humans and ecological toxicity in general can be established based on the main characteristics of compounds to be employed in an analytical procedure. In the case of the analytical work, the threshold

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limit value (TLV), the airborne concentration of a substance below which all workers are believed to be protected while exposed to it day after day for 8-h periods, together with other indicators of exposures to chemicals, like the TLV–TWA, time weighted average concentration for an 8-h workday or 40-h work week, the TLV–STEL or short term exposure limit or maximum concentration for a continuous 15-min exposure or 4-min exposure periods per day with at least 1 h between exposure periods, inform us about the risk of working with a chemical substance. Thus, the aforementioned data encourage us to replace the toxic reagents by innocuous ones.

Material safety data sheets (MSDSs) provide important information to evaluate the chemical compounds and their relationship with the risks involved in their handling [2]. Table 3.1 summarizes the main information reported by the MSDS and from this summary it seems clear that the

Table 3.1 Information available from MSDS

Section	Information
1	Product information
2	Ingredients
3	Hazardous identification
4	First aid measures
5	Fire fighting measures
6	Accidental release measures
7	Handling and storage
8	Exposure controls/Personal protection
9	Physical and chemical properties
10	Stability and reactivity
11	Toxicological information
12	Ecological information
13	Disposal considerations
14	Transport information
15	Regulatory information
16	Other informations

MSDS of involved reagents and solvents in a proposed analytical method are of great value to establish the greenness of a procedure.

Nowadays, there are many sites available on the Internet to obtain the MSDS of any chemical compound or chemical formulation. In addition to the product information, regarding product and chemical name and formulation, the Chemical Abstracts Service (CAS) and EP registration number and the ingredients (in the case of formulated compounds), MSDS provides information on the hazard category of compounds, their acute and/or chronic hazardous properties together with information about fire, pressure, or reaction risks involved in their use. Odor, aspect, and deleterious effects are also included.

A relevant aspect to be considered concerns the first aid measures suggested in case of intoxication through contact, inhalation, or ingestion routes and the reported recommended actions must be known before the operator starts working with a compound. Fire measures include flash point temperature, if required, together with the fire-extinguishing media and special fire-fighting procedures. Accidental release measures together with handling and storage precautions to be taken provide a list of solutions suggested to avoid risks both to operators and the environment. Regarding the exposure controls, the MSDS provides information regarding ventilation requirements and personal protective equipments with special attention to dermal, eye, and respiratory protection.

The physical and chemical properties section of a MSDS reports the melting and boiling point, the evaporation rate, as compared with butyl acetate, the vapor pressure, vapor density (compared with that of air), specific gravity, solubility in water, and others, but stability and reactivity data are reported in a separate section.

Toxicological information concerns the medium lethal doses (LD_{50}) established as the mass of a substance administrated per unit of mass of test subject (e.g., grams of substrate per kilogram of body mass) which can kill half of the members of a tested population, and thus, this parameter concerns the acute toxicity of chemicals. In the ecological information section, the risks to aquatic biota and toxicity toward some species, like trees in the case of pesticides used to treat crops, are summarized.

Disposal considerations and transport information provide guides for selecting the best way for disposal, the containers to be selected, and guidelines for domestic ground transport and shipping of compounds and related formulations. The aforementioned information is complemented with regulatory information and other informative sections.

Table 3.2 summarizes some of the MSDS on freely available Internet sites [3] with a note about the number of compounds included in each site. Some of these are general sites which provide general information about chemicals also providing MSDS but others, like Oxford University, do not provide MSDS but just chemical substance information. Some government and nonprofit sites are the concern of chemical societies and international and government services like the World Health Organization (WHO) or Environmental Protection Agency (EPA). In other cases, chemical manufacturing services and suppliers provide complete information about their products and there are specific sites for agrochemicals including pesticides, insecticides, fungicides, nematocides, rodenticides, and also fertilizers and products employed in different fields like cleaning or adhesives, natural products, or automotive compounds.

Based on the aforementioned information, method developers and analysts can compile a complete picture of the hazards and risks involved in the use of a method and can identify the reagents to be replaced or controlled and the problems related with treatment and disposal of the analytical wastes. Solvent-free and innocuous-based methodologies must be selected if possible. However, in other cases, the automation of processes and the reduction of reagent consumption and waste generation, together with the on-line treatment and minimization of residues, would be the way for greening the methods, and to do this as completely as possible information about the chemicals considered is necessary.

It is also important to know which reagents are the most hazardous or toxic ones, because the primary intention of green analytical methods is to avoid or reduce the use of these substances. In this regard, the reagents and solvents listed in the hazardous waste list of the EPA is highly informative [4].

The National Waste Minimization Program focuses efforts on reducing 31 priority chemicals (PCs) by finding ways to eliminate or substantially reduce their use, not only in production but also in analytical laboratories. The 31 PCs are listed in Table 3.3. A fact sheet including a summary of the potential health effects of each chemical can be accessed at http://www.epa.gov/wastes/hazard/wastemin/priority.htm. This list of 31 PCs replaces the list of 53 chemicals identified by EPA in its 1998 Federal Register "Notice of Availability: Draft Resource Conservation and Recovery Act (RCRA) Waste Minimization Persistent, Bioaccumulative, and Toxic (PBT) Chemical List" [5].

Table 3.2 Free MSDS available Internet sites

	Internet site	Number of MSDS
1.	MSDSonline	>3,500,000
2.	MSDS Solutions 3E Company	>3,500,000
3.	MSDSXchange	>1,000,000
4.	Seton MSDS Hazard Communication Library	350,000
5.	Vermont SIRI, hazard.com Backup site	180,000
6.	CambridgeSoft, ChemBioFinder.com	153,000
7.	Docstoc	44,000+
8.	Oxford University	28,059 (some repeats)
9.	University of Akron	25,009
10.	ChemExper	10,000
12.	Conform-Action Data Systems	5000
13.	Iowa State University	295
14.	PubChem, US National Institutes of Health	>48,700,000
15.	Chemspider, Royal Society of Chemistry	>21,000,000
16.	Scorecard, Environmental Defense Fund	11,200
17.	OHSAH MSDS Database	>9500+
18.	Envirofacts Chemical Databases (U.S. EPA)	6864
19.	North American Emergency Response Guidebook	3714
20.	Household Products Database	~2000 chemicals
	National Library of Medicine (National Institutes of Health)	~7000 brands
21.	The National Toxicology Program (National Institutes of Health)	>2000
22.	The National Toxicology Program (National Institutes of Health)	1000
23.	The National Toxicology Program (National Institutes of Health)	228
24.	New Jersey Hazardous Substance Fact Sheets (NJHSFS)	>1700 English, >600 Spanish

Continued

Table 3.2 Free MSDS available Internet sites—cont'd

	Internet site	Number of MSDS
25.	CDC/NIOSH/WHO International Chemical Safety Cards	1264
26.	International Agency for Research on Cancer, IARC	900
27.	OSHA/EPA Occupational Chemical Database	>800
28.	NIOSH Pocket Guide to Chemical Hazards	677
29.	Agency for Toxic Substances and Disease Registry	185
30.	Health Canada	176
31.	Sigma, Aldrich, Fluka, Supelco, RdH-Lab	90,000
32.	Acros Chemicals, Fisher Scientific	61,000
33.	VWR Scientific Products	25,929
34.	Alfa Aesar	14,000
35.	Merck KGaA	>12,000
36.	EMD Chemicals, (formerly EM Science)	7000
37.	Roche Applied Science	3600
38.	Airgas	3283
39.	Gelest Inc	2408
40.	J. T. Baker, Inc.	2100
41.	Mallinckrodt Laboratory Chemicals	2100
42.	Air Products and Chemicals, Inc.	1862
43.	Lamotte Company	1712
44.	Eastman Kodak	1500
45.	E. I. du Pont de Nemours and Company	>1400
46.	Science Stuff, Inc.	1400
47.	Flinn Scientific	1300
48.	Redox Chemicals Pty Ltd.	1158
49.	Rhodia Silicones	>1000
50.	ExxonMobil Corporation	750

Continued

Table 3.2 Free MSDS available Internet sites—cont'd

	Internet site	Number of MSDS
51.	Electronic Space Products International	425
52.	Matheson Tri-Gas, Inc.	419
53.	Dojindo Molecular Technologies (Japan)	396
54.	Scott Specialty Gases	387
55.	United Laboratories	361
56.	USB Corporation	358
57.	BOC Gases (part of the Linde Group)	>350
58.	Bristol-Myers Squibb, BROKEN LINK as of 03-Jan	338
59.	BD Diagnostics Systems (formerly Difco)	274
60.	Linde Gas (Linde Group)	>200
61.	Accepta Ltd.	~100
62.	Crop Data Management Systems	4400
63.	Greenbook	1500
64.	EXTOXNET	193
65.	Dow AgroSciences	177
66.	Syngenta Crop Protection, Inc. (Merger of Novartis and Zeneca), Crop Protection Chemicals, Professional Products	150, 59
67.	Aubuchon Hardware	>5000
68.	JohnsonDiversey (Merger of Johnson Wax Professional and DiverseyLever)	4000
69.	W. W. Grainger, Inc.	3734
70.	Loctite Corporation	1838
71.	Cytec Industries	>1633
72.	Procter and Gamble	695
73.	Johns Manville	503
74.	Xerox Corporation	~500
75.	The Sherwin-Williams Company	497

Continued

Table 3.2 Free MSDS available Internet sites—cont'd

	Internet site	Number of MSDS
76.	Center for Advanced Microstructures and Devices at LSU	450
77.	ABC Compounding	433
78.	Lincoln Electric, Airco + Murex, too	356
79.	Butcher's	298
80.	Rentokil Initial plc	200
81.	Liberty Natural Products	181
82.	Hewlett-Packard	175
83.	ProSciTech (Australia)	157
84.	Falcon Safety Products, Inc.	150
85.	The Essential Oil Company	144
86.	Hercules Plumbing Products	132
87.	5 Star Autobody Products	103

3.2. EVALUATION OF THE CONTACT OF OPERATORS WITH REAGENTS AND WASTES

Analytical methodologies that use toxic, bioaccumulative and persistent chemicals, or solvents are dangerous for the environment but also for the analysts. The ways that a chemical toxic substance can enter the body concern three main routes, as indicated in Figure 3.1, involving (i) contact, (ii) inhalation, and (iii) ingestion. In fact, in the analytical laboratory ingestion has been reduced by eliminating mistakes based on using old practices like oral pipetting.

It is evident that contact risks can be easily avoided by an appropriate application of the standards of laboratory safety. Skin and eyes, which are the most exposed organs, must be protected by wearing appropriate gloves, protective glasses, and clothing to safely handle materials. Contact risks can be avoided during the in-field operations of sampling and during the sample pretreatment in the laboratory (see Figure 3.2). Special attention must be paid to the fact that clinical and environmental analysis put operators in contact with samples which can contain toxic and hazardous components and thus the aforementioned operations must follow a

Table 3.3 Priority Chemicals (PCs) to be eliminated or reduced as defined by the National Waste Minimization Program

Chemical name and summary fact sheet	CASRN
Organic chemicals and chemical compounds	
1,2,4-Trichlorobenzene	120-82-1
1,2,4,5-Tetrachlorobenzene	95-94-3
2,4,5-Trichlorophenol	95-95-4
4-Bromophenyl phenyl ether	101-55-3
Acenaphthene	83-32-9
Acenaphthylene	208-96-8
Anthracene	120-12-7
Benzo(g,h,i)perylene	191-24-2
Dibenzofuran	132-64-9
Dioxins/Furans	1746-01-6
Endosulfan, alpha and Endosulfan, beta	959-98-8, 33213-65-9
Fluorene	86-73-7
Heptachlor and Heptachlor epoxide	76-44-8, 1024-57-3
Hexachlorobenzene	118-74-1
Hexachlorobutadiene	87-68-3
Hexachlorocyclohexane, gamma-(Lindane)	58-89-9
Hexachloroethane	67-72-1
Methoxychlor	72-43-5
Naphthalene	91-20-3
Pendimethalin	40487-42-1
Pentachlorobenzene	608-93-5
Pentachloronitrobenzene	82-68-8
Pentachlorophenol	87-86-5
Phenanthrene	85-01-8
Polycyclic Aromatic Compounds (PACs)/PAH Group	
Polychlorinated Biphenyls (PCBs)	1336-36-3

Continued

Table 3.3 Priority Chemicals (PCs) to be eliminated or reduced as defined by the National Waste Minimization Program—cont'd

Chemical name and summary fact sheet	CASRN
Pyrene	129-00-0
Trifluralin	1582-09-8
Metals and metal compounds	
Cadmium	7440-43-9
Lead	7439-92-1
Mercury	7439-97-6

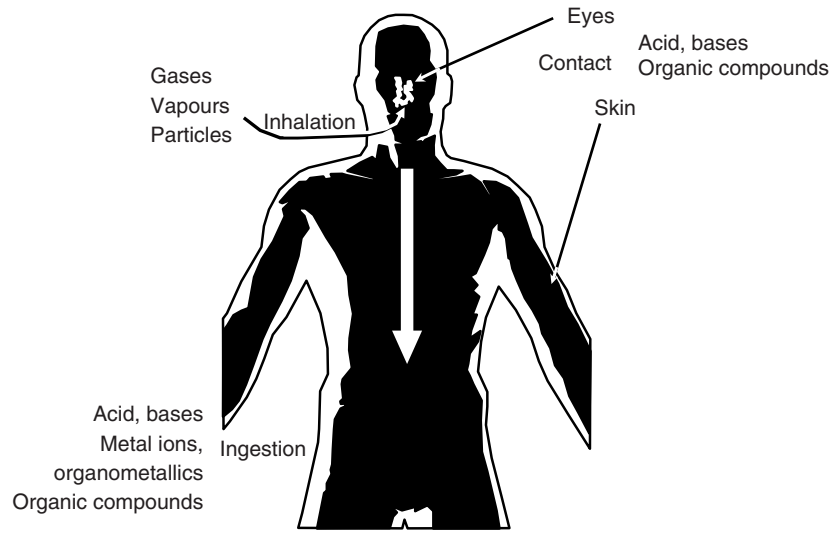

Figure 3.1 Exposure risks of operators working with chemicals.

detailed protocol that considers the potential risks. Additionally, these first steps of analysis are difficult to automate and thus the contact of the operator with the samples is mandatory.

Inhalation is commonly the single route of exposure to toxic materials of the operators during sample transport and storage and inhalation must be also considered during sampling and sample treatment. It is particularly dangerous for gaseous compounds but also for particles and vapors. Attention must be paid to the fact that the transition between the solid and the liquid state is done at a fixed temperature. However, vapors remain in

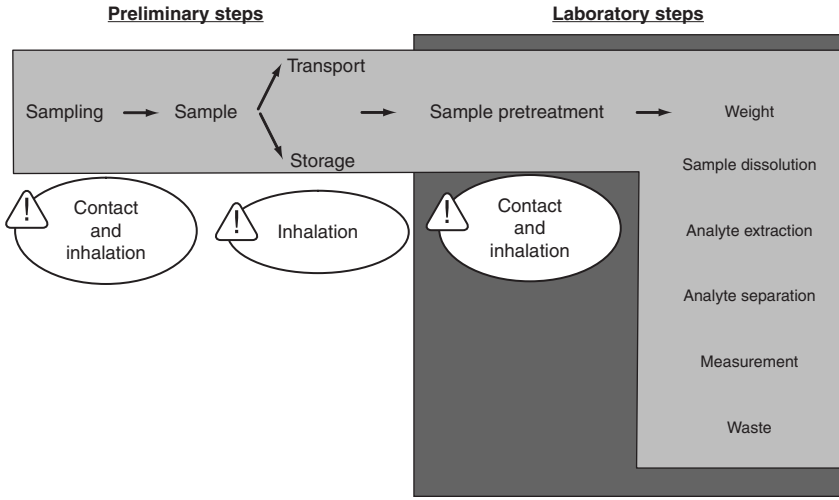

Figure 3.2 Exposition risks associated to the different steps of an analytical procedure.

equilibrium with any solid or liquid compound and transition from solids or liquids to gaseous molecules depend on the boiling or sublimation temperatures and additionally on the vapor pressure and thus, at relatively low temperatures, operators could be in contact with toxic vapors, especially when dealing with volatile compounds and easily volatile elements. Protection masks must be used when the presence of volatile or semivolatile compounds, both in the field and during transport operations, is suspected. Moreover, volatile solvents, reagents, and samples should be manipulated in fume hoods, preferably equipped with filters to avoid or reduce the emission of toxic substances to the environment.

On the other hand, waste handling and waste disposal are not at all free of risks because these operations involve the contact with mixtures of samples, analytes, and reagents and in addition to specific risks, the possibilities of synergistic effects must be considered also. In this regard, good laboratory practice (GLP) as applied to nonclinical laboratories is conducted for the assessment of the safety of chemicals to man, animals, and the environment. The accepted definition of GLP embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported, and archived. These studies are undertaken to generate data by which the hazards and risks to users, consumers, and third parties, including the environment, can be assessed for pharmaceuticals in preclinical studies, agrochemicals, cosmetics, food,

and feed additives and contaminants, novel foods, biocides, detergents, etc. GLP helps assure regulatory authorities that the data submitted are a true reflection of the results obtained during the study and can therefore be relied upon when making risk/safety assessments.

In short, in order to make analytical methods greener it is strongly recommended to automate as many as possible steps to avoid the contact of operators with samples, reagents, and solvents, and working in closed systems to avoid emissions to the environment. As will be discussed in detail in Chapter 4, remote sensing or fiber optics-based methodologies followed by direct analysis of samples inside vials or blisters are the preferable methodologies compared to those methods which involve sample damage and others requiring chemical treatments in order to avoid risks to operators and the environment, through the elimination of dangerous sample treatment steps.

3.3. EVALUATION OF ENERGY CONSUMPTION

It has been mentioned in Chapter 2 that energy requirements and consumption of the analytical methods must be reduced to avoid the side effects of the general energy demand. Thus, for a correct evaluation of green alternatives, reagents, and solvents reduction or replacement must be done but also the energetic aspects must be considered to provide a complete picture of the environmental side effects. Energy consumption depends on the voltage and required maximum power but also on the consumption, in watts, of the apparatus and instruments for the chemical measurements and to guarantee the best experimental conditions.

In fact, on considering the different steps of a method, it is clear that sample storage and preservation involve long and continuous energy consumption. Refrigerators and freezers are continuously switched on and, thus, once again, it can be demonstrated that on-field methods of analysis reduce the energy consumption. Moreover, the reduction of the amount of sample needed for the analysis will indirectly reduce energy consumption by reducing the number of freezers or refrigerators per laboratory.

Sample treatment steps that involve high temperatures are also responsible for the energy consumed and there is interest in moving from high temperature treatments during long periods of time to short time treatments at a reduced temperature or power consumption and to use room temperature treatments. On considering sample digestion and analyte extraction, the tendency today is to move from high temperature, long

and high power consuming classical methods of dry ashing and wet ashing to microwave-assisted treatments, pressurized solvent extraction (PSE), and sonication [6,7]. So, it can be concluded that Analytical Chemistry is moving from hard to soft and this tendency reduces drastically the energy consumption of sample preparation apparatus and provides additional advantages on considering new opportunities for trace element speciation and a drastic reduction of matrix effects during the separation and measurements steps [8].

Regarding the power consumption of instruments, it is clear that this value is relatively low compared with that of heating apparatus. However, some atomic and ionic techniques have high power consumption and the tendency to miniaturization will also provide a reduction of energy consumption. In this regard, the development of energetically self-sufficient portable instruments for the direct analysis of untreated samples has a positive effect on greening the analytical methods and facilitating the direct measurement of the parameters of interest [9], the power requirements of the miniaturized and portable equipment being the key limitation to the in-field use of instrumentation (see Figure 3.3).

So, for greening an analytical method from the point of view of the energy requirements, we must move from high to low voltage in order to improve method miniaturization and portability and from high to low power to reduce consumption.

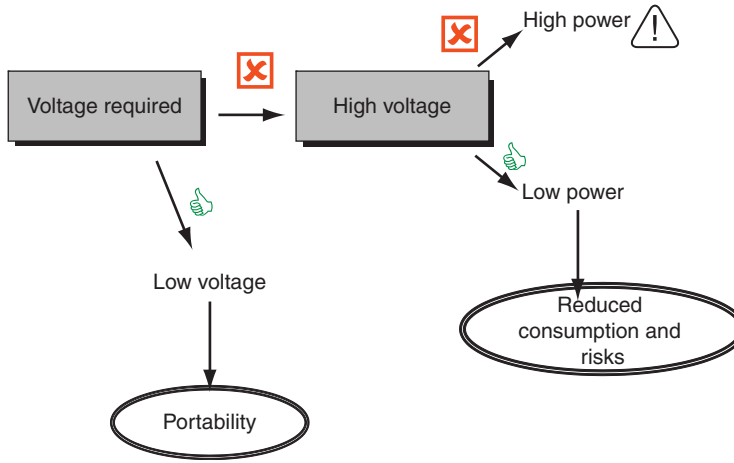

Figure 3.3 Evaluation of analytical methods as a function of the power supply required.

3.4. EVALUATION OF REAGENT CONSUMPTION AND WASTE GENERATION

Reduced consumption of solvents and reagents is one of the main purposes of Green Analytical Chemistry and thus one must first evaluate the amount of reagents involved in the different methods to be compared and in addition determine the amounts of samples, reagents, and solvents, also including cleaning solutions, required for an analytical procedure.

It is clear that sampling and sample treatment are the analytical steps which generate bigger amounts of wastes. Regarding the sampling step, the amount of sample should be enough to assure the representativeness of the data but in any case must involve the transport to the laboratory of high amounts of crude samples. In fact, nowadays, the development of methods which allow the reduction of the size of the original sample, permitting the analysis of microsamples and the achievement of specific information in the case of macrosamples, is rapidly increasing. In this regard, in-field methods provide important advantages, for instance in the case of environmental incidents. If on-site analytical capability is used, only the contaminated material would be removed, reducing substantially the costs of clean-up. On the other hand, from an environmental point of view, it must be considered that the remaining samples in the laboratory after method application are also an analytical waste which must be disposed of or processed to avoid landfill or water pollution and for this reason it is very convenient to downsize the scale of samples and to obtain as much as possible information in-field.

Sample treatment is probably the green limiting analytical step. In many cases, it is absolutely necessary the digestion, extraction, dissolution, or derivatization of the analytes from the sample by using solvents and reagents which are expensive materials with a negative side effect on the environment. Moreover, the increase of their required amount has no positive effects on the main analytical features of the method. Thus, the drastic reduction of reagents and solvents is very convenient not only from the environmental, but also from the economical view-point. Once again, the use of alternative sample treatments, such as microwave-assisted extraction, PSE, or sonication reduces the amount of solvent from hundreds of milliliter, used in Soxhlet or liquid–liquid extractions, to tens of milliliter in the modern methodologies.

Another question to be considered is the advantage provided by the use of sample treatments in closed systems which avoid the contact of

operators with the sample and reagents, thus minimizing the risks of accidents and also downsize the level of required amounts of solvents and acids also enhancing the time required for sample treatment. On the other hand, mechanized or automated approaches offer safe and green alternatives. These methods reduce the reagent consumption by injection of small amounts in a flow stream and also, due to the fact that unmixed reagents can be stored for relatively long periods of time, avoid the disposal of unused reagents which creates additional wastes. On employing traditional batch systems, the washing and cleaning of all glass and plastic materials and that of measurement cells creates additional low contaminated wastes, which increase the total amount of laboratory wastes thus increasing the cost per analysis and the side effects of the methods, making them unsustainable practices. With respect to the aforementioned problems, the systematic use of flow approaches [9–11] avoids the use of an excess of reagent mixtures and provides a self-cleaning of the experimental setups with a reduced volume of the carrier solutions, thus providing the best way for a drastic reduction of laboratory wastes. In recent years, a series of works have been published in which parameters like laboratory productivity and time of analysis have been completed with data about reagents consumed per 100 analyses or waste volumes generated per session [10,11] and these approaches clearly identify automation as one of the ways for highlighting the green approaches with respect to unsustainable ones (see Figure 3.4).

3.5. COMPATIBILITY OF GREEN CHEMISTRY PRINCIPLES AND THE MAIN ANALYTICAL FIGURES OF MERIT

Greening a method implies an assessment of the safety of both the environment and the operators, and the milestones of green methods concern the replacement of toxic and hazardous reagents, the reduction of operator risks, energy consumption, reagent consumption, and waste generation. However, these aspects, which in some cases must be balanced to obtain the best compromise between them, cannot be overestimated with respect to the classical analytical features like accuracy, representativeness, traceability, precision, selectivity, and sensitivity (see Figure 3.5). In fact, accuracy and selectivity could be affected by the replacement of toxic reagents, miniaturization can affect the representativeness of samples and the method traceability and automation reduces the attainable sensitivity in many cases. So, attention must be paid to these deleterious effects and we need to

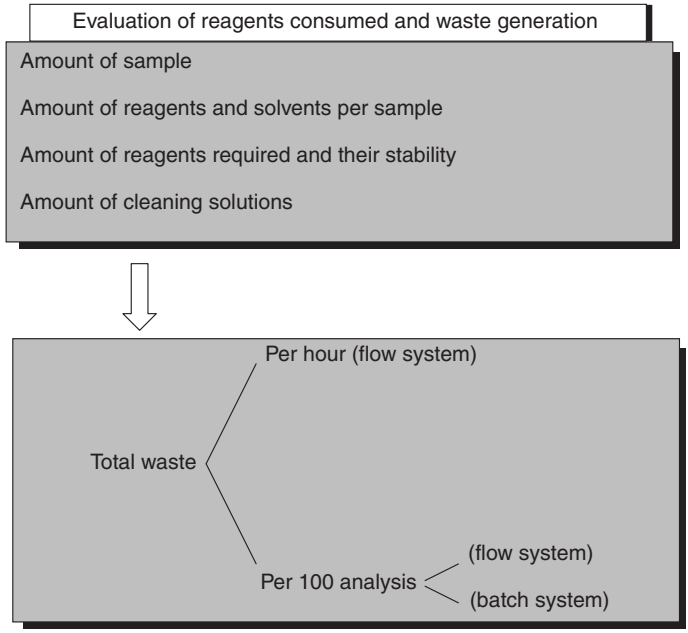

Figure 3.4 Criteria employed to evaluate reagents consumed and waste generation.

evaluate whether the precision and selectivity enhancement provided by automation of a method largely compensates for the lack of sensitivity and that the miniaturization continues to provide representative data.

The main disadvantage presented by the on-site methodologies relates to the reliability of measurements. Normally, in-field methods do not have the ability to build in checks such as the simultaneous analysis of standards or there are no easy means to run a calibration plot. So, reliability of in-field measurements should be tested or improved by building procedures that also include running standards between samples or other means of calibration.

Regarding the replacement of reagents, the selectivity and accuracy of the method must be assured before it is carried out. If these main properties cannot be guaranteed on using alternative nontoxic reagents, the replacement is not acceptable and additional efforts must be made to use as small as possible quantities of hazardous reagents and to avoid their associated risks. However, on concerning the use of alternative sample treatments, it is well demonstrated that green alternatives should provide similar extraction recoveries to the classical extraction procedures, when the accuracy of the methodology is not modified.

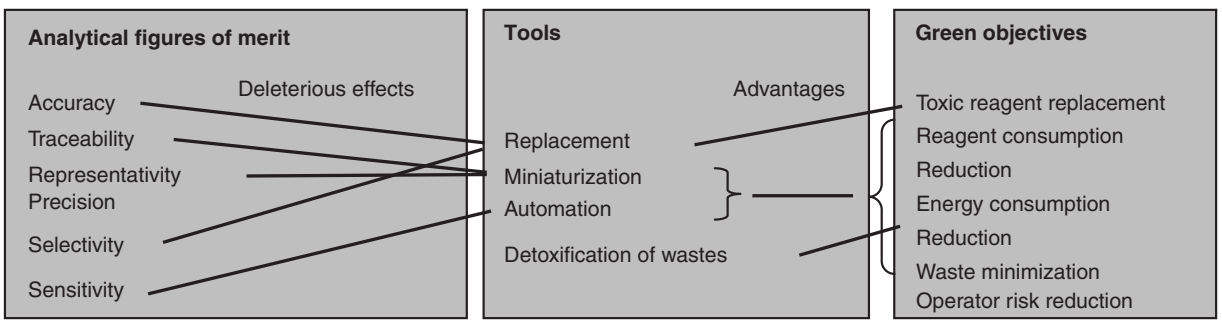

Figure 3.5 Compatibility between the main parameters of the method and green characteristics paying attention to green advantages and probable deleterious effects on the main method characteristics.

Miniaturization of analytical systems has been one of the most important issues in recent analytical chemistry. It realizes high performance, rapid analysis with low-cost operation and environmental compatibility, reducing sample, energy, waste, and reagents consumed, but it could affect the representativeness of data. The increase and development of new microfluidic systems for accurate fluid handling and control allows automating sample analysis in portable multianalyte sensors and improves the representativeness of data. However, it is clear that green alternatives that are unsuitable for solving the problems with as much as possible guarantees of accuracy and representativeness do not make sense and should not be adopted.

On the other hand, automation provides the easiest way for greening a method and improves the economy of consumption. Additionally automation increases the precision and selectivity in many cases, and enhances the traceability by avoiding manual handling. Only sensitivity might deteriorate. However, on increasing the sample size until reaching the steady-state of transient signals, the signal reduction could be avoided and thus we can preserve the sensitivity of the method.

In short, we can conclude that the green aspects of analytical methods cannot be evaluated separately from the main analytical features. On the contrary, the greening tasks must be compatible with the preservation of the best possible accuracy, selectivity, and sensitivity while increasing the comfort of operators, the speed of analysis and reducing costs and deleterious side effects. So, greening a method is our own responsibility as analytical chemists and we must proceed with the final objective of any analytical method in mind—to solve the problems of the customers by maximizing the information and minimizing costs and risks. Any greening activity which could damage the quality of the analytical information must be considered as the wrong way to go.

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CHAPTER 4

Avoiding Sample Treatments

Contents

As mentioned in the previous chapters of this book, sample treatment is one of the most energy consuming and contaminating analytical steps, due to the large amounts of organic solvents or inorganic acids or bases needed to dissolve, extract, or digest samples under study and the thermal sample treatment required in many cases. It is clear that one of the most direct ways for greening analytical methods would be to avoid the sampling and sample treatment steps, thus avoiding at the same time the use of large amounts of acids and organic solvents and strongly reducing waste generation, reagent and energy consumption, and risks to the operators, thereby contributing to increased laboratory productivity. These ideas were discussed in depth in 2007 by He et al. in a review paper entitled

Comprehensive Analytical Chemistry, Volume 57
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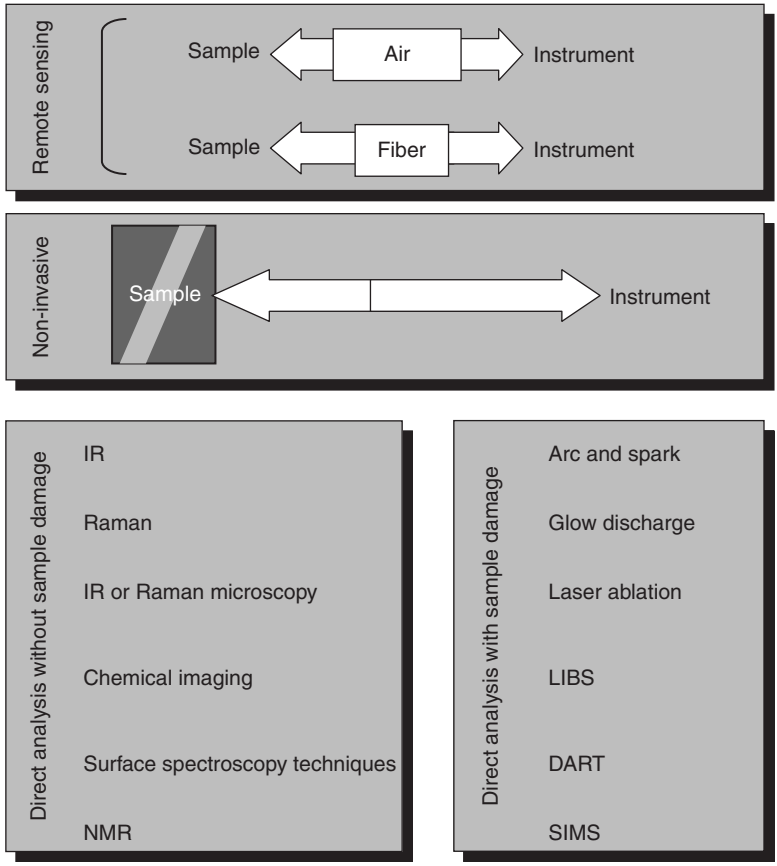

Figure 4.1 Strategies to avoid sample treatment.

"Spectroscopy: the best way toward Green Analytical Chemistry?" [1]. In this chapter, the strategies to avoid sample treatment—from remote sensing and noninvasive determinations to direct analysis, without and with sample damage—will be reviewed (see Figure 4.1) paying special attention to their contribution to greening the analytical methodology.

4.1. REMOTE SENSING

Remote sensing provides analytical information about the sample composition without the need for sample pretreatment nor to take the sample and transport it to the laboratory. It is possible to distinguish different remote sensing techniques as: (i) direct remote sensing in which both the source

and signal are used along an open atmospheric path and (ii) indirect remote sensing, in which the source or the signal is brought, through fiber optics or other means, in contact with or near to the target sample.

4.1.1 Open-path sensors

Open-path available instrumentation covers the range from Fourier transform Infrared (FTIR) spectroscopy and Raman spectroscopy to ultraviolet differential optical absorption spectrometry (UV-DOAS), tunable diode lasers (TDLs), and laser induced breakdown spectrometry (LIBS). Table 4.1 provides a summary of the available instrumentation characteristics, indicating the analytes suitable to be determined by remote sensing, the typical limits of detection domain in which these techniques can be employed, some information about the typical interferents, the effective pathlength, their advantages, and limitations. As can be seen, these instruments provide valuable tools for the determination of organic and inorganic compounds, low molecular weight gases, and trace elemental composition at ppm and ppb levels.

Open-path FTIR spectrometers are able to transport the IR radiation along an open beam path (up to 1 km) to simultaneously identify and measure a wide range of contaminants directly in the atmosphere [2]. There are different ways by which open-path FTIR spectra can be measured (see Figure 4.2).

In bistatic measurements, the source is located in a different place to the detector, the pathlength being the distance between them. On the other hand, for monostatic measurements all components that require a power supply are mounted at the same location. A monostatic open-path FTIR spectrometer uses a remotely placed retroreflector to reflect an infrared beam across an open distance to the detector. As the open-path FTIR spectrometer scans the area, it measures the amount of light absorbed by air contaminants that are present in a volume of air. However, because atmospheric water vapor and CO_2 cannot be removed from the spectra, absorption of those molecules severely limits the available spectral range. In this regard, different alternatives have been studied to minimize the effect of water vapor and carbon dioxide on the spectra such as synthetic, equidistant, and short path backgrounds [3].

Like FTIR, open-path Raman spectroscopy is capable of measuring the presence of many chemicals, achieving low detection limits. However, the very weak signals measured can affect its resolution capabilities. The expected relative humidity of a site also can be an aspect to be considered

Table 4.1 Summary of open-path instrumentation characteristics

Technology	Analytes	LOD	Interferents	Effective Pathlength	Advantages	Limitations
Open-path FTIR	Numerous organic and inorganic compounds, and acids	Low ppb	CO_2, H_2O	1 km—bistatic; 500 m—monostatic	Quantitative measurement of multiple chemicals at one time Large linear range	For best LOD requires cryogenic cooling Only molecular species can be detected
Raman	Numerous organic and inorganic compounds	ppm	Depend on the target chemical	100 m; 10 km fixed LIDAR equipment	Quantitative measurement of multiple chemicals at one time	High LOD
UV-DOAS	SO_x, NO_x, NH_3, HF, O_3, BTEX, and aldehydes	Low ppb	O_2 with BTEX	10 or more km	Continuous monitoring Relatively low detection limits	Limited number of chemical species detected
TDLs	Low molecular-weight gases	ppb-ppm	Depend on the target chemical	1 km	Light and easily deployed	Measures one chemical compound per tuning May require cryogenic cooling for best detection limits
LIBS	Numerous organic and inorganic compounds	Low ppb	–	10 m–10 km	Quantitative analysis of trace elements can be easily done	Very long distances limits sensitivity

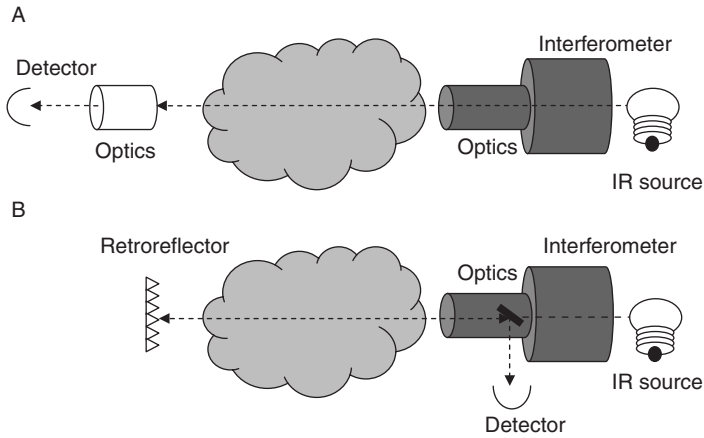

Figure 4.2 Scheme of open-path FTIR systems for bystatic (A) and monostatic (B) measurements.

when choosing between Raman and FTIR since Raman spectra are insensitive to water vapor while it is a strong interferent in FTIR. However, Raman scattering produces a very weak signal and therefore the source must be very intense and monochromatic. Several types of lasers have been successfully applied and the best laser depends on the application and wavelength requirements for the target compounds. The shorter the wavelength, the most intense the scattering; therefore, blue and ultraviolet light produce the strongest Raman scattering in spite of the fact that fluorescence could be the main problem on using high energy wavelength lasers [4].

DOAS uses the unique absorption of specific electromagnetic energy wavelengths by chemicals in the UV, visible and near IR spectrum to identify and quantify individual chemicals. The DOAS technique relies on the measurement of absorption spectra, it being possible to separate the absorption structures of several atmospheric species from each other as well as from the extinction due to scattering on molecules and aerosols. This technology has found its most widespread use in detecting inorganic gases and vapors (SO_x, NO_x, NH_3, H_3S, HF), monoaromatics (BTEX), and aldehydes (HCHO) [5].

In open-path spectroscopy, TDLs are designed to focus on single absorption wavelengths specific to a compound of concern in the gaseous form. They are capable of achieving low detection limits and are virtually interferent-free. Open-path TDLs are used in atmospheric pollutant studies, process line/tank leak detection, industrial gas-purity applications, and monitoring and control of combustion processes [6].

Commercially available diodes are semiconductors, fabricated from exact combinations of ultra pure materials. The basic materials of construction of these diodes include gallium, indium, arsenic, antimony, phosphorus, aluminum, lead, tin, selenium, tellurium, and sulfur. Generally, TDLs used for measurement in the near-infrared (NIR) can be thermoelectrically cooled or operated at room temperature and pressure. There are some diodes of this class [7] that can produce frequencies in the near mid-infrared (MIR; 3–5 μm) and therefore can access a small number of fundamental bands, which greatly improves their detection limits for those chemicals having absorption bands in this range.

In the open-path LIBS configuration, the laser beam and the returning plasma light are transmitted through a transparent atmosphere. This approach allows analysis of physically inaccessible targets, which may be located in hazardous environments where optical access is possible through a transparent atmosphere.

Open-path LIBS analysis requires generating analytically useful plasma at distance and collecting a sufficient amount of light, thus imposing constraints on the specifications of the laser, the optical system used for laser focusing and plasma light collection as well as on the spectrograph and detector. Two different methods of stand-off LIBS measurements have been developed. The first method uses nanosecond laser pulses with the required energy dependent on the target distance and the characteristics of the optical system to produce power densities sufficiently high to form the plasma. It has been used for the analysis of solid samples at a distance range of tens of meters or for liquid samples at a few meters. The second open-path LIBS method employs femtosecond laser pulses either in conventional LIBS or by using the self-guided filaments induced in the transparent medium that propagates over long distances and produce excitation of the sample. This method can extend the analysis to very long distances in the kilometer range.

4.1.2 Fiber devices

A fiber is a cylindrical waveguide that transmits light along its axis, by the process of total internal reflection. The transmission of light through an ordinary glass fiber (optical fiber) is limited by the spectral transmission of glass. It is not transparent in the UV region, at wavelengths below 0.3 μm, and in the infrared region above 2.5 μm. Various materials are needed to produce fibers which can be used for the transmission of UV or IR wavelengths. Some of them are: sapphire, fused silica (quartz), diamond, SiO_2

glass, potassium chloride, fluoride glass, silver halide, zinc selenide, or chalcogenide glasses. Another problem for transmitting all radiation wavelengths appears when extremely high power needs to be delivered. The maximum energy transmitted through the fiber is determined by the air breakdown and by the damage threshold of the fiber front surface and the bulk. A possible solution could be the use of hollow waveguides fibers having their core formed by air.

Fiber devices can be classified into two categories: intrinsic devices, in which the interaction of the light with the analyte occurs within an element of the fiber; extrinsic devices are those where the fiber is only used to couple light. The last type of waveguides are particularly useful in acquiring IR or UV spectra when samples are situated in a remote location or when the unusual size or shape of samples prevents them from fitting well in a standard sample compartment. Many analyses in hazardous or process environments use these devices [8].

Fiber devices can be used with Raman spectroscopy in a similar way to IR or UV spectroscopy, where the fiber probe is coupled to the laser and to the monochromator of the Raman instrument. Fiber-optic cables up to approximately 100 m connect each measurement point to the instrument. The cables typically contain four single fibers; a primary pair to carry laser light to the sample and another to return the Raman signal to the spectrometer, and a spare pair. In such studies, the probe head is linked by the fibers to the portable micro-Raman apparatus [9] to facilitate measurements of spectra of large objects under a conventional Raman microscope. This possibility of easy separation of the probe head from the main part of the spectroscope is much easier for devices utilizing visible radiation, such as Raman spectroscopes with VIS excitation sources, since waveguides for other parts of the electromagnetic spectrum are not so simple and flexible.

A liquid-core waveguide (LCW) can be made from tubing in which the wall material has a lower refractive index than the liquid-core material. The major advantage of using an optical waveguide for Raman spectroscopy is that the signal is significantly enhanced by the integration of Raman scattered light along the length of the waveguide. On the basis of the observed signal intensity and signal-to-noise enhancement, the waveguide should be well suited for use in biological, environmental, or other chemical analysis applications with attributes such as small scattering cross-section, sensitivity to laser power, and utility for analysis of low analyte concentration.

Remote LIBS can indeed be implemented by transporting the laser pulses to the target through a fiber optic and by collecting the plasma light and transporting it back to the detection system using either the same optical fiber or a second fiber optic cable. Due to the breakdown threshold of the optical fiber material, optical fibers were initially used in LIBS only to deliver the plasma emission to the detection system [10]. The use of fiber-optics was then proposed also for laser beam delivery, in a configuration suitable for operating in hostile environments, allowing the separation of the focusing and collecting head from the most delicate parts of the instrumentation, such as the spectrometer and detector. Moreover, this configuration also offers an obvious advantage in terms of safety with respect to possible eye damage. This method, which has been demonstrated over distances up to 100 m, requires that the optical fiber be positioned adjacent to the sample. Therefore, the use of fiber optics is of restricted application and even impossible in cases where contaminated or aggressive chemical or temperature environments may affect the probe, or when large areas must be analyzed.

4.2. NONINVASIVE MEASUREMENTS ON BLISTERS, BOTTLES, OR VIALS

The noninvasive approach is only possible with optical techniques if the vessel is made of a transparent material or has a suitable window. It is possible to measure packaged samples through the packaging material. By using Raman spectroscopy it is possible to obtain the Raman spectra of a sample inside the blister package, since many blister materials provide a suitable spectral window for both excitation and scattering radiations. Clear glass or plastic bottles, bags, blister packs, or ampoules are suitable package forms. Thus, tablet components can be often analyzed through the packaging [11]. Moreover, liquid samples can be analyzed inside glass or plastic bottles. For instance, the concentration of an active pharmaceutical ingredient (povidone) in a commercial eyewash solution has been measured directly through a plastic (low-density polyethylene: LDPE) container using Raman spectroscopy [12].

The fact that samples can be measured directly in the sample bottle or packaging, without sample preparation makes FT-Raman spectroscopy an ideal technique for quality control methods.

Furthermore, the method termed spatially offset Raman spectroscopy (SORS) provides a new capability to analyze diffusely scattering media

such as pharmaceutical drugs through their blister packs with much higher clarity than conventional Raman approaches. The SORS approach is based on the collection of Raman spectra from spatially offset regions away from the point of illumination on the sample surface and subsequent scaled subtraction of the spectra (or multivariate data analysis) to separate the signals of individual layers within the interrogated sample [13]. In this regard, SORS has been successfully applied in the identification of counterfeit pharmaceutical tablets and capsules through different types of packaging [14].

On the other hand, finished pharmaceutical formulations can be also identified using NIR spectroscopy directly from the package [15]. It is possible to perform an identity check for 100% of the pharmaceutical products on the packaging line at full line speed, avoiding mix-ups. The possibility to measure through blisters is very useful to identify clinical trial samples [16]. Moreover, the composition of pharmaceutical oral liquids in polyethylene terephthalate (PET) bottles had been directly measured using NIR transmission spectroscopy [17].

The suitability of noninvasive NIR and Raman spectrometries for determination of the percentage of ethanol in whisky, vodka, and sugary alcoholic drinks, 200 mL (flat) and 700 mL (round) glass bottles has been investigated [18].

X-ray powder diffraction (XRPD) is a key analytical technique in the pharmaceutical industry because it penetrates easily through commercial blister packs, enabling the analysis of tablet components, without the need to remove tablets from their packaging [19].

In short, noninvasive measurements provide green analytical alternatives that are free of risks to operators and do not require solvents, reagents, or sample handling thus providing fast, sustainable, and waste-free methodologies.

4.3. DIRECT ANALYSIS WITHOUT SAMPLE DAMAGE

The direct analysis of samples by using a nondestructive methodology provides several advantages in terms of Green Analytical Chemistry, such as the elimination of organic solvents to extract the analytes from the sample matrix or the use of inorganic acids to digest the sample prior to its analysis. Moreover, the nondestructive nature of these methodologies permits storage of the samples for further analysis, in this way reducing sample consumption as well as waste generation.

In the following subsections the main techniques that provide a direct analysis of samples will be discussed, from vibrational to surface spectroscopy and nuclear magnetic resonance (NMR).

4.3.1 Vibrational spectroscopy

Vibrational spectroscopy encompasses NIR, MIR, and Raman spectroscopy. Based on the continuous advances in instrumentation, these complementary techniques have now entered common use for the study of solid-state samples. They allow both qualitative and quantitative analysis and can also be deployed in-line. The NIR and MIR sample spectra, in the absorbance or reflectance mode, can be obtained in a wide spectral range and processed at each of the numerous characteristic wavelengths of the target analyte. NIR spectra arise from recording molecular overtone and combination vibrations. The MIR spectrum records the absorbance or reflectance of the incident radiation at the vibrational and rotational frequencies of the atomic bands within the molecule. Raman spectroscopy involves the scattering of a monochromatic source.

On the other hand, the main problem that is presented by vibrational spectroscopy-based techniques is their poor sensitivity, which normally reduces their usefulness to the analysis of major and minor sample components. They are not useful for trace analysis without a previous preconcentration step.

4.3.1.1 Infrared spectroscopy (IR)

IR is one of the most common spectroscopic techniques. It is based on the interaction of the sample with the IR beam, which provides a unique fingerprint of the different compounds present in the sample. Depending on the region of the electromagnetic spectrum, we have the following classifications: NIR ranging from 0.8 to 2.5 μm (12500–4000 cm^{-1}), MIR from 2.5 to 50 μm (4000–200 cm^{-1}) and far infrared (FIR) from 50 to 1000 μm (200–10 cm^{-1}).

Infrared spectroscopy is routinely used for the analysis of organic and inorganic samples in the gas, liquid, and solid state, thus offering a flexible tool in molecular analysis.

MIR spectroscopy

One of the main advantages that MIR offers is that it is possible to obtain the IR spectrum using different sampling techniques and commercially available accessories from samples in many different forms such as liquid or solutions, solid, and gas or vapors, practically without any sample pretreatment.

Liquid cells are used for diluted solutions of solid and liquid samples. Solutions are introduced by using a peristaltic pump or syringes in fixed pathlength cells containing two transparent material windows (NaCl, CaF_2, ZnSe, ...) separated by a lead or teflon spacer and held in a stainless steel frame. By changing the spacer, pathlengths from 0.010 to 1.0 mm can be obtained. In some cases, the measurement of IR spectra of water solutions can be made using spacers of 25 μm or less. The main problem when reducing the pathlength is the associated reduction of the sensitivity and also cell blockage. Traditionally, solvents such as carbon tetrachloride, tetrachloroethylene, methylene chloride, chloroform, and carbon disulfide are preferred to dissolve or dilute the samples. However, all of them are quite toxic, and thus there is great interest in replacing the aforementioned solvents by greener ones.

Another approach to obtain infrared spectra on aqueous solutions is to use attenuated total reflectance (ATR) instead of transmission cells. ATR accessories are useful for obtaining IR spectra of thick or highly absorbing solid and liquid materials, including films, coatings, powders, threads, adhesives, polymers, and aqueous samples. ATR requires little or no sample preparation and is one of the most versatile sampling techniques.

Gas cells can be used to examine gases or vapors obtained from low-boiling molecules. These cells consist of a glass or metal body, two IR-transparent end windows, and valves for filling gas from external sources. They provide vacuum-tight light paths from a few centimeters to 120 m. Longer pathlengths are obtained by multiple reflections of the beam through the sample, using internal mirrors located at the ends of the cell.

The diffuse reflectance technique is mainly used for acquiring IR spectra of solid samples, for example, powders and rough surface solids such as coal, paper, and textiles. IR radiation is focused onto the surface of the solid in a cup and results in two types of reflections: specular reflectance, which directly reflects off the surface and has equal angles of incidence and reflectance, and diffuse reflectance, which penetrates inside the sample and then scatters in all directions. Special reflection accessories are designed to collect and refocus the resulting diffusely scattered light by large ellipsoidal mirrors, while minimizing or eliminating the specular reflectance, which complicates and distorts the IR spectra.

Figure 4.3 shows the scheme of the measurement process in IR through transmission, ATR and diffuse reflectance.

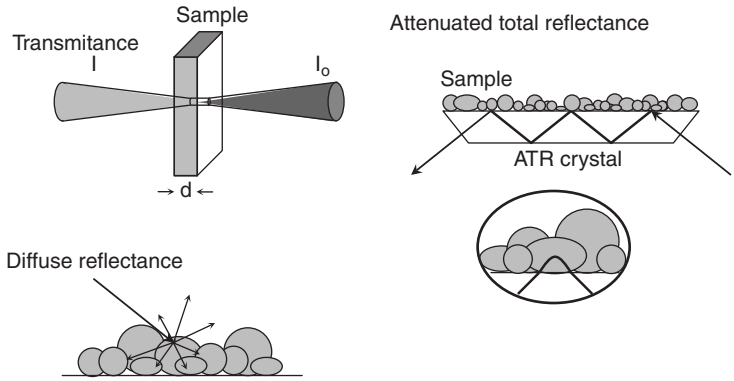

Figure 4.3 Scheme of the measurement process by transmittance, attenuated total reflectance, and diffuse reflectance.

NIR spectroscopy

NIR spectra are due to molecular vibrations (overtones and combinations of fundamental IR vibrations). In the last two decades, NIR spectroscopy was found to offer many possibilities for industrial analytical applications because it is a very fast technique and permits us to obtain chemical and physical information about samples. Another interesting advantage of this technique is its nondestructive character and the minimal or nonexistent sample preparation, avoiding dissolution, extraction, or similar sample treatment steps, which imply organic solvents use. However, NIR also present some drawbacks such as its high limit of detection, which means that the technique is not suitable for trace analysis, and the reduced number of bands present in the main part of sample spectra. Another drawback of the technique is the complexity of the spectra. In a complex matrix, the resulting NIR spectrum is the combination of a broad range of overlapping absorption bands, which makes it difficult to obtain relevant specific information about the target analytes without a chemometric treatment. With the recent development of chemometrics a wide range of applications of NIR in different scientific and industrial areas has opened up.

4.3.1.2 Raman spectroscopy

When monochromatic radiation is focused on a sample, the scattered radiation comprises radiation with the same frequency as the incident radiation (Rayleigh radiation) and radiation of different frequencies (Raman radiation). Analogously to spectra obtained with other vibrational

techniques, a Raman spectrum can be treated as a molecular fingerprint, providing specific information on the analytes under study. In contrast to MIR, Raman scattering of water is quite weak and Raman spectra of water solutions can be easily obtained.

The amount of sample necessary to obtain a Raman spectrum is really small. This is because the scattering volume is limited by the diameter of the laser beam at the focal point, this diameter being lower than 1 mm.

Some applications of Raman spectroscopy in the analysis of intact samples are found in the pharmaceutical and forensic areas [20]. Raman analysis provides a rapid, green and accurate way for the identification and quantification of cocaine, heroin, ecstasy, and various other phenethylamine analogues. Bell et al. have demonstrated that from the Raman composition profile of ecstasy tablets it may be possible to distinguish the tablets prepared by various major manufacturers, and hence, to track its illegal distribution [21].

Another important field for Raman spectroscopy is cultural heritage analysis, where each art object is unique, and sampling is generally forbidden. Due to the intrinsic characteristics of Raman spectroscopy, it has become an important tool in the surface analysis of art works (especially the identification of pigments used). Raman spectroscopy provides information about the technique implemented in their construction, the authentication of samples and possible restoration undertaken in earlier times, as well as helping to designate appropriate procedures for future restoration and conservation [22].

In the last few years many reports have been published dealing with Raman spectroscopy analysis of pigments in ancient manuscripts [9], paintings [23] including prehistoric rock paintings [24] and ancient glass [25].

4.3.1.3 Infrared and Raman microscopy

To obtain the infrared spectra of microsamples a beam condenser can be mounted in the sample compartment. In this device, the diameter of the beam is reduced by a factor of X, reducing the sampled area by a factor of X^2. In this case, a microsample of 1 mm diameter can be effectively measured with minimal loss in optical efficiency.

However, to examine small regions of larger samples, beam condensers are less beneficial. In these cases, the infrared microscope is the option of choice where a remote aperture is used instead. Infrared microspectroscopy has become a popular technique for different analyses; such as trace contaminants in semiconductor processing, multilayer laminates, surface defects, and forensic samples with a reduced or no sample pretreatment.

Microspectroscopy systems consist of four main parts [26]: (i) the light source, (ii) the splitters and filters, (iii) the detector, and (iv) the optics.

The light source is a simple polychromatic thermal source generally used for MIR and NIR spectroscopy and a laser in the case of Raman spectroscopy [27].

Different types of splitters can be found in those systems: Fourier transform spectrometers, which provide rapid acquisition, high spectral resolution, high energy available to the detector and high wavelength repeatability. Filters are useful for focusing on specific wavelengths. An alternative is a tunable filter [28], which electronically controls spectral transmission by applying a voltage. Filters are mainly used in NIR and Raman hyperspectral imaging. The diffraction grating system has a large number of parallel lines or slits that disperse the light in several directions depending on the angle of diffraction and the wavelength.

Photon detectors are the most widely used in MIR and NIR spectroscopy to record the signal after wavelength separation being 2D charge coupled detectors (CCDs) the most commonly used Raman detectors [29,30].

The microscope is fitted with optical elements for selecting spatial resolution. Typically 6×, 15×, and 32× objectives are used on a MIR or NIR microscope [31], and 50× and 100× objectives on a Raman microscope.

4.3.1.4 Chemical imaging (CI)

In 1988, Harthcock and Atkin [32] obtained the first chemical map in the MIR range using a microscope and moving stage. By revealing information that is both spectral and spatial, the technique can identify and localize compounds. Since then, subsequent refinements have been exponential. Development of the first microscope-mounted focal plan array (FPA) detectors increased enthusiasm for CI [33]. Fast and robust acquisition is now possible in the NIR and MIR ranges and also with Raman spectroscopy, almost all chemical compounds in a sample being visualized within minutes. Their applications have increased in various fields, from waste sorting [34] to biological tissue [35], food quality [36], and pharmaceuticals [37].

CI has also raised new data-processing challenges. A single acquisition may record thousands of images across numerous wavelengths and the resulting image stack forms a three-dimensional (3D) matrix, or data cube, spanning two spatial dimensions with a series of wavelengths making up

the third (spectral) axis. The data cube may be viewed as spatially located spectra, with the processing tools of classical spectroscopy being applied to single spectra; and secondly, the data may be viewed as images, with image-processing tools being used to extract high-quality spatial information. CI thus combines the techniques of spectroscopy and signal and image processing, making it a truly multidisciplinary discipline.

4.3.2 Surface spectroscopy techniques

The most commonly used surface spectroscopy techniques for analyzing the composition and chemistry of solid sample surfaces are X-ray photoelectron spectroscopy (XPS), Auger electron spectroscopy (AES) and ion scattering spectroscopy (ISS). All of these techniques involve bombarding the sample surface with a particle probe (electron, photon, or ion) and analyzing the energy of an outgoing particle. In XPS, the probe is an X-ray photon and the detected particle is the photoelectron emitted by it. In AES, the probe is an electron and the detected particle is a lower-energy electron. In ISS, both types of particles are ions. Sample preparation in those techniques implies just surface cleaning or *in situ* surface creation.

X-ray fluorescence (XRF) spectrometry is a technique based on the detection of emitted X-ray radiation from excited atoms. The fluorescent emitted photon is characteristic of the element and can thus be identified by measuring the energy of the emitted photon. Moreover, the intensity of the emitted photon determines the concentration of the element in the sample, providing qualitative but also quantitative information. Although XRF is a mature technique, new developments in instrumentation, including synchrotron XRF, total reflection XRF and X-ray microfluorescence have improved the capabilities of the technique within analytical chemistry [38].

Depending on the analytical requirements, different sample preparation approaches result in different analytical information. Liquid samples can be analyzed directly without sample pretreatment and solid samples can also be analyzed directly, without any treatment and in a nondestructive way.

Table 4.2 summarize the main characteristics of the surface spectroscopy techniques discussed above, including the elements determined, depth, width, probing and detected radiations, advantages, and drawbacks and Figure 4.4 shows the interaction process between the incident radiation and the samples, also including the process involved in secondary-ion mass spectroscopy (SIMS), which could also be considered as a surface technique but as it involves sample damage it will be considered in Section 4.4.

Table 4.2 Main characteristics of surface spectroscopic techniques

Technique	AES	XPS	ISS	SIMS[a]
Elements detected	Li–U	Li–U	Li–U	H–U
Depth (nm)	0.5–10	0.5–10	One monolayer	0.5–500
Width	50 nm–30 μm	10 μm–1 mm	1 mm	1 μm–1 mm
Probing radiation	1- to 10-keV electrons	X-rays	He^+ ion	0.5- to 10-keV ions (Ar^+, O^+, etc.)
Detected radiation	1- to 2000-eV electrons	1- to 1500-eV electrons	He^+ ion	Secondary ions
Advantages	Fast, semiquantitative	Minimal damage, sensitive to chemical states, quantitative	Exclusively top monolayer	H–He detection, very high sensitivity
Drawbacks	Very limited chemical information	Depth profiling slow	Charging effects and contamination extremely critical	Quantification unreliable, destructive

[a]Secondary-ion mass spectroscopy.

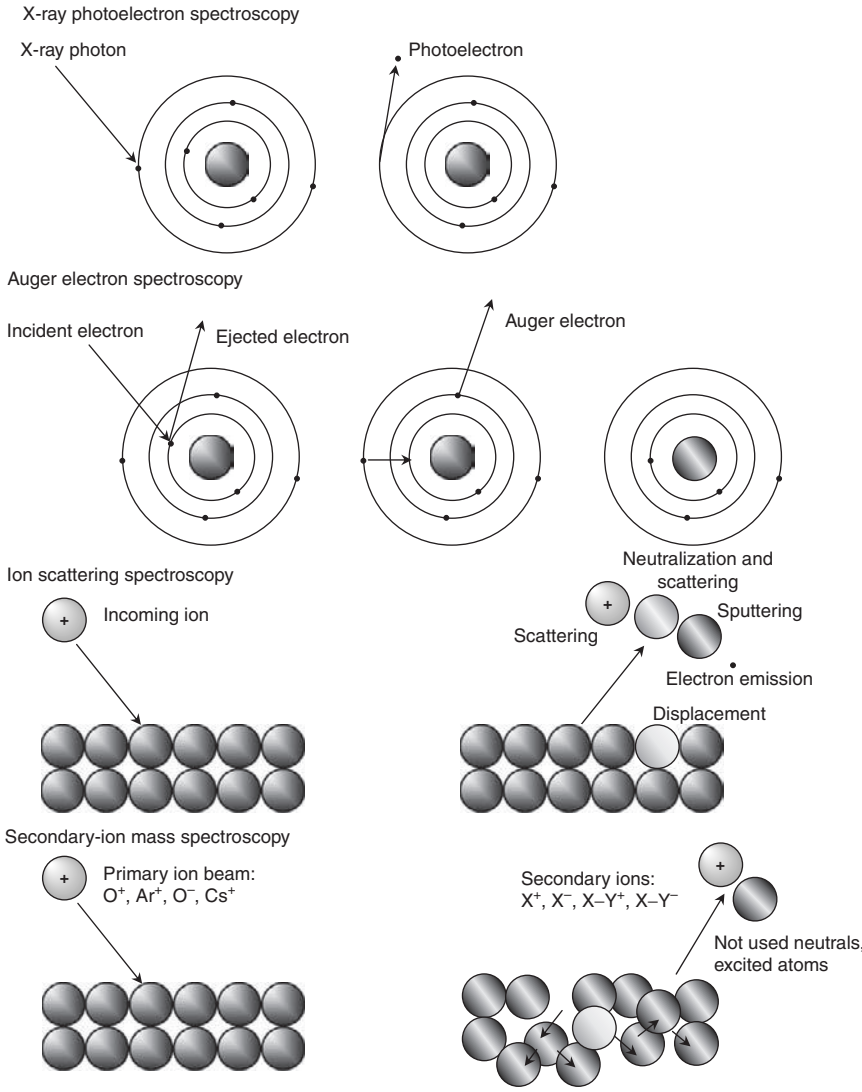

Figure 4.4 The interaction process between incident radiation and simple surface in techniques involving no simple damage and SIMS.

4.3.3 Nuclear magnetic resonance

NMR spectroscopy provides information about atomic environments based on the different resonance frequencies exhibited by nuclei in a strong magnetic field. Many different nuclei are observable by NMR but those of hydrogen and carbon atoms are the most frequently studied.

NMR spectroscopy of solutions is commonly used for structure elucidation. However, solid-state NMR measurements are extremely useful for characterizing the crystal forms of solid samples without requiring any sample treatment.

Nuclei that are typically analyzed with this technique include those of ^{13}C, ^{31}P, ^{15}N, ^{25}Mg, and ^{23}Na. Different crystal structures of a compound can result in perturbation of the chemical environment of each nucleus, resulting in a unique spectrum for each form. Once resonances have been assigned to specific atoms of the molecule, information on the nature of the polymorphic variations can be obtained. Long data acquisition times are common with solid-state NMR. So, it is often not considered for routine analysis of samples. However, it is usually a sensitive technique and sample preparation is minimal. NMR spectroscopy can be used either qualitatively or quantitatively, and can provide structural data, such as the identity of solvents bound in a crystal.

Solid-state NMR spectroscopy has become a popular method of analyzing pharmaceutical dosage forms [39,40]. The technique offers little interference from many excipients but the data acquisition can be complicated and lengthy. Quantitation can also be performed with NMR spectroscopy [41,42]. A number of parameters need to be determined for each system in order that signal intensities are representative of the number of nuclei being measured.

4.4. DIRECT METHODS WITH SAMPLE DAMAGE

In some cases, it is possible to do a direct analysis of samples without requiring any previous chemical treatment or dissolution. However, a part of the samples must be volatilized or decomposed in order to obtain a population of atoms or ions which can be measured or provide an associated radiation.

In this section, analytical methodologies providing direct measurements of untreated samples after electron, ions, laser, or electrical discharges will be considered.

4.4.1 Arc and spark ablation

By using an electrical discharge between an electrically conductive sample and a counter electrode, sample material can be ablated. Sample is used as the cathode of a direct current discharge and a high-melting metal, such as tungsten, can be used as the anode.

The sample volatilizes from a molten phase in the burning crater. When the burning voltages are high, as with discharges under reduced pressures or sparks, the particles impacting on the cathode have high energies and mechanically remove material from the sample, which depends much less on the boiling points of the individual components [43].

Arc ablation has long been proposed for producing an aerosol at the surface of electrically conducting samples and has been used in combination with various sources. The area, which is subjected to the discharge, is usually restricted to 6–8 mm and a flowing gas stream transports the aerosol particles to the detector. The analysis of electrically nonconductive samples is also possible, mixing it with and metal powder in a ratio of about 1:5.

The use of internal standardization may also be very helpful, both with respect to the analytical precision as well as for obtaining comparable data from different sessions. In many cases, the sample matrix element can be taken as the internal standard reference element. However, when an internal reference is added, as for powders and briquetting pellets, a reference element with thermo chemical behavior similar to the analytes should be selected [44].

The ablation rates are considerably enhanced by using the jet electrode, where the argon, used as carrier, is blown through the electrode, which has a narrow bore gas channel. It results not only in a very efficient transport of the ablated material away from the arc channel, but its particle size might even be favorably decreased, as a result of an immediate dilution of the atomic vapor produced, which makes analyte condensation into large droplets less likely.

In the case of arc ablation wandering effects may make the sampling irreproducible. To avoid these difficulties, the use of magnetic fields, as known from classical dc arc spectrometry, may be very helpful [45].

For the favorable use of sparks as ablation devices, it must be guaranteed that condensed sparks and not diffuse spark discharges are obtained. The particle diameter in the case of a medium voltage spark is of the order of a few millimeter, through which the particles can easily be volatilized in many sources, ranging from flames, through microwave discharges and inductively coupled plasmas. When a high-voltage spark is used the particle diameter may be considerably increased, which is due to an increase in the ablation of larger particles as a result of the highly energetic impacting gas and metal species.

The high temperatures obtained in spark sources anticipate that ion lines in particular will be excited, the norm temperatures of which are often beyond 10,000 K, whereas in arc sources atom lines will be predominant.

Spark chambers with special features for small samples, such as wires and chips, have also been developed. Here, the sample is cooled less efficiently and thermal volatilization has to be limited by using low-frequency sparks and an appropriate choice of the reference element. The gliding spark, which is formed through an high frequency discharge superimposing sparks along the surface of electrically nonconducting samples, can also ablate electrically nonconducting materials as plastics and even ceramics [46].

4.4.2 Glow discharge (GD) sources

GD sources that have been widely exploited in analytical chemistry for the direct analysis of solid samples permits the direct determination of traces, impurities, and depth profiling of solids. GD is a low-energy plasma sustained between two electrodes that are immersed within a reduced pressure, inert gas environment. For analytical applications, argon is most commonly used discharge support gas even though other gases are sometimes used. The plasma is created by inserting two electrodes in a cell filled with the discharge gas at a low pressure and is initiated when a high potential typically of the order of 1 kV is established between the two electrodes.

Glow discharge optical emission spectrometry (GDOES) is recognized as a rapid method for depth profiling, capable of surface analysis [47–49], interface and bulk qualitative, and quantitative analysis of solids [50]. Glow discharge mass spectrometry (GDMS) provides a way for the direct insertion in the mass spectrometer of vapors generated from the glow chambers and permits the direct determination of impurities and depth profiling of solids [51–53]. Glow discharge mass spectrometers, which are commercially available with fast and sensitive electrical ion detection, allow direct trace elemental determination in solid materials with good sensitivity and precision in the concentration range lower than ng g^{-1} [54].

4.4.3 Laser ablation sources for atomic and mass spectrometry

Laser desorption occurs when the analyte absorbs the incident laser radiation and is subsequently volatilized into the gas phase and ionized. It is partly a result of thermal evaporation through the local heating of the sample because of the surface impact of the highly energetic photons

from the laser. The main characteristics of this process are a localized heating at the surface, both in space and in time, and a fast process evolution. The evaporation occurs with a plasma formation, involving several successive processes such as: surface melting, vaporization, vapor ionization, surrounding and evaporating gas breakdown, free electron acceleration within the evaporated cloud, plasma heating.

Laser ablation is independent of the electrical conductivity of the sample and thus, has become increasingly important for solid analysis. Solid state lasers in the UV and IR ranges are of particular interest for laser ablation because almost all molecules absorb in those electromagnetic regions.

Laser ablation is a microsampling technique and thus enables microdistributional analyses to be made. Laterally resolved measurements can be taken with a resolution of around 10 μm [55]. Moreover, the sampling depth can be varied from 1 to around 10 μm by suitably adjusting the laser and the amounts of material sampled are of the order of about 0.1–10 μg. When operated in a scanning mode, laser ablation permits measurement of microspatial variations in sample composition along selected transect axes.

However, elemental fractionation and calibration in laser ablation remain as limiting factors in quantitative analyses of a wide variety of sample types. Elemental fractionation has been studied intensively and the sources of this problem remain a subject of controversy [56].

In many of the applications of laser ablation accurate quantification remains a challenge due to problems related to calibration. Several calibration strategies have been developed [57] that rely on calibration with solid standard samples. In general, the use of matrix-matched standards for calibration is advantageous, but this approach is limited by the lack of suitable materials. Furthermore, matrix-matched calibration usually also requires correction for signal drift, and an internal standard element has to be added to samples and standards. Internal standardization is also frequently used for nonmatrix-matched calibration if a standard of the same matrix is not available.

A powerful alternative calibration strategy in laser ablation inductively coupled plasma-mass spectrometry (ICP-MS) is isotope dilution, in which the ideal internal standard is used for each analyte since an enriched isotope of each analyte is added to the sample. The advantages of isotope dilution for laser ablation ICP-MS have been established for accurate and precise determinations of trace elements in powder [58].

4.4.4 Laser induced breakdown spectroscopy (LIBS)

Since 1963, where was published the first analytical use of a laser plasma for spectrochemical analysis of surfaces [59], the LIBS technique has demonstrated its potential in qualitative analysis and quantitative determination of the trace element composition of solids even in hostile environments, liquids, gases, aerosols, and environmental and geological samples. LIBS uses have grown steadily proving to be a relatively dynamic research activity for performing direct spectrochemical elemental or metal analysis of a variety of materials with none or little sample pretreatments. The analytical interest of LIBS is its multielement capability, its applicability to all sample types, its low sample requirements, its lack of sample preparation and its speed of acquisition allowing real-time measurements. Moreover, one of the unique capabilities of LIBS is to perform remote measurements in field environments where the sample may be many meters from the instrumentation.

There are many applications of LIBS for qualitative and quantitative elemental measurements in a wide range of samples such as metallurgical and solid samples, environmental samples, colloidal, and liquid samples, particles and gases, and advanced materials. While the qualitative analysis of a sample is rather a straightforward task, quantitative results on elemental compositions emerging from LIBS measurements require much more effort [60].

4.4.5 Direct analysis in real time (DART)

When using MS-based methods, the resulting fingerprints are preprocessed before being eventually pretreated (centering, scaling, etc.) and finally processed. Processing generally relies on multivariate statistical tools, which facilitate data visualization through data dimensionality reduction and highlight relevant information. Finally, identification of the discriminating signals is undertaken by combining mass spectrum analysis and database consultation.

Recently, it has been developed a new sample ionization source, called DART [61]. DART allows the direct and rapid identification of analytes in solid samples after no sample treatment. DART source consists of a tube divided in three chambers with a gas such as nitrogen or helium flowing through the tube. The first chamber is the discharge zone where the ions, electrons, and metastable species are generated by applying an electrical potential. The second stage is comprised by an electrode which removes

ions and electrons, the metastable molecules being the only working reagents in DART. In the third chamber the metastable gas molecules can be heated and finally exit through a grid electrode to interact with the sample generating sample ionization. In this configuration, the solid sample is placed in an open air ambient close to the spectrometer inlet (several mm).

This technique has permitted the determination of flavors and fragrances in real samples [62], melamine in pet food [63] and pharmaceuticals, pesticides and environmental relevant compounds [64].

4.4.6 Secondary-ion mass spectroscopy

SIMS involves the bombardment of the sample surface with a beam of ions (primary ions) and produces a localized ion emission characteristic of the material itself (secondary ions) [65]. This provides severe limitations for quantitative analysis of organic and inorganic species since the ionized fraction of the emitted flux is low and varies greatly from sample to sample and with bombardment conditions.

Typical primary ions are Cs^+, Ga^+, and O_2^+. The secondary ions emitted by the specimen are analyzed by mass spectrometry (magnetic sectors, quadrupole mass filter or time of flight (TOF) analyzers).

Depending on the ion dose applied to the sample, the SIMS technique can be used in two distinct modes of operation, which led to two different types of instruments defined as dynamic and static SIMS. Dynamic SIMS is mostly used as a depth-profiling tool for elemental composition and as a tool for the analysis of a nano/microvolume and has, in most favorable circumstances, a high sensitivity down to the ppb level. Combination of depth profiling and imaging allows 3D compositional analysis of the sample to be achieved. Static SIMS, on the other hand, is specifically designed for the detection of the elemental and molecular composition of one or few monolayers at the surface of a solid sample.

In short as it has been commented through this chapter avoiding sample treatment the analyst can be followed the main recommendations of green analytical chemistry because they avoid the use of reagents and solvents, reduce the energy consumption and avoid or just create a small volume of gaseous wastes also increasing the laboratory productivity and reducing or avoiding the operator risks. On the other hand, when samples were not damaged the aforementioned strategies permit the reuse of samples for analysis confirmation and thus we can conclude that the

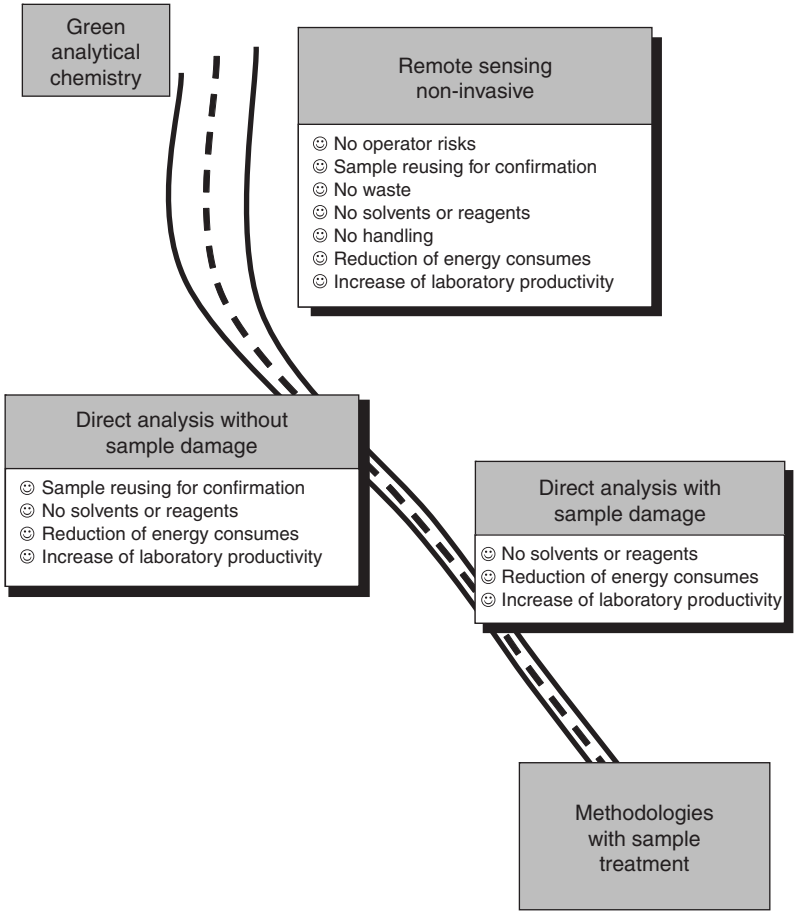

Figure 4.5 Green advantages of avoiding simple treatment from direct analysis with simple damage to remote sensing.

aforementioned methodologies must be strongly recommended (see Figure 4.5). Unfortunately, in many cases the sensitivity level attainable is not enough for trace or ultratrace analysis and they cannot be used for the determination of many organic compounds in complex samples and thus, alternative strategies based on green sample treatments and miniaturization and automation greener procedures, also including detoxification or recovery steps for on-line waste treatment must be employed to explore as many as possible components of different kind of samples.

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CHAPTER 5

Greening Sample Treatments

Contents

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As we have indicated in the previous chapter, from the Green Analytical Chemistry viewpoint it is highly desirable to avoid sample treatment and sample damage. However, it is clear that remote sensing, noninvasive, and direct analysis of samples cannot be done in all cases and therefore in many cases the greening of an analytical method also involves consideration of the side effects of sample treatments.

Sample preparation is a key step in any analytical procedure that requires it. Many classical dissolution or extraction methods are, unfortunately, still in use, and efforts must be made to develop alternative green sample preparation tools. The evolution of these sample preparation tools pursues the following objectives: (i) use of reduced amount of sample, (ii) reduction or elimination of organic solvents, (iii) simultaneous multiclass compound extraction, and (iv) potential for automation and/or high-throughput determination.

A closer look at the scientific literature shows that sample treatment has been the most evaluated analytical step in terms of greenness. For instance, we find reviews devoted to: minimization of solvent consumption in pesticide residue analysis [1], membrane extraction for biomedical and environmental analysis [2], passive sampling [3] as well as general reviews describing the present situation of sample preparation techniques, including solventless ones [4,5].

Automated extraction techniques are generally more reproducible than manual ones and will also decrease the time spent on sample preparation, which is often the bottleneck in analysis.

In the following sections the state-of-the-art of green alternatives for sample treatment will be discussed. We have classified the different alternatives according to the physical state of the samples, solids or liquids and also considering the generation of a gas or vapor phase directly from the samples. Many different objectives like analyte isolation from the matrix, analyte enrichment and/or sample clean-up and selective isolation of target analytes will be considered here.

5.1. SOLID SAMPLE EXTRACTION TECHNIQUES

Extraction of analytes from solid samples is commonly a preliminary step of the analytical procedure. It is expected to be performed in a reproducible

manner, in the fastest way, at a low cost and without risks to operators. So, from the green and sustainable point of view, it should avoid as much as possible the use of toxic organic solvents, acids, and energy consumption.

The choice of an extraction method is often made on the basis of the properties of the matrix and the analyte (analyte–matrix interactions), the requirements of the analytical method used for the determination of those analytes, and the analyte concentration levels in the sample. However, a change of mentality is also important and we need to incorporate new green variables to select the most appropriate extraction method in each case. So, we will focus on the modern clean, fast, low temperature and partial dissolution methods suitable to be carried out in closed systems with respect to the classical Soxhlet and wet and dry ashing procedures based on the use of convective heating of samples together with solvents or acids for long periods of time.

5.1.1 Supercritical fluid extraction (SFE)

SFE employs the unique feature of supercritical fluids to facilitate the extraction of organic compounds from solid samples. The choice of a supercritical fluid, usually carbon dioxide, as an extraction solvent allows for a more selective extraction, and provides faster reaction kinetics than the use of most liquids or solvents. The solvation power of the fluid can be manipulated by changing pressure and/or temperature and by adding small volumes of solvents as modifiers.

As well as a reduction in the use of organic solvent, carbon dioxide has the advantage that it is inexpensive, nonflammable, and nontoxic. Another advantage is that the supercritical CO_2 can be easily removed after extraction by reducing the pressure.

Nonpolar supercritical CO_2 produces high extraction efficiency for nonpolar to low polarity compounds. Combining CO_2 with one or more modifiers ($\leq 15\%$) extends the utility of CO_2 to more polar compounds and even ionic compounds extraction can be improved.

One of the main problems with SFE is the robustness of the method compared with other techniques and extraction conditions which must be consistent for reproducible extractions.

SFE can be accomplished in both static and dynamic mode. In the static mode sample and solvent are mixed and kept for a fixed time at constant pressure and temperature. In the dynamic mode, the fluid flows through the sample in a continuous way. The extracted analytes can be collected inside an off-line device, this step being performed by depressurizing the supercritical fluid and absorbing analytes into a solvent or a solid phase, or transferred to an on-line chromatographic system for direct analysis using special interfaces.

The chromatographic counterpart of SFE was developed in 1962 [6] emerging in the mid-1980s as a promising tool to overcome the difficulties of solid sample extraction [7] (http://www.sciencedirect.com/science?_ob=RedirectURL&_method=outwardLink&_partnerName=655&_targetURL=http%3A%2F%2Fwww.scopus.com%2Finward%2Frecord.url%3Feid%3D2-s2.0-33947479623%26partnerID%3D10%26rel%3DR3.0.0%26md5%3D4ac39674eb77dcd2b49d7494358f4b02&_acct=C000053935&_version=1&_userid=1647180&md5=75632cf6b9ac14ebbac8d5cd1431f9c3) and becoming a serious alternative for safe food processing [8], to improve the green extraction of organic compounds from solid sediments and also from foods and biota, being applied in polyaromatic hydrocarbons extraction [9] and also in the speciation analysis of organometallic compounds like organotins [10].

Figure 5.1 shows a scheme of an SFE system operated in static and dynamic modes but it should be noted that modern devices can perform both operation modes under well controlled automatic conditions.

5.1.2 Pressurized solvent extraction (PSE) and superheated water extraction (SHWE)

PSE, also called accelerated solvent extraction (ASE), pressurized liquid extraction (PLE), high-pressure solvent extraction (HPSE), high-pressure, high temperature solvent extraction (HPHTSE), pressurized hot solvent extraction (PHSE), and subcritical solvent extraction (SSE), involves analyte extraction from solid and semisolid sample matrices under elevated temperature (50–200 °C) and pressure (500–3000 psi) conditions for short time periods (5–10 min) [11]. It is becoming increasingly important as a preparation technique in analytical chemistry, combining the benefits of high throughput, automation, and low solvent consumption, although expensive lab-equipment is required [12].

Elevated temperatures can lead to significant improvements in extraction efficiency, because they can increase the solubility of target analytes, assist in breaking down analyte–matrix interactions and encourage the diffusion of the analyte to the matrix surface and mass transfer of organic compounds to the solvent. Under these conditions, solvents have enhanced solvation power and increased extraction rates. Rapid extraction rates are possible, compared to conventional techniques, such as Soxhlet extraction. High pressure allows maintaining the solvent in a liquid state at high temperature and may increase the penetration of the solvent in the sample matrix.

However, a drawback of PSE is that the presence of relatively high water percentages in the samples to be treated strongly decreases analyte extraction

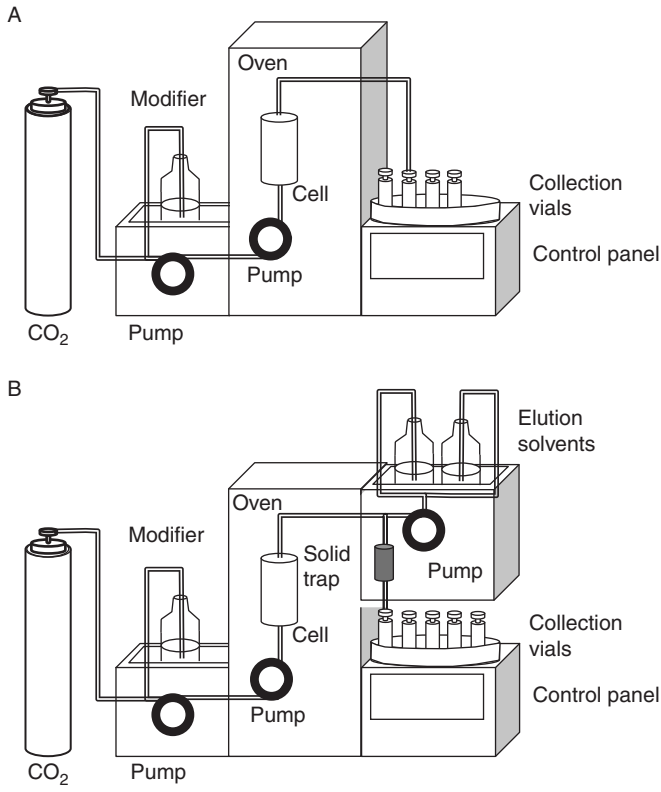


Figure 5.1 SFE system operating in static (A) and dynamic (B) mode.

efficiency when using hydrophobic organic solvents as water hinders contact between the solvent and the analyte. The efficiency on the PSE is dramatically influenced by the pressure and temperature conditions. Furthermore, the sample matrix can also affect the extraction efficiency.

PSE can be performed in both static and dynamic modes (see Figure 5.2). In static mode, the sample is enclosed in a stainless steel vessel filled with an extraction solvent, and following extraction the remaining solvent is purged with N_2 into a collection vial. Flow-through systems continuously pump solvent through the sample, but this has the disadvantage of using larger volumes of solvent and of diluting the extract. A desiccant; such as sodium sulfate, diatomaceous earth, or cellulose, can be added directly to the extraction cell or alternatively sorbent materials can be used to provide *in situ* clean-up [4].

When water is employed as the extraction solvent, a different name, such as SHWE, is often used to highlight the use of this environmental-friendly

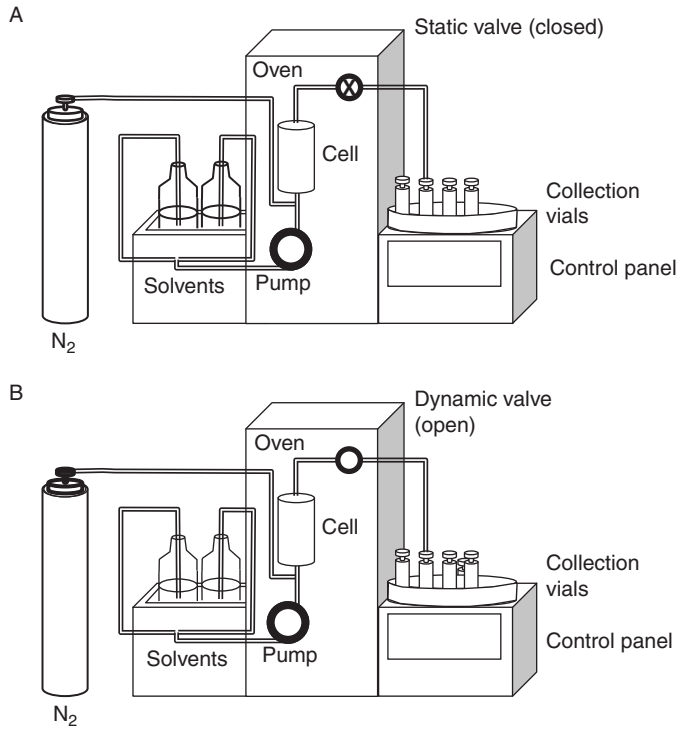


Figure 5.2 Scheme of a PSE system working in static (A) and dynamic (B) mode.

solvent, but it is essentially a variant of PLE. It is clear that the interest in the use of water as the solvent for PSE can reduce or eliminate the use of organic solvents. This technique uses water in the condensed phase between 100 °C and the critical point, and has also been called subcritical water extraction (SWE), hot water extraction (HWE), pressurized hot water extraction (PHWE), or high temperature water extraction (HTWE). The main advantages are that it is cleaner, faster, cheaper, and more environmentally friendly than conventional methods.

Increasing the temperature of water under pressure reduces its polarity and therefore extraction becomes more selective and at 100–200 °C, it can act as a medium/nonpolar solvent. The useful temperatures and pressures of water are lower than its critical point, in contrast to SFE with carbon dioxide.

The main disadvantage of SHWE, particularly for trace analysis, is that the extract obtained is a diluted aqueous solution, a concentration/extraction step being required prior to its analysis.

5.1.3 Ultrasound-assisted extraction for sample dissolution and analyte extraction

Ultrasonic extraction, also known as sonication, can be used to enhance extraction, through using the ultrasonic vibration to ensure an intimate contact between the sample and the solvent or the reagent solution (see Figure 5.3). Sonication is relatively fast as compared with traditional extraction or dissolution systems. However, the extraction efficiency is not as high as some of the other extraction techniques and frequently it involves the use of several sequential steps on the same sample. The solvent type or mixture can be selected to obtain maximum extraction efficiency and required selectivity. Several extractions can be performed simultaneously and as no specialized laboratory equipment is required the technique is relatively inexpensive compared to most modern extraction methods.

Thus, sonication is an expeditious, inexpensive, and efficient means to innovate some conventional extraction techniques such as SFE, PLE, and Soxhlet extraction. Sonication is usually performed in the static mode, although it is possible to perform the dynamic or on-line combination with analytical systems [4–15]. One disadvantage of ultrasound-assisted extraction is that this on-line combination cannot be easily achieved and automation is not easily obtained, except when high power probes were used [16].

5.1.4 Microwave-assisted extraction and digestion (MAE)

MAE involves the use of microwave energy to heat a solvent in contact with a sample, in order to remove the analytes from the sample matrix into the solvent or the acidic solution. MAE is based on the heating of the system due to the absorption of the microwave energy by polar molecules. The efficiency of MAE depends on several factors, such as solvent properties, sample material, the components being extracted, and, specifically, on

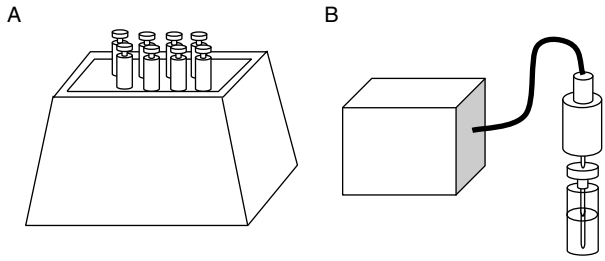


Figure 5.3 Scheme of an ultrasound water bath (A) and an ultrasound probe (B) used for the simultaneous extraction and for the on-line extraction, respectively.

the respective dielectric constants. The higher dielectric constant, the increased amount of energy is absorbed by the molecules and the faster the system reaches the extraction temperature.

The patent on the use of microwaves for heating is from the domestic appliances field. In 1946, the Raytheon Company patented the first microwave oven called the Radarrange [17] but the preliminary studies on their use for sample digestions were done by Abu-Samra et al. in 1975 [18] and for extraction of organic compounds by Ganzler et al. in 1986 [19].

Figure 5.4 shows the scheme of the two available configurations of microwave ovens adapted for laboratory experiments: multiposition ovens and focused systems. Both of the aforementioned devices have been applied in sample digestion in order to carry out trace element determinations of samples [20] and for the extraction of organic compounds [21]. The on-line applications are especially interesting [22].

MAE is well suited for routine analysis and offers a considerable reduction in time and acids consumption for trace element analysis. Additionally, with regard to organic compounds extraction MAE offers a high throughput of samples as compared to Soxhlet and ultrasound-assisted extraction, but it is only applicable to thermally stable compounds due to the increase in temperature during extraction. Figure 5.5 shows a comparison of the aforementioned systems applied to the determination of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated pesticides (DDTs), and hydrocarbons. From the aforementioned scheme it can be concluded that SFE and pressurized MAE are the best alternative to save solvents and time. As nonpolar solvents do not absorb microwave energy, at least some polarity must be ensured in the system and for this purpose mixtures of polar and apolar solvents or nonpolar solvents with water have been proposed for organic compounds extraction.

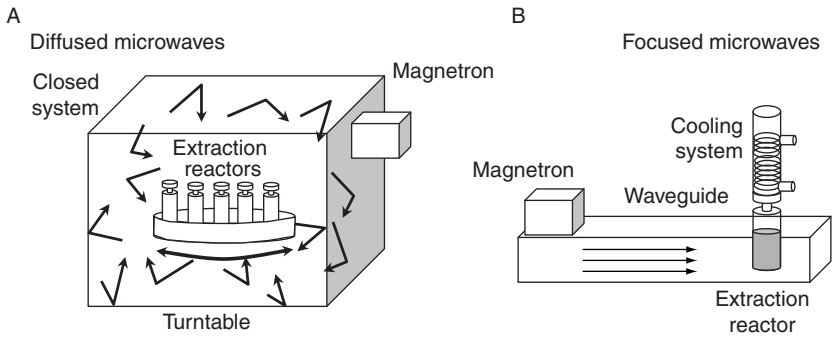
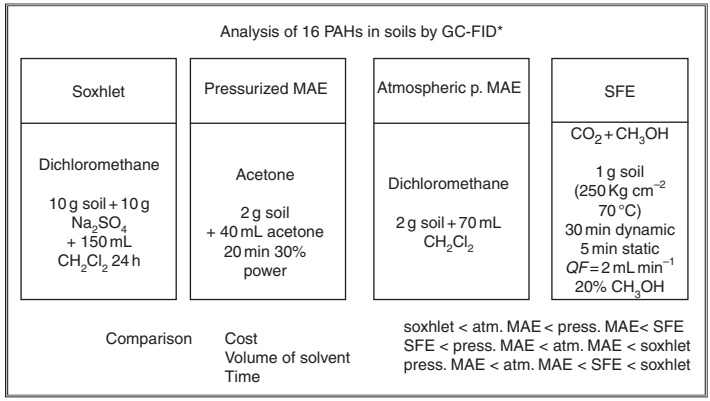


Figure 5.4 Scheme of the microwave system employed for multisample (A) and focussed (B) treatments.



*GC-FID: Gas chromatography-flame ionization detector

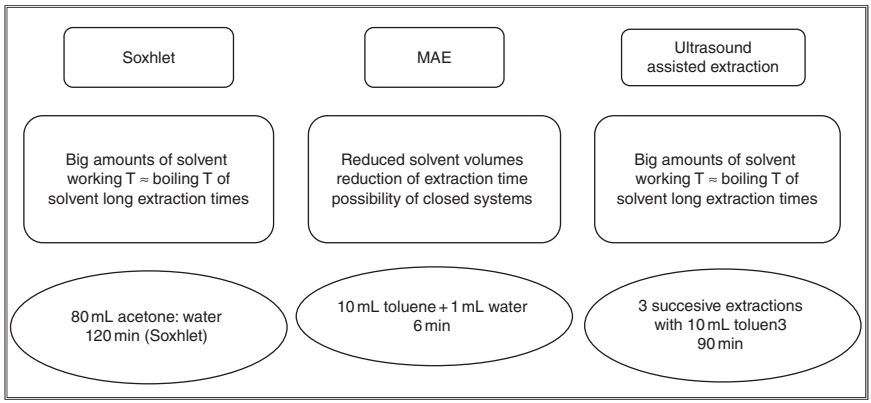


Figure 5.5 Comparison of extraction procedures based on traditional systems like Soxhlet and green ones for 16 PAHs in soils [23] and from 15 linear hydrocarbons, 19 PAHs, 4 DDTs, and 6 PCBs in sediments [24].

Table 5.1 summarizes the main advantages and drawbacks of SFE, PSE, sonication, and MAE as green alternatives for analyte extraction from solid samples taking into consideration the time of extraction, solvent or acid requirements, cost of equipment, and necessary additional treatments for analyte determinations.

5.2. EXTRACTION OF LIQUID SAMPLES

The following sections will deal with the extraction of analytes from liquid samples or from solutions or extracts of solid ones. The majority of liquid samples are aqueous, where matrix effects are minimal or

Table 5.1 Advantages and drawbacks of green alternatives for analyte extraction from solid samples

Technique	Advantages	Drawbacks
Supercritical fluid extraction (SFE)	Fast extraction (30–75 min) Minimal solvent use (5–10 mL) Nontoxicity, non-flammability of CO_2 Controlled selectivity Filtration not required Evaporation not needed	Matrix dependent Expensive equipment Limited applicability
Pressurized solvent extraction (PSE)	Fast extraction (12–18 min) Small amount of solvent (15–40 mL) Large amount of sample (up to 100 g) Automated Easy to use Filtration not required	Expensive equipment Clean-up required
Ultrasound based extraction	Not matrix dependent Relative inexpensive equipment Fast extraction (10–45 min) Moderate amount of sample (2–30 g)	Large amount of solvent or acids (10–300 mL) Mandatory evaporation of extracts Limited extraction efficiency Intensive Labor and attention Filtration required
Microwave assisted extraction (MAE)	Fast extraction (20–30 min) High sample throughput Small amount of solvent or acids (5–30 mL) Moderate amount of sample (0.5–20 g)	Polar character needed Clean-up mandatory Filtration required Moderately expensive equipment Possibility of thermal degradation and chemical reaction

nonexistent, greatly simplifying the extraction step. As the techniques involved in a liquid sample extraction are similar to those used for cleaning up a liquid extract from a solid sample, both will be treated together in this section.

5.2.1 Solid phase extraction (SPE)

SPE is one of the most popular sample preparation techniques [25]. The principle of SPE is based on the sorption of analytes on a solid phase from the original sample. The aqueous sample solution passes through the SPE column, and the analytes are first trapped on the sorbent and then eluted with a suitable small volume of an appropriate organic solvent, simultaneously achieving extraction and enrichment of the analytes [26]. The aforementioned process can be also applied to the extraction of polar compounds from nonpolar solutions based on the use of polar solid sorbents or on the selective extraction of cationic or anionic species from aqueous solutions.

The main advantages of SPE over liquid partitioning procedures are its low solvent consumption, high precision and throughput. Additionally, SPE can be easily automated with relatively simple and inexpensive equipment, which can lead to improved accuracy, precision, and laboratory throughput.

A wide range of sorbents have been used, including C8 and C18 bonded phases on silica, polymeric resins (polystyrene/divinyl benzene copolymer), Florisil (activated magnesium silicate), polar sorbents such as alumina, charcoal, silica and cyano, and amino bonded. Ionic functional groups, such as carboxylic acid or amino groups, can also be bonded to silica or polymeric sorbents to create ion-exchange sorbents. Mixed-mode sorbents are also available that use both primary and secondary mechanisms for selective retention of analytes and some very specific selective sorbents have been designed. These different phases enable interactions based on adsorption, H-bonding, polar and nonpolar interactions, cation, anion exchange, or size exclusion to be utilized for the separation and enrichment of polar and nonpolar analytes from different media.

One of the drawbacks of SPE is that the packing must be uniform to avoid poor efficiency, so the use of prepacked commercial cartridges is desirable. The sample matrix can also affect the ability of the sorbent to extract the analytes due to competition processes for retention. Many traditional sorbents are limited in terms of selectivity and insufficient retention of very polar compounds can also be a problem. Additionally, it must be taken into consideration that both the retention and elution processes must be quantitative to guarantee an accurate determination.

The SPE units are commercialized in different forms such as packed syringe-shaped cartridges, 96 well plates or 47- or 90-mm flat disks, which can be mounted on commercially available extraction manifolds that allow the simultaneous processing of many samples. A typical cartridge SPE manifold

can accommodate up to 24 cartridges, while a typical disk SPE manifold can accommodate at least 6 disks, thus contributing to save time in applied analysis.

5.2.2 Solid phase microextraction (SPME)

SPME is a very simple and efficient, solventless sample preparation method, developed in 1989 by Belardi and Pawliszyn [27], which integrates sampling, extraction, concentration, and sample introduction in a single step, thus being clearly compatible with the Green Analytical Chemistry principles.

In SPME a coated fiber is placed in contact with the sample matrix, by direct immersion or headspace, during an established period of time. If it is long enough, concentration equilibrium is established between the sample and the extraction phase. After the extraction step, the SPME fiber is transferred to the injection port of the selected instrument (gas chromatograph, liquid chromatograph, mass spectrometer) where desorption of the analytes takes place and analysis is carried out.

The main advantages of SPME are that it reduces the sample preparation time, decreases purchase and disposal costs of solvents, and can improve detection limits. However, it presents some drawbacks such as the precision and accuracy being dependent on the quality of the fibers, which in turn depends on the manufacturer, and the performance of the different batches. Some fiber degradation can occur during repeated usage, and the system is relatively fragile and easily broken while handling. Finally, it can be concluded that SPME is relatively expensive and presents possibilities of carryover between analyses.

5.2.3 In-tube SPME

An alternative to the use of coated fibers is the use of internally coated capillaries, through which the sample flows, or is drawn repeatedly. With the in-tube SPME technique, organic compounds in aqueous samples are directly extracted from the sample into the internally coated stationary phase of a capillary column, and then desorbed by introducing a moving stream of mobile phase or static desorption solvent when the analytes are strongly absorbed to the capillary coating. This technique was developed due to the difficulties of interfacing SPME with liquid chromatography (LC) systems [28]. The first technique based on this approach was known as the inside needle capillary adsorption trap (INCAT) and was introduced in 1997 by McComb et al. [29]. INCAT is a preconcentration device consisting of a hollow needle, with either a short length of gas chromatography (GC) capillary column placed inside it, or an internal coating of carbon.

In-tube SPME is suitable for automation, and can continuously perform extraction, desorption, and injection using a standard autosampler. Although the theories of fiber and in-tube SPME methods are similar, the main difference between these methods is that the extraction of analytes is performed on the outer surface of the fiber in the first case and on the inner surface of the capillary column for the latter. Therefore, with the in-tube SPME method it is necessary to prevent plugging of the capillary column and flow lines during extraction, and typically particles must be removed from samples by filtration before extraction. However, peak broadening is comparatively smaller than in SPME because analytes are completely desorbed before injection [30].

5.2.4 Solid phase dynamic extraction (SPDE)

SPDE is a recent variant of dynamic in-tube SPME, where a special internally coated syringe needle repeatedly draws up the sample before injection into a GC line. It was first described by Lipinski [31] for the analysis of pesticides in water samples and was compared to SPME.

SPDE utilizes a headspace syringe with a needle coated with an immobilized extraction phase. The SPDE needle coatings possess around four to six time's larger extraction phase volumes as compared with a 100-μm SPME fiber [32]. For the extraction, the needle can be immersed directly inside the sample or in the headspace above it and the plunger is moved up and down several times for a dynamic extraction of the sample, the analytes being sorbed in the internal coating. After several extraction cycles (aspirating and dispensing), the analytes are thermally desorbed from the coating in the GC injector.

There are many single, mixed, and custom-made phases available, including polar and nonpolar coatings; such as Polydimethylsiloxane (PDMS), PDMS/activated charcoal, PDMS/vinyl modified OV 225, PDMS/phenylmethylpolysiloxane, polyethylenglycole (PEG), and PDMS, 7% phenyl, 7% cyanopropyl (OV 1701) and they can be applied in various thicknesses.

5.2.5 Stir bar sorptive extraction (SBSE)

SBSE was introduced in 1999 [33], as a solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices. The method is based on sorptive extraction, whereby the solutes are extracted into a polymer coating on a magnetic stirring rod. Sorptive extraction is by nature an equilibrium technique, where the extraction is controlled by the partitioning coefficient of the solutes between the polymer coating and the sample matrix and by the phase ratio

between the polymer coating and the sample volume. The basic principles of SBSE are thus identical to those of SPE or SPME using PDMS-coated fibers, but the volume of extraction phase is 50–250 times larger than in the case of SPME. The increased recovery obtained by SBSE in comparison to SPME has been demonstrated by different groups using PAHs [34] and pesticides [35,36] as test solutes. Also, in headspace sampling mode, SBSE results in higher recovery than in SPME [37].

After extraction, the stir bar is removed, dipped on a clean paper tissue and introduced in a thermal desorption unit. In some cases, it is recommended to rinse the stir bar with distilled water to remove adsorbed sample components. Because more sorptive extraction phase is used, the desorption process is slower than for a SPME fiber (10 min desorption time; 10–100 mL/min desorption flow). Special thermal desorption units are available for optimum desorption, reconcentration, and GC analysis [38].

Alternatively, liquid desorption can be used. Typically, the stir bar is placed in a small vial (2 mL, or vial with insert) and desorption is performed with apolar solvents (hexane), followed by GC analysis, or with polar solvents (methanol, acetonitrile), followed by LC analysis.

The main drawback of SBSE is that only PDMS stir bars are commercially available for the extraction phase. Attempts have been made to apply other coatings, but problems with irreproducible coating, excessive bleeding in thermal desorption or only a small advantage in comparison to PDMS have hindered their commercial introduction. Thus, *in situ* derivatization is absolutely necessary to retain relatively polar solutes by means of SBSE with PDMS-coated stir bars and thus, from the sustainable point of view, SPME remains the best alternative for polar compounds. Figure 5.6 provides a comparison of the basic setups employed for SPE in the modalities commented on in the previous sections.

5.2.6 Single drop microextraction (SDME)

SDME is a simple, low cost, and fast extraction technique applicable even for headspace extraction. In SDME, analytes in the donor phase are extracted inside a single drop of the acceptor phase of nanoliter to microliter volume, thus providing a strong reduction of organic solvent consumption. However, it requires careful and elaborate manual operations, presenting problems of drop stability, with associated poor sensitivity and precision in many cases. This is because prolonged extraction times and faster stirring rates are not recommended, since they typically result in drop dissolution and/or instability [39].

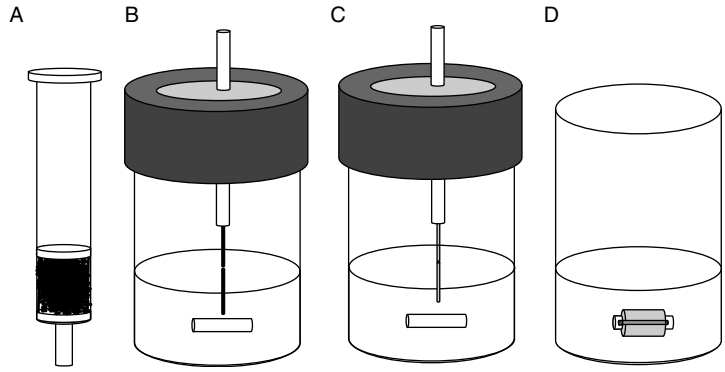


Figure 5.6 Scheme of the different SPE system available in the market from SPE cartridges (A) to SPME (B), in-tube SPME (C), and SBSE (D).

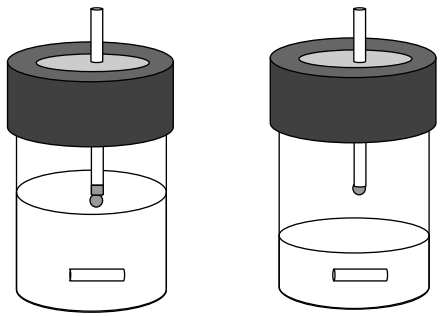


Figure 5.7 Scheme of the SDME system.

Since the introduction of SDME in 1996 [40], different modes of SDME have been developed, catering to various analytical applications, such as direct immersion (DI)-SDME, headspace (HS)-SDME, and continuous-flow microextraction (CFME). Based on these applications, different methodologies have been developed to improve selectivity, ease of automation, and expand the application range to make it compatible with more analytical techniques [41].

Figure 5.7 provides a scheme of the SDME system employed in both the headspace and the liquid–liquid extraction modes.

5.2.7 Liquid phase microextraction (LPME)

One of the emerging techniques in extraction of analytes from liquid samples is the so-called LPME, where a fiber impregnated with an organic solvent is used to accommodate or protect microvolumes of acceptor solution [42].

In LPME, the organic (acceptor) phase is protected by the fiber and it appears that the presence of the hollow fiber decelerates the process of organic solvent dissolution into the bulk solution. Moreover, the surface area for the rod-like configuration of the LPME system is larger than the spherical one adopted by the drop-based SDME method, improving the sensitivity of LPME due to the increased rate of analyte extraction.

A major advantage of LPME over SPME is that the range of compounds amenable to this technique can be extended by simply changing from the two-phase to the three-phase LPME mode and by adjusting the composition of the different phases. Furthermore, the low cost per unit allows the use of each fiber for only one extraction, eliminating the possibility of sample carryover.

There are two sampling modes in LPME, namely two-phase and three-phase (See Figure 5.8) [43]. In the two-phase LPME sampling mode, the analyte is extracted from an aqueous sample (donor phase) through a water-immiscible solvent immobilized in the pores of the hollow fiber into the same organic solvent (acceptor phase) present inside the hollow fiber. In the three-phase sampling mode, the analyte is extracted from an aqueous solution (donor phase) through the organic solvent immobilized in the pores of the hollow fiber (organic phase) into another aqueous phase (acceptor phase) present inside the lumen of the hollow fiber. The organic phase acts as a barrier between the acceptor and the donor aqueous solutions, preventing mixing and additionally reducing consumption of the organic solvent.

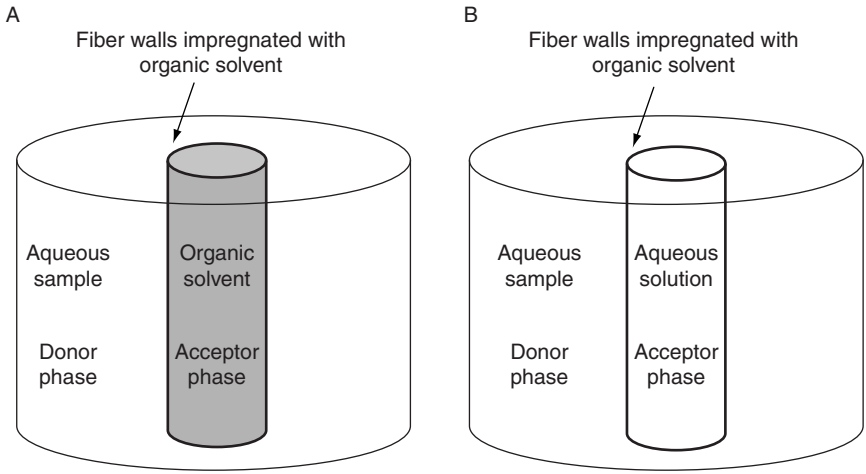


Figure 5.8 Liquid phase microextraction sampling modes using two phases (A) and three phases (B) system.

5.2.8 Continuous-flow microextraction

CFME evolved from conventional SDME, and was first described by Liu and Lee in 2000 [44]. An aqueous sample (3 mL or less) is pumped continuously at a constant flow rate (0.05 mL/min), using a LC solvent delivery system, into a 0.5-mL glass extraction chamber. After the chamber is filled with the sample solution, the required volume of an organic extraction drop (1–5 μL) is introduced in the system via a conventional LC injection valve. The drop remains attached in the outlet of the polyether ether ketone (PEEK) tubing and the sample solution is pumped around the drop, allowing analytes to be extracted efficiently. At the end of the extraction, the extract is collected for analysis with a microsyringe needle.

CFME differs from other micro liquid extraction approaches in that a drop of solvent fully and continuously makes contact with a fresh and flowing sample solution. Other advantages are the high preconcentration factor that can be achieved and the smaller volumes of aqueous samples needed for extraction.

Xia et al. [45] made some modification to the basic CFME setup and developed a recycling-flow system, in which the sample waste from the chamber was returned to the sample vial, allowing a reduction in sample consumption.

5.2.9 Membrane-based techniques

Membrane extraction from liquid (water and biological fluids) and air samples plays an important role among the solvent-free techniques of isolation and/or preconcentration of analytes, and are really useful methodologies in our quest towards greener analytical chemistry.

The membrane-based extraction methods can be differentiated based on the type of membrane involved: porous and nonporous or semipermeable membranes. In the case of porous membranes separation of components is accomplished as a result of the sieving effect (size exclusion), the selectivity being dependent on the membrane pore diameter. In the case of nonporous membranes separation is based on differences in solubility and diffusion rates of individual analytes in the membrane material.

5.2.9.1 Microdialysis

Microdialysis is the miniaturized version of a separation technique that uses the mass flux through a semipermeable inert membrane which separates two chambers, one containing the acceptor solution and the other

which acts as sample reservoir (donor chamber). Microdialysis is a sampling technique extensively used in clinical research, medicine development, and the life sciences. A microdialysis system is essentially composed of a micropump, a microdialytic probe with a semipermeable membrane at the tip and liquid delivery and collection devices.

Dialysis membranes of a particular molecular-weight cut-off (MWCO) provide an effective tool for molecular separation based on molecular hydrodynamic dimensions, where only species with molecular weight lower than the MWCO will diffuse across the membrane as a result of the concentration gradient between the two streams.

The main disadvantage of the technique is the temporal resolution, determined by the time required to remove a detectable quantity of analyte, with typical sampling times of 10–30 min.

In order to increase the recovery of microdialysis, an enhanced technique has been developed by chemically converting the target species to other forms once they diffuse into the receiving solution. This conversion can maintain the highest driving force of diffusion required for analyte transportation. For example, metal ions can efficiently be collected by converting them into complexes with chelating agents and/or biopolymers added in the receiving solutions [46]. Similarly, β-cyclodextrins are used to extract some drugs through host–guest complexation [47]. By introducing affinity solid particles into the receiving solution, a solid-support-enhanced microdialysis method has been developed [48]. On the other hand, micellar ultrafiltration offers a unique way to improve the selective collection of species suitable to be incorporated inside the micelles [49].

5.2.9.2 Membrane-assisted solvent extraction (MASE)

In MASE, an organic solvent is immobilized in the pores of an inert support material, separating the aqueous donor and the acceptor phases. This enables the use of larger volumes of samples than those employed in dialysis but also acts as a physical barrier between the phases. The principle of MASE involves transfer of organic compounds across a polymeric membrane to a small volume of organic solvent, thus providing a green alternative.

The membrane is usually made of polypropylene or other porous hydrophobic material. Polypropylene is highly compatible with a broad range of organic solvents, and, owing to a pore size of approximately 0.2 μm, the membrane strongly immobilizes the organic solvent in the pores. A further benefit of the use of a membrane is that, owing to the pore structure, the concentration of high-molecular-mass compounds in

the sample extract is reduced. Membrane-assisted liquid extraction can thus be considered as a subtechnique of LPME.

The main disadvantage of all membrane extraction techniques is that the extraction tends to be nonexhaustive. The recoveries are usually at a similar level to those in SPME, and calibration must be carefully carried out under the same conditions as those used for samples. Quantitative recoveries are obtained for highly hydrophobic analytes, also when the flow rate of the donor is kept either sufficiently low or the sample is circulated across the extraction system. If quantitative recoveries are not required, the extraction time can be decreased by increasing the flow rate of the sample, or the sensitivity can be increased by using an excessive amount of sample [50].

5.2.9.3 Polymeric membrane extraction (PME)

In PME an entirely solid membrane is used to separate the donor and the acceptor solutions. Silicone rubber is mostly used because it is hydrophobic and thus highly permeable for small hydrophobic molecules [51]. The difference in the solubility and diffusion of various analytes into the polymer is the basis of selectivity. The solid nature of silicone rubber nonetheless makes it a versatile technique as it allows aqueous [52], organic [53], and gaseous samples to be processed. Selectivity can be enhanced by modifying the conditions in the acceptor phase, such as analyte ionization. Its major advantage is that the solid nature of silicone rubber means that phase breakthrough is minimized. The major disadvantage is that the solid membrane does not allow the incorporation of other functional groups (carriers) that can enhance both the mass transfer and the selectivity of the compounds of interest. It is an especially ideal method for extracting analytes in complex samples with high amounts of organic materials such as lipids [54] since the instability associated with liquid membranes does not exist.

5.2.9.4 Membrane extraction with a sorbent interface

This technique is based on membrane extraction into a gas followed by trapping of the analytes on a solid sorbent (cryofocusing) and subsequent thermal desorption into a gas chromatographic system [55]. The technique is suitable for volatile or semivolatile organic compounds either in air or aqueous samples. The receiving phase is always a carrier gas that continuously transports the analytes onto the sorbent. A more detailed theory of the technique and the type of sorbents that are used to trap the analytes has been described by Luo et al. [56]. The basis of selectivity of the method is differences in solubility and diffusion of various analytes into the nonporous polymer.

The main drawback of the technique is that it has a narrow application window for environmental analysis; only volatile organic compounds can be extracted.

5.2.9.5 Semipermeable membrane devices (SPMDs)

In SPMDs, organic analytes passively diffuse from the aqueous or gaseous donor phase through a polymeric membrane such as polyethylene into the acceptor phase filled with a thin film of a synthetic lipid such as triolein [57,58]. It is used as a passive field sampler, being an important technique in exposure risk assessment of pollutants. Generally, this technique is suitable for extraction of hydrophobic nonpolar compounds such as polycyclic aromatic hydrocarbons [59], pesticides [60,61], and PCBs [62] with partition coefficients (log Kow) in the range 3.0–6.0. SPMDs are also not very selective as they rely on the differences in solubility and diffusion of the various analytes into the membrane and lipid. For quantitation, very little data is available for sampling rates of various pollutants into the SPMDs [63], making it difficult to estimate accurately the true concentrations of analytes in the original environmental compartment. The methodology necessitates additional clean-up of the extracts, increasing the time of analysis, and the consumption of organic solvents. However, recent advances in the extraction of analytes from SPMDs based on the use of PSE [64] and MAE [65] have contributed to improving the sustainable aspects of this membrane-based extraction technique, which is highly efficient for passive sampling.

Figure 5.9 provides comparative schemes of the different membrane-based separation techniques.

5.2.10 Surfactant-based analyte separation

In recent years, extraction and concentration procedures based on substrate solubilization in the supramolecular pseudophases of surfactant aggregates have been extensively developed as alternatives to solvent-based extraction [66].

5.2.10.1 Liquid–coacervate extraction

At temperatures above the cloud point, aqueous solutions of nonionic surfactants separate into a micellar concentrated or coacervate phase and a diluted phase. When an organic solute or compound is present in an aqueous solution and nonionic surfactant is added to the water, at temperatures above the cloud point, the organic solute will tend to partition into the

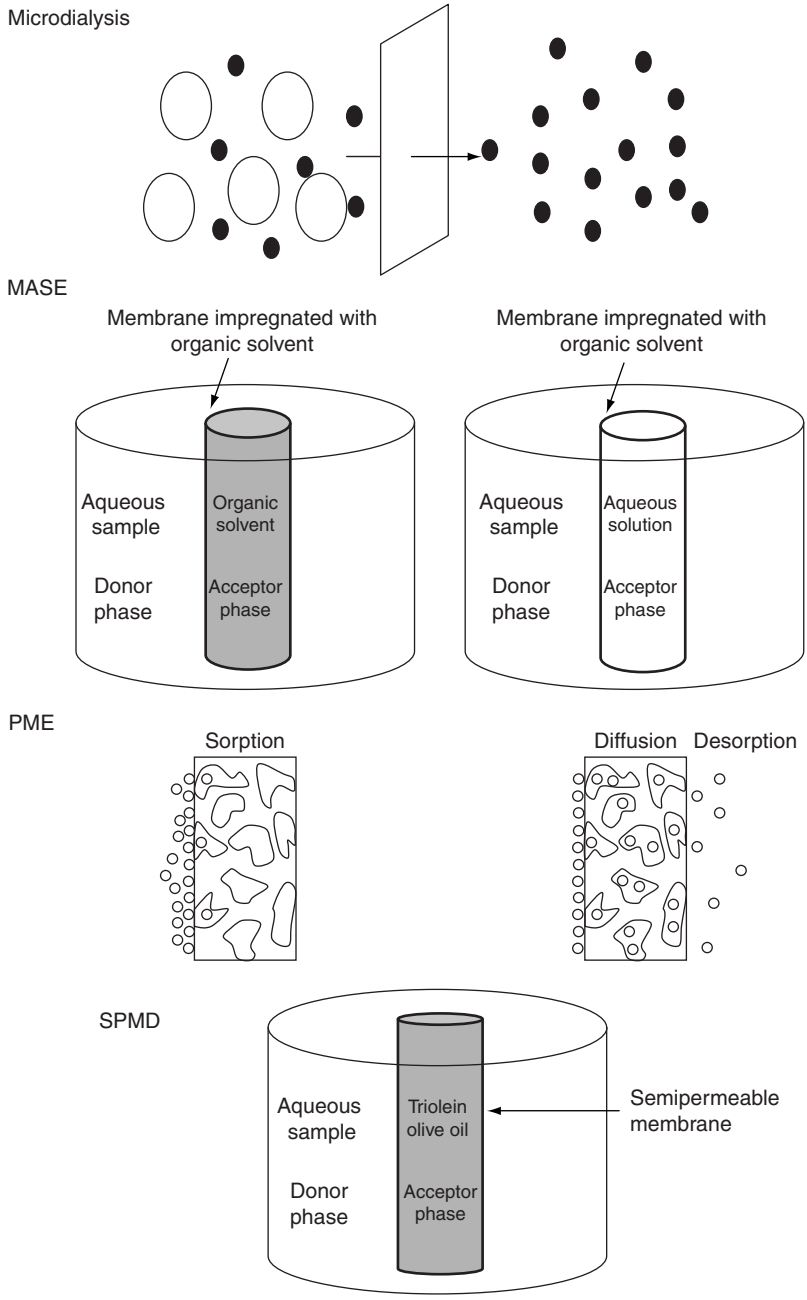


Figure 5.9 Schematic representation of the membrane-based extraction techniques.

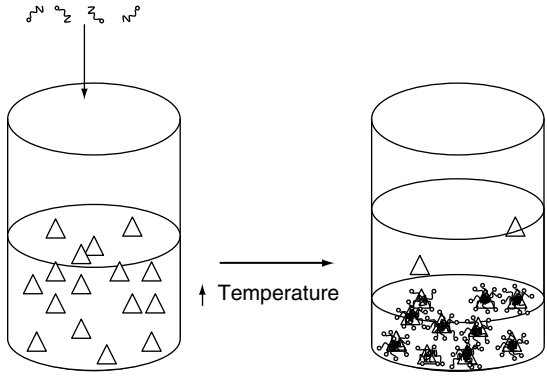


Figure 5.10 Scheme of a cloud point extraction system.

coacervate phase. This liquid/coacervate extraction, sometimes referred to as cloud point extraction, shows great potential for removing toxic solutes from polluted water [67].

Figure 5.10 provides a scheme of the cloud point extraction process and Table 5.2 indicates the critical micellar concentration and cloud point temperature of the main surfactants used in the literature for analytical liquid–coacervate extraction.

From the analytical point of view, one of the most important properties of the aforementioned organized structures is their good capacity to solubilize solutes of different types and nature. The small volume of the surfactant-rich phase allows us to preconcentrate and extract the analytes in one step, prior to their analysis. The capacity of the micelles to solubilize different compounds has been used to develop the extraction and the preconcentration of organic compounds.

In addition, a new extraction approach has been recently proposed [68]: the coacervate phase separation into lamellar vesicles, termed vesicular liquid coacervate extraction (VLCE). This novel method is based on the formation of vesicles by precipitation of an insoluble phase through a charge neutralization chemical reaction in the presence of a water miscible cosurfactant. The most attractive feature of this method is that it allows for both electrostatic forces with polar analytes (ionic species) and hydrophobic interactions, involving nonpolar analytes (hydrophobic compounds or metal-chelates) to be encountered simultaneously in the same procedural scheme. In this manner, the isolation and preconcentration of both metal ions and metal-chelates can be pursued within a single experimental procedure.

Table 5.2 Main characteristics of surfactants commonly used in cloud point extraction

Surfactant	Commercial name	CMC[a]	Cloud point (°C)[b]
Polyoxyethylene 4 lauryl ether	Brij 30 (C12E4)	0.064	2.0
Polyoxyethylene 6 lauryl ether	C12E6	0.068	50.3
Polyoxyethylene 9 lauryl ether	Polidocanol (C12E9)	0.069	86.6
Polyoxyethylene 10 lauryl ether	Pole (C12E10)	0.09	77
Polyoxyethylene 8 dodecanol	Genapol X80 (C12E8)	0.05	75
Polyoxyethylene 7.5 nonylphenyl ether	Ponpe 7.5	0.085	5.0
Polyoxyethylene 9.5 octylphenyl ether	Triton X-100	0.24	64
Polyoxyethylene 10 cetyl ether	Brij 56 (C16E10)	0.0006	69
Polyoxyethylene 10 stearyl ether	Brij 97 (C18E10)	0.0017	72

[a]Critical micellar concentration (CMC) values given in mM/l.
[b]Values given are the critical temperatures for micellar solutions in which the surfactant concentration is 1% (w/v).

5.2.10.2 Microemulsion-based separations

Microemulsions are mixtures of oil, surfactant, and water that form spontaneously on bringing the three components together [69]. Microemulsions are thermodynamically stable, and their formation is facilitated by the ultralow interfacial tension of these systems, which leads to the formation of extremely small droplets of the dispersed phase. These microemulsions can take different forms which include, among others, oil, water, and oil/water bicontinuous microemulsions.

Microemulsions, for instance oil-in-water (Winsor I type) microemulsion, provide a potential approach for the remediation of soils and groundwater due to the high solubilization capacity and extraction power of organic pollutants, compared to the surfactant micellar solutions [70] and they could be used for the extraction of organic pollutants prior to their determination.

5.2.10.3 Separations using aphrons

Predisperded solvent extraction, which uses colloidal liquid aphrons (CLAs) as a predispersed solvent, was first proposed by Sebba [71] in order

to account for the enhanced stability of aphrons relative to oil droplets in conventional oil–water emulsions. CLAs are micron-sized solvent droplets encapsulated by a thin aqueous film stabilized by a mixture of nonionic and ionic surfactants [72].

Aphrons have been investigated in relation to the predispersed solvent extraction of a range of organic molecules [73,74] and as a novel support for enzyme immobilization in multiphase biocatalytic processes [75,76] providing environmentally friendly alternatives to the use of organic solvents.

Table 5.3 provides a comparison of the aforementioned green extraction methods for liquid samples and solvents treatment considering the extraction

Table 5.3 Comparison of green extraction methods for liquid samples and solutions

	On-line SPE	**SPME**	**SBSE**
Extraction time (min)	15–30	15–60	30–200
Type of analytes best suited for	Semi- to nonvolatile Nonpolar to rel. polar	Very to semivolatile Nonpolar	Very to semivolatile Nonpolar to rel. polar
Complexity	Low	Low	Low
Cost of equipment	Low	Moderate	Rel. High
Repeatability	Excellent	Good	Good
Remarks		Suitable for on-site sampling	Suitable for on-site sampling
	LPME	*MMLLE*	*Surfactant-based extraction*
Extraction time (min)	5–60	30–200	15–30
Type of analytes best suited for	Semi- to nonvolatile Nonpolar to rel. polar	Semi- to nonvolatile Nonpolar to rel. polar	Nonvolatile Nonpolar to rel. polar
Complexity	Low	Moderate	Low
Cost of equipment	Low	Moderate	Low
Repeatability	Moderate	Good	Good
Remarks		Suitable for on-site sampling	

time required, the type of analytes for which these extraction methods are suitable, the complexity, cost, repeatability, and some other remarks.

5.3. EXTRACTION OF VOLATILE ANALYTES; DIRECT THERMAL DESORPTION

For the analysis of volatile or semivolatile analytes, the use of solvents to recover those compounds directly from the samples or from the solid phase in which they have been preconcentrated can be avoided by a thermal desorption [77], thus avoiding the extraction of the nonvolatile matrix components. The challenge is to extract the analytes quantitatively or at least reproducibly, to enable accurate quantitation and to select properly the technique, which depends on the sample type and the analytical information (qualitative/quantitative) and sensitivity required.

Vapor generation at relatively low temperature (less than 200 °C) has been proposed in order to improve the analytical features of vibrational spectrometry [78] and it has permitted the direct determination of ethanol in blood [79] and the discrimination by Fourier transform Infrared (FTIR) spectroscopy of methanol and ethanol in alcoholic beverages [80].

However, it has been in the field of GC and mass spectrometry (MS) where the main developments of direct thermal desorption of analytes have been made on the basis of today's frequently employed systems of headspace and purge-and-trap.

The headspace and purge-and-trap techniques involve simple sample preparation and, as only the vapor phase (gas) is injected and practically no organic solvents are required at all, provide green techniques. Both static and dynamic systems can be used and the headspace can also be sampled using sorption techniques, such as SPME and headspace sorptive extraction (HSSE).

5.3.1 Static headspace

Static headspace involves heating an aliquot of a liquid or solid sample in a sealed vial at a given temperature, for a given amount of time, and then analyzing a portion of the headspace directly by GC or MS. The increase in temperature leads to an increase in the analyte vapor phase concentration. The effects of sample volume, temperature, and modifications to the sample matrix must be considered [81] as reproducible analysis requires exact replication of analytical conditions. In recent years this technique has been applied to benzene, toluene, and xylene (BTEX) monitoring in sediments directly analyzed by MS [82].

5.3.1.1 Headspace-solid phase micro extraction (HS-SPME)

A variation on static headspace is to trap volatile analytes in a SPME fiber hung above the sample. In this sampling mode, extraction is based on the equilibrium between sample matrix, vapor, and fiber. Optimization of the temperature is important because in HS-SPME a higher temperature may result in less analyte deposition with the volatile compounds remaining in the vapor phase.

5.3.1.2 Headspace sorptive extraction

Another variation of static headspace analysis is to concentrate the volatile or semivolatile analytes by using SBSE in the headspace of a sample. This is very similar to HS-SPME, but a coated stir bar is held in the headspace in place of the fiber, being generally more robust [83].

5.3.1.3 Headspace-single drop microextraction (HS-SDME)

In a similar manner to SDME, a single drop of solvent can be suspended from the tip of a syringe in the headspace above the sample to trap volatile analytes [84]. Selectivity can be achieved through the choice of solvent, which must have a boiling point high enough to avoid evaporation.

The main advantage of this method over immersed SDME is that it allows rapid stirring of the sample solution with no adverse impact on the stability of the droplet. Additionally, as in HS-SPME, nonvolatile matrix interferences are reduced, if not eliminated. In this mode, the analytes are distributed among the water sample, the headspace and the organic drop. Aqueous phase mass transfer is the rate-determining step in the extraction process as explained by Theis et al. [85]. Hence, a high stirring speed of the sample solution facilitates mass transfer among the three phases. The use of an internal standard is recommended when manual injection is performed. Practical difficulties with the technique include a limited choice of solvents due to the required viscosity.

5.3.2 Dynamic headspace

In dynamic headspace the sample is continuously purged with an inert gas until all volatile compounds are removed from it. During this step the gas effluent is conducted through a trap either cooled to low temperature or containing an adsorbent to retain the volatile analytes purged from the sample. Once the extraction is completed, the condensed or adsorbed analytes are rapidly released by heating the trap purged with a carrier gas.

5.3.2.1 Purge-and-trap

Dynamic headspace sampling was first described by Teranishi et al, who did not use an adsorber but only a cooled trap to retain the volatile analytes [86]. The purge-and-trap technique started to be utilized more generally after the introduction of Tenax—poly(2,6-diphenyl-*p*-phenylene oxide—as a universal adsorbent for dynamic headspace by Zlatkis et al. [87] in 1973. They also demonstrated the reproducibility achieved by purge-and-trap GC analysis. In short, purge-and-trap offers the advantage of direct thermal volatilization of analytes and the separation from matrix compounds and SPE.

5.3.2.2 Headspace-solid phase dynamic extraction (HS-SPDE)

The SPDE, recently developed by Chromtech (Idstein, Germany), is the first commercially available inside-needle device for headspace analysis using GC–MS. Stainless steel needles (8 cm) internally coated with a 50-mm film of PDMS and 10% activated carbon are used. The dynamic sampling is performed by passing the headspace gas through the tube actively by a syringe. The volume of the stationary phase of the SPDE needle is approximately 4.40 mm^3, compared with the 0.94-mm^3 of a 100-mm PDMS SPME fiber. A great advantage of the SPDE technique over SPME is the robustness of the capillary. In contrast to the fragile SPME fibers, the SPDE device is nearly impossible to damage mechanically. The SPDE has been successfully applied to the analysis of amphetamines and synthetic designer drugs in hair [32].

All the aforementioned strategies (see Figure 5.11) provide green alternatives for the direct determination of volatile and semivolatile compounds and are free from the use of organic solvents and provide safe and relatively low temperature strategies, thus reducing the side effects of chemical methods of analysis.

5.3.2.3 Pervaporation

Analytical pervaporation can be defined as the integration of continuous evaporation and gas diffusion processes in one module. It consists of two steps, firstly the evaporation of volatile analytes or volatile reaction products from a heated donor liquid or solid phase; once the species are sorbed and permeated through a porous hydrophobic membrane, they are desorbed in a cool acceptor solution, or gaseous stream on the other side of the membrane, which may be flowing or stationary. The main advantage of pervaporation is that the sample never comes into contact with the membrane, and so the membrane pores never become blocked [88].

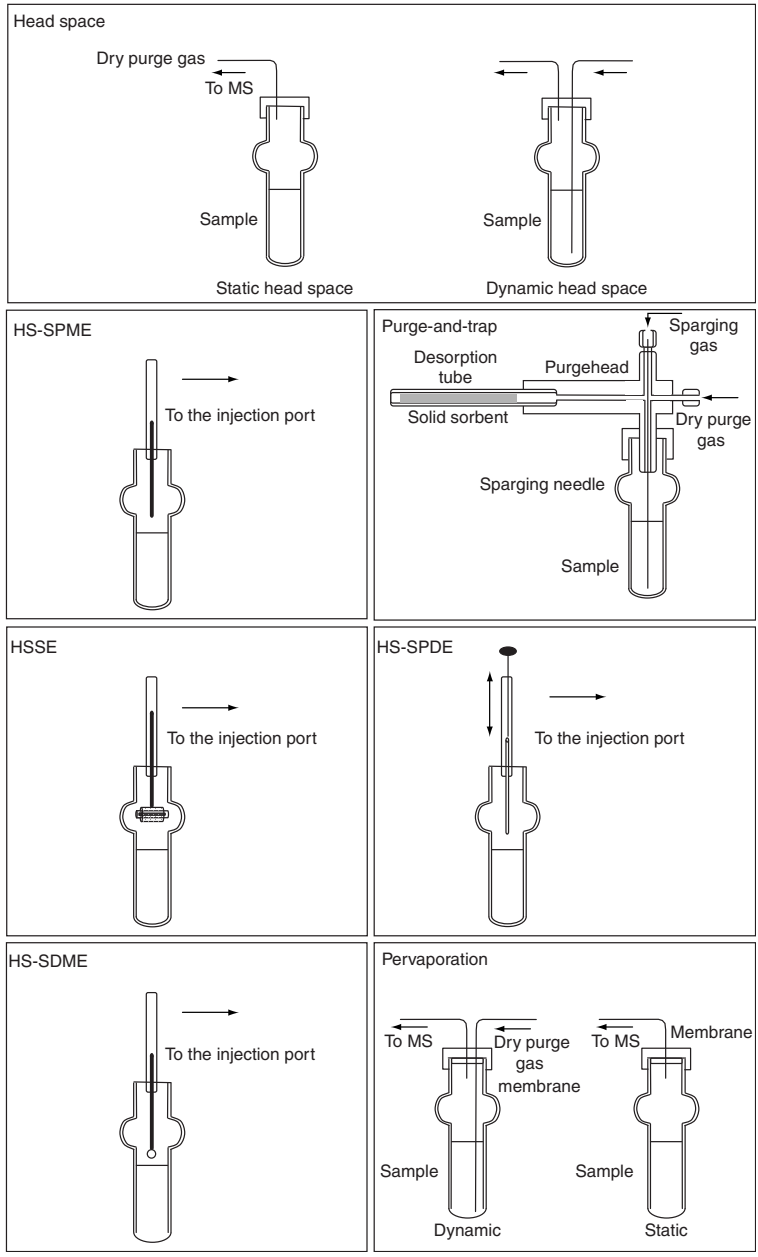


Figure 5.11 Setups employed for direct thermal desorption of analytes from solid or liquid samples.

Analytical pervaporation can be used in a continuous manifold, allowing a great simplification and miniaturization of the preliminary operations, facilitating the automation of the overall analytical process and reducing the organic solvent consumption.

In recent years, pervaporation has been successfully used for the extraction of volatile analytes or volatile reaction products in different areas, such as environmental [89], clinical and pharmaceutical [90] and in food and industrial analysis [91].

5.4. CONCLUDING REMARKS

As compared with the remote sensing and local sample damage strategies discussed in Chapter 4, it is clear that the methodologies discussed in this chapter involve an increase in the time of analysis and additional analytical steps. However, the main objective of the sample extraction and analyte separation and preconcentration processes discussed here is to dramatically reduce the tremendous amounts of acids and/or organic solvents employed in classical sample treatments through wet or dry ashing digestion and Soxhlet extractions and thus the advances in these soft and fast sample treatments could provide valuable tools for greening the methods of analysis, especially those which require the obtention of a solution of the analytes from complex samples.

In general, we can see a tendency from hard to soft sample treatments by reducing the temperature and time of digestion and extraction processes to dissolve the analytes from solid samples. On the other hand, solid phase and miniaturization liquid–liquid extraction are the methodologies of choice for matrix removal and analyte preconcentration.

Special attention must be paid to the generation of the vapor phase for the direct determination of volatile or semivolatile compounds through the use of GC or MS, but also in FTIR. The improved selectivity provided by volatilization processes is of great interest to improve the main figures of merit of analytical methods and additionally they provide a good way to avoid the use of organic solvents.

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CHAPTER 6

Multianalyte Determination Versus One-at-a-Time Methodologies

Contents

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In today's modern society the tremendous demand for analytical information has created a growing need for the development of techniques capable of measuring and quantifying a large variety of different analytes in complex matrices, such as clinical, environmental, or food samples in the same sample run. This has created new challenges concerning a fast analytical response and also new problems concerning the side effects of the increased number of determinations being carried out.

Multianalyte procedures are thus relevant tools in the Green Analytical Chemistry field because they allow analysis of several compounds or elements with a single sample pretreatment while avoiding the use of several chemistries and methodologies for each analyte, thus saving time and resources. Nowadays, multianalyte analysis is a primary goal of analytical laboratories in the regulatory, industrial, and academic environments throughout the world. So, in this chapter, we will focus on available techniques which provide the simultaneous or sequential determinations of many compounds. In addition to vibrational spectrometry, we consider mass spectrometry (MS)-based techniques, like MS, inductively coupled plasma optical emission spectroscopy (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), ion mobility spectrometry (IMS), but specially chromatography, both liquid chromatography (LC) and gas chromatography (GC), and also the well established capillary electrophoresis (CE) techniques with all the modern modifications which have contributed to make the aforementioned techniques more green.

6.1. MULTIANALYTE DETERMINATION IN SPECTROSCOPY

It is clear that the possibility to accomplish simultaneous multicomponent analysis without sample separation in spectroscopy-based methodologies is highly dependent on the selectivity provided by the spectroscopic technique. In this regard, all the methodologies described in Chapter 4 which are able to measure a sample while avoiding any type of sample pretreatment are also capable of providing enough selectivity to perform multianalyte determinations. However, in this chapter we will focus on the possibilities offered by spectroscopy-based procedures to perform multianalyte determinations after a sample pretreatment step such as extraction or digestion.

Spectroscopy techniques like X-ray fluorescence (XRF), infrared (IR) spectroscopy in both the near IR (NIR) and middle IR (MIR) domains, and Raman spectroscopy offer unique tools for multianalyte determination, especially because of the specific molecular information provided

and the large wavelength range covered by their spectra and additionally they are suitable for direct determinations of untreated samples. However, in many cases the lack of sensitivity or selectivity of an analytical procedure necessitates the use of a previous analyte extraction or matrix correction and thus a series of analytes are determined after the preprocessing of samples, taking advantage of the multianalyte capabilities of these techniques.

On the other hand, there are some typical multianalyte spectroscopy techniques, for example, optical emission spectroscopy in plasmas such as ICP-OES, which require a previous dissolution of samples.

6.1.1 X-Ray spectroscopy

XRF spectroscopy measurements of solid samples permit the direct determination of elements present in samples at percentage values. However, in some cases the direct analysis through XRF is just semiquantitative due to problems related to the use of appropriate calibrations, which can compensate matrix and interelement interferences. The prior grinding of samples, their dilution with a nonabsorbing compound like mannitol, bone and/or cellulose [1] or the prior fusion of samples or standard salts with acid, alkaline, or neutral melting materials is therefore common practice in XRF in order to obtain fused glass beads [2] which allow us to avoid calibration problems.

In spite of the extra consumption of energy and time and the risks to operators, it is true that XRF analysis consumes a reduced amount of reagents and provides reduced volumes of solid wastes thus preserving its intrinsic character of an environmentally friendly methodology [3].

6.1.2 UV-visible and IR spectroscopy

The extended spectral range of ultraviolet (UV), visible, and IR regions permits the determination of several compounds in one sample based on their different absorption behavior. Moreover, the IR capability to perform multianalyte determinations is due to the specific molecular information provided, especially in the case of the mid IR region. The broad bands associated with UV and visible regions due to the presence of chromophore and anxocrome groups reduces the multianalyte capabilities of this range to the simultaneous determination of two or three compounds based on direct processing of spectral data at specific wavelengths in zero order or derivative spectra [4] or the use of chemometric data treatments [5].

However, on considering IR methods, both in the mid and near regions, the multianalyte capabilities of transmission or reflection sampling

techniques are quite a bit higher than in the UV-visible range due to: (i) the extended spectral range, (ii) the excellent resolution attainable in this region, (iii) the relatively high number of bands associated with the different vibrational modes of practically all bonds present in a molecule, and (iv) the lower bandwidth of IR bands as compared with the UV ones. All the aforementioned reasons (see the scheme in Figure 6.1) enhance the multianalyte character of vibrational techniques [6] even after sample dissolution or analyte extraction with a suitable solvent [7].

On the other hand, the multianalyte capabilities of spectroscopic techniques have been improved through the development of modern instrumentation based on the use of focal plane detectors [8], diode arrays [9], and Fourier transform (FT) [10].

6.1.3 Raman spectroscopy

The multianalyte capabilities of Raman offer unique environmentally friendly tools for the direct determination of major and minor compounds in solid samples without any sample handling as was discussed in Chapter 4. After an appropriate sample pretreatment the Raman technique allows the determination of a mixture of compounds at levels of a few mg Kg^{-1}. It is thus highly productive and provides a reduced volume of wastes.

FT Raman quantitative analysis of mixtures of well-defined systems with the application of chemometric methods is a common procedure. The application of Raman spectrometry is far less common, but owing to instrumentation development and chemometric advances in the last few decades, it will no doubt be more widely adopted for the qualitative and quantitative analysis of the active ingredients in solid samples [11].

Moreover, measurement of Raman scattering on liquid or diluted samples preconcentrated on solid supports offers an enhanced sensitivity which this technique requires to be able to determine trace compounds [12].

On the other hand, surface enhanced Raman scattering (SERS) involves a prior dissolution of analytes and their absorption on silver or gold colloids on which the Raman spectra is enhanced by a factor near to 1000 [13].

6.1.4 Inductively coupled plasma-optical emission spectrometry (ICP-OES)

In ICP-OES, samples are generally introduced as acid solutions and then nebulized to form a fine aerosol that is transported into the plasma where it undergoes desiccation, vaporization to molecular gases, and

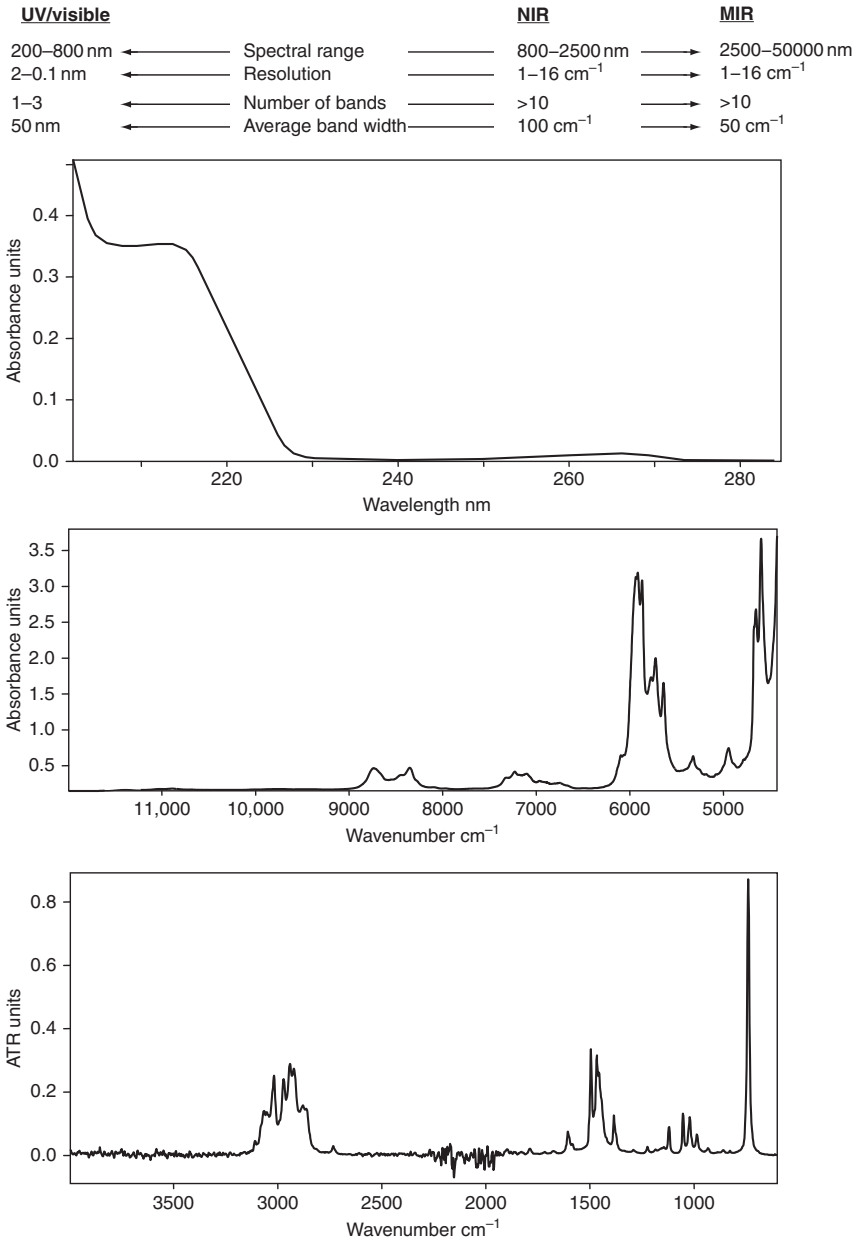

Figure 6.1 Comparison of UV/visible and IR concerning their possibilities for multianalyte determination.

dissociation into atoms that can be ionized. Both atoms and ions become excited in the plasma, reverting to their ground state in with the emission of light, which is measured using an optical spectrometer. All elements present in the radiation source emit their characteristic spectra at the same time. Thus, from the principles of OES it is clear that it is a multielement method, which can be operated in simultaneous or sequential mode [14].

Other advantages of ICP-OES for elemental analysis are: (i) it can be used to measure almost all the elements in the periodic table with detection limits in the range of micrograms per liter to milligrams per liter, (ii) the technique has a wide dynamic concentration range, and (iii) it is able to perform multielemental quantitative analysis in a period of 1 min. So, we can conclude that XRF and ICP-OES are quite complementary with XRF analysis being faster and less labor intensive for major and minor compounds whereas ICP-OES is tremendously sensitive and thus highly appropriate for trace element analysis.

6.2. MULTIANALYTE DETERMINATION IN MS

MS is a powerful analytical technique for both elemental and molecular analysis and the advances in the development of robust, compact, and low price instrumentation have contributed to extend its use in applied analysis. In this section, we will discuss the main aspects of current MS-based methods which have contributed to greening analytical procedures by providing fast multianalyte determination in samples of different types, such as foods, clinical materials, and industrial products.

6.2.1 Mass spectrometry

MS-based methodologies, also known as mass sensors, are based on the introduction of a fraction of the sample into the ionization chamber of the mass spectrometer without any prior chromatographic separation. This provides a mass spectrum which serves as a fingerprint of the injected fraction of the sample. Most applications based on MS have focused on qualitative analysis [15], although quantitative analysis is also possible [16]. A mass spectrum contains enough information for a fast characterization or recognition of a wide range of analytes. However, multivariate statistical techniques are absolutely necessary to extract this information from the spectral fingerprints comprising the complex mixture of volatilized compounds.

The main advantages of MS e-noses are their adaptability in selecting the optimum set of fragment ions, their sensitivity due to rejecting

fragment ions from potentially interfering components, their versatility in that they can be applied to a wide range of samples, and, of course, the absence of the use of organic solvents.

There are different types of inlets for direct sampling as summarized in Figure 6.2: (i) Membrane introduction mass spectrometry (MIMS) where a semipermeable membrane is placed between the liquid or gaseous sample and the vacuum of the mass spectrometer. Analytes of interest pass through the membrane inside the mass spectrometer in a three-step process called pervaporation. (ii) Static headspace [17] which allows a drastic reduction in analysis time, increasing the sample throughput. However, it implies no preconcentration step, (iii) purge-and-trap [18], (iv) solid phase microextraction (SPME) [19], which implies a preconcentration step, and (v) pyrolysis [20]. Additionally the combination of a pyrolysis inlet system with a MS has been employed to study thermal degradation of sample materials.

Direct injection MS has found application in a wide range of analytical fields, such as the study of polymers [21], geochemistry [22], forensic [23], food [24], and environmental areas [25].

Different ionization sources are used in combination with direct injection MS. Electron impact ionization MS is not widely employed for three reasons related to the interference of N_2, O_2, and CO_2, which overwhelm the instrument response at the low mass end of the spectrum; extensive fragmentation of many ions, which makes their identification difficult and, finally, the complications in the quantification due to the choice of inlet conditions and by the variation in the ionization cross-section from one molecule to another.

Chemical ionization (CI) has been used as an alternative ionization source because it is more selective and softer in the ionization process than electron impact, eliminates contributions from N_2, O_2, and CO_2 and yields a reduced ion fragmentation. However, a quantification problem still remains.

This problem has been avoided by the combination of CI with negative ion sources and reaction kinetics to determine concentrations of specific compounds [26]. On the other hand, the modus operandi of proton transfer reaction-MS (PTR-MS) is the CI of a gas sample inside a drift tube through a proton transfer. The main advantages of PTR-MS are its fast response, high detection sensitivity and strength in quantitative determination. PTR-MS is a technique developed for the detection of volatile gaseous organic compounds which has demonstrated great versatility as can be seen from the different applications developed in atmospheric chemistry, food science, botany, medicine, and process monitoring research fields [27].

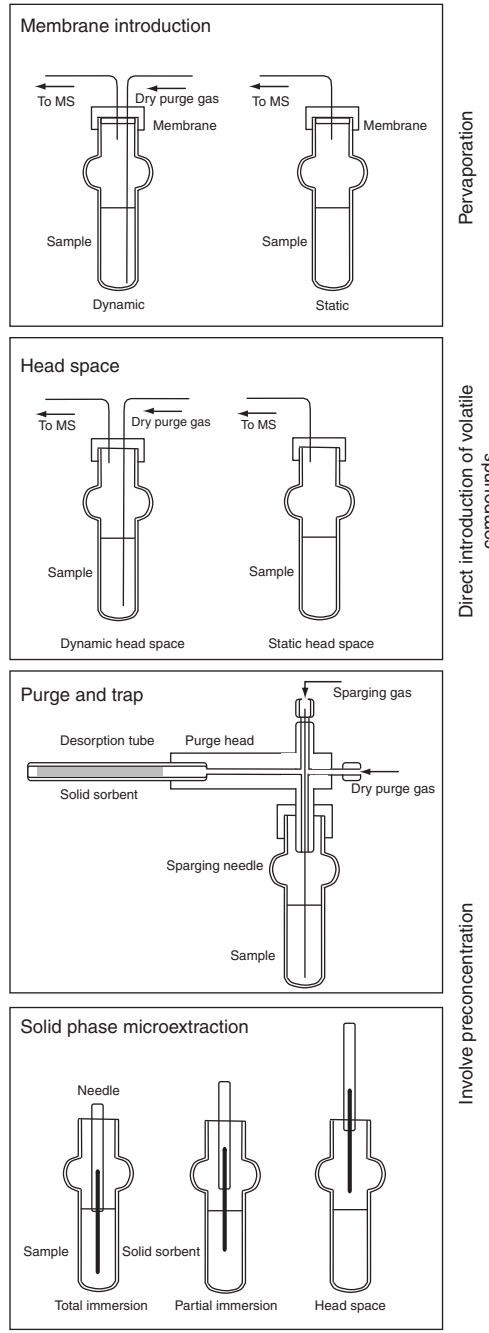

Figure 6.2 Strategies proposed for the multianalyte determination through direct mass spectrometry.

6.2.2 Inductively coupled plasma-mass spectrometry (ICP-MS)

In this technique, the sample must be introduced as a solution into the ICP where it is ionized at the temperature of the argon plasma. The hot sample aerosol emerging from the plasma contains a mixture of atoms in both fundamental and excited states, ions, nondissociated molecular fragments, and nonvolatilized particles which are extracted into the mass spectrometer through a sampler cone followed by a skimmer cone, which provides an appropriate interphase between the ICP working at the atmosphere pressure and the MS working at a reduced pressure. An electrostatic lens system placed behind the skimmer, in the region of high vacuum and the quadruple system, acts as a mass filter. Ions of selected masses are detected in a sequential mode using usually electron multiplier detectors (see Figure 6.3).

In short, ICP-MS has been accepted as a rapid, accurate technique for multielement analysis at trace and ultratrace levels in liquid samples. ICP-MS today is widely used for trace metal analysis due to its wide linear dynamic range and essentially simultaneous multielement analytical capability [28]. In principle, spectral interferences are not as common as in ICP-OES, but sometimes they can be very severe; polyatomic ions, doubly charged ions, refractory oxides, and isobaric ions can affect the selectivity and accuracy of ICP-MS determinations. Furthermore, nonspectroscopic interferences are frequently observed when the sample matrix is very complex, for instance, the effect of sample density or viscosity and low tolerance to high dissolved salts in sample solutions.

The main disadvantages of ICP-MS with regard to the Green Analytical Chemistry concept are those related to the high consumption of energy

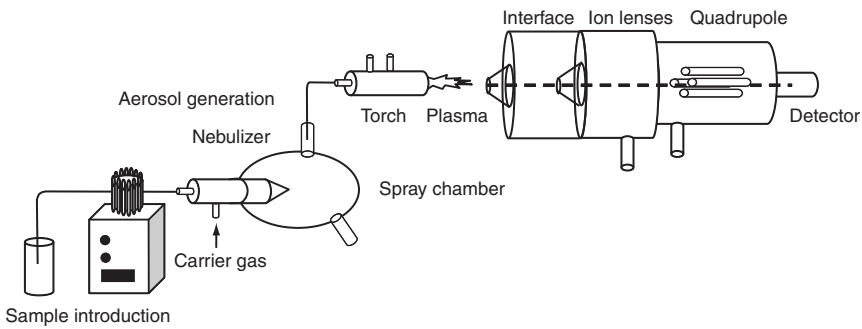

Figure 6.3 Scheme of an ICP-MS system employed for the determination of ultratrace elements in dissolved samples.

and pure gases and that the conventional sample introduction methods are continuous flow-based procedures which need considerable amounts of samples and standards. Usually, when analyzing samples through ICP-MS and ICP-OES, the nebulizer coupled to the spray chamber operates at a flow rate of 0.5–2 mL/min and, taking into consideration the time required to carry out a complete signal reading, the volume of sample required ranges from 1 to 10 mL.

However, the last drawback can be minimized by using micronebulizers [29], which are used to generate stable aerosols at liquid flow rates below 100–200 $\mu L\ min^{-1}$. In comparison to conventional nebulizers operating at delivery rates of the order of a milliliter per minute, the design of micronebulizers must be modified to cope with very low delivery rates.

On the other hand, wastes generated from ICP-based methodologies are metallic vapors that also contain molecular species and solutions with toxic elements at $\mu g\ L^{-1}$ and $mg\ L^{-1}$ levels. Regarding the toxic vapor generation it is true that the hot fumes condensate in the extraction system and toxic molecules cannot reach the atmosphere. On considering the liquid wastes note that during the nebulization process the main parts of the sample or standard solutions go to the waste and the final solution obtained corresponds to a diluted acid mixture of essential and trace elements. The alkalinization of these wastes after adding an appropriate amount of Fe (III) permits the precipitation and coprecipitation of all the elements present in the waste with a drastic reduction of their mass from the kilogram level to a few grams in which the toxic elements are coprecipitated by $Fe(OH)_3$ thus providing a passivation.

The aforementioned process is very cheap and easy and the strong reduction of the waste volumes also reduces the cost of their treatment outside the laboratory.

6.2.3 Ion mobility spectrometry

IMS is an instrumental technique for chemical analysis capable of separating ionic species at atmospheric pressure, and has been the subject of much research work for almost 40 years. It is a method that has been systematically used in practice for 20 years. As can be seen in Figure 6.4, in IMS, solid or liquid samples are directly introduced to the analyzer by thermal desorption, where the resulting vapor is selectively ionized by atmospheric pressure chemical ionization (APCI) to produce ions. Those ions are gated by an electronic shutter into a drift tube. There they collide with neutral gas molecules at atmospheric pressure before striking the collector and generating an

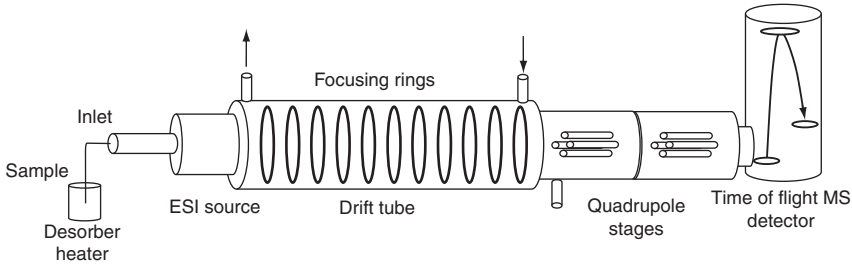

Figure 6.4 Scheme of an ion mobility spectrometer.

electric current. Ions with greater mobility reach the collector electrode earlier than heavier ions having lower mobility. The current induced in the ion collector is an output signal for an IMS detector. Its time dependence is called a drift time spectrum. With regard to the direction of the electric field, it is possible to measure spectra of positive or negative ions [30].

One of the main advantages of IMS is that it can be used with liquid, gas, and solid samples, the amount of sample material required is very small and no sample treatment is required. IMS is an ideal screening tool due to its high sensitivity, instrumental simplicity, low cost, analytical flexibility, real-time monitoring capability, and, moreover, green characteristics.

Moreover IMS can be used as detector in GC [31]. By monitoring positive background reactant ions, it can obtain essentially the same information from IMS as that from a flame ionization detector, whereas by monitoring electron current with nitrogen gas in the spectrometer the obtained response is similar to that of the electron capture detector.

From the standpoint of sample size IMS provides another advantage because for many analytes appropriate sample sizes are in the range of a few picograms to a few nanograms. Although vapor-phase samples are most common, condensed phases can also be introduced in the spectrometer using nebulization/ionization techniques. In this regard, effluents from supercritical fluid chromatography (SFC) [32] and LC [33] have also been introduced into the ion mobility spectrometer.

Another advantage of IMS from the green analytical chemistry point of view is that nowadays several micro IMS instruments are commercially available which consist of a millimetric internal diameter drift channel of less than 50 mm in length and use UV laser radiation at 266 nm or micro helium plasmas as sources, providing short pocket devices able to deliver information about chemical species.

6.3. MULTICOMPONENT DETERMINATION IN CHROMATOGRAPHY AND CE

Chromatography and CE techniques provide excellent tools for the separation and quantification of compounds of the same type or with similar polarity or chemical behavior and, based on this fact, the aforementioned techniques are suitable tools for multiresidue analysis of compounds in foods or for the determination of as many compounds as possible in the same fraction of a sample. In the following sections, the main strategies proposed in the literature to greener the separation multianalyte techniques will be discussed.

6.3.1 Liquid chromatography

Environmentally conscious or "green" chemistry has outgrown the buzzword stage and has begun to provide significant returns on investment in the area of LC. Although it is clear that most separations require the use of a stronger and thus less polar solvent than water even at very high temperatures, it is highly desirable to replace organic solvents in LC separations. This section summarizes the different ways that analytical chemists have developed to help advance green analytical methodology when using LC. Those ways range from organic solvent replacement to more sophisticated solutions such as high temperature or thermal gradients for LC or the use of monolithic columns or two dimensional LC (2D LC) as summarized in Figure 6.5.

6.3.1.1 Replacement of organic solvents

In the book entitled "The HPLC solvent guide" [34], P.C. Sadek stated "To effectively differentiate solvents in terms of the benefit that one offers over another, or the trade-off the chromatographer faces in choosing one solvent versus another, three fundamental factors need to be considered: i) physical properties of the solvent, ii) chemical properties of the solvent (specially in terms of system compatibility and safety aspects) and iii) the effects of the aforementioned properties have on the chromatographic process". Nowadays, it seems to be mandatory adding the effect of the solvent on the environment as a factor to be considered also by the chromatographer to choose the appropriate solvent to perform the analyses.

At present approximately 90% of all LC separations are carried out by reversed phase liquid chromatography (RP-LC), using as mobile phase mixtures of methanol:water (MeOH:H_2O) or acetonitrile:water (ACN:H_2O),

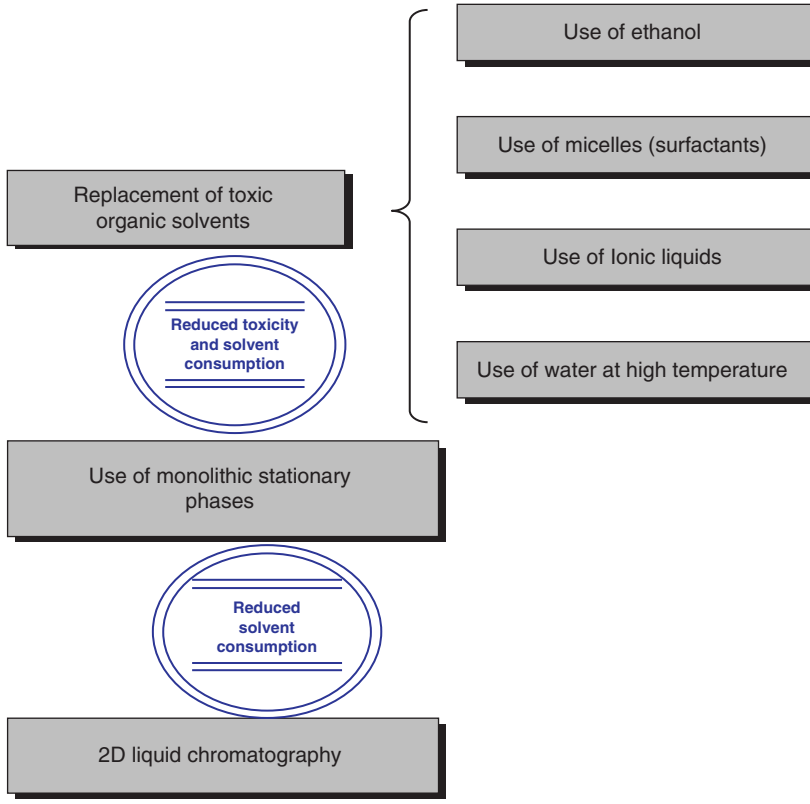

Figure 6.5 Strategies for greener liquid chromatography.

despite the fact that both of these organic solvents are quite toxic. Thus, disposal of these mobile phases requires special treatment steps, especially for acetonitrile, which has to be detoxified through chemical treatment since the combustion route produces highly toxic HCN. Table 6.1 shows the properties of the most commonly used solvents in RP-LC. Methanol and acetonitrile have many favorable properties for RP-LC applications, such as miscibility with H_2O, excellent UV transmission, relatively low viscosity of the aqueous solutions, high purity, and low chemical reactivity. However, they present also some important disadvantages such as high toxicity and very high disposal costs. It is obvious that reduction of the amount of organic solvents is the most advantageous approach to waste management and the replacement of organic toxic solvents by "greener" solvents is one of the goals of Green Analytical Chemistry in this field.

Other mixtures, such as acetone:water, ethanol:water, and isopropanol:water which were used in the past present problems of high pressure, but

Table 6.1 Characteristic of the most commonly employed solvents in RP-LC

Solvent	Polarity index	UV cutoff (nm)	Eluotropic value on C18	EPA applicable waste code
Water	10.2	191	-	–
Dimethyl sulfoxide	7.2	275		D001
Ethylene glycol	6.9	210		J120
Acetonitrile	5.8	190	3.1	U003, D001
Methanol	5.1	210	1.0	U154, F003, D001
Acetone	5.1	330	8.8	U002, F003, D001
Dioxane	4.8	215	11.7	U108, D001
Ethanol	4.3	210	3.1	D001
Tetrahydrofuran	4.0	220	3.7	U213, D001
n-Propyl alcohol	4.0	210	10.1	D001
Isopropyl alcohol	3.9	210	8.3	D001
1-Butanol	3.9	210		U031, F003, D001

Missing value indicate data was not available.
J120, Waste oil/hydrocarbons mixtures/emulsions in water; D001, Ignitability; F section, Hazardous Waste from nonspecific sources; U-section, Toxic (NonAcute) Hazardous Waste; EPA, Environmental Protection Agency.

can be reevaluated because today's newer equipment supports higher pressures. Among organic solvents available with high purity and low absorbance in the UV region, ethanol is an interesting solvent, especially considering that it is much less toxic than methanol and acetonitrile.

A simple and rapid analytical method based on a gradient LC program with ethanol as organic modifier was developed for the determination of phthalates, usually employed in nail cosmetic products [35] and a similar green procedure based on RP-LC with gradient elution using ethanol, 1% acetic acid or 1% sodium acetate buffer pH 4.75, has been developed to determine 18 UV filters in cosmetics [36].

6.3.1.2 Micellar liquid chromatography (MLC) and ion-pairing chromatography (IPC)

In 1976, Knox and Laird [37] developed IPC by adding a small amount of ionic surfactant, below its critical micellar concentration (CMC), in water to the mobile phase in RP-LC. In these conditions, surfactant monomers are adsorbed on the stationary phase as they are able to associate with ionic solutes bearing an opposite charge, thereby increasing their retention time. In MLC, neutral and charged surfactants are used in a concentration exceeding the CMC, which has major implications in both stationary and mobile phases. In 1980, Armstrong and Henry [38] demonstrated the usefulness of replacing organic modifiers in RP-LC with an aqueous micellar solution. In MLC, the aqueous solution of surfactant varies the polarity of the water and changes the characteristics of the stationary phase. Since its origin in 1980, its popularity has been demonstrated by the large number of published papers [39].

The idea of using pure micellar solutions as mobile phases in RP-LC is very attractive owing to the lower cost and toxicity of surfactants as compared with the solvents, and their reduced environmental impact. Using this approach, a green chromatographic analytical method for determination of Tartrazine, Brilliant Blue, and Sunset Yellow in food samples was proposed [40]. The method is based on the modification of a C18-column with a 0.25% (v/v) Triton X-100 aqueous solution at pH 7 and the usage of the same surfactant solution as mobile phase without the presence of any organic solvent modifier.

However, in many cases, the presence of an organic modifier, such as a short or medium chain alcohol, is necessary to reduce the retention times in MLC and to improve peak efficiency and resolution [41]. For instance, a simple isocratic reversed-phase method for five antioxidant, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl, octyl, and dodecyl gallates determination using cationic or anionic surfactants and short-chain alcohols in the mobile phase has been developed [42]. In a similar way, a green chromatographic analytical method for determination of fat-soluble vitamins (A, E, D_3, and K_1) in food and pharmaceutical supplement samples was developed by modifying a C18-column with sodium dodecyl sulphate (SDS) and using the same surfactant solution as mobile phase in the presence of 15.0% (v/v) butyl alcohol as an organic solvent modifier [43].

6.3.1.3 Use of ionic liquids as mobile phase modifiers in RP-LC

In recent time, room temperature ionic liquids (RTILs) like ethylammonium nitrate imidazolium halogenaluminated salts or molten salts are receiving much attention as environmentally benign solvents for organic

chemical reactions, separations, and electrochemical applications. RTILs have been used as novel materials in various separation methods, such as stationary phases in GC, buffer electrolytes in CE and mobile phase additives in LC.

The main problem of the RTILs to be used as mobile phase modifiers in LC is their lack of transparency in the UV region. Furthermore, the density and viscosity of RTILs is appreciably higher than usual LC solvents. However, when used as additives in RP-LC mobile phases to improve basic compounds separation, both the anionic and cationic components of RTILs contribute to solute retention and peak shape improving in addition to their simple "salting-out" and ion pairing effects. RTIL cations can interact and compete with silanol groups (specific electrostatic interactions) on the alkyl silica base surface with the polar group of the analytes, improving the peak shapes and decreasing the retention time of the analytes. At the same time, the nonpolar alkyl groups of the stationary phase can interact with different alkyl groups of the heterocyclic ring or quaternary cation (unspecific type of interactions and hydrophobic interactions). Table 6.2 shows some of the characteristics of ionic liquids employed in RP-LC.

Using RTILs and without adding any volatile organic solvents, a green chromatographic procedure was developed to determine octopamine, synephrine, and tyramine. The problems of the adrenergic amines separation, such as band tailing, low retention, and low resolution were solved successfully by using RTIL in aqueous solution [44]. The effect of 1-ethyl-3-methylimidazolium tertafluoroborate ([EMIM][BF4]) was the best in the six investigated RTILs.

To learn more about the use, the properties and the potential applications of ILs as modifiers in LC, see the two recently published interesting reviews regarding the properties of RTILs in analytical chemistry [45] and especially focusing on their capability of modifying mobile phases [46].

6.3.1.4 High temperature and thermal gradients for RP-LC

The use of solvent heating in LC has been described by a variety of authors [47–50] and it is nowadays an area of increasing interest. The advantages of applying elevated temperature in LC separations are numerous, the most obvious being the gain in speed and reduced backpressure. Due to the decreased retention, the amount of organic modifier in the mobile phase can be reduced significantly, and in some cases can be eliminated completely, resulting in pure aqueous mobile phases. Hot water used as an eluent exhibits reduced viscosity, increased ability to dissolve

Table 6.2 Characteristic of the ionic liquids employed as mobile phase modifiers in RP-LC

Ionic liquid					
Cation	**Anion**	**Melting point (°C)**	**Density (25 °C)**	**Viscosity (25 °C cPa)**	**Toxicity EC50 (μM)[a]**
Tetrafluoroborate (BF_4)	EMIM	6	1.248	66	–
	BMIM	−82	1.208	233	2512, 707[b]
	HMIM	−82	1.208	310	–
Hexafluorophosphate (PF_6)	BMIM	10	1.373	400	1318
	HMIM	−61	1.304	800	–
Halogenate	EMIM Cl	89	1.12	Solid	13573[b]
	BMIM Cl	65	1.10	Solid	2884, 2224[b]
	HMIM Cl	−75	1.05	7500	753[b]
Halogenaluminates	EMIM $AlCl_4$	9	1.3	20	–
	BMIM $AlCl_4$	−10	1.24	26	–

EMIM, 1-Ethyl-3-methyl imidazolium; BMIM, 1-Butyl-3-methyl imidazolium; HMIM, 1-Hexyl-3-methyl imidazolium.

[a]The effective concentrations of various ionic liquids and alkali salts to the alga, *Selenastrum capricornutum*, in 96-h chronic toxicity tests. Data extracted from C.-W. Cho, T.P. Thuy Pham, Y.-C. Jeon, Y.-S. Yun, Green Chem. 10 (2008) 67–72.

[b]The effective concentrations of various ionic liquids and alkali salts to the alga, Oocystis submarina (green alga). Data extracted from A. Latała, M. Nedzi, P. Stepnowski, Green Chem. 11 (2009) 580–588.

nonpolar compounds, and decreased polarity. All of these properties result in the liquid having properties comparable to an organic solvent. The reduced backpressure also enables the replacement of methanol or acetonitrile by ethanol. Water and ethanol are both nontoxic liquids and analysis with these substances can be considered as "green chromatography." In summary, the combination of the previous factors leads to the conclusion that increasing the temperature in LC is a powerful tool to reduce its environmental impact in spite of the fact that the use of high temperatures rather than room temperature increases the energy consumption.

A factor of more concern in using high temperatures in conventional LC is the stability of the stationary phases. In 2004, an issue of the *Journal of Chromatography A* devoted to developments in stationary phases, including phases for high temperature, was published [51]. Silica-based stationary phases usually are stable at temperatures up to 60 °C and in some instances up to at least 90 °C. Some novel reverse phase material can even be used up to 120 °C. A new generation of silica-based phases has recently become available that is stable at temperatures up to 200 °C under certain reverse phase conditions [52].

Stationary phases with the highest temperature stability are based on materials other than silica, for example, graphitized carbon types, zirconium oxide-based phases, and polystyrene/divinylbenzene phases. Such columns are stable at temperatures of 150 °C or higher provided that all polyether ether ketone (PEEK) present in the column hardware is replaced by stainless steel.

Of great interest is the review by Sabine Heinisch and Jean-Louis Rocca entitled "Sense and nonsense of high-temperature liquid chromatography" where the different effects of high temperatures under reversed-phase conditions have been evaluated and described, giving an overview of related papers and the most recent studies on the use of high-temperature LC [53].

Let us consider in detail the possibilities and limitations of the technique called either "superheated water chromatography", "subcritical water chromatography," or "chromatography in very hot water", depending on the authors. As it has been mentioned above, at high temperatures water has many of the characteristics of aqueous-organic eluents in terms of eluotropic strength, its polarity being controlled by changing temperature [54]. RP-LC using superheated water as the mobile phase, at temperatures between 100 and 250 °C, offers a number of advantages for the analyst such as improved detection, allowing UV spectra to be

monitored down to short wavelengths, as well as a compatibility with the universal flame ionization detection and MS as well as being an environmentally clean solvent, thus reducing solvent usage and disposal costs. The use of pure water as mobile phase has been investigated in depth, mostly at temperatures higher than 100 °C [55,56].

Temperature programming could be an alternative to phase gradient elution. Undoubtedly, temperature programming is not capable of fully replacing solvent programming. However, in superheated water a temperature gradient represents the only way of increasing the eluent strength during the analysis. A review entirely dedicated to temperature programming and its related problems of instrumentation has been published in 2004 [57]. More recently, Vanhoenacker and Sandra [58] reviewed some applications.

The use of capillary columns is usually recommended for temperature programming due to a better heat transfer with small inner diameters [59]. However, there are some specific problems associated with temperature programming which limit its applications. For instance, the compositions needed for a gradient is consistent with a range of temperatures wider than 200 °C which unfortunately is not possible to cover with commercially available equipment. Furthermore, the heat transfer in liquids is very slow compared with that in gas and the heating of the steel associated with LC columns is also too slow as well. As a result, temperature programming is mostly used to improve isocratic isothermal analysis rather than to replace gradient elution conditions.

6.3.1.5 Monolithic and nonporous stationary phases

Monolithic columns are a recent acquisition of analytical chromatography. Monoliths can be described as integrated continuous porous separation media without interparticular voids. The mobile phases are forced through the porous monolithic media, which results in a convective flow and consequently an enhancement of the mass transfer rate.

The essential advantages of monolithic columns stem from the possibility to control and optimize separately the average sizes of the throughput channels or macropores and of the porons (lumps of porous silica) while these two dimensions are closely related to the average diameter of the particles packed in conventional columns.

Combined with the fast mass transfer kinetics across monolithic columns, this high permeability facilitates the achievement of fast separations, hence of a high analysis throughput. Alternatively, long monolithic

columns can be used to achieve high column efficiency, hence separations exhibiting high resolution. However, the number of monolithic columns that are currently commercialized is still pitifully small compared to packed columns.

From the environmental point of view the use of monolithic columns provides a strong reduction of the analytical wastes based on the fast separation of compounds by a factor between 4 and 10 and thus on the reduction of organic solvents consumption.

6.3.1.6 2D Liquid chromatography (2D LC)

2D LC is defined as the technique in which two independent liquid phase separation systems are applied to a sample. 2D LC can be done by "heart-cutting" chromatography, which implies the transfer of only a portion of the eluate of the first column to the second column, or by sequentially transferring all the first dimension eluent in many small aliquots, to the second dimension. This is also known as "comprehensive" 2D chromatography.

Because of its high resolving power, 2D LC has received a great deal more attention over the past few years, especially for dealing with complex samples.

One requirement that should be met in 2D LC is that the second dimension has to be fast. This requirement for much faster separation of the second dimension than the first was realized in two dimensional GC (2D GC) a relatively long time ago. Fortunately, high speeds in GC have been well known for many years and are relatively easier to implement in GC than in LC, from both the theoretical and practical perspectives.

It is obvious that to do fast 2D LC one must speed up the separation on the second dimension column since this separation is repeated many times and thus it is effectively the rate-controlling step. One way to achieve this is by increasing the temperature. However, a thermally stable phase is an absolute necessity for success. There are many thermally stable normal phases with silica, alumina, zirconia, or titania as the substrate, but unfortunately, there are not many commercially available, thermally stable RP-LC columns.

6.3.2 Gas chromatography

GC can be considered as the greenest of the chromatography modalities because it only consumes sample and pure carrier gases. However, the extraction of those gases from the atmosphere carries a measurable

carbon footprint and GC is only limited to samples that are thermally stable and easily volatilized.

Since the introduction of GC in 1952 [60], there has been an ongoing interest in improving separation speed. Increasing separation speed in capillary GC is, in the first instance, dictated by the problem at hand, the primary objective being the complete separation of the compounds in a mixture. Most of the time, the capillary column plate number is too high for a given separation problem, the solutes being too well separated, paying for this "over-resolution" in separation speed.

In a really interesting discussion article entitled "Some Remarks on Gas Chromatographic Challenges in the Context of Green Analytical Chemistry" [61], Wardencki and Namieśnik presented three approaches for the implementation of principles of green chemistry into GC; (i) solventless sample preparation techniques, (ii) preconcentration of pollutants followed by thermal desorption, and (iii) high-speed (fast) GC. The first two approaches are focused on the reduction of the sample treatment, the third on the instrumental technique.

Speeding up GC analysis provides unquestionable benefits over conventional GC, such as high laboratory throughput, good analytical precision by the possibility of more replicable analyses, and, of course, reduced GC operating costs, including a reduction of energy consumption.

In the available literature different ways to further increase the speed of capillary GC analysis have been reported, and they can be classified into three general routes; (i) minimization of the resolution to a "just enough" value, decreasing the length of the column; increasing the carrier gas flow-rate above the optimum; using higher isothermal temperature, higher initial/final temperature, and higher temperature programming rates or using pressure/flow programming, (ii) maximization of the selectivity of the chromatographic system by application of coupled columns, 2D GC, or by selective detection (i.e., MS detection), and (iii) implementation of the method that reduces the analysis time at constant resolution (i.e., reducing the column inner diameter, using hydrogen as carrier gas or applying vacuum-outlet conditions).

6.3.2.1 High-speed GC using narrow bore columns

Technological innovations in GC instrumentation have greatly contributed to making high-speed capillary GC popular. The wide majority of high-speed GC applications have been carried out by means of reduced I.D. columns [62].

The reduction of column I.D. is usually combined with other strategies such as modification of the column geometry or its operating parameters to optimize the speed of sample analysis. The narrower I.D. leads to the shorter analysis time at constant resolution. Thus, the increase of analysis speed does not compromise the separation efficiency. The decrease of column diameter results in a proportionally decreased value of minimum plate height [63]. Therefore, the column length can be decreased by the same factor in order to yield the same plate number in a shorter time.

However, the drawbacks of the methodology are those related to a much lower sample capacity, which results in higher limits of detection (LODs) and quantification (LOQs), column performance deterioration represented by peak broadening, adsorption and tailing [64], and injections of complex extracts very quickly deteriorate the performance of microbore columns. The effect of sharper peaks may improve detectability for injection of clean samples and standards, but it does not hold true in real-world analysis where the need for high spectral acquisition rates limits the degree of selectivity that can be achieved, thus chemical noise from the matrix is still likely to be the limiting factor.

On considering the environmental benefits it is clear that we must focus on increasing the possibilities of real time analysis and the consideration of additional analytes in the same sample.

6.3.2.2 Fast temperature programming

Faster temperature programming is an option used to minimize resolution to a "just enough" value. Increasing the temperature programming rate is a simple way to increase the speed of the GC separation without the need for special instrumentation. Like the use of short or narrow columns, fast temperature programming is used in combination with other strategies to reduce the analysis time. In practice, fast temperature programming is achieved by resistive heating, where an electrical current is employed to heat a metal which contains the analytical column, the temperature being determined by resistance measurements. Commercial systems are available that are able to achieve temperature programming rates up to 20 °C s^{-1}. The main advantages of these systems are related to a fast cool down rate, resulting in a higher sample throughput, and very good retention time repeatability. A practical drawback of the approach is the difficulty in accessing the column to perform routine maintenance.

6.3.2.3 Low pressure gas chromatography (LP-GC)

As has been previously mentioned, speeding up analysis time has always been a need in GC, because shorter analysis times provide higher

throughput and reduced costs and wastes (in this case reduced pure gas consumption). In GC, the application of a vacuum-column outlet is an attractive way to speed up the analysis which, however, has not been extensively studied [65].

The main advantages of using LP-GC can be summarized as follows: First, using increased velocities and shortening the column, it is possible to reduce the analysis time by a factor of 3–10. Second, LP-GC allows analytes to elute at much lower temperatures (30–60 °C lower than those used in GC), and is beneficial for the analysis of thermally labile compounds and offers a reduction of energy consumption. Low elution temperatures of target analytes result in low signal intensity of the column bleed and less interferences with masses of the target analytes. The last advantage is high sample loadability, which is of particular interest if traces have to be analyzed.

6.3.2.4 Comprehensive 2D GC (GC × GC)

GC can typically separate 100–150 analytes in one run. However, it is sometimes not enough to separate the individual constituents of many different types of samples. One way to improve the separation power of a GC system is to couple two independent columns.

Comprehensive 2D GC has attracted the attention of the scientific community since the first study was published in 1991 [66]. Nowadays, petrochemical, food, air, and environmental analysis are the main areas of research using 2D GC [67].

When two similar columns are used, the large majority of applications of 2D GC are of the heart-cutting type. It means that only one, or a few, narrow fraction(s) of the eluate from the first column is/are transported to the second column for further separation. However, if the screening of an entire sample is required, 2D GC becomes a laborious and time-consuming technique, the analysis of all fractions and reconstruction of the chromatograms being the major problems.

The alternative is to separate the sample on two different columns, keeping the fractions narrow to avoid loss of information gained during the first separation and ensuring that the total 2D separation is completed within the run time of the first-dimension analysis. Thus, 2D GC provides substantially improved resolution for all sample constituents with no loss of analysis time.

A common phase selection strategy uses a first dimension separation of analytes according to boiling point and the second dimension separates

analytes according to polarity. In the second dimension a very fast separation should be obtained in the order of seconds.

6.3.3 SFC on packed columns

SFC has attracted wide interest in analytical chemistry as a useful separation technique [68]. SFC is considered to be a type of normal-phase chromatography that exhibits unusual retention behavior and selectivity relative to traditional normal phase LC (NP-LC) and RP-LC. SFC has several advantages over traditional LC, such as increased diffusivity, which results in sharper peaks and, thus, increased resolution; reduced viscosity, which decreases the pressure drop across the column, allowing faster separation and lower solvent consumption. The procedures of SFC are thus greener than those based on LC.

There are a number of possible fluids which may be used in SFC as the mobile phase. However, based on its low cost, low interference with chromatographic detectors, and good physical properties (nontoxic, nonflammable, low critical values) carbon dioxide is the standard fluid employed. The nonpolar character of carbon dioxide favors the solubility of hydrophobic compounds in the mobile phase, it being possible to replace RP-LC by SFC with alkyl bonded stationary phases. Moreover, numerous separations of polar compounds can be also performed on polar stationary phases in SFC, replacing NP-LC. However, very polar compounds, inorganic ions and proteins cannot be separated and analyzed by SFC.

So, the main disadvantage of carbon dioxide is its inability to elute very polar or ionic compounds. However, this can be overcome by adding a small portion of a second fluid, called a modifier fluid. This is generally an organic solvent which is completely miscible with carbon dioxide (alcohols, cyclic ethers) but can be almost any liquid, including water. The addition of the modifier fluid improves the solvating ability of the supercritical fluid and sometimes enhances selectivity of the separation. Mixed-phase SFC solvents are theoretically not as environmentally benign as single-phase CO_2, but they are significantly easier to dispose of or to recycle than mixed organic-aqueous LC solvents.

Another challenge lies in the variety of columns available, which while improving, is still limited. The column contains a highly viscous liquid (called a stationary phase) into which the analytes can be temporarily adsorbed and then released on the basis of their chemical nature. Different types of stationary phases are available with varying compositions and

polarities. Two types of analytical columns are used in SFC, packed and capillary. Packed columns contain small deactivated particles to which the stationary phase adheres. On the other hand, capillary columns are open tubular columns of narrow internal diameter made of fused silica, with the stationary phase bonded to the wall of the column.

6.3.4 Capillary electrophoresis

Special attention should be paid nowadays to CE, which provides the opportunity to move toward a greener analytical chemistry by replacing many chromatographic reference methodologies that consume large volumes of solvents. CE is a separation technique in which analytes are separated based on their ability to move through a conductive medium, usually an aqueous buffer, in response to an applied electric field.

CE is a very attractive separation method because of its low sample and electrolyte consumption, short analysis time, high efficiency, ease of operation, and automation. Generally the use of free solution CE, in which simple electrolytes such as phosphate or borate are employed with no additives, is enough to achieve most separations. CE is also a versatile separation method as it can be applied to a wide variety of analytes because of the different modes that can be used [69].

Micellar electrokinetic chromatography (MEKC) was designed to separate neutral solutes which were impossible to separate by FSCE. In MECK, the separation occurs via partitioning into surfactant micelles where the extent of partitioning is related to solute solubility. However, MEKC has extended its applicability to the analysis of charged compounds, providing an increase of the selectivity compared to free solution CE including solubility and ion-pairing factors.

Microemulsion electrokinetic chromatography (MEEKC) has recently appeared as a complementary technique to MEKC, the principal difference being that solutes interact with an oil droplet in MEEKC [70].

Capillary isotachophoresis (cITP) is a technique in which the analytes are focused along the capillary based on their mobility compared to leading and terminating added solutes. The sample analytes focus to match the concentration of the added solutes. In this way, the concentration of the solutes can be dramatically increased inside the capillary, which is useful to preconcentrate samples prior to conventional CE in multidimensional separations.

In recent years, routine CE methods have been applied in different scientific areas and environments and it has become recognized as an

acceptable and reliable alternative to traditional analytical methods. Thus, it is clear that CE often offers an alternative to LC and, although it is not widely implemented, CE is well adopted in niche areas such as chiral separations and indirect UV absorbance determinations of inorganic anions and metal ions.

Technological developments have continued to occur in CE, such as multiplexing and miniaturization, offering significant advantages over chromatographic methods. The relatively simple mechanism of separation in CE, migration speed based on solute size and charge under the influence of applied voltage, allows very successful miniaturization to be achieved on chip-based devices. Furthermore, the multiplexed CE instruments allow simultaneous separation of 96 samples/standards, which has significantly impacted on analytic speed and throughput.

In this regard, multiplexed and microchip CE systems offer and will continue offering key operational advantages over LC, such as speed and simplicity of analysis and huge sample throughput. Thus, it can be assumed that these factors, in combination with the green capabilities of CE, will serve to push CE more into the forefront of analytical chemistry and that it will gradually replace LC methods.

Figure 6.6 provides a scheme of the comparison of LC and CE based on the green analytical chemistry principles.

6.3.4.1 Multiplexed CE instruments

One of the main advantages of CE is the ease of multiplexing, for instance using several capillaries simultaneously within a single instrument. Parallel analysis can be achieved in CE by using instruments containing arrays of capillaries. Each capillary can be loaded with sample and then analyzed. This multiplexed format does not have the precise fluid pumping and high pressure requirements of LC.

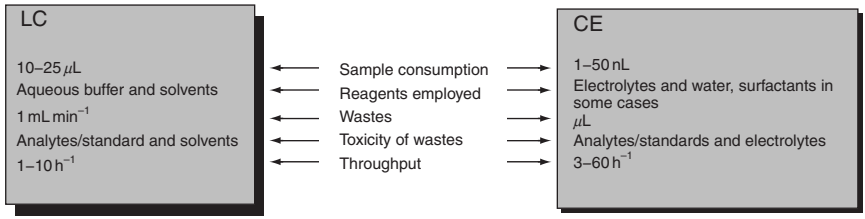

Figure 6.6 Comparison of HPLC and CE based on the green analytical concepts.

Multiplexed CE offers a number of advantages over conventional techniques such as shorter analysis and method development times, reduced consumable and solvent expenses, simplicity of operation and the possibility of implementing a single set of operating conditions for the analysis of several different samples. However these operational advantages have not resulted in the widespread implementation of multiplexed CE in routine analytical laboratories where traditional LC continues to predominate.

The introduction in 1999 of a 96-capillary multiplexed CE-UV absorption detection instrument [71] provided a generalized approach for high-throughput analytical applications. The performance of this system has been demonstrated in several applications including comprehensive peptide mapping [72] and DNA sequencing [73], enantiomeric separations [74], enzyme activity [75], organic reaction monitoring [76], and different applications in pharmaceutical analysis [77] and drug discovery areas [78].

6.3.4.2 On-line sample preconcentration techniques

One of the disadvantages of CE is generally thought to be the poor concentration sensitivity of photometric detectors, which are the most popular among CE detectors. The volume of the injected sample zone in the capillary is very small (nL) to avoid deterioration of the separation efficiency. The capillary detection causes a short pathlength of the light, equal to the diameter of the capillary, and its detection capability is 100 times less than conventional detection in LC. Thus, the sensitivity of the UV detection in CE is much lower than in LC.

One way to solve the problem of low sensitivity in CE is by using on-line sample preconcentration techniques. The principle and background of most on-line preconcentration techniques is based on the velocity change of the analytes between the sample zone and the separation solution zone and will be reviewed in this chapter considering: (i) field-amplified sample stacking (FASS), (ii) large-volume sample stacking, (iii) dynamic pH junction, (iv) isotachophoresis stacking, and (v) sweeping as on-line preconcentration strategies in CE (see Figure 6.7).

The simplest preconcentration technique is FASS which only requires the use of a background electrolyte (BGE) of higher conductivity than the sample. The fact that a local electric field is inversely proportional to conductivity results in a high-electric field in the sample zone, injected hydrodynamically, providing a high-analyte velocity in this low-conductivity region. Consequently, when analytes penetrate into the low-electric field of the BGE zone, they slow down and are concentrated into narrow bands. The conductivity

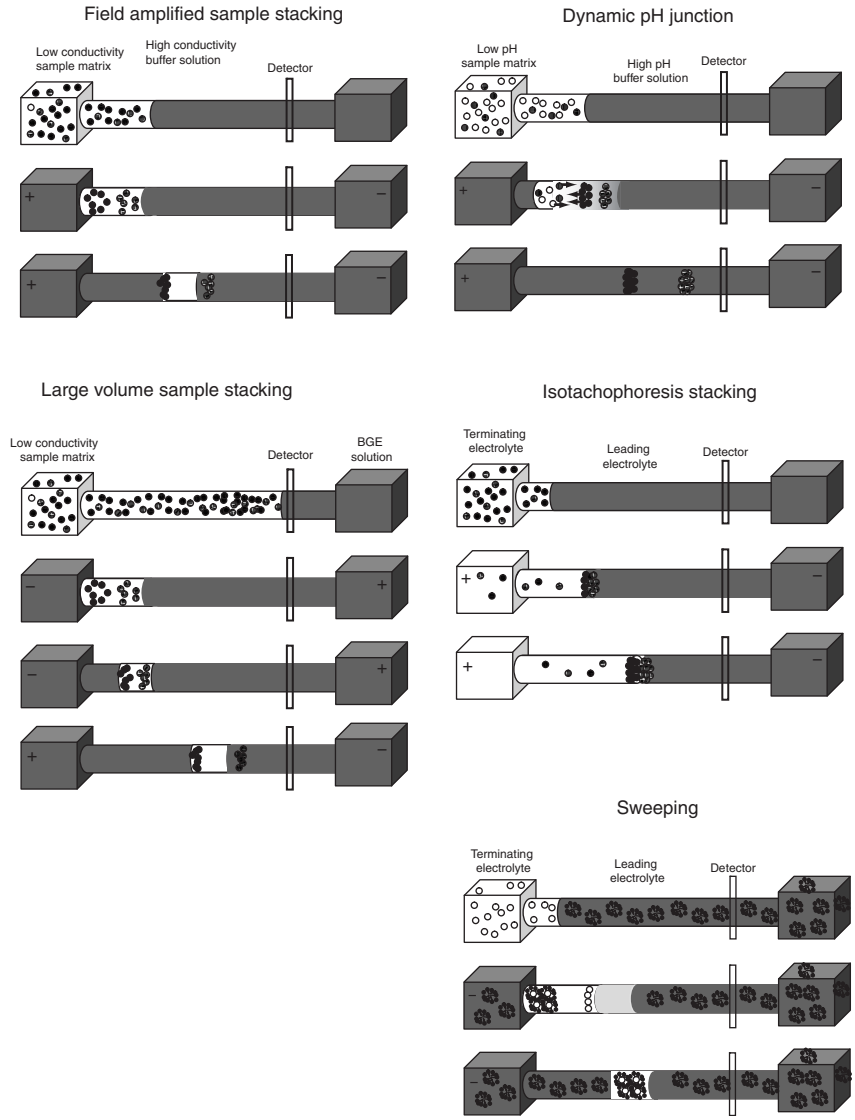

Figure 6.7 Scheme of the different on-line preconcentration strategies proposed in the literature for CE.

ratio between the BGE and the sample strongly influences the performances of FASS with respect to sensitivity enhancement and resolution.

Several techniques have been developed by utilizing the field-enhanced sample stacking preconcentration principle technique, such as

the normal stacking mode (NSM), which is the simplest among the techniques but concentration efficiency is not really high (10-fold) [79].

To obtain a higher concentration efficiency than in NSM, the large volume sample stacking (LVSS) technique is available, providing more than 100 times preconcentration. In LVSS, the sample matrix must be removed from the capillary before CE separation. In order to remove sample matrix, two techniques are available, with or without polarity switching.

In the switching mode, a negative voltage is first applied to remove the sample matrix for the preconcentration of anions under a strong positive electroosmotic flow (EOF), after that the polarity is switched to positive. To successfully concentrate the analytes, their electrophoretic velocity in the sample solution must be faster in the opposite direction than the velocity of EOF. For the preconcentration of cations by LVSS, an additive is used to reverse EOF.

LVSS without polarity switching must be performed under low EOF having an opposite migration direction to the electrophoretic migration of the analyte which is obtained under acidic conditions.

The main disadvantage of the methodology is that only anions (or cations) can be concentrated and separated in LVSS in a run [80].

The junction between two electrolytes with different pH is formed in dynamic pH junction and the preconcentration is achieved due to a divergence in the dissociation of the analytes in the electrolytes. When an electrical potential was applied, the H^+ and OH^- ions migrated toward each other, converting the original sample zone into a low-conductivity zone through which the analytes could migrate quickly. The dynamic pH junction technique utilizes significant changes in ionization states of the analytes or electrophoretic velocities between different pH values. For instance, a weakly acidic analyte dissolved in an acidic matrix is injected as a long plug and the capillary is filled with an alkaline background solution. After application of positive voltage at the injection end, the acidic sample zone is gradually titrated by the hydroxide ion of the background solution and the analyte will be ionized in the neutralized zone. The negatively ionized analyte will migrate toward the anode until it enters into the acidic sample zone, where it will be protonated again to neutral and stop the electrophoretic migration. Thus, the weakly acidic analyte can be focused at the neutralization boundary during the neutralization of the sample zone [81].

In isotachophoresis stacking, the sample zone is sandwiched between two electrolytes, the leading and terminating electrolytes, which consist

of co-ions with, respectively, faster and slower electrophoretic mobilities than that of the analytes. Upon voltage application, a potential gradient and a constant current develops over the three (terminating, sample, leading) zones. In each zone, the field strength is inversely proportional to the mobility of the ions within that zone, and therefore ions in all three zones migrate at the same velocity. When a steady state is reached, the analytes position themselves in discrete bands according to their mobilities. The concentrations of the leading and tailing buffers determine the overall current, and in turn the final concentration of the analytes in each band [82].

An alternative approach to stacking (known as sweeping) involves the use of charged additives; such as surfactants, to interact with sample solutes causing a focusing inside the capillary. The sweeping technique was introduced to preconcentrate and, thus, to improve the sensitivity, either for neutral or charged molecules. In principle, it is based on partitioning of analytes between the nonmicelle electrolyte and the pseudostationary micellar phase; analytes are swept and then they are separated by common MEKC [83].

A very interesting way to concentrate analytes is using a combination of the main preconcentration techniques. As has been mentioned, each on-line concentration technique is itself useful. However, the combination of two of the proposed techniques is more efficient in increasing detection sensitivity.

6.4. MS AS DETECTOR IN SEPARATION SYSTEMS

MS has become the most powerful detector for separation science. MS detection is capable of providing a high degree of specificity for reliable identification and quantification of every compound of interest. The main advantage of the MS detector is that selectivity and almost universal applicability are not mutually exclusive. In principle, the selectivity of an MS detector can be varied from nonselectivity (all ionizable compounds are detected) to extremely high selectivity for only one compound in special applications. MS detection allows a reduction in sample cleanup and chromatographic separation compared to the use of less selective detection methods. Thus, the combination of chromatographic separation with MS and MS^n detection yields a particularly specific detection, which is a major reason for the popularity of the technique.

LC is the most commonly used technique for coupling with MS. Due to its universality, selectivity, and sensitivity, MS is considered the gold

standard detector for LC. The first coupling of LC to MS was performed in the 1970s but became widely used only with the appearance of atmospheric pressure ionization (API) sources in the early 1990s. API sources provide high sensitivity, stable performance, and good repeatability and, as a result, have almost totally replaced the earlier interfacing techniques such as continuous flow fast atom bombardment, thermospray, and particle beam. Electrospray ionization (ESI), APCI, and atmospheric pressure photoionization (APPI) are currently the most widely employed API sources [84]. ESI is particularly adapted to the analysis of polar molecules, and its ionization occurs in the liquid phase, while APCI and APPI allow the ionization of less polar molecules in the gas phase.

A typical RP-LC mobile phase contains a high percentage of organic solvent and a low concentration of buffer ions, making it highly volatile for efficient desolvation and analyte ionization. However, MS detection is not compatible with all solvents and eluent additives that are commonly used in LC separation. For example, nonvolatile mobile-phase additives cannot be used in practice since they cause excessive background noise and rapid contamination of the ion source. Thus, the selection of eluent composition for LC–MS is usually a compromise between LC separation and ionization efficiency.

The limitations of LC–MS are mainly related to the poor spectral information (in single-stage apparatus), thus unknown identification is difficult, there is poor reproducibility of the ionization and a susceptibility to matrix effects (ion suppression or enhancements).

The use of MS as a detector in GC was developed during the 1950s by Roland Gohlke and Fred McLafferty [85]. GC–MS is the most commonly employed technique today for the analysis of volatile organic pollutants in environmental samples. The main advantage of GC–MS is related to the fact that the MS fragmentation pattern can often provide unambiguous component identification by comparison with spectral libraries. Huge mass spectral libraries are commercially available, such as the NIST Library and the Wiley Library. However, a major prerequisite for GC/MS analysis is that the compound should be volatile and thermally stable.

At present, direct coupling of capillary columns to the ion source of the mass spectrometer is by far the most common interfacing method in use, with electron ionization (EI) being the most popular because it often produces both molecular and fragment ions. However, in some cases, the extensive fragmentation of EI does not provide the sensitivity required and softer ionization techniques such as CI are applied.

CE is also widely used in conjunction with MS via ESI interfaces. However, some difficulties can be found in the design of the interface between CE and MS. The challenge is to maintain a stable electrical contact at the ESI-end of the capillary, exposed to air. Moreover, the high concentration of salts and buffers used in CE separations and extremely low flow rates make the technique less compatible with the MS detector. Another reason for the lack of attractiveness of CE is its limited loading capacity resulting in poorer overall sensitivity. In addition, most commercial CE–MS systems use sheath flow setups that lower the sensitivity even more by analyte dilution in the make-up liquid. The most widely used interface for commercial CE–ESI-MS instrumentation exhibits a configuration that uses a coaxial sheath liquid [86], usually an organic solvent, which primordially serves to establish the electrical contact for CE. In this setup, electrical contact is established through the coaxial sheath liquid which mixes with the CE effluent, providing a higher flow rate that promotes stable potential application for both electrophoresis and ESI. Other CE–ESI-MS interface designs include sheathless [87] and liquid-junction interfaces [88].

In short, the use of MS as a detector in separation science is of high value for LC and GC and less so for CE. However, in all the cases the on-line tandem between MS and the separation system offers a highly selective detector with tremendous possibilities for both screening and quantitative analysis, thus contributing to the obtaining of a wide range of information on samples with a low consumption of reagents (MS does not need any derivatization of the analytes to be determined) and to the greening of analytical methods, especially in the environmental field.

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CHAPTER 7

Downsizing the Methods

Contents

When the strategies to make analytical methodologies greener that have been mentioned in previous chapters cannot be adopted, general alternatives to minimize reagent consumption and waste generation are readily available. These minimization approaches also have the advantage of reducing operational costs, including those spent on waste treatment and disposal. In this regard, miniaturization and automation of analytical instruments has attracted wide interest over the past decade. There is an increasing demand for in-field low-cost instruments capable of rapidly analyzing one or several compounds at very reduced sample volumes with a high level of automation, also providing a drastic reduction of solvent and reagent consumption, and a concomitant reduction of waste generation.

7.1. MINIMIZATION OF THE REAGENTS CONSUMED THROUGH AUTOMATION

Since the inception of flow analysis in the earlier 1950s [1], flow systems have proved to be excellent tools for solution handling and automation of measurements and avoids operator contact with toxic solutions, reduces

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sample, and reagent consumption. Consequently, it has replaced methods related to classical batch chemical analysis.

In 2001 Rocha, Nóbrega and Fatibello Filho reviewed the different flow analysis strategies for a greener Analytical Chemistry [2]. The paper deals with reagentless procedures, replacement of hazardous chemicals, strategies for waste minimization as well as on-line waste treatment or recycling. They highlight the potential of flow approaches such as sequential injection, multicommutation, and monosegmented flow.

Since the advent of the first flow injection analysis (FIA) systems, in which the reagent solution was also employed as a carrier stream [3] to those based on the use of merging zones [4] and the intermittent reagent addition approaches [5], reagent consumption has been substantially reduced.

Another alternative in FIA systems to reduce reagent consumption is the use of reagent injection systems, where the reagent is inserted in a sample carrier stream [6]. This strategy is very attractive when the sample volume is not a limiting parameter and allows an increase in sensitivity while reducing the reagent volume to the microliter level.

In monosegmented flow analysis, the sample aliquot is inserted in the carrier stream sandwiched by two air bubbles, reducing axial sample dispersion [7]. In this case, the introduction of small sample aliquots in tandem with reagent aliquots between the air bubbles provides good analytical performance with minimum reagent consumption and waste generation [8].

Sequential injection analysis (SIA) is considered the second generation of FIA, and was established by Ruzicka and Marshall in 1990 [9]. SIA systems involve a high precision bidirectional pump, a holding coil, a multiposition valve, and a flow through detector. Once the system is filled with a carrier stream, sample, and reagent(s) plugs are sequentially aspirated into the holding coil. The multiposition valve is then switched to the detector position, and the flow direction is reversed, propelling the sample/reagent zones through the flow-through cell. The main advantage of SIA over classical FIA is the strong reduction of reagent consumption and waste generation. However, the main drawback is that it provides a lower sample throughput than FIA.

In multicommutation, sample and reagent volumes are controlled by the time during which the stream is allowed to circulate, the uncertainty in the sample volume being dependent mainly on the time measurement precision [10]. As explained in the review paper by Reis et al. [11], the main advantage of multicommutation-based procedures is that the low size and low cost of solenoid micropumps make them ideal tools to build compact,

environmentally friendly analytical systems. Moreover, single commutators, like sliding bars or rotary valves, which are able to operate in two resting positions, are used in the same manifold, improving the versatility of the system [12]. So, a multicommutated system can be considered as an analytical network that involves the actuation of n active devices on a single sample, allowing the establishment of up to $2n$ states. Multicommutation uses solenoid valves as differential elements and replaces insertion volumes by insertion times. With electronic timing devices, the associated error is minimal [13]. Minimization of the reagent consumption is another of the favorable characteristics of the multicommuted flow systems, achieving a considerable reduction in reagent consumption and waste generation [14]. It has been recently reported that the main advantage of multicommutated systems is the potential to provide new tools for in-field analysis by spectrometric and electroanalytical methods [15].

A step forward in miniaturization and automation of the sequential injection concept was achieved with micro sequential injection lab-on-valve (SI-LOV) equipment [16]. The SI-LOV concept resulted from the incorporation of the detection system in the selection valve which is possible due to the use of fiber optics technology. This downscale of SIA results in an even lower consumption of reagents and samples and, of course, waste generation. Those advantages make SI-LOV a perfect tool for assays where reagent consumption is a key aspect, such as enzymatic assays [17] or inmunoassays [18] and thus offers a good way for greening all of these methods.

In flow systems, solid-phase reagents present some advantages in comparison with reactions performed in homogeneous media, including the improvement of the radial mass transference and possibly reaction with the highest concentration of the reagent [19] but reduces the amounts of waste generated. Because only the necessary reagent amount is consumed, greener and less expensive procedures are inherently achieved.

A variant of the use of immobilized reagents is the so-called bead injection technique, where beads replace the reagent solution and the assay is carried on the beads' surface [20]. The main advantage of bead injection is that it allows us to renew the beads after each single analytical run [21].

When the solid support is placed inside the flow cell, the technique is called solid-phase spectrophotometry, performing analyte retention and detection simultaneously. The main goal is to increase sensitivity, to improve the selectivity and allow sequential analyte determination. This technique will be extensively described in Section 7.5.

In short, it can be concluded that automation in all the available strategies offers a unique way of reducing the amounts of solvents employed, thus improving the sustainability of the analytical methods. Figure 7.1 provides a comparison of the basic strategies of manifolds employed for FIA, SIA, multicommutation, and LOV analysis automation, indicating the main advantages offered for greening the analytical procedures. A drastic reduction of the amount of reagents consumed and waste generated together with an increase of sample throughput and portability of the systems based on the use of elements suitable to be driven through 12 V power battery are the main advantages offered by these systems, which also avoid undesirable side effects for both the environment and operators. Additionally, automation components are mainly low cost elements and, therefore, once again the sustainability of green procedures also includes a reduction of the costs, thus providing economic opportunities which clearly are the basis of the generalization of automated methods of analysis.

Table 7.1 summarizes the order of magnitude of sample volume, reagents consumed, and waste generated in the different automation strategies commented on throughout this section.

7.2. MINIATURIZATION OF THE SAMPLE PREPARATION SYSTEMS

Sample preparation, in particular sample digestion and analyte extraction, is the slowest and most tedious step in many analytical procedures and often involves an extensive use of reagents and implies tremendous attention and personal risks to operators. Due to the long time and intensive sample handling, sample preparation is the Achilles heel of the procedures in order to obtain the appropriate accuracy.

In recent years, much effort has been devoted to eliminating these drawbacks and, as has been mentioned in Chapter 5, this has led to the development of faster and more powerful and/or more versatile extraction techniques, including on-line solid-phase extraction (SPE), solid-phase microextraction (SPME), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and subcritical water extraction (SWE), and microwave-assisted extraction (MAE).

The miniaturization of sample pretreatments integrated in general analytical procedures has been regarded as one of the most attractive techniques for the treatment of complex samples, especially in "Micro-Total Analysis Systems (μTAS)". An effective on-line coupling of the miniaturized sample

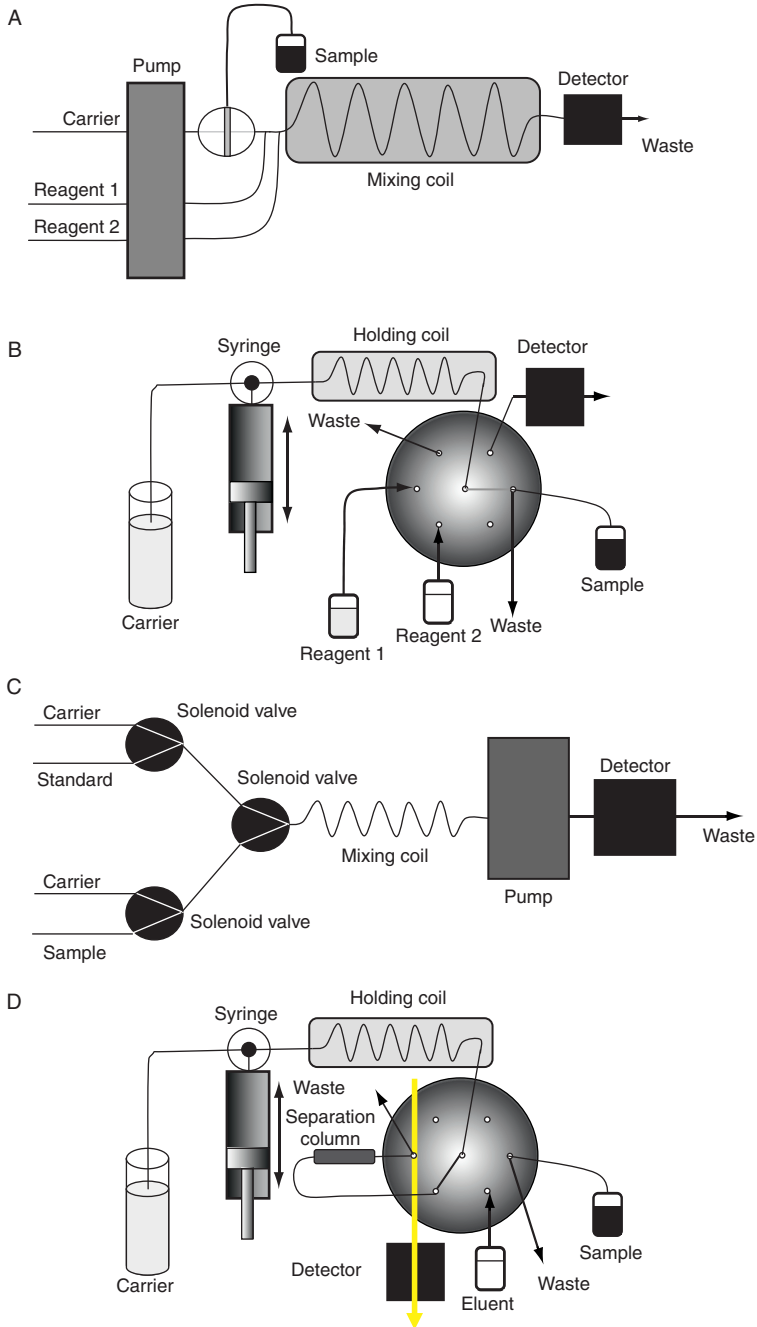

Figure 7.1 Basic manifolds employed for automation of analytical procedures, based on classical FIA (A), SIA (B) and multicommutation based on solenoid valves (C), and lab-on-valve approach (D).

Table 7.1 Microscale Liquid Chromatography (LC) columns classified according the flow rate used

Column ID (mm)	Column category	Flow rate range (μL min^{-1})	Analyte capacity
1.0	Microbore	100–500	~10 μg
0.5	Micro	10–100	~2.5 μg
0.3	Capillary	1–10	~1 μg
0.1	Nano	0.25–2.5	~100 ng
0.075	Nano	0.1–1	~25 ng

preparation and the determination procedure provides several advantages, such as: (i) high speed of analysis with high efficiency and (ii) low operation cost due to extremely low or no solvent consumption, which provides an excellent way for greening analytical procedures.

Miniaturization of a procedure can be achieved simply by reducing the dimensions of the systems used in earlier approaches or by developing completely new setups or techniques.

Applications dealing with miniaturized PLE have, until now, been rather limited, probably due to the relatively large size of the extraction cells of commercial systems. One way to analyze small-size samples in a commercial 11 mL extraction cell is simply to fill the rest of the cell with purified sea sand, XAD-7 HP resin, silica modified. However, in these cases, the amount of sample is reduced but the large dimensions of these cells oblige one to use amounts of sorbent and solvent similar to those of conventional PLE applications.

At present, the only way to solve the above problem is to design a home-made miniaturized PLE system. In this regard, the use of a heatable 10 mm x 3.0 mm i.d. stainless-steel holder as extraction cell enabled quantitative extraction of the 16 Environmental Protection Agency (EPA) polycyclic aromatic hydrocarbons (PAHs) from 50 mg soil with only 100 μL toluene with quantitative recoveries and relative standard deviation (RSD) values similar to those found using traditional methods [22]. A significant reduction of the preparation time to only 10 min and minimum consumption of reagents were also achieved.

Recently, a miniaturized PLE device has been developed [23]. The mini-PLE includes a small thermostatic oven in which up to three extraction cells of variable size can be simultaneously mounted, the valves allowing

the assembly and communication of those cells with the fluid supplier, and a coil-based refrigeration system prior to sample recollection. This system allows a precise control of the extraction fluid volume and the pressure. Using this device, extraction of arsenic species with an aqueous mixture of 1% sodium dodecyl sulphate (SDS) and 5% isopropanol from hair was carried out [24], dispersing 50 mg of sample in 350 mg of Teflon balls, and then applying a temperature of 150 °C at 14 MPa during 15 min.

As with PLE, no miniaturized MAE system is commercially available; there are thus only a few studies regarding miniaturized dynamic MAE coupled on-line with SPE reported in the literature [25,26]. Water was continuously pumped through the MAE system and 1 mL of the aqueous slurry of the sample was injected at a flow rate of 0.75 mL min^{-1}. After passing through the microwave cavity, the slurry was in-line filtered to separate the solid particles from the liquid fraction [25]. As an alternative to this approach, a preheating column was inserted in front of the extraction cell in the microwave cavity. Using this configuration the authors demonstrated the feasibility of dynamic MAE coupled on-line with SPE for accurate determination of PAHs in a reference sediment (recoveries 88–104%, RSDs 1–10%) although only 60 mg sample was used [26].

Finally, it should be mentioned that the potential of sonication for miniaturized rapid, relatively inexpensive and quantitative sample extraction has already been demonstrated [27].

The integration of techniques for introducing samples, pumping, storing, mixing, and metering out fluids is the basis to achieve the miniaturization of laboratory instruments. In this regard, automated techniques for distributing reagents in parallel microfluidic channels compete with expensive liquid handling robots that dispense fluids in 96-, 384-, or 1536-well plates.

Microfluidic diluters, or microdilutors, are systems in which solutions or reagents are carried through a series of controlled dilutions, and then used in assays [28]. These dilutors perform some of the functions of 96-well plate assays, but use smaller quantities of reagents, and are less labor-intensive.

7.3. μTAS OR "LAB-ON-A-CHIP"

The concept termed μTA, or "Lab-on-a-chip," aims to develop integrated microanalytical systems [29], where complete analytical determinations are performed on the same microdevice [30].

As described in a really interesting review article by Ríos et al. entitled "Challenges of Analytical Microsystems" [31], a μTAS is a device that improves the performance of an analysis by virtue of its reduced size. The aim of those microdevices is to offer enhanced performance in terms of fast response and increased analysis throughput together with multifunctionality; decreased consumption of sample and reagent, and production of waste, as well as portability and disposability of the devices [32]. The most significant benefits of these lab-on-a-chip devices would be the analytical improvements associated with the scaling down of the size, minimized consumption of reagents, increased automation, and reduced manufacturing costs [33].

The high degree of integration offered by "lab-on-a-chip" devices implies that the principles of Green Analytical Chemistry can be applied to all steps of the analytical process [34].

Typical analytical microdevices are glass-, silicon-, or polymer-based planar chips ranging in overall size from the mm- to cm-scale with individual structures in the μm-scale.

One area in which the μTAS is becoming more and more important is in drug discovery in the pharmaceutical sector. In this area, ultra-high throughput screening (uHTS) methodologies, in which many compounds are screened at lower cost and in less time than with classical processes, are a major goal. Miniaturized assays consume less solvents and reagents, thus reducing the cost of screening, and can also be performed faster. Assay miniaturization has followed an evolutionary process, from the milliliter volumes used in test tubes to the microliter volumes in standard 96-well microplate format [35] and finally to the increased utilization of 384-well plates, which enable assays to be performed in the 10–20 μL range, the next step being the development of assays in the submicroliter volume range, performed in 1536-well plates [36].

The main difference between a μTAS microfluidic systems and a microfabricated LOV unit is in the channel dimensions. In the first case, the depths of the channels are of the order of 10–100 μm while in the second case the corresponding channel dimensions typically range from 0.5 to 2.0 mm. The downscaling of the flowpath in μTAS has undoubtedly revolutionized the volume requirements of (bio)chemical assays, leading to chips able to process samples within the nL to pL range, thus facilitating the implementation of single-molecule detection methods [37].

Moreover, the difference in channel dimensions produces the crucial difference between microfluidic systems, which is associated with the propelling device. Liquid manipulation in μTAS systems is normally based on

electroosmotic or electrophoretic forces [38]. Although pressure-driven flow is also applicable by implementation of micromechanical pumps within the microdevices, electroosmotic pumping has the inherent advantages of being pulse free, with none of the backpressure effects that occur with integrated pumps, and offering an extra degree of freedom as regards to improved miniaturization [39]. On the other hand, fluid movement in LOV systems capitalizes on mechanically driven flow as precisely executed via an external microsyringe pump, which provides the advantage of applying flow programming based on bidirectional flow or on stopping-flow approaches.

Figure 7.2 shows a schematic diagram of a multireagent system integrated on a chip for analytical purposes. A wide range of detection techniques has been employed in microfluidic devices, such as laser-induced fluorescence [40], mass spectrometry (MS) [41], electrochemistry [42], and optical sensing [43], which will be described in the following sections.

There has been a large amount of research directed toward the integration of microfluidic technologies with different aspects of biochemistry, such as drug development [44], tissue engineering [45], sample preparation for molecular diagnostics [46], and biosensors [47].

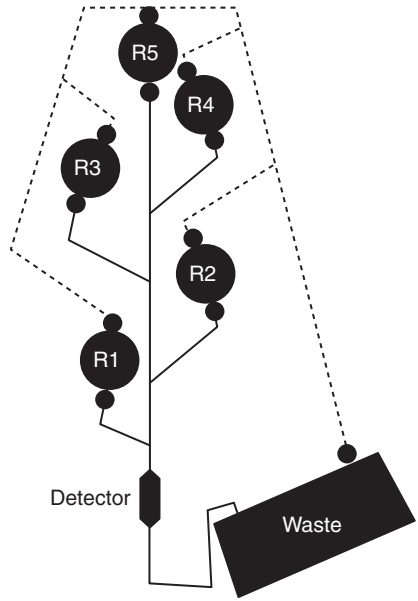

Figure 7.2 Multireagent system on a chip for analytical purposes.

7.3.1 CE on a chip

One of the first major applications of modern microfluidics was separation based on electrokinetic processes [48]. Capillary electrophoresis (CE) microchips have received much attention because of their high degree of integration, portability, minimal solvent/reagent consumption, high performance, and speed. They hold considerable promise for applications such as environmental monitoring, biomedical, and pharmaceutical analysis, clinical diagnostics, and forensic investigations [49,50].

In general, the mechanisms of on-chip separation and techniques for performing them are reasonably well understood. As such a large amount of current research is directed toward integrating detection mechanisms, or creating chips for highly parallel analysis. Microchip CE in its various modes of operation offers advantages from the viewpoint of high resolving power, high separation efficiency, and unique selectivity.

Optical [51], electrochemical [52], and MS [53] techniques are routinely used as detectors in microfluidic chips. The main disadvantage of laser induced fluorescence (LIF) as a detector is that it typically requires pre- or postcapillary derivatization. On the other hand, commercially available MS systems are not inherently portable and are more costly and less sensitive than LIF. Moreover, both techniques need sophisticated and expensive instrumentation. These are the main reasons why electrochemistry offers major advantages for such microsystems, including sensitivity, inherent miniaturization of detector and control instrumentation, independence of sample turbidity or optical pathlength, low cost, minimal power demands, and high compatibility [54].

At the beginning, most CE microchips were made from glass or quartz using photolithography, wet etching, and thermal bonding [55,56]. The main disadvantages were their high cost, the harmful and complicated fabrication procedures, and the limitation on the geometric modification of the chip channel. Therefore, polymers became the most promising materials for microsystem technology because they can be produced with mass-replication technologies, such as injection molding and hot embossing [57,58].

Figure 7.3 shows a typical scheme of a CE on a chip system.

7.3.2 Liquid chromatography (LC) on a chip

On-chip LC was introduced as early as 1990 by Manz et al. [30], who demonstrated the possibility of fabrication of open-tubular chromatography on a silicon wafer. The development of on-chip LC has been more

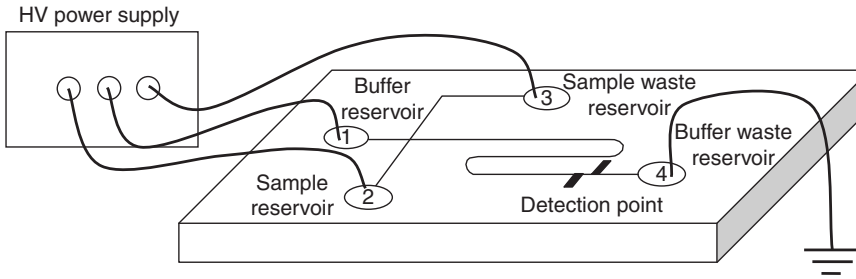

Figure 7.3 Schematic representation of a setup of CE on a chip.

difficult to realize than on-chip CE, probably due to the difficulties of introducing the stationary phases inside the microchannels of the chip. However, some advances have been achieved toward a miniaturized LC system such as the development of a partially integrated picoliter injector [59], the packing of microparticle suspensions by a pressurized flow [60] and *in situ* polymerization inside microchannels [61,62].

Microfabrication plays an important role in on-chip LC because it can create miniaturized devices containing the needed fluidic components in an integrated manner. Initial work in the microfabrication of nano-LC columns was published by He et al. [63], who used a lithographic process to create a monolithic support structure acting as the stationary phase for chromatographic separation. The design of an adequate microscale pumping system for chip-based LC is challenging. Nonmechanical pumping systems, employing electroosmosis as the driving force, have found the greatest application in chip-based systems [64,65]. Sandia National Laboratories (http://www.ca.sandia.gov) and Eksigent Technologies (http://www.eksigent.com) developed an electrokinetic high-pressure pump [66], which uses electroosmosis in charged porous media to generate pressure and flow in microdevices. It is realized by applying voltage across a monolithic porous polymer or a bed of packed silica particles. Moreover, micropumps that utilize an expanding bubble of gas created by electrolysis as the driving force have been investigated [67]. The on-chip electrochemical pumping system offers some advantages over designs that utilize electroosmotic-driven flow such as (i) the possibility to deliver aqueous and mixed aqueous–organic solvents, (ii) a broad range of flow rates achieved by regulating the current to the pump electrodes, and (iii) the possibility to perform gradient mode separations by combining the output of two pumps. Nevertheless, the control of the gradient formation is imprecise and further development is needed.

The amount of waste generated is reduced by approximately 4–5 orders of magnitude in comparison to conventional liquid chromatographic assays (10 μL vs. 1 L per daily use). Such significant improvement in the rate of waste generation and material consumption has enormous implications for Green Analytical Chemistry.

The results of several chip-based approaches published in the past formed the basis for the appearance of a commercial microfluidic nano-LC/MS device. The LC-Chip/Ion Trap or time of flight (TOF) MS system has demonstrated its ability and maturity for the analysis of complex peptide mixtures in the context of different proteomic studies like biomarker discovery, glycopeptide profiling, differential phosphoproteome studies, and nucleolar proteome investigation.

An alternative technique to conventional on-chip chromatography, hydrodynamic chromatography (HDC), has recently been introduced on a chip [68]. HDC utilizes pressure-driven flow for separation of macromolecules or particles in a wide and flat microchannel. Larger molecules or particles are transported faster than smaller ones, as they cannot fully access the slow-flow regions near the channel walls. On-chip HDC has been proposed as an attractive alternative to the classical separation methods such as size exclusion chromatography (SEC) or field-flow fractionation (FFF).

Figure 7.4 provides the scheme of an LC on a chip system in which can be identified the different internal and external components.

7.3.3 GC on a chip

The first μ-TAS was a gas chromatography (GC) etched on a 5-cm silicon wafer, which was developed in the late 1970s [69]. Samples were injected into a gas stream in a silicon capillary and analytes were detected with a thermal-conductivity detector integrated in the microsystem.

However, only few reports of on-chip GC have been published since then, probably due to the difficulties faced in the integration of stationary phases on a chip in a homogeneous way [50,70,71].

7.4. ELECTROCHEMICAL DETECTION

The development of sensors or instruments with real-time field measurement capability is highly desired in order to replace sample collection, transport, and subsequent laboratory analysis [72]. These sensors offer a rapid return of the chemical information, minimizing costs, and the environmental side effects associated with laboratory-based analyses. The

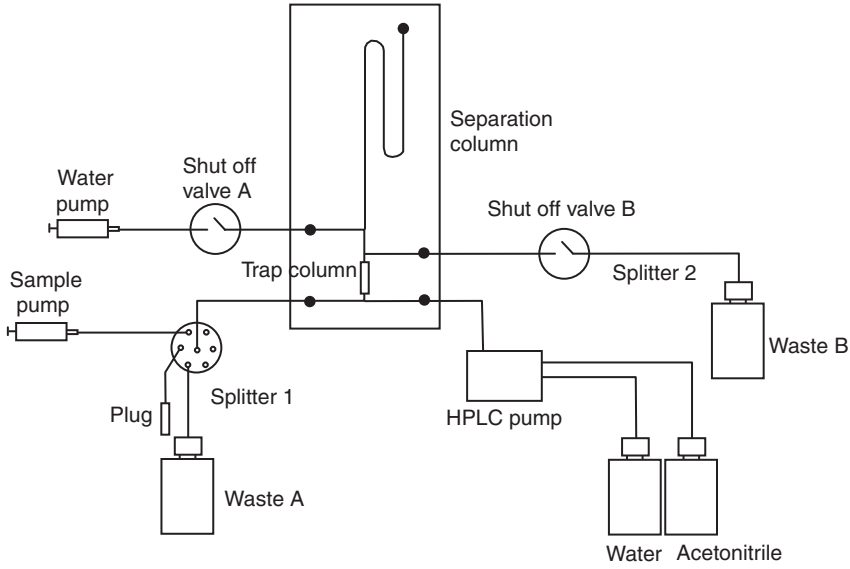

Figure 7.4 Scheme diagram of a setup of LC on a chip.

capabilities of real-time electrochemical monitoring have been summarized in an excellent review article by Wang in 2002 [73].

The coupling of modern electrochemical detection principles with recent advances in microelectronics and microfabrication has led to powerful and compact analytical devices for real time, in-process monitoring [74]. Electrochemical detection [75], specially conductivity detection [76], offers great promise for microfluidic systems, with features that include high sensitivity, intrinsic miniaturization capability, low-power requirements, compatibility with advanced microfabrication technologies, and low cost [51]. The power and scope of electrochemical microsystems have been further enhanced through the coupling of on-chip enzymatic or immunochemical reactions [77].

The lab-on-cable concept refers to a device which incorporates several functions into a single sealed, submersible package [78]. This submersible microanalyzer integrates *in situ* microdialysis sampling, reservoirs for reagents, waste, and calibration/standard solution, along with the micropump and necessary fluidic network on a cable platform (see Figure 7.5). On-cable metal complexation reactions or enzyme-inhibition ones have thus been demonstrated in connection measurements of trace chromium [79], cyanide [80], long-term oceanographic monitoring [81], or process control [82].

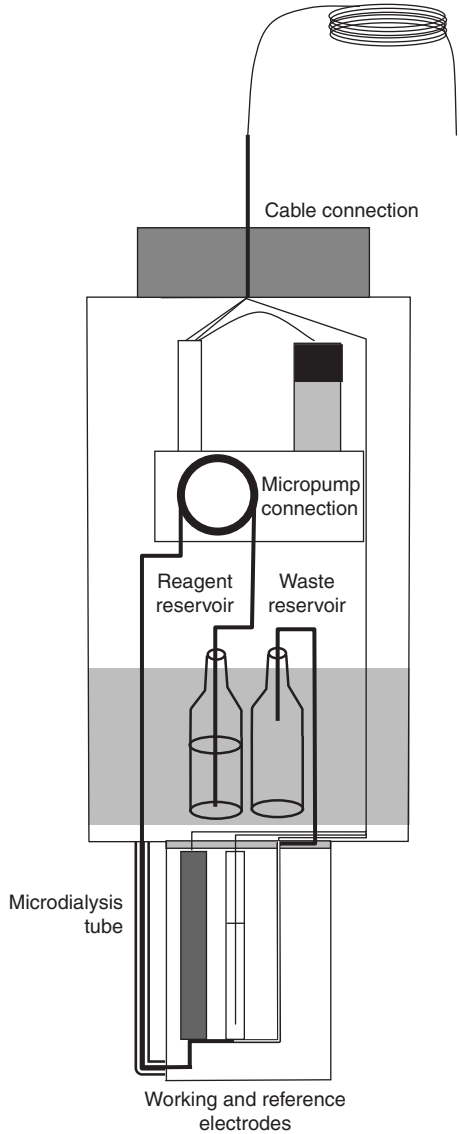

Figure 7.5 Scheme of a lab-on-cable for submersible microanalysis.

Conductivity detection is a simple and universal detection technique normally used in electrophoresis. It involves the measurement of the conductance between two or four electrodes through which an alternating current is passed, allowing the detection of ionic species down to the

nanomolar level [83]. Conductivity detection can be accomplished either by a direct contact of the run buffer and the sensing electrodes or by a contactless mode.

Sensing conductivity by a galvanic contact of the electrolyte solution with the electrodes can generally be performed either using electrodes which are placed in holes through the capillary wall [84] or in an end-column wall jet arrangement [85] (see Figure 7.6). For microchip arrangements, different on-column contact electrode geometry modes [86,87] and wall-jet conductivity [88] have been reported.

Contactless conductivity detection (CCD) was employed as a detection technique in isotachophoresis in the 1980s [89] and in CE in 1998 [90,91]. It provides several advantages, including the absence of problems associated with the electrode-solution contact, effective isolation from high separation voltages, a simplified construction and alignment of the detector, and the use

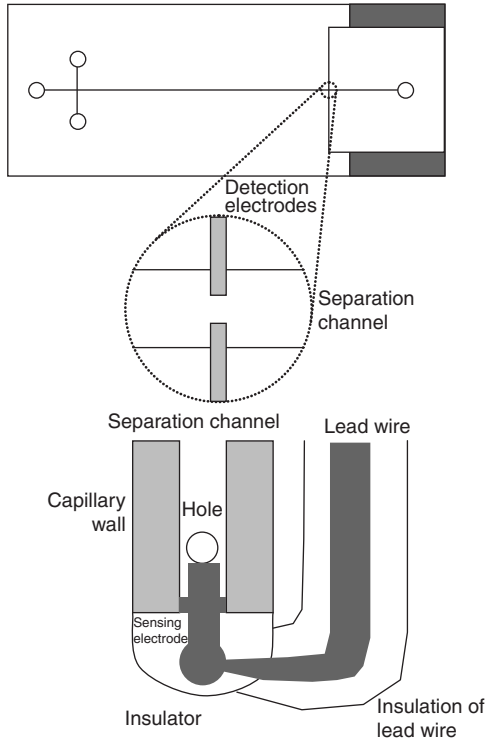

Figure 7.6 Different setups employed in microchip devices for conductivity sensing by galvanic contact of the electrolyte solution with the electrodes.

of narrow micro/nanochannels [92]. First reports on integration of CCD to the microfluidic format emerged in 2001 and 2002 [93,94].

Since then, three main schemes of CCD have been implemented in microfluidics: (i) CCD electrodes are placed along the microchannel from outside of the microchip and they are insulated from the channel by the thickness of the covering microchip lid; (ii) the electrodes are placed across the microchannel in the same plane and they are insulated by a thin layer of chip material, and (iii) electrodes are buried in a widened part of microchannel and they are insulated from the solution by an ultrathin layer of silicon carbide (see Figure 7.7).

On the other hand, as has been highlighted in Chapters 2 and 3, the elimination of hazardous materials from the analytical protocol is a major thrust of green analytical chemistry. Thus, the development of sustainable alternatives to mercury electrodes, which were for many years the choice for routine applications in polarographic and voltammetric techniques, is highly desirable, and in downsized electrochemical detectors nontoxic metals and composite materials have replaced mercury-based electrodes.

For instance, bismuth-film electrodes offer high-quality trace-metal measurements that compare favorably with those of mercury electrodes. The analytical power and scope of carbon and other solid electrodes can be greatly enhanced through a deliberate modification of their surface [95]. The miniaturization of solid electrode offers several fundamental and practical advantages [96], including a dramatic reduction in sample consumption and a significant reduction of resistance effects, which greatly facilitates voltammetric measurements in low-ionic-strength water samples.

7.5. SPECTROSCOPIC DETECTION

Macro-scale optical detection, especially spectrometric detection, is commonly employed due to its wide range of applications. To focus micron-sized detection areas using macro-scale spectrometric systems with pinholes or optical fibers is commonly called the "off-chip approach". This approach provides very low levels of background signal which, combined with very sensitive photon detection techniques, results in very low limits of detection. However, the reduction of the optical pathlength decreases the sensitivity of the method, in particular for absorbance measurements. Currently, there are a wide range of intense light-emitting diodes available and photodiodes that can be coupled directly to microfluidic devices to provide a miniaturized technique of on-chip detection. The existing

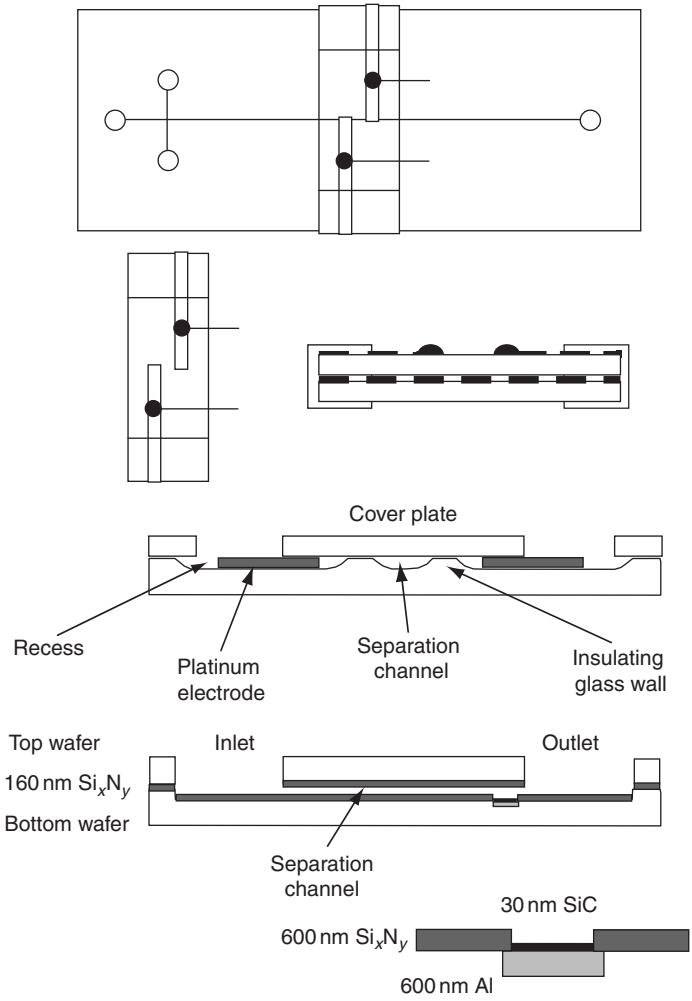

Figure 7.7 Contactless conductivity detection schemes employed in microfluidic systems (Adapted from Ref. [86]).

devices in the "off-chip approach" are generally well developed either as homemade detection systems or commercial instruments, such as Shimadzu MCE-2010, Hitachi SV1100, and Agilent Bioanalyzer 21000.

UV/vis absorbance detection is the most widely used detection method in macro sensing devices. However, the small dimensions of a microchip channel are a severe limitation for a sensitive and reliable absorbance measurement. This is the reason for the few examples of absorbance-based detection systems in microfluidic devices [97].

As mentioned before, fluorescence detection is today still the most widely used optical method for microsensing systems, due to its superior selectivity and sensitivity [98]. Although a variety of excitation sources are available, laser-induced fluorescence is most easily adapted to the dimensions of microchips.

Chemiluminescence as a detection method for microsensing systems has the advantages of a high sensitivity, low detection limits, and simple instrumentation as compared with other spectrophotometric techniques, due to the exclusion of an external light source [99]. However, the main drawback of this technique is that chemiluminescence reagent needs to be mixed with the separated analytes before detection and thus a rather complex microchip setup is required.

On the other hand, the integration of optical components or functions in a microfluidic platform that should be able to perform all chemical functions and detection in a single device, requires increased integration of fluidic elements, with electrical and other types of elements, thus providing an "on-chip approach".

Direct electronic optical sensors like the silicon photodiode operate by converting absorbed photons directly into electronic carriers which are ultimately detected. Alternatively, many semiconductors and combinations of them form the basis of laser diodes and light-emitting diodes, a class of devices producing light in different wavelength regions from the blue to the near infrared. From both a materials and technology standpoint, the integration of optical functions inside a microchip is very promising. However, the existing devices in the "on-chip approach" are generally still in their infancy [100].

The integration of microlenses and planar waveguides in microfluidic devices is useful for the improvement of detection in sensing systems. For instance, by using a planar waveguide the optical pathlength can be increased for absorbance measurements, or by focusing the light into the channel in order to increase the excitation power for fluorescence measurements.

Applications of on-chip absorbance measurements using a commercially available complementary metal oxide semiconductor imager chip, bonded together with a microfluidic channel network cast in polydimethyl siloxane, can be found in the literature [101].

Moreover, microlenses have been fabricated directly into a glass chip for the collection of fluorescence light by melting islands of photoresist into a hemispherical shape [102]. In order to have a compact system, a pinhole, an interference filter, and a photodetector were placed in close proximity to the microlens.

Organic light-emitting diodes (OLEDs) have been successfully integrated as a lightweight flat panel light source. Their main advantage compared to LEDs is their flat film-like shape. It makes it easier to incorporate them into microfluidic devices and to bring them into close proximity of the channel. However, their broad emission spectra require additional excitation filters. OLEDs placed on the rear side of a glass substrate and a channel cast in polydimethylsiloxane (PDMS) on the front side showed the emission peak centered at 520 nm and had a relatively wide peak of 70 nm [103].

Photodiodes have been fabricated in the same microchip at the bottom of the microfluidic channels via a monolithic approach [104] as an integrated detector to perform chemiluminescence. In order to lower the detection limit, the photodetector sensitivity was improved by fabricating diodes with a shallow junction to reduce the recombination of photocarriers and thereby to increase the quantum efficiency.

The incorporation of solid particles into microfluidic devices for SPEs and immunoassays, by fabricating a retaining feature in the microchannel which confines the particles in a specific location inside the microfluidic network [105,106], or by trapping and manipulating beads within fluid streams using special channel geometries incorporating planar diverging and converging channel elements [107] has been also employed. Solid particles are used as sensing particles points and thus contribute to the development of new and more sensitive sensing microfluidic systems. The aforementioned strategy can be considered a miniaturization of the solid phase spectroscopy (SPS), proposed in 1976 by K. Yoshimura [108] and consists of a combination of an active solid support to preconcentrate the analyte and the direct measurement of the light absorption of the analyte sorbed on the solid phase. The main advantages of this technique are those related to increased sensitivity and selectivity, improved sample throughput, commodity, automation and reduction of reagents, and solid supports consumption [109].

7.6. UPLC, MICRO-, AND NANO-LC

From about 1965 until today there has been a continuous development in miniaturization in chromatographic techniques, especially for LC. Basically the use of smaller dimensions within LC has three major advantages: (i) better resolving power in shorter time; (ii) less sample volume is necessary for the analysis; and (iii) costs and side effects of solvent consumption are reduced.

Miniaturization in LC can be achieved by downsizing the size of the particles of the stationary phase. Reducing the size of the particles allows faster separations, thus reducing the consumption of mobile phase and therefore that of organic solvents.

Interest in microscale LC has gradually increased since its introduction and miniaturized columns are now commercially available from several manufacturers. Microscale LC columns are often classified according to their packing state as: densely packed columns (6–0.5 mm), loosely packed columns (0.05–0.2 mm i.d.), and open-tubular columns (0.005–0.01 mm id.). On the other hand, they can also be classified on the basis of their flow rate (see Table 7.1): conventional LC (0.5–2.0 mL min^{-1}), microbore LC (100–500 μL min^{-1}), micro-LC (10–100 μL min^{-1}), capillary LC (1–10 μL min^{-1}), and nano-LC (10–2500 nL min^{-1}).

Relatively short columns packed with sub-2 μm particles provide high speed and efficient separations. Ultrahigh or very high pressure pump systems have been used to overcome the high pressure drop generated by small particles [110,111].

7.6.1 Micro high performance liquid chromatography

Micro-LC has a number of advantages over standard-size LC because the smaller inner diameter of the micro-LC columns leads to a reduction of all volume-based system characteristics. The reduction of the flow rate reduces drastically the mobile phase consumption, thus providing obvious economic consequences. Micro-LC offers a drastic reduction in organic solvents consumption compared to conventional LC. For example, a 1.0-mm ID column will reduce the mobile phase waste by a factor of 21 compared with a standard 4.6 mm ID column.

Column miniaturization reduces the amount of stationary phase that is required, which is a special advantage in enantioseparation applications using expensive chiral stationary phases. Another advantage is the reduction of the required sample volume, which can be a limiting factor for biological analysis. Micro-LC offers a higher eluted peak concentration and consequently a higher sensitivity than conventional LC due to the reduction of peak volume. However, this advantage is minimized due to the use of shorter detection pathlengths in micro-LC. Other benefits of micro-LC include easier interfacing with MS and simpler hyphenation with other microseparation techniques [112].

Micro columns are also available for Gel Permeation Chromatography (GPC). Recently, the Polymer Science Service Company developed a new

Table 7.2 Main analysis conditions and solvent consumption of GPC/SEC procedures depending on the size of the column

	Micro GPC/SEC	**Analytical GPC/SEC**	**High speed GPC/SEC**	**Semipreparative GPC/SEC**
Flow rate (mL/min)	0.33	1	6.25	6.25
Recommended columns	Micro columns	Analytical columns	High speed columns	Preparative columns
Column dimensions (mm)	4.6×250	8×300	20×50	20×300
Analysis time (min)	10	12.5	2	12.5
Eluent consumption (mL)	3.5	12.5	12.5	78.1

concept of micro GPC/SEC columns. According to the company, micro columns allow users to save solvent without sacrificing resolution. Micro columns are available as single porosity columns, linear columns or in-line combinations and almost all PSS columns materials are available so the applications are easily transferable. Table 7.2 shows the analysis conditions and the solvent consumption reduction offered by these micro columns.

7.6.2 Ultra performance liquid chromatography

Ultra Performance Liquid Chromatography (UPLC) could be considered a new and green alternative to conventional LC. It differs from conventional LC in two important respects: average particle size for UPLC is 1.7 μm versus 5.0 μm for ordinary columns, and the system runs at 15,000 psi rather than the usual 5000. In conventional LC the choice of particle size must be a compromise—the smaller the particle size, the higher the column back-pressure—this being a serious limitation of the use of small column diameters like 2.1 or 1.0 mm under conventional conditions. UPLC instruments are specially designed to resist higher back-pressures than those supported in conventional LC, providing higher speed, resolution, and sensitivity.

In UPLC, sample introduction has been particularly challenging because of the difficulty in constructing a valve that satisfies the seal requirements at high pressure while accurately transferring a small volume of sample into a column. Several strategies have been developed, such as (i) the static-split injection technique, which is too irreproducible for quantitative purposes, (ii) the pressure-balanced injection which provides better reproducibility, shorter injection time, reduced sample consumption ,and greater ease of use, (iii) the air-actuated needle valve injection system, and (iv) a modified diamond coated six-port automated switching valve with sample loops of different volumes.

Regarding the packing material, most stationary phases used for early capillary UPLC are 1–1.5 μm silica-based nonporous particles [113,114]. It was believed that nonporous particles provide an alleviation of band-broadening due to sample diffusion in the stagnant mobile phase within the pores, leading to overall faster mass transfer [115]. However, the study of Wu et al. [116] who compared efficiencies of columns packed with 1.5 and 3.0 μm nonporous and porous particles showed that nonporous particles provide higher efficiency at high linear velocities but the efficiency difference diminished significantly when the particle size was reduced from 3 to 1.5 μm. Furthermore, the sample loading capacity for porous C18 particles was 15 times higher than that for nonporous C18 particles and the average retention factors for a 1.7-μm porous C18 column were significantly higher than those for a 1.5-μm nonporous C18 column.

The narrow peaks produced by fast UPLC require a small detection volume and fast acquisition rate to ensure high efficiency. In capillary UPLC, on-column detection is used to minimize the detection volume. Amperometric detection has a near-zero dead volume and provides very high sensitivity to electroactive compounds [117]. On-column LIF detection has been used for peptide analysis [118] and on-column UV detection has been also employed [119] by removing an approximately 1 mm wide band of the polymeric coating from the fused silica tubing immediately after the outlet frit of the capillary column. The drawback of this detection mode is low sensitivity as the pathlengths for the UV detection are equivalent to the column internal diameters (30–150 μm) of capillaries.

Table 7.3 summarizes the main characteristics and operating conditions of miniaturized LC systems as compared with classical LC and CE and shows that miniaturized LC systems can drastically reduce the side effects of LC but CE still remains a greener separation technique than LC.

Table 7.3 Main characteristics and operation conditions of miniaturized LC systems comprising classical liquid chromatographic and capillary electrophoresis

	LC	Micro-HPLC	UPLC	CE
Injected volume	1–100 μL	0.5–5 μL	0.5–5 μL	1–100 nL
Flow rate (mL/min)	0.2–10	0.001–0.005	0.01–2	–
Number of peaks separated	<100	<100	<200	<100
Analysis time (min)	10–60	10–60	1–10	1–20
Separation efficiency (plates)	$>10{,}000$	$>10{,}000$	$\sim 10{,}0000$	$>10{,}0000$
Maximum pressure (psi)	6000	6000	15000	–
Particle size (μm)	3.5–10	2–5	1.7	–

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CHAPTER 8

Moving from Wastes to Clean Wastes

Contents

8.1. THE PROBLEM OF ANALYTICAL WASTES

It should be considered that most chemical assays are reagent based, and since many reagents are toxic and/or carcinogenic, corrective action needs to be taken. Also, the majority of Environmental Protection Agency (EPA) and Food and Drug Administration (FDA)-certified analytical methods use corrosive and toxic chemicals, with no currently available alternatives.

So, in cases in which remote sensing or direct spectrometric measurements made directly on untreated samples are not available we must pay attention to the fact that, in addition to results, analytical methods provide wastes as a side product.

It is true that laboratory wastes are not of the same magnitude as industrial ones but if we consider the sum of sample and standards solutions mixed with reagents and solvents used for the determinations, and the washing solutions employed in the different steps, it can be concluded that the volume of wastes created by an active laboratory can be at the level of several kilograms a day. This is exacerbated by the fact that laboratory

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wastes in concern single products in only a few cases and are often complex mixtures that are difficult to treat, especially when they are cumulated and left in the hands of external waste management companies.

So, it is important that green analytical methods incorporate chemical solutions for solving the problems related to waste generation. In Chapter 7 some ideas have been provided in order to reduce the waste volumes as much as possible through method miniaturization (Section 7.2 and 7.3) and automation. However, in this chapter, additional ideas regarding on-line waste decontamination will be discussed in order to solve the waste generation problems just by adding a little bit of chemistry to the proposed methods.

8.2. REPLACEMENT OF TOXIC REAGENTS

Green Analytical Chemistry aims to explore the use of alternative reagents that minimize the use of toxic chemicals. In this regard, a primary goal stated in 1995 by M. de la Guardia and J. Ruzicka in a guest editorial of the *Analyst* [1], should be "the replacement of toxic chemicals by harmless ones, however, it can be considered as an initial goal to reduce the use of toxic chemicals by a factor of ten". From then until now this effort has been taken up by a large number of scientific groups that have proposed alternative, environmentally acceptable, and reagentless chemistries. However, wastes from the analytical laboratories are often complex mixtures, composed of relatively small individual amounts of a wide variety of chemicals, and individual reagent replacement, as it has been mentioned throughout this book, is not the most effective approach, but sometimes is the unique way for greening Analytical Chemistry.

For instance, in Thailand there is some local usage of guava leaves as an indicator of the presence of iron in groundwater. Local villagers used the water color change to make pretreatment decisions such as adding alum to precipitate the iron. This fact is the basis to use Guava leaf extract as an alternative natural reagent for quantification of iron using a flow injection manifold which enables the use of the extract in acetate buffer solution without further purification being needed. The guava leaf is retained in a column for flow injection analysis (FIA) spectrophotometric determination [2] (see Figure 8.1).

Formaldehyde determination is normally based on the spectrophotometric chromotropic acid method. However, this method uses concentrated H_2SO_4, which is hazardous and corrosive. Thus, the spectrophotometric method for formaldehyde determination was modified to avoid the use of

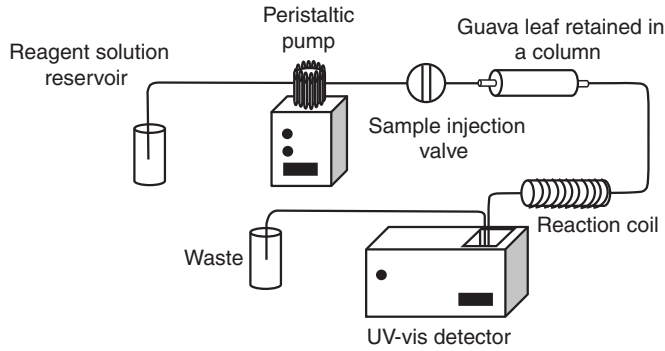

Figure 8.1 The use of guava leaf as an alternative iron reagent in FIA-spectrophotometric determinations.

concentrated H_2SO_4. The method is based on the reaction of formaldehyde with chromotropic acid in the presence of magnesium sulfate, producing a colored complex with maximum absorption at 535 nm [3].

Another example of toxic reagents replacement can be found in liquid–liquid extraction and chromatography, but it corresponds really to the use of alternative solvents and will be discussed in the next section.

8.3. USE OF ALTERNATIVE SOLVENTS

One of the first approaches for greening Analytical Chemistry was the replacement of hazardous and toxic organic solvents. In this regard, in 1995 at the DOE Pollution Prevention Conference XI held in Knoxville, Tennessee, Green et al. presented a paper entitled "Waste minimization in Analytical Methods" in which different mixtures of organic solvents such as 50:50 methylene chloride:acetone and 50:50 hexane:acetone were evaluated to replace methylene chloride from the EPA Method 8270B as SVOC extraction from soil, sediments, and sludge samples [4]. It is clear that this paper is far from Green Analytical Chemistry goals, but it was a milestone that indicated the direction and concerns that analytical chemists were following.

As has been mentioned in Chapter 5, sample pretreatment methods have evolved to reduce sample manipulation, solvent and energy consumption, and time of treatment. In this regard, liquid-phase microextraction (LPME) is a really promising technique which reduces the consumption of organic solvent to approximately 15 μL per sample. Moreover, since the organic phase only serves as an intermediate extraction medium, the use of natural plant oils as organic phase has been evaluated to propose a green direction

for LPME [5]. The procedure demonstrated that in spite of the multicomponent nature of the oils tested, they do not contaminate the acceptor phases during extraction, and are a serious alternative to the use of hazardous organic solvents in LPME.

A solution of surfactants provides a micellar microenvironment that is highly useful for the dissolution of nonpolar organic compounds [6] and is also the basis for new extraction and separation methods such as cloud point extraction [7] and micellar ultrafiltration [8]—see Section 8.5. The use of micellar solutions in chromatography (see Section 6.3.1) and electrophoresis has provided new analytical tools such as micellar chromatography [9] and micellar electrokinetic chromatography [10] in which organic pollutant solvents are replaced by diluted solutions of surfactants. On the other hand, the incorporation of micelles and vesicles to detection systems has contributed to the improvement of the limits of detection of spectrometric [11], fluorimetric [12], and electroanalytical methods [13] and also to the improvement of the analytical performance of atomic spectrometry [14]. So, it is clear that this system when used as alternative solvents in Analytical Chemistry enhances both the method characteristics and their environmentally friendly nature.

Subcritical water and supercritical fluids [15] provide another type of alternative solvents that have been extensively used in extraction (see Section 5.2.1) and in chromatography (see Section 6.3.3) enhancing the characteristics of many determinations and reducing drastically the toxicity of wastes obtained from the use of common organic solvents.

Nowadays, a novel type of solvents, room temperature ionic liquids (RTILs), is gaining wide acceptance as a replacement for organic solvents in several analytical fields. RTILs are formed by the combinations of organic cations and various anions that are liquids at room temperature; they are salts with melting points of ca. 100 °C. The main applications of RTILs in analytical chemistry are summarized in the review of Liu et al. entitled "Application of ionic liquids in analytical chemistry" [16], and can be divided into three areas: (i) sample preparation; (ii) chromatographic and electrophoretic separations (see Section 6.3.1) and, (iii) detection, this area being the most important from a green and environmentally friendly perspective as it involves the replacement of organic solvents in the sample preparation step.

The extraction and preconcentration of heavy metal ions from water to RTILs has been successfully investigated by using different chelating agents as extractants. Sr^{2+} has been extracted from the aqueous phase into

disubstituted imidazolium hexafluorophosphates and bis(trifluoromethyl) sulfonylamides by using dicyclohexano-18-crown-6 [17], Na^+, Cs^+, and Sr^{2+} were extracted from water to 1-alkyl-3-methylimiazolium hexafluorophosphate ([CnMIM][PF6], $n=4$; 6; 8) by crown ethers [18], selective extraction of Hg^{2+} and Cd^{2+} was performed from water by task-specific RTILs [19], and Ag^+, Hg^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, and Zn^{2+} were successfully extracted into [C4MIM][PF6] by employing dithizone as chelator [20].

Moreover, RTILs have also been applied for the extraction of various organic compounds including substituted benzene derivatives [21], biofuels [22], and erythromycin-A in bioprocess operations [23].

The unique properties of nonvolatility, adequate viscosity, and immiscibility with water allow the aforementioned RTILs to be conveniently adopted as extraction solvents in both direct-immersion and headspace LPME. For instance, RTIL-based LPME was applied to determine formaldehyde in mushrooms [24], EPA priority PAHs [25] and for the screening of 45 typical environmental pollutants including benzene, toluene, ethylbenzene, and xylene (BTEX), polycyclic aromatic hydrocarbons (PAHs), phthalates, phenols, aromatic amines, herbicides, organotin, and organomercury compounds [26].

Table 8.1 provides a comparison of the structure, chemical properties, advantages, and drawbacks of the different alternative types of reagents proposed in the literature to replace organic solvents.

8.4. ON-LINE DECONTAMINATION OF WASTES

In different parts of this book we have insisted on the use of automation as one of the main tools for greening analytical methods (see Chapters 1–3 and Section 7.1) in order to reduce operator contact with toxic substances and to minimize the reagent consumption and waste generation. In fact, the evolution of mechanization and automation of methods from classical FIA to sequential injection analysis (SIA), multicommutation, and lab-on-valve (LOV) can be identified, in our opinion, as the search for a fast, versatile, and waste minimization strategy as indicated in Figure 8.2. However, all the efforts made in the way of automation of the different steps of the analytical process and those concerning the miniaturization and portability of systems have not resolved the problems associated with the toxicity of wastes obtained after analyte detection.

In fact, the idea to move from wastes to clean wastes was an original aim of the pioneering studies developed in the field of clean methods [27] and thus the on-line decontamination of wastes is a key tool for greening those methods

Table 8.1 Main characteristics of alternative solvents proposed to replace organic solvents

Solvent type	Structure	Characteristics	Advantages	Drawbacks
Natural products	Mixture of essential oils or natural compounds	Nonpolar solvents with low density	Biodegradable, cheap	Increase noise levels and can cause interferences
Micellar solutions	Aqueous surfactant solutions	Amphiphilic compounds with nonpolar characteristics	Biodegradable, aqueous solutions, cheap, versatile tool	Increase of noise levels and specific interactions
Subcritical water	Water at temperature and pressure below its critical point	–	Low toxicity and environmental impact, aqueous solution, cheap	Reduced solubility of apolar compounds and expensive instrumentation
Supercritical CO_2	CO_2 at temperature and pressure above its critical point	–	Low toxicity and environmental impact	Expensive instrumentation
RTILs	Liquid salts at room temperature	Favorable solvating properties for polar and nonpolar compounds	Reduced environmental impact due to their nonvolatility	Highly aquatic toxicity

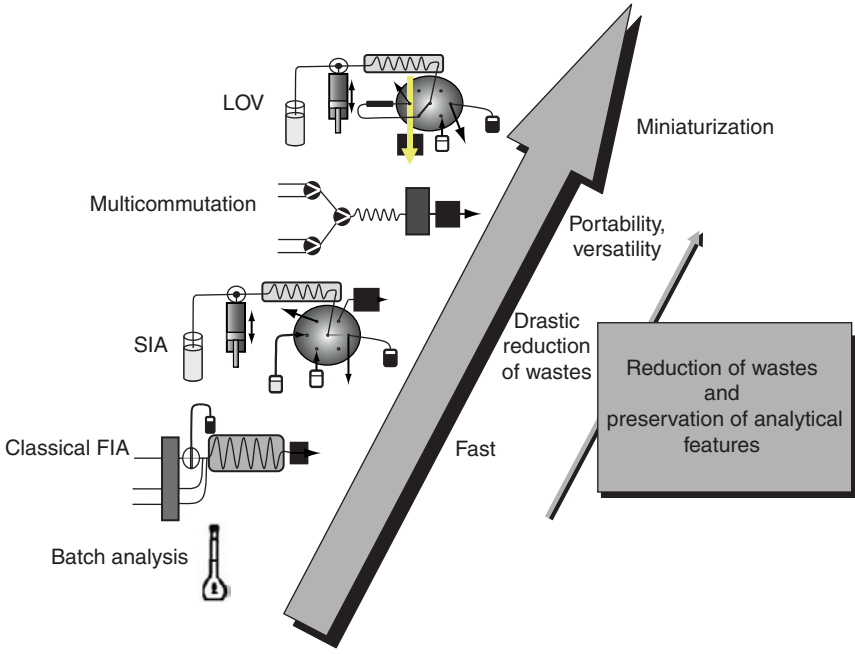

Figure 8.2 Evolution of the mechanization and automation strategies as a search for a fast, versatile, and waste minimization strategy.

which involve the use of any kind of toxic or environmentally dangerous reagent. The main objective of the studies commented upon throughout this section has been the development of integrated systems in which the toxic wastes, normally obtained as end products in the analytical procedures, can be turned into clean or innocuous wastes [28].

It is obvious that the on-line decontamination of toxic analytical wastes will reduce the amount or, at least, the toxicity, and thus the risks to operators and the environment of chemical wastes. At the same time it also reduces the analytical costs by avoiding the external management of residues. In this regard, it is clear that effective methods of treatment of toxic reagents must be sought.

Nowadays, different strategies have been successfully applied to decompose chemical residues or, at least, to passivate and drastically reduce the amount of waste. Most of those methodologies can be classified (see Figure 8.3) as being chemical, physical, or biological in nature. Chemical degradations involve the application of reagents and reaction conditions to transform toxic target species to innocuous ones. Physical and biological methods are generally slower than chemical ones, but they do not require the presence of excess of reagents or harsh conditions such as extreme pH to accomplish the desired transformation.

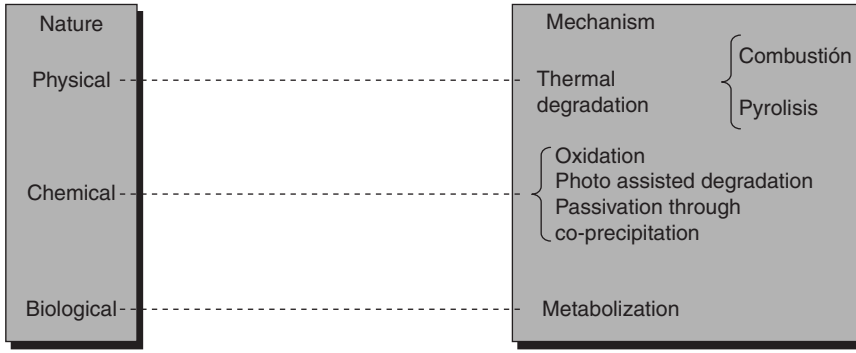

Figure 8.3 Main on-line decontamination strategies suitable for analytical use.

8.4.1 Thermal degradation

Thermal degradation of organic wastes involves incineration/combustion or pyrolysis, the main difference being the presence or absence of oxygen in the reactor. This degradation process is mainly used to decompose solid organic matter in the food, petrochemical, and polymer industries. This degradation process is normally carried out off-line, which means that the solid residues are stored, transported to the incineration plant and then destroyed by combustion thereby generating heat, water vapor, nitrogen, carbon dioxide, and oxygen. Depending on the composition of the waste, other emissions may be formed including carbon monoxide, hydrogen chloride, hydrogen fluoride, nitrogen oxides, sulfur dioxide, volatile organic carbon, dioxins and furans, polychlorinated biphenyls, aerosols containing heavy metals, etc. [29].

Obviously, this degradation method is not the best alternative to clean analytical laboratory wastes. However, it is the most commonly used procedure for waste treatment throughout the world and can also be incorporated on-line when the toxic compounds to be degraded can easily be decomposed by heating. For this purpose, the schemes included in Figure 8.4 can be useful. Note that the use of an accepting solution could be convenient to retain gases generated from organic compounds degradation.

8.4.2 Oxidation

Wastewater management is a major concern for many industrial sectors. The development of useful and effective degradation processes of organic materials in wastewater is an area of significant current interest. These degradation processes are employed to improve the quality of water

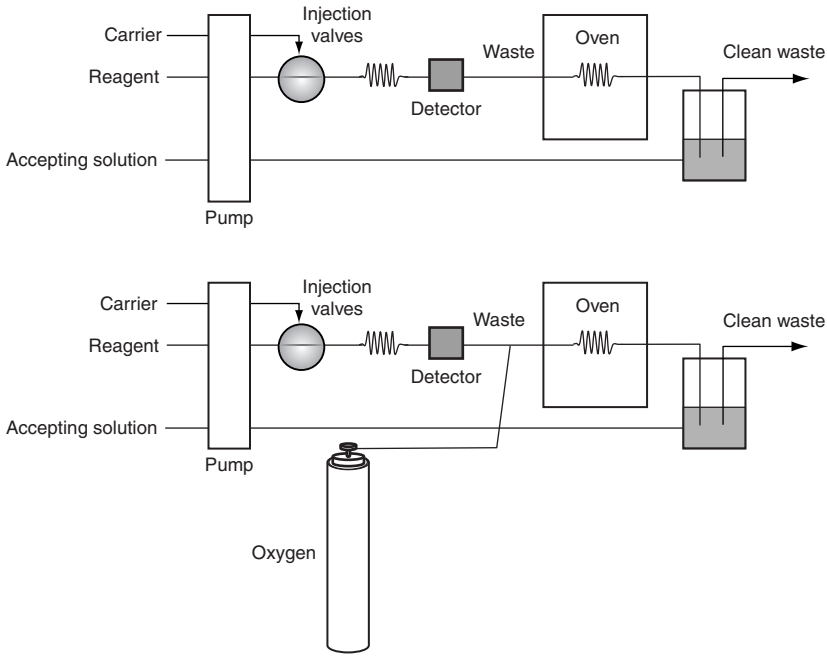

Figure 8.4 Schemes proposed for on-line thermal degradation of analytical wastes.

streams and can also be used for the on-line treatment of wastes in analytical laboratories. Commonly, oxidative processes involving the use of reactive oxygen species are used for treatment of such effluents, and generate free radicals at the structural backbone of the organic moieties.

Peroxydisulfate is a strong oxidant which has long been used industrially for the destruction of hydraulic fluids in the petroleum industry as an industrial bleach and reaction initiator. Recently, it was suggested as a useful reagent for the destruction of organic compounds in wastewater through a process named direct chemical oxidation, where the oxidant could be regenerated by electrolytic methods [30].

The on-line mixing of analytical wastes with strong oxidants can provide a fast and easy way for the destruction of organic compounds $KMnO_4$, $K_2Cr_2O_7$, H_2O_2. Chlorine compounds can also be used. However, metal ion containing compounds must be avoided because of their persistent nature and chlorine and H_2O_2 are the compounds of choice. Another alternative for on-line oxidation is the use of ozonolysis which can generate the oxidant on-line and does not provides any residues at all. Figure 8.5 indicates setup schemes suitable for on-line oxidation of wastes.

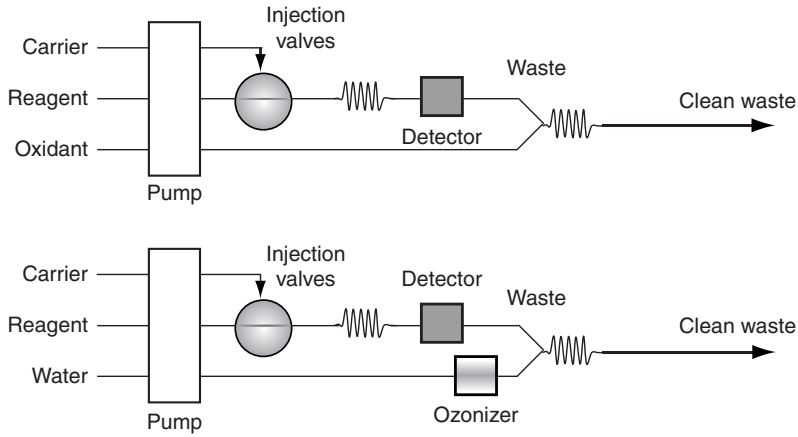

Figure 8.5 Setups suitable for on-line oxidation and on-line ozonization of analytical wastes.

8.4.3 Photocatalytic oxidation

In some cases, microbial degradation and naturally occurring hydrolysis are very slow processes. Sometimes, direct photolysis can actually lead to more toxic products, for instance direct photolysis of *p*-chlorophenol has been reported to lead to octachlorodibenzo-*p*-dioxin, an even more toxic species than its precursor. Photo-assisted catalytic procedures are a very promising, efficient, and economically feasible approach that can be used to detoxify organic molecules until their mineralization. Several semiconductors, such as ZnO, CdS, WO, and TiO_2, have been used in this field in order to provide the formation of electron–hole pairs on illumination with UV radiation and hence allow the photo-assisted catalytic oxidation of aromatic molecules on the surface of the catalyst, opening the aromatic ring, and, subsequently, their complete mineralization to carbon dioxide and hydroxyl radicals [31,32].

Chlorinated aromatics can be decomposed to form harmless CO_2 and HCl in a process referred to as total mineralization, using TiO_2 as semiconductor catalyst [33].

Additionally, small nanosized clusters of MoS_2 have demonstrated their ability for visible light-driven photooxidation of phenol and *p*-chlorophenol, achieving complete mineralization [34]. Moreover, the use of certain cationic surfactants on the surface of the nanosize materials was successfully developed to enhance the photocatalytic activity through accumulation of the organic compounds of anionic nature and improving the solid surface-dissolved molecule interaction.

The use of *p*-aminophenol as a reagent in spectrophotometric determinations has been widely extended. In general, the composition of the final products in these spectrophotometric methods is very dangerous and polluting, because not only the compound to be determined is toxic but also the reagents employed. Based on the photocatalytic degradation of organic compounds using TiO_2 slurries, different manifolds for the spectrophotometric determination of propoxur [27] and formetanate [35] by derivatization with *p*-aminophenol followed by the photo-assisted catalytic detoxification were proposed. Figure 8.6 shows the manifold proposed for photo-assisted catalytic oxidation of phenolic compounds based on the on-line use of UV-radiation in the presence of a TiO_2 slurry. In this case, it is important to note that the Teflon tubes are transparent to the UV radiation and thus the compatibility between classical automation setups and the photodegradation units is really good.

8.4.4 Biodegradation

Biological processes are naturally occurring processes in natural water regeneration and have been successfully used for the degradation of organic molecules for many decades. Such processes make use of the natural metabolism of living cells to degrade or transform chemical species by means of a sequence of reactions catalyzed by enzymes. The main advantage of these methods is that they do not need the harsh conditions required in many chemical degradations. However, the use of microorganisms is beset with many rate-limiting factors, such as the costly and time-consuming methods necessary to produce microbial cultures. Furthermore, the microbial cells stability or activity can be severely affected by changes in pH, temperature, and the presence of toxins, making their preservation in contact with analytical wastes difficult.

Taking all the aforementioned factors into consideration, the biodegradation processes are commonly used to remove the bulk organic load in wastewater, but they often have important difficulties in removing toxic organic pollutants [36].

For on-line biodegradation of analytical wastes the use of a manifold integrating a well stirred tank was proposed as can be seen in Figure 8.7.

8.4.5 Passivation of residues

Sometimes, degradation of the investigated compounds is not possible or is an expensive or slow process. In such cases, passivation of the residues is an alternative option to reduce the potential risks of analytical wastes.

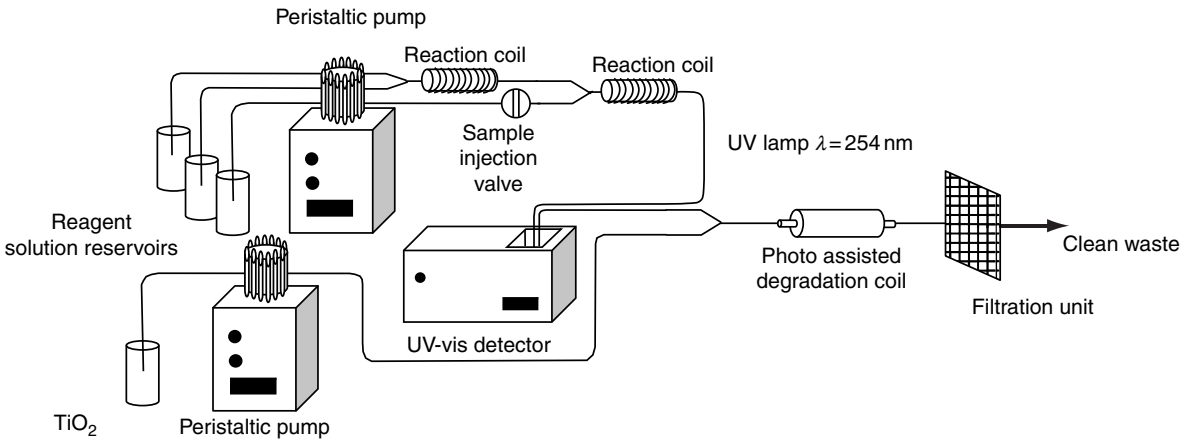

Figure 8.6 Manifold proposed for the on-line photo-assisted catalytic oxidation of phenolic compounds.

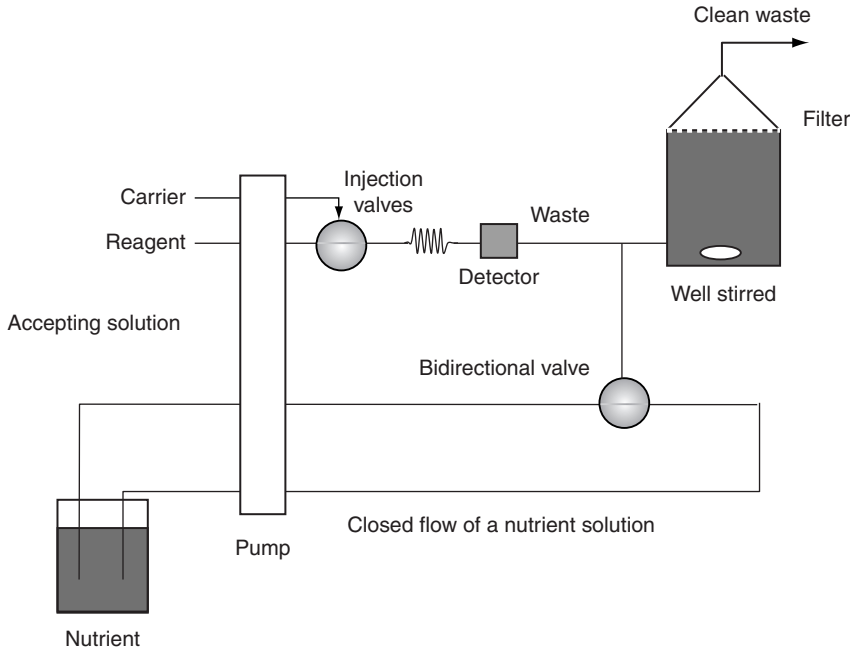

Figure 8.7 Setup proposed for the on-line decontamination of analytical wastes by biodegradation.

For instance, an activated charcoal filter has been used to remove ethidium bromide from water, various buffers, CsCl solutions, and ethanol. Since ethidium bromide is thermally degradable by heating at temperatures above 262 °C, the contaminated charcoal filters should be incinerated to provide a simple and cheap decontamination procedure [37].

Another approach for the on-line passivation of the toxic residues can be found in the example of the coprecipitation of heavy metals in the wastes obtained after cold vapor-atomic fluorescence spectroscopy (CV-AFS) determination of Hg by means of $Fe(OH)_3$ [38]. The liquid effluent obtained from the CV-AFS system with a very acidic pH (0.5) and probably containing dissolved heavy metals (Cr, Cd, Pb, ...) is merged with a solution of Fe(III) and a NaOH solution providing the on-line coprecipitation of heavy metals with $Fe(OH)_3$ (see Figure 8.8).

Metal removal from diluted wastes generated by atomic and ionic spectroscopy methods like flame atomic absorption spectroscopy (FAAS), inductively coupled plasma optical emission spectroscopy (ICP-OES), or inductively coupled plasma mass spectrometry (ICP-MS) provides a drastic reduction of the volume of wastes to be treated outside the laboratory and

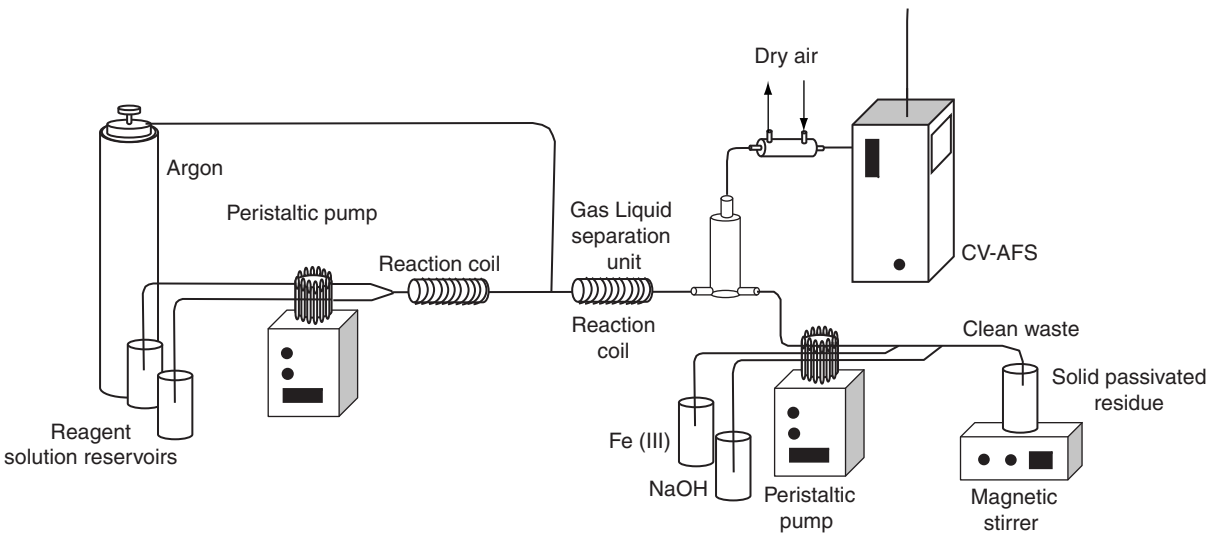

Figure 8.8 Setup employed for the green analytical determination of Hg by CV-AFS including the on-line passivation of liquid wastes.

thus provides an economic benefit. On the other hand, the coprecipitation of trace pollutant elements with iron provides a passivated solid residue which also minimizes the risks of contamination during their transport. However, it must be taken into consideration that the most toxic forms of metals are the gaseous ones, which can be directly absorbed through the respiratory system or by the skin, and thus special attention must be taken on refrigerating metal vapors obtained after FAAS, electrothermal atomic absorption spectroscopy (ETAAS), ICP-OES or ICP-MS atomization, excitation or ionization of sample components. The hot fumes which escape from the spectrometer contain gases, vapors, and fine solid particles which must be condensed by refrigeration and retained in appropriate filters. The common practice to just incorporate a mechanized extraction system of a variable length does not ensure prevention of the emission of toxic gases and particles to the outside laboratory atmosphere and thus can create risks to both the environment and the community.

8.4.6 Macromolecule-based ultrafiltration

To remove metal ions and/or organic contaminants from aqueous solutions is a frequent problem in the treatment of industrial wastewaters. Traditional ultrafiltration is limited to the separation of high molecular weight molecules, being ineffective in removing metal ions or small molecular weight organic solutes [39]. Although it is possible to reduce the membrane pore size for the purpose of removing metal ions or organic pollutants, the operating costs will be increased significantly.

Several techniques have been developed to effectively remove metal ions and small organic contaminants from wastewater, such as micellar enhanced ultrafiltration (MEUF) [40], ion-expulsion enhanced ultrafiltration [41], polymer enhanced ultrafiltration (PEUF) [42,43], dendrimer enhanced ultrafiltration (DEUF) [44], and polyelectrolyte enhanced ultrafiltration (PeEUF) [45].

MEUF is an effective separation technique to remove metal ions and/or soluble organic solutes from aqueous environments. In MEUF a surfactant is added to the polluted solution, forming large amphiphilic aggregates, at a concentration higher than its critical micelle concentration. The metal ions are attracted by the micelle surface and the organic molecules are solubilized in the micelle core and then the solution can be filtered through an ultrafiltration membrane having pore sizes small enough to block the micelles. The MEUF technique has been applied for the removal of copper ions and dissolved phenol from water [46], naphthalene, and trichloroethylene [47].

In ion-expulsion ultrafiltration, a water-soluble colloid with the same charge as the ion to be removed is added to water stream and then it is filtered through an ultrafiltration membrane with pores small enough to block the colloid. This technique has been employed to remove chromate from water eluents using polystyrene sulfonate as colloid [41].

PEUF has emerged as a promising process for recovering metal ions from contaminated water. In PEUF, a water-soluble polymer with strong binding affinity for the target metal ion is added to waste water and the resulting solution is passed through an ultrafiltration membrane with pore sizes smaller than those of the metal ion-polymer complexes. For instance, a semicontinuous system based on PEUF has been developed for removal of Pb and Cd and operates at industrial and laboratory scales [48]. It is divided into two stages: metal retention and polymer regeneration. Using this methodology, the separation of mercury from aqueous solutions by complexation—ultrafiltration was achieved using polyethylenimine as polymeric complexing agent [49].

Advances in macromolecular chemistry such as the invention of dendritic polymers provide unprecedented opportunities to develop high-capacity nanoscale chelating agents. This dendrimer technology provides nanoparticles which can be functionalized with surface groups that make them soluble in appropriate media or bind onto appropriate surfaces [50]. Thus different studies based on the use of dendrimers as chelating agents have been proposed to remove metal ions from aqueous solutions. For instance Cu(II) ions were successfully removed from aqueous streams using Poly(amido amine) dendrimers with ethylene diamine core and terminal NH_2 groups [51].

In PeEUF, a polyelectrolyte having an opposite charge to that of the multivalent ion to be removed is added to the stream. Those ions are concentrated in the vicinity of the polyelectrolyte and when the solution is filtered through an ultrafiltration membrane the polyvalent ions are retained and the permeate solution only contains small concentrations of salts. PEUF has been used to remove Cd ions from aqueous streams [52].

8.5. RECYCLING OF WASTES

The amount of wastes derived from analytical procedures can be reduced by on-line or off-line recycling with an additional benefit obtained by the recovery of costly or dangerous reagents. The treatment of analytical wastes to obtain pure reagents or solvents that can be reused should not

sacrifice the accuracy and precision of the methodologies, nor reduce the sampling throughput.

As has been mentioned in previous sections, liquid chromatography (LC) is one of the most popular analytical techniques in use around the world, due to its wide range of applications to many fields of science and industry. However, classical LC has one important disadvantage in terms of Green Analytical Chemistry, namely the large amount of wastes generated.

A direct solution for greening LC-based analytical procedures by a considerable reduction of the amount of hazardous chemical waste generated is mobile-phase recycling. In this respect, Abreu et al. evaluated the effect of recycling the mobile phase in isocratic LC on successive analyte quantifications using an UV detector [53]. When the mobile phase is recycled, the analyte exiting the detector is fed back into the solvent reservoir and diluted in the remaining mobile phase, considerably diluting the concentration of analyte. Automated LC systems set the detector signal to zero at the start of each sample run to correct for any changes in background absorbance. The results obtained in those experiments demonstrate that when analyte concentration in the MP exceeds that in the sample, a negative peak is observed, and when its concentration in the MP is equal to that in the sample, no peak is observed for that analyte. The slopes of the linear regression lines for standards in MPs with different concentrations of analyte did not change, although the y-intercept values decrease with increasing concentration of analyte in the mobile phase. In this study it was suggested that when analyte concentration in the MP approaches the lowest concentration of analyte in samples, is time to discard recycled mobile phase.

On the other hand, most of the analytical procedures developed for infrared (IR) determinations require the use of organic solvents, the chlorinated ones such as CCl_4, $CHCl_3$, and CH_2Cl_2 being frequently preferred due to their transparency in the mid-IR (MIR) range. However, these solvents are highly toxic and are considered as ozone depleting agents and hence it is essential to control the analytical waste from this type of measurement.

Taking into consideration all these factors, the on-line recycling of chlorinated solvents from IR measurements seems to be highly desirable to reduce the environmental side effects of those methodologies, reducing the risks to operators and the environment but also the costs of the analysis.

In this respect, Sanchez-Dasi et al. incorporated a semimicro distillation unit in a FIA-IR manifold for the on-line recovery of the solvent used as

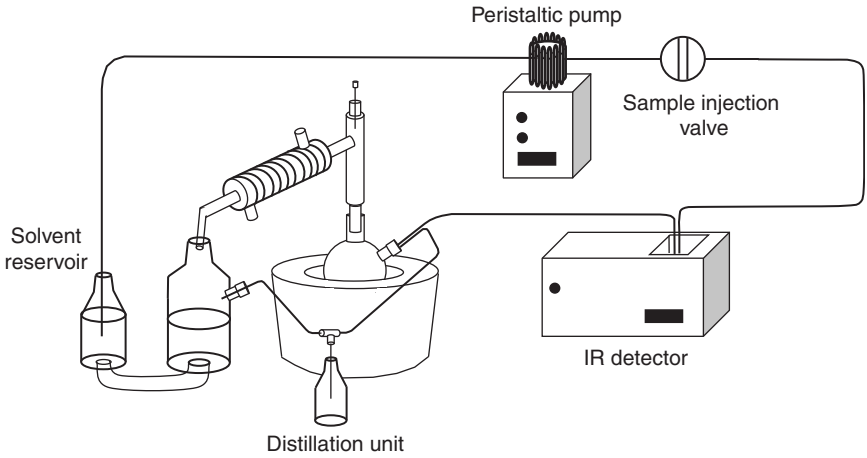

Figure 8.9 Manifold employed for the on-line recycling of carrier solvents in FTIR determinations.

the carrier. In this case, chlorinated solvents such as CCl_4, $CHCl_3$, and CH_2Cl_2 can be recovered after the ketoprofen [54] and propyphenazone and caffeine [55] determination in pharmaceuticals. Figure 8.9 shows a scheme of the employed manifold.

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CHAPTER 9

Ideas for a Change of Mentality and Practices

Contents

One of the main objectives of this chapter is to contribute to a change of the mentality of analytical chemists about the importance of their work the deleterious effect of laboratory wastes, and risks posed by the analytical methods to their operators.

As shown by a recent scientometric study about Green Analytical Chemistry [1], one of the problems in correctly identifying green practices in this field is the absence of the use of a clearly identified term in the scientific literature. This is in spite of the fact that the environmental conscience of method developers existed in many cases prior to the theoretical developments in this field and thus there are many green determinations available in the literature that are not well identified as such.

Four aspects—(i) the use of sustainability concepts and practices, (ii) the consideration of environmental aspects in the economic balance of methods, (iii) the downscaling of side effects of the methods, and (iv) the consideration of operator risks—will be discussed within the framework of this change in both mentality and practices of the analytical method developers and users, which is the key argument of all the aforementioned chapters. We need to pay attention to the input and output of analytical methods and their environmental side effects (see Figure 9.1).

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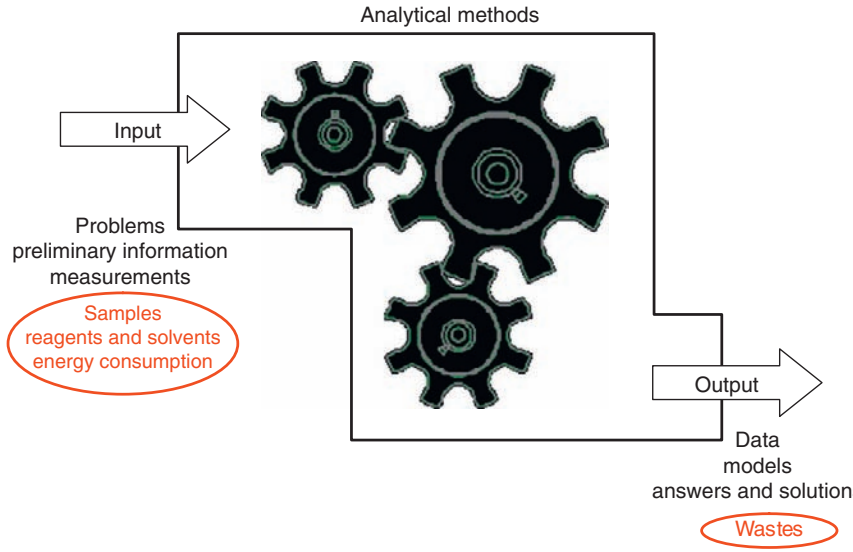

Figure 9.1 Inputs and outputs of analytical methods from both the metrological and sustainable points of view, paying special attention to aspects that can provide environmental side effects.

9.1. INTRODUCING SUSTAINABLE PARAMETERS IN THE EVALUATION OF METHODS

In the Chemiurgy period of the development of Chemistry [2] the fundamental characteristics of the analytical methods focused on their academic characteristics like accuracy, sensitivity (limit of detection), selectivity, and precision. Later on, economic and safety aspects were considered, thus providing a metrological hierarchy of analytical features [3], whereby accuracy and representativeness of methods were considered capital analytical properties with precision, sensitivity, and selectivity as basic properties and expeditiousness, cost effectiveness and personal safety/comfort considered as accessory properties [4].

In the above mentioned it is unfortunate to note the absence of sustainable aspects and thus one of the primary tasks for greening analytical chemistry concerns the introduction of sustainability-based properties in the evaluation of a method's features.

A first approach to sustainability conditions will include environmental/operator hazards, reagent and energy consumption, and waste generation as the main aspects to be considered. These parameters must be incorporated in method evaluation together with the classical ones used in method validation.

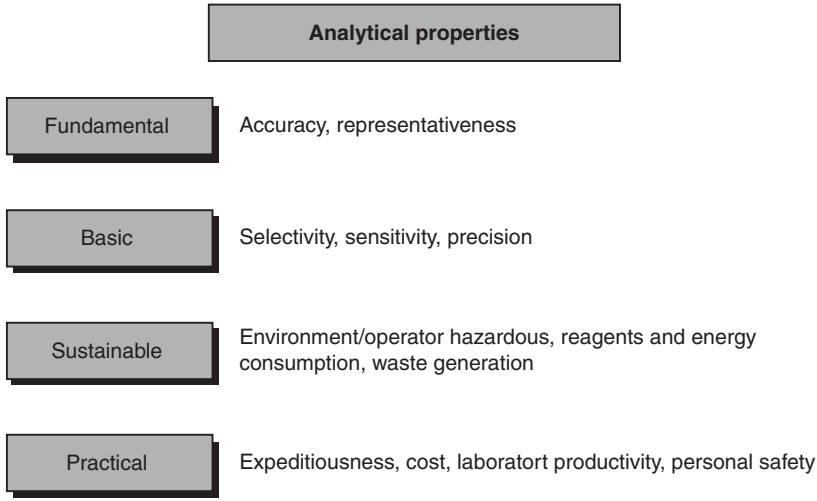

Figure 9.2 Hierarchy of analytical properties including sustainable ones.

As indicated in Figure 9.2 there is no reason to remove any of the previously considered features but we should include the new aspects in order to think about new green methods and not only new approaches.

In fact, we are absolutely convinced that there is no contradiction between the search for an accurate, selective, sensitive, and precise procedure and to improve, at the same time, the environmental side effects. Additionally, we are convinced that the ecological mentality provided by Green Analytical Chemistry does not involve necessarily an increase in the costs and thus Green Chemistry can be seen as a business opportunity for our future.

As it was clearly indicated in the 12 principles of Green Chemistry [5] and in the greenness symbols of the National Environmental Methods Index (NEMI) [6], environment/operator hazards and waste generation are two of the fundamental sustainable characteristics of a method to be considered for both evaluation of existing procedures and for greening them. It is obvious that avoiding the use of permanent bioaccumulative, toxic, or hazardous reagents is a key factor to inhibit the deleterious effect of any analytical procedure and the absence of wastes provides environmentally friendly methodologies. However, in many cases, remote sensing or noninvasive physical methods are not sensitive or selective enough to solve the analytical problems at hand and, therefore, additional aspects such as reduction of reagent and energy consumption must be taken into consideration as part of the evaluation of the sustainability of methods.

Regarding the control of the use of toxic and hazardous chemicals in the analytical laboratory, international organizations have created restrictions and legal regulations on the emission of volatile organic compounds, as in the 1985 Vienna Convention and in the 1987 Montreal protocol [7], the regulations controlling persistent organic pollutants and air contaminants in the 2001 Stockholm protocol and the 1990 USA Clear Air Act [8] but additional efforts must also be made to replace toxic reagents by innocuous ones or by employing the lowest amounts of the least toxic available reagents. To achieve this, the use of material safety data sheet (MSDS) is mandatory as indicated in Chapter 3.

Concerning the reduction of wastes, Koel and Kaljurand have suggested that for medical and state laboratories that analyze a huge number of samples daily, the E-factor criterion concerning the ratio of by-products to desired products can be employed, thus bringing these laboratories in line with the fine chemical industry [9,10].

In short the change of mentality concerning the features of the analytical methods must involve new criteria for method evaluation and new objectives and, as it has been shown in Chapters 4 and 6, the avoidance of sample treatments together with multianalyte capabilities of methods are some of the objectives to be reached.

On the other hand, a correct evaluation of reagent consumption and waste generation, by working hour or per analysis, must be done in order to provide new comparative parameters for the appropriate selection of methods. We therefore propose that alternative green methodologies be fully validated and evaluated from the aforementioned criteria and comparative data provided to allow a comparison between sustainable figures of merit of available methods and those proposed.

9.2. ECONOMIC BALANCES OF SUSTAINABILITY

Pfizer, Glaxo Smith Kline and Bristol-Myers Squibb have incorporated the Green Chemistry ideas to their research and development programs [11] and, in particular Pfizer has recognized the benefits obtained in reduced waste management cost, reduced energy consumption cost, reduced time requirements, increased yields and simple processing, reduced compliance cost, increased worker, community and environment safety, and increased operational flexibility [12].

The aforementioned comments agree well with our viewpoint on the opportunities offered by Green Analytical Chemistry in economic terms.

For an adequate balance of the cost of the analysis, the amount of reagents employed is, of course, one of the main outputs together with the cost of energy and waste management. In all these points the greening of available methods reduces their amounts and associated costs, especially when remote sensing or multianalyte or multisample approaches are selected. So, just from an economic perspective, the reduction of costs as a result of the adoption of Green Analytical Chemistry justifies the selection of these methodologies which are also suitable candidates for improvement through miniaturization and automation (see Figure 9.3). However, the main advantages of green methods are associated with

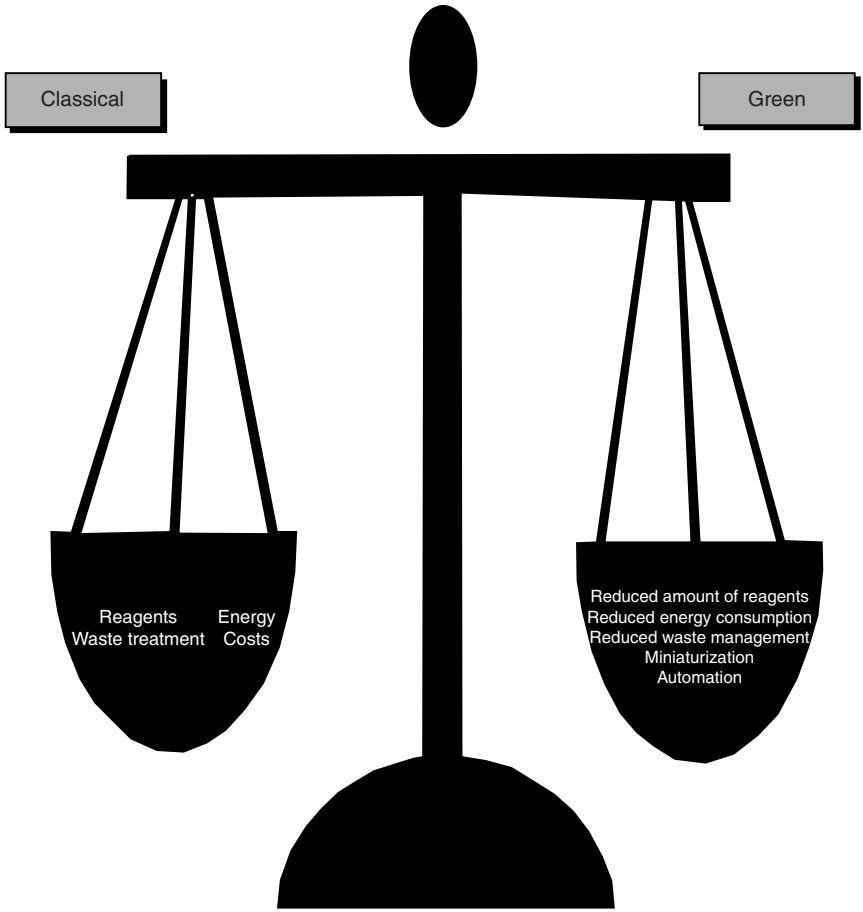

Figure 9.3 Economic balance of green analytical methods as compared with traditional ones.

operator and environmental health and safety because green alternatives can strongly reduce risks of significant human and economic damage.

Concerning the cost of waste management, the examples provided by Dane Mooney are very clear.

In Chapter 1, in discussing the ecological mentality (see Figure 1.3) we noted that the bad conscience period involved the creation of waste management programs in both industrial and academic laboratories, and over time the academic authorities recognized the tremendous economic effort that was involved in waste management. This led, in 1991, to the University of California at Berkeley implementing a charge-back program for approximately 1000 laboratories in its schools. This program was implemented gradually to minimize the risks that researchers improperly disposed of their hazardous waste and thus, at the beginning the charge-back was 25% of disposal cost and the authorities performed laboratory inspections and analyzed effluents, training garbage and pickup crews to identify properly the hazardous wastes. After the first period the charge-back cost of disposal gradually increased to reach 100%. In another case, in the University of Wyoming (which established this charge-back program in 1994) the 25% of the cost of waste management of its 450 laboratories was financed by the university but 75% of this cost was assumed by the different departments and research laboratories.

So, it is clear that a new ecological mentality in academic and applied laboratories involves the introduction of new variables in their economic balances and provides additional reasons for the implementation of a Green Chemistry program, which necessarily must involves all the members of the community.

9.3. DOWNSIZING THE SCALE OF PROBLEMS

As shown in Chapter 4, in order to avoid sample treatments, the use of remote sensing or noninvasive methods of analysis are the best alternative to greening the analytical methods because the aforementioned systems completely avoid the use of hazardous chemicals. However, in many cases samples must be analyzed using classical schemes of sample pretreatment and sample dissolution followed by separation and detection of the analytes under study. In such cases, a change in the mentality of method developers would involve the drastic reduction of reagents consumed, operator risks and waste generation. Downsizing the scale of problems is a clear option from both the economic and safety points of view. In a story, which was

included in a Spanish manual for the education of children, a young man visits a holy man who lives as a hermit in the mountains and he asks the hermit about the secret of life. During the conversation, the old man suggests to the young man that he pull out of the ground a green shoot, a small plant, then a shrub, and finally a tree. In the beginning the young man had no problem performing the actions suggested by the holy man and thought that the old man was completely out of his senses and did not understand his question. However, when he was unable to pull the tree out of the ground the old man told him that this tree was once a shrub, before that a small plant and began as a little green shoot growing out the ground, and the secret of life is to not let problems to grow to such a size that it is impossible to find an easy solution [13]. This story could be used at this point to try to modify our behavior in the lab and to support the wisdom of not letting problems grow, thus avoiding waste accumulation.

The best way to avoid the problems of waste with the associated high cost and risk to operators and the environment is, in our opinion, to reduce drastically the consumption of reagents and the generation of wastes and, whenever possible, to carry out the decontamination or passivation of wastes on-line to prevent the accumulation of residues in industrial and academic laboratories.

The miniaturization and automation of laboratory operations can be considered as the simplest way of greening analytical methodologies because this reduces the amount of samples, reagents and contact with the operators, and the risks can be minimized (see Chapter 7 for a discussion about available strategies proposed in the literature). Additionally, the full automation of methods must include the treatment of wastes. In fact, after the measurement step the wastes could be conveniently treated on-line to recover the solvents employed or to destroy the hazardous molecules. There are many available strategies to do so (see Chapter 8) and in fact the change of mentality required to adopt this kind of approach is simply to try and solve problems at the earliest possible stage in order to avoid risks and expenses.

The mentality for downsizing problems requires method developers and users to take responsibility for both data and wastes, and not compromise the environment. They can do so by avoiding the side effects of chemistries employed in the laboratory. Additionally, it provides new economic opportunities because of the reduction of costs (see Figure 9.4).

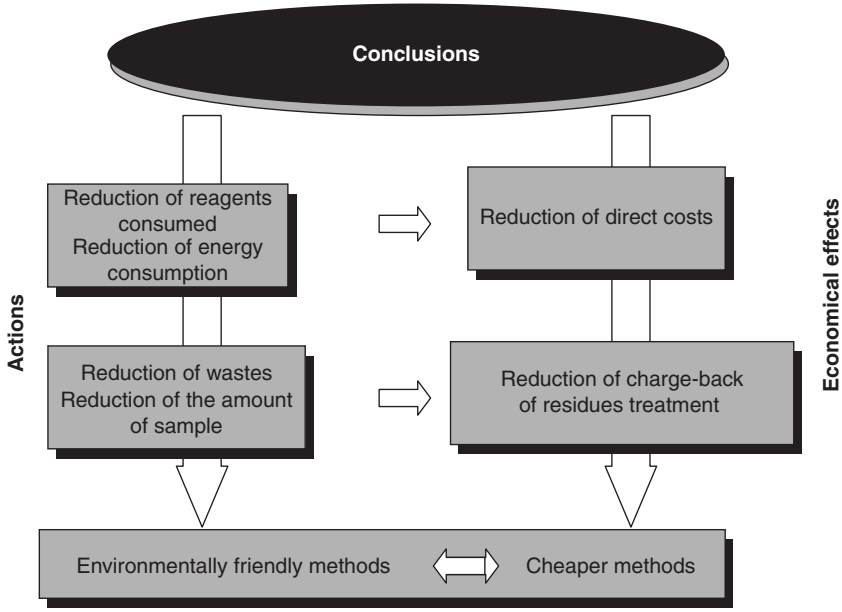

Figure 9.4 Correlation between downsizing environmental side effects of analytical methods and their costs.

9.4. CREATING NEW RELATIONSHIPS BETWEEN SAMPLES AND OPERATORS

Green analytical methods involve a drastic reduction of the amounts of samples to be taken on site and transported to the laboratory. In fact, the greenest methods are those which can obtain the required information about the samples without removing any amount of material and provide the necessary data by remote sensing or in-field noninvasive measurements. So, the green methods are cognizant of the Heisenberg principle and try to modify the system about which the information needs to be obtained as little as possible.

However, in many cases neither open cell remote procedures nor appropriate portable instrumentation are available to obtain the information required and, in these cases, efforts should concentrate on: (i) taking the minimum amount of sample which could be representative of the system, (ii) avoiding the problems related with sample transport and sample storage, (iii) carrying out in-field preconcentration of the analytes to be determined and modifying both their concentration and their physical state in order to avoid contamination, losses, and stability problems, (iv) avoiding, if possible, the

addition of stabilizers which could contribute to increasing the blank values and affect some of the determinations to be made in the laboratory, and (v) not accumulating large volumes of samples in the laboratory, keeping just a representative, well-preserved amount for confirmatory analysis.

All the aforementioned actions try to avoid accuracy problems in sample analysis and take into consideration the fact that the transport of excessive amounts of samples and their accumulation in the laboratory clearly increases the cost of analysis, does not provide any advantage, creates transport and storage issues and significantly increases the amount of wastes to be treated.

The minimization of the amount of samples together with the reduction of sample mass transported could be completed with a general practice of avoiding operator contact with samples wherever possible. Contamination and adsorption effects, but specially the reduction of operator risk, are the basis for creating new relationships between samples and operators.

In a recent paper about the use of near-infrared (NIR) spectroscopy in monitoring industrial food processes, Pedersen and Engelsen [14] made a comparison between explorative multivariate spectroscopic measurements and traditional deductive univariate and slow physicochemical methods in terms of a comparison of two ways of handling the (nature) dragon, thus either being invasive, destructive, slow, environmentally harmful and univariate or being remote, nondestructive, rapid, environmentally friendly, and multivariate. This is a good description of the change of mentality of today's analytical chemistry with respect to the problems and our preference for the soft multiinformative strategies over aggressive monoparametric and univariate methods.

The risks to operators can be minimized by introducing fully mechanized and automated procedures, from the sampling step through to the final waste treatment, and it provides, once again, advantages from the environmental, and economic points of view.

As an example of the change of mentality regarding the interaction between operators and samples, Figure 9.5 shows a setup created to do in-field sampling of water in deep lagoons and *in situ* measurement of as many parameters as possible (pH, redox potential, dissolved O_2, conductivity, and activity of anions like NO_3^- or S^{2-}), after the samples are filtered on-line without the deep layer of water being in contact with the surface [15]. This strategy avoids problems of exchanges between deep samples and the surface medium and provides a clean and safe way to take representative data about the system. The on-line collection of solid particles can be done by filtration and trace and ultratrace amounts of cations,

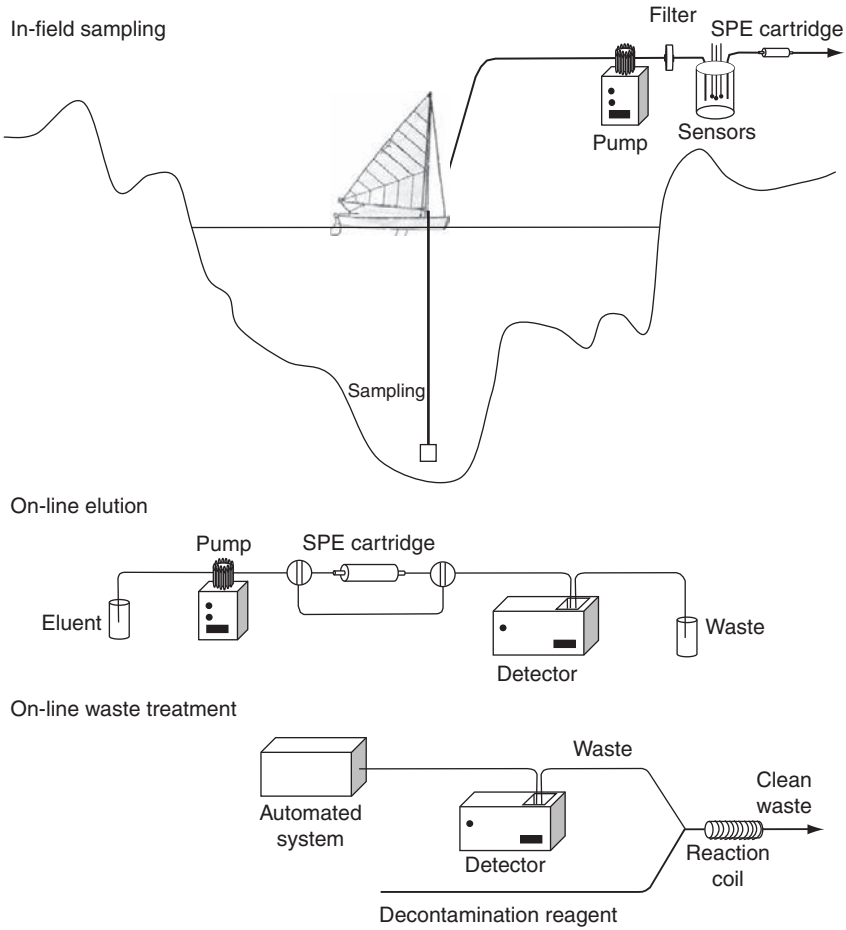

Figure 9.5 In-field sampling, on-line elution, and on-line waste treatment as examples of the new relationships between samples and operators created by the Green Chemistry principle.

anions, and dissolved organic molecules could be retained on-line. Attention must be paid to the fact that dissolved species could be selectively retained by using this approach in combination with appropriate solid phase extraction (SPE) cartridges. The mass of analytes present in volumes of 100–1000 mL can be recovered in less than 100 mg of a solid sorbent, thus providing a preconcentration and a stabilization of the species to be measured because inside the cartridge the stability of cationic, anionic, or organic compounds is enhanced and contamination or losses are minimized.

After in-field sampling, cartridges can be easily and safety transported to the laboratory and the analytes eluted on-line with the minimum amount of appropriate solvent, thus saving costs and improving the sensitivity, representativeness, safety, and repeatability of operations.

After the complete analysis of samples the on-line treatment of wastes assures the obtention of a clean waste through the use of the different strategies commented in Chapter 8, thus integrating the ideas of a sustainable analytical chemistry in all the steps of the analytical methods [15].

In a general conclusion of this chapter, we agree with J.C. Warner, the editor of *Green Chemistry Letters and Reviews* [16] that we are living the coming of age of Green Chemistry. This is supported by the increasing interest in this topic in the scientific literature but also in the evolution of the European Union legislation on registration, evaluation, authorization, and restriction of chemical substances (REACH) and by the fact that the state of California rolled out the first U.S. State Green Chemistry laws last year and as Peoples, the director of the American Chemical Society (ACS) Green Chemistry Institute in Washington DC, said in a recent editorial of the Green Chemistry journal, we are now far from the real and perceived barriers to the greater adoption of Green Chemistry as identified by the Royal Society of Chemistry (RSC) Environment Health and Safety Committee in 2002 [17] and this change of scenario is clearly due to a change in the mentality of chemists, industries, and teachers in order to identify Green Chemistry as a business and investment opportunity for the future. Therefore the principles discussed throughout this chapter offer a means to avoid hazards to the environment, an opportunity to create workforce development and a way to improve science literacy in classrooms.

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CHAPTER 10

Practical Consequences of Green Analytical Chemistry

Contents

The main objective of the present chapter is to convince the reader about the need for greener analytical methods based on two arguments: ethical reasons, which support the compromise of all persons with the environment and future generations, and the fact that a green method can be substantially less expensive than a nongreen one. From our point of view this mixture of idealism and realism provides the reason of the success of Green Analytical Chemistry and the guarantee of its future (see Figure 10.1).

In the previous chapters of the book, we have exposed the origins and connections between Green Analytical Chemistry and Green Chemistry and in many of these chapters, we have presented different advances and techniques which have contributed to the development of efficient tools for greening the available methods.

It is clear that many of the main scientific contributions on automation, miniaturization, alternative solvents, and low energy consumption systems have been produced outside of the framework of Green Analytical Chemistry. However, we are convinced that the Green Chemistry principles, the ethical component of the ecological paradigm of chemistry and the economic reasons, which offer new business opportunities in the Analytical Chemistry field, will guarantee a series of changes in both the mentality

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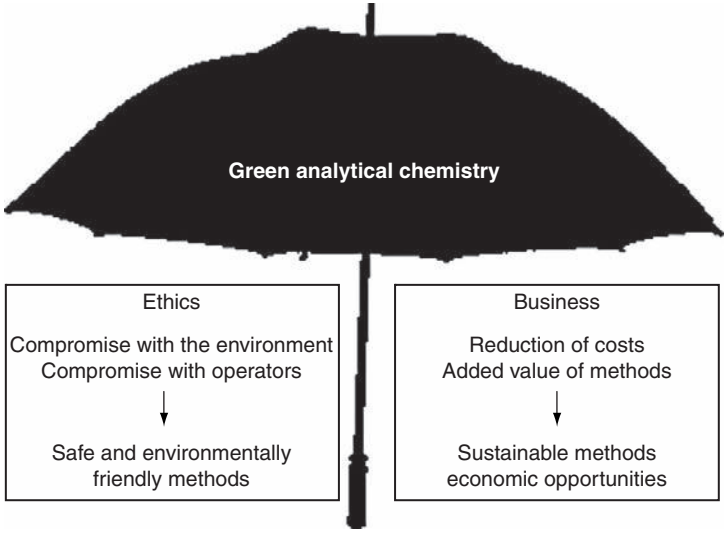

Figure 10.1 Green Analytical Chemistry an equilibrium between idealism and pragmatism.

and practice of Analytical Chemistry that will change the panorama of analytical methodologies in the near future.

In the following sections, we will describe some of the aspects of the practical work in the research and application laboratories which will be drastically affected by the development of Green Analytical Chemistry, from the use of green terms and featured criteria to evaluate the methods that are common practice in routine laboratory practice, also those affecting the publication habits and the teaching practices.

10.1. THE USE OF GREEN TERMS

As has been commented on in Chapter 1, one of the main difficulties in writing about the development of Green Analytical Chemistry or in preparing a review on this topic is that use of this term is not common. In the past, terms like clean analytical methods, environmentally friendly methods, or sustainable analysis were used together with the term green, thus making it difficult to identify the relevant papers in this field. Additionally, many basic and methodological developments, which are of a great importance in greening analytical methods, were simply to increase the selectivity of available procedures or to improve the repeatability of laboratory operations, to facilitate the analysis of extremely small samples or to increase the analysis

speed or overcome problems related to sample transport or analyte stability. For these reasons in fundamental studies about the use of microwave-assisted sample treatments, ultrasound-assisted extractions, supercritical fluids applications in Analytical Chemistry, solid phase extraction (SPE), flow injection analysis (FIA), sequential injection analysis (SIA) or multicommutation, in-field sampling, and miniaturization or remote sensing, there is no reference to Green Analytical Chemistry, only some comments about the reduction of solvent or reagent consumption and waste generation. Nevertheless, it is the aforementioned studies which today we consider as milestones in the development of Green Analytical Chemistry.

So, one of the practical consequences of the crystallization of Green Chemistry in the scientific literature and research institutes and laboratories (see Chapter 1 and Table 1.2 to confirm the influence of this term in today's panorama of the global literature and research) is that nowadays Green Analytical Chemistry seems to be the best framework in which to integrate all the aforementioned studies and efforts and, therefore, it is highly desirable to incorporate this term into the keywords of papers including advances on greening the available methodologies, on both the replacement of toxic reagents by innocuous ones and on aspects regarding reduction in reagents and energy consumption, operators, and environment risks or waste generation.

The use of the term Green Analytical Chemistry in the literature will contribute to easier identification of methodological advances in this field and, additionally, could contribute to expansion of the ethical component of Green Chemistry, encouraging new practitioners.

Green is just a color and perhaps a term like sustainable analytical chemistry would be more appropriate to describe the objectives and practices presented throughout this book. However, the general use of the term green by ecological groups throughout the world and its identification with environmental health have definitely contributed to the success of the term Green Chemistry as defined by Paul Anastas [1] and therefore Green Analytical Chemistry is probably the best choice to reflect in the literature the advances in sustainable analytical chemistry.

We strongly recommend the use of Green Analytical Chemistry and associated terms like green parameters, green consequences, green aspects, or greening to describe efforts toward avoiding the undesirable side effects of Analytical Chemistry practices and we hope that the general use of the aforementioned terms in the scientific literature will help create a snowball effect as schematized in Figure 10.2.

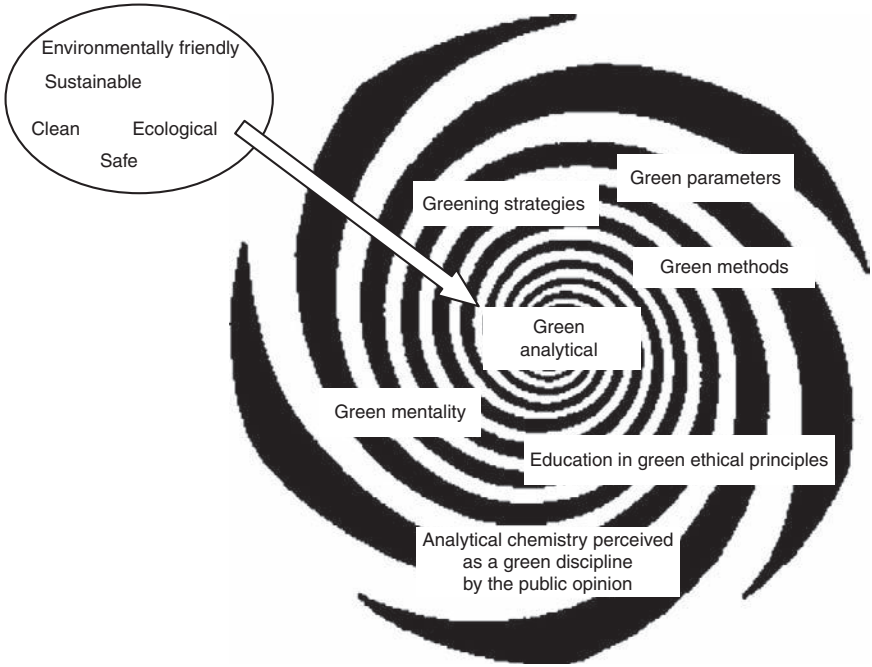

Figure 10.2 The importance of the use of green terms.

So, ultimately we hope that public opinion will perceive Analytical Chemistry as a friendly tool to preserve the health of the planet. This complements the recommendations of the Pimentel report [2] which was, as indicated in Chapter 1, the foundation for the environmental compromise made by chemistry societies.

10.2. THE NEED OF CLASSIFICATION CRITERIA FOR ANALYTICAL METHODS CONCERNING SUSTAINABILITY

In Analytical Chemistry, validation of methods has always been the main challenge. So, from the analytical view point it was never enough for a method to be new, because the reason for replacing old methodologies by new ones has been based on the improvement of their analytical features.

In the past, efforts to enhance the sensitivity, selectivity, and precision of methods, in addition to accuracy validation, were the basis of the advancement of Analytical Chemistry.

Nowadays, the ethical compromise represented by Green Analytical Chemistry involves the necessity of using new parameters concerning

sustainability to evaluate alternative methods. Thus the full validation of proposed methodologies must consider the evaluation of the toxicity of reagents involved, the potential risks to operators, an in-depth consideration of the amount of reagents and solvents required together with the evaluation of energy consumption and power requirements, as well as the quantification of the amounts of waste generated and its management. So, validation of green methods involves complete information for potential users and this must be presented in an easy way. In this regard, the greenness symbols established by the NEMI [3] offer a good way to clearly show those methods which can be classified as green. However, it is clear that the aforementioned symbols were established just to differentiate green methods from nongreen ones. At the present stage of the development of Green Analytical Chemistry they must be complemented with additional indicators that are able to differentiate green methods using quantitative aspects concerning operator risks, energy and reagents consumed, and volumes of waste generated. So, as can be seen in Figure 10.3, we propose an additional pictogram to classify, using a color scale, three levels of evaluation of methods for how green they are. Based on the same principle of the green symbols, a circle with four quadrants could be used to quantify—from red to orange and green—the high, medium, or low risk involved for operators and the levels of reagent and energy consumption and wastes. This new pictogram could be used to evaluate the greenness level of a proposal. The limits of high, medium, and low consumption, risks or waste volumes must be established by consensus within and between scientific societies.

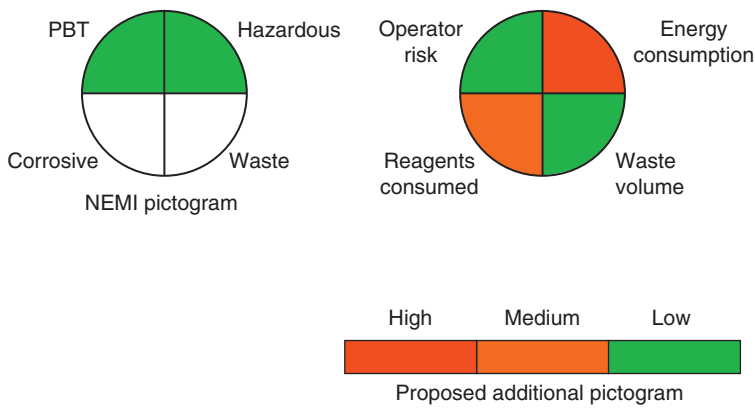

Figure 10.3 Green pictograms for method classification.

We are convinced that the generalization of green parameters in method evaluation and the general use of greenness symbols will contribute to expanding the concepts and general use of Green Analytical Chemistry and to improve the safety of analytical chemistry operators.

As indicated in Section 10.1 green parameters cannot be contradictory to classical ones, the search for accurate, selective, and sensitive methods being irrevocable but not at the price of the use of dangerous practices nor based on the use of large amounts of hazardous chemicals and creating large volumes of toxic wastes. Once again, we would like to stress that the environmentally friendly character of methods is not at all contradictory with reduction of costs. On the contrary, the reason for the advance of Green Chemistry ideas is that they offer economic opportunities for business as green methods can be less expensive than traditional ones thus providing sustainable tools from both the environmental and economic viewpoints.

10.3. PRACTICES TO BE AVOIDED IN ANALYTICAL LABORATORIES

As the main principles of Green Chemistry indicate, the use of toxic or hazardous reagents should be avoided in any kind of analytical determination. So, toxic reagents proposed in available methods must be replaced by innocuous or less toxic ones. However, it is clear that many of the analytes and sample constituents could be toxic themselves and, thus, it is impossible to practice a hazard-free analytical chemistry and, therefore, efforts to replace traditional chemical and physicochemical methods, based on an extractive sampling and sample transport and sample treatments by remote or noninvasive methodologies, must be a priority task in Green Analytical Chemistry.

Table 10.1 summarizes the good and bad practices in analytical laboratories, examined from a Green Analytical view point.

Ordered as a function of the different steps of the analytical process, from sampling to waste management, it is clear that the extensive sampling and sample transport must be avoided for safety considerations but also, from the scientific point of view, to minimize sample contamination risks and changes in the composition of samples due to the modification of their environment.

From an analytical viewpoint the development of *in situ* analysis tools and in-field sampling methods can affect operator and environment safety by eliminating the deleterious effect of the transport of high volumes of samples that are potentially polluted and thus containing dangerous

Table 10.1 Good and bad practices to be done or avoided in the analytical laboratory

	Avoid	Do
Sampling	Extensive sampling and sample transport	Remote sensing *In situ* parameters measurement In-field sampling and analyte preconcentration
Sample treatment	Extensive physical and chemical treatment	Noninvasive analysis Reduction of sample size Increase of fast sample treatments
Separation	Low pressure techniques Involving big solvent volumes	Miniaturization techniques
Determination	High volume in "batch" measurements	Automation and miniaturization of methods
Waste management	Accumulation and disposal	On-line decontamination Recycling Passivation and reduction

materials. Additionally, the replacement of traditional in-batch off-line methods can reduce dramatically the analytical costs and the time required to obtain relevant data for making decisions, thus improving the environmental and economic aspects of our discipline.

Concerning sample treatments, in Chapters 4 and 5 we showed that these steps can be the Achilles heel of the methods as they include time consuming, extensive physical, and chemical handling of samples. So, efforts to avoid sample dissolutions and analyte extractions, matrix removal and analyte preconcentrations, or at least to make the processes greener, can contribute to eliminating or reducing the amounts of reagents and solvents required and to improving the accuracy and precision of analytical methods. Once again it can be seen that the interest from the fundamental and environmental sides is complementary.

Soxhlet and traditional wet digestion in open systems, together with liquid–liquid classical extraction systems could be replaced by microwave-assisted extraction procedures, improving the classical methods and providing unexpected solutions for trace and speciation analysis due to the drastic reduction of blank values and the good preconcentration levels obtained using the modern green sample treatment alternatives.

Separation processes of the analytes to be determined in complex samples frequently require the use of chromatography or electrophoresis. In this field the miniaturization strategies offer exciting alternatives to the use of classical techniques involving large volumes of samples and solvents without reducing the accuracy, selectivity, and precision of the determinations.

High volumes in batch determination methods must be avoided to drastically reduce the amounts of solvents and reagents and to avoid the generation of large volumes of wastes. On the contrary, automation of the whole analytical method, or at least of the determination steps, is strongly recommended to improve operator safety and to increase the sampling throughput, in addition to minimizing reagent consumption and waste generation.

On the other hand, the old practice of waste accumulation for later treatment must be replaced by processing wastes as they are generated. If we consider waste generation as a step in the analytical method, a simple and low cost strategy for the *in situ* treatment of wastes must be proposed together with the operations to obtain quantitative data in order to avoid safety problems related to the storage of large amounts of residues and the elevated cost of their management.

The recycling of solvents and reagents and the decontamination of wastes can be considered as just an additional chemical effort to compensate the side effects of the activities of chemical laboratories and the incorporation of these aspects to the new proposed methods could increase the feeling that chemistry is not the cause of environmental problems but that, on the contrary, chemical processes could be an important part of their solution.

10.4. PRACTICES TO BE IMPROVED IN ANALYTICAL LABORATORIES

Information is the keyword for a Green Analytical Chemistry. The correct knowledge about the toxicity and associated risks of analytes and reagents is the basis for taking decisions about the best way for greening analytical methods.

Replacement of hazardous and toxic reagents and solvents is one of the principles of Green Chemistry and in this respect the development of remote sensing and noninvasive procedures is strongly recommended in order to avoid the use of chemicals and contact between operators and samples. An additional benefit of the aforementioned procedures is their

nondestructive character and they can be of great importance in cultural heritage analysis and for fast diagnostics in clinical and industrial chemistry.

The *in situ* analysis, or at least in-field sampling, reduces the risks of sample transport and avoids chemical treatments for analyte stabilization. For this purpose the developments in portable instrumentation and automation are of great importance and Green Analytical Chemistry derives some of its best tools from the automation and miniaturization advances in both sample treatment and measurement steps, and also miniaturization of the separation processes.

Regarding waste management it is evident that the reduction of residues and their detoxification or passivation concern analytical methods users and not external organizations. A green conscience with respect to the importance and environmental impact of wastes could benefit laboratories by leading them to incorporate on-line solutions to deal with the problems created by the generation of residues.

It is evident that the treatment of reduced quantities of toxic wastes involves lower costs and offers less social risks than the accumulation of residues from different laboratories. In this respect we should emphasize that our knowledge about the synergistic effect of mixtures of chemicals is somewhat deficient and that problems related to the toxicity of mixtures of reagents and solvents can be several orders of magnitude worse than the risks associated with each reagent alone. Therefore it is highly desirable to treat the wastes as a part of the analytical method itself in order to avoid the increase the magnitude of the chemical-related problems.

The aforementioned recommendations must be considered within the framework of a change of mentality of method developers and users in the direction of an ethical compromise with society and the environment. Only on the basis of this new green mentality can we easily apply the available tools in the literature to greening our analytical procedures.

10.5. GREENING THE ANALYTICAL PUBLICATIONS

There are two aspects that contribute to the implementation of Green Analytical Chemistry which will be of a great importance in the coming years: The incorporation of a green mentality into the teaching of Analytical Chemistry in schools and universities and the consideration of the green principles in scientific publications concerning new methods.

As indicated in Chapter 1, the terms green method or Green Analytical Chemistry are rare in the literature in spite of the fact that there have been

so many efforts to save reagents and reduce wastes and risks and this contributes to a lack of conscience about problems related to the deleterious effects of analytical methods. So, the introduction of green principles in both teaching and publishing activities will contribute to the best integration of efforts in this field, to modifying the deleterious environmental practices in research and development laboratories, and to changing the social perception of chemistry as a dangerous practice.

Regarding the publication of new methods involving advantages in their environmental aspects, Table 10.2 summarizes the recommendations to provide the reader with an adequate idea of the objective of the method proposal.

The use of Green Analytical Chemistry as a keyword is highly desirable if the method really contributes to avoiding the use of hazardous chemicals or to alleviating the collateral effects of analytical procedures. It will favor the identification of new tools of general use and sustainable analytical methods as well as contribute to spreading the green mentality amongst our colleagues.

When the objective of a study is to green a method, the incorporation of green terms into the title and/or the abstract will contribute to attracting the attention of readers with an environmentally friendly viewpoint and we are sure that it will contribute positively to the impact factor in the literature concerning Green Chemistry.

Table 10.2 Aspects to be considered on greening analytical publications

Title and abstracts	Introduction, if possible, the green terms as green method or greening of previous ones
Keywords	Incorporate the terms Green Analytical Chemistry
Procedure	Advertisement about reagent toxicity Advertisement about operator risks Incorporate, if possible, waste treatments for on-line measurements
Figures	Incorporate green pictograms
Tables	Quantify reagents consumed, energy required and waste generation
Complementary material	Add links to MSDS Incorporate information about personal safety media Add recommendation for waste treatment

However, from our perspective, the main aspects to be included in the publication of a green analytical method concern the incorporation into the procedure of information about reagent toxicity, operator risks, and the consideration of waste hazards and waste treatment recommendations. If possible, the on-line treatment of analytical wastes must be included in the recommended procedure in order to provide an actual green method.

Other important aspects of Green Analytical Chemistry publications are figures and tables. To clearly show the green character of the proposed procedures the use of green pictograms, as indicated in Figure 10.3, is strongly recommended. The aforementioned pictogram (included in one of the manuscript figures, as an inset) could show the main advantages and remaining drawbacks of the proposed method with regard to its sustainability. The common use of the aforementioned symbols can raise consciousness about the deleterious effects of the use of reagents and solvents and it could contribute to spreading the green mentality.

Quantifying the reagents consumed in milligram per determination or grams per 100 determinations together with a clear reference to the power requirements of all the apparatus and instruments involved in the proposed method, and the evaluation of the energy consumption of the procedures and the volume of wastes generated per 100 determinations or per hour (when automated or mechanized methods are involved), is a necessity in evaluating the greenness of an analytical procedure and thus, the aforementioned aspects must be included in both the summary and tables to provide objective quantifiers about the environmentally friendly character of the method.

The on-line publication of analytical journals offers additional possibilities for authors to include complementary materials which, of course, are not necessary to evaluate the original contribution of their studies but can be of great importance for those who would like to apply the proposed methodologies. Material safety data sheets (MSDS) or links to free web pages including data about toxicity and risks of the use of all reagents and solvents involved in the analytical procedure, together with specific information about the personal safety media required for safe use of the proposed procedures and complete recommendations for waste treatment, could be necessary for the application of methods. Therefore our proposal to journal editors is to encourage their authors to provide the aforementioned complementary material when the accepted manuscript involves a new or a modified analytical procedure suitable to be used in routine analysis by nonspecialized operators.

These recommendations are easy to follow and will provide an increase of the available chemical information for practitioners, thus improving the safety of analytical method operations for both analysts and the environment.

10.6. TEACHING GREEN ANALYTICAL CHEMISTRY

The incorporation of green chemistry principles into common practice in research and development laboratories strongly depends on the education of the new generations of chemists and thus, the teaching of chemistry disciplines needs to change and the younger generations must be aware of the ideas of sustainability and environmentally friendly chemistry and analytical chemistry in particular. The objective is not the indoctrination of the younger generations not the introduction of new matters as green ones, but just to put the things in their proper context.

Green Chemistry and Green Analytical Chemistry are an ethical compromise of chemistry practitioners with society and the environment and involve all aspects of chemistry in such a way as to increase the safety of Analytical Chemistry practices and to avoid the collateral effects of the use of chemical reagents and solvents. So, our proposal is to incorporate the main principles and examples of Green Chemistry at the beginning of chemistry studies in secondary schools and also in primary schools, in the study of the relationships between man and biota.

The introduction of the Green Chemistry principles together with the fundamentals of chemistry in the early education of students [4] could contribute to improving public opinion about chemistry, which is generally perceived as a symbol of everything that is artificial, dangerous, and toxic. It is our responsibility to emphasize the fact that chemistry is not essentially good or bad. The atomic and molecular knowledge of our surrounding environment, the reactions involved in natural processes and in bodily functions are the basics of a science, which, of course, permits us to produce and manage both natural and synthetic products. We must not forget to transmit the ethical and environmental aspects so that our students can identify chemistry as a part of the solution of environmental problems and not only as one of the causative agents of environmental change.

It is in the high schools and universities where Green Analytical Chemistry must play an important role in the formation of future practitioners. As has been mentioned in the first chapter of this book and can

be confirmed in many of the references, the interest of analytical chemists in undesirable environmental side effects of their practices began some time prior to the formalization of Green Chemistry.

Within the framework of Green Chemistry all the efforts to reduce sample handling, reagent consumption, the risks to operators, energy consumption, and waste generation, became integrated in a new concept for analytical methodologies, starting from the consideration of the problems and finishing by proposing explanatory models based on the chemical data. However, new problems associated with energy, reagents and solvents demand, and waste management required before, during, and after application of the method have arisen. The original contribution of Green Analytical Chemistry is that all of the aforementioned questions are considered as analytical aspects of both problems and methodologies.

So, an important basic education of young analytical chemists on the principles and practices of Green Analytical Chemistry will be of a great interest for their future professional activities and will provide tools for an appropriate selection of the best method for both obtaining the best possible data and from a waste management point of view.

Nowadays, journals devoted to chemical education [5] and papers concerning studies in chemistry [6] have incorporated laboratory practices [7] and specific programs [8] for the introduction of green chemistry principles in university studies and an increase in this kind of initiative could be very important for the general acceptance of Green Analytical Chemistry.

In summary, based on the ethical and philosophical aspects of Green Analytical Chemistry, and supported by the available tools for avoiding sample treatments and reagents through the use of remote sensing and noninvasive physical methods, automation and miniaturization strategies, and on-line waste decontamination, it is clear that Green Analytical Chemistry could contribute to improve the social reputation of chemistry. In addition, when the basic and applied aspects of Green Analytical Chemistry permeate scientific publications and teaching practices, we shall be able to improve the available methods. Last but certainly not least, the economic aspects, which have been highlighted many times throughout this book, offer opportunities for saving reagents and to reduce energy consumption and waste management costs.

We hope that in this book the reader has found reasons, ideas, and examples for greening his or her own activity and that this text will contribute to the silent revolution of an environmentally conscientious science.

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