2nd Edition

Green Chemistry Series

Challenges in Green Analytical Chemistry

Edited by Salvador Garrigues
and Miguel de la Guardia

ROYAL SOCIETY OF CHEMISTRY

Challenges in Green Analytical Chemistry
2nd Edition

Green Chemistry Series

Editor-in-chief:
James H. Clark, *Department of Chemistry, University of York, UK*

Series editors:
George A. Kraus, *Iowa State University, USA*
Andrzej Stankiewicz, *Delft University of Technology, The Netherlands*
Peter Siedl, *Federal University of Rio de Janeiro, Brazil*

Titles in the series:

1: The Future of Glycerol: New Uses of a Versatile Raw Material
2: Alternative Solvents for Green Chemistry
3: Eco-Friendly Synthesis of Fine Chemicals
4: Sustainable Solutions for Modern Economies
5: Chemical Reactions and Processes under Flow Conditions
6: Radical Reactions in Aqueous Media
7: Aqueous Microwave Chemistry
8: The Future of Glycerol: 2nd Edition
9: Transportation Biofuels: Novel Pathways for the Production of Ethanol, Biogas and Biodiesel
10: Alternatives to Conventional Food Processing
11: Green Trends in Insect Control
12: A Handbook of Applied Biopolymer Technology: Synthesis, Degradation and Applications
13: Challenges in Green Analytical Chemistry
14: Advanced Oil Crop Biorefineries
15: Enantioselective Homogeneous Supported Catalysis
16: Natural Polymers Volume 1: Composites
17: Natural Polymers Volume 2: Nanocomposites
18: Integrated Forest Biorefineries
19: Sustainable Preparation of Metal Nanoparticles: Methods and Applications
20: Alternative Solvents for Green Chemistry: 2nd Edition
21: Natural Product Extraction: Principles and Applications
22: Element Recovery and Sustainability
23: Green Materials for Sustainable Water Remediation and Treatment
24: The Economic Utilisation of Food Co-Products
25: Biomass for Sustainable Applications: Pollution Remediation and Energy
26: From C-H to C-C Bonds: Cross-Dehydrogenative-Coupling
27: Renewable Resources for Biorefineries

28: Transition Metal Catalysis in Aerobic Alcohol Oxidation
29: Green Materials from Plant Oils
30: Polyhydroxyalkanoates (PHAs) Based Blends, Composites and Nanocomposites
31: Ball Milling Towards Green Synthesis: Applications, Projects, Challenges
32: Porous Carbon Materials from Sustainable Precursors
33: Heterogeneous Catalysis for Today's Challenges: Synthesis, Characterization and Applications
34: Chemical Biotechnology and Bioengineering
35: Microwave-Assisted Polymerization
36: Ionic Liquids in the Biorefinery Concept: Challenges and Perspectives
37: Starch-based Blends, Composites and Nanocomposites
38: Sustainable Catalysis: With Non-endangered Metals, Part 1
39: Sustainable Catalysis: With Non-endangered Metals, Part 2
40: Sustainable Catalysis: Without Metals or Other Endangered Elements, Part 1
41: Sustainable Catalysis: Without Metals or Other Endangered Elements, Part 2
42: Green Photo-active Nanomaterials
43: Commercializing Biobased Products: Opportunities, Challenges, Benefits, and Risks
44: Biomass Sugars for Non-Fuel Applications
45: White Biotechnology for Sustainable Chemistry
46: Green and Sustainable Medicinal Chemistry: Methods, Tools and Strategies for the 21st Century Pharmaceutical Industry
47: Alternative Energy Sources for Green Chemistry
48: High Pressure Technologies in Biomass Conversion
49: Sustainable Solvents: Perspectives from Research, Business and International Policy
50: Fast Pyrolysis of Biomass: Advances in Science and Technology
51: Catalyst-free Organic Synthesis
52: Hazardous Reagent Substitution: A Pharmaceutical Perspective
53: Alternatives to Conventional Food Processing: 2nd Edition
54: Sustainable Synthesis of Pharmaceuticals: Using Transition Metal Complexes as Catalysts
55: Intensification of Biobased Processes
56: Sustainable Catalysis for Biorefineries
57: Supercritical and Other High-pressure Solvent Systems: For Extraction, Reaction and Material Processing
58: Biobased Aerogels: Polysaccharide and Protein-based Materials
59: Rubber Recycling: Challenges and Developments

60: Green Chemistry for Surface Coatings, Inks and Adhesives: Sustainable Applications
61: Green Synthetic Processes and Procedures
62: Resource Recovery from Wastes: Towards a Circular Economy
63: Flow Chemistry: Integrated Approaches for Practical Applications
64: Transition Towards a Sustainable Biobased Economy
65: Transportation Biofuels: Pathways for Production: 2nd Edition
66: Challenges in Green Analytical Chemistry: 2nd Edition

How to obtain future titles on publication:
A standing order plan is available for this series. A standing order will bring delivery of each new volume immediately on publication.

For further information please contact:
Book Sales Department, Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge, CB4 0WF, UK
Telephone: +44 (0)1223 420066, Fax: +44 (0)1223 420247
Email: booksales@rsc.org
Visit our website at www.rsc.org/books

Challenges in Green Analytical Chemistry
2nd Edition

Edited by

Salvador Garrigues
University of Valencia, Spain
Email: salvador.garrigues@uv.es

and

Miguel de la Guardia
University of Valencia, Spain
Email: miguel.delaguardia@uv.es

ROYAL SOCIETY OF CHEMISTRY

Green Chemistry Series No. 66

Print ISBN: 978-1-78801-537-0
PDF ISBN: 978-1-78801-614-8
EPUB ISBN: 978-1-83916-029-5
Print ISSN: 1757-7039
Electronic ISSN: 1757-7047

A catalogue record for this book is available from the British Library

© The Royal Society of Chemistry 2020

All rights reserved

Apart from fair dealing for the purposes of research for non-commercial purposes or for private study, criticism or review, as permitted under the Copyright, Designs and Patents Act 1988 and the Copyright and Related Rights Regulations 2003, this publication may not be reproduced, stored or transmitted, in any form or by any means, without the prior permission in writing of The Royal Society of Chemistry or the copyright owner, or in the case of reproduction in accordance with the terms of licences issued by the Copyright Licensing Agency in the UK, or in accordance with the terms of the licences issued by the appropriate Reproduction Rights Organization outside the UK. Enquiries concerning reproduction outside the terms stated here should be sent to The Royal Society of Chemistry at the address printed on this page.

Whilst this material has been produced with all due care, The Royal Society of Chemistry cannot be held responsible or liable for its accuracy and completeness, nor for any consequences arising from any errors or the use of the information contained in this publication. The publication of advertisements does not constitute any endorsement by The Royal Society of Chemistry or Authors of any products advertised. The views and opinions advanced by contributors do not necessarily reflect those of The Royal Society of Chemistry which shall not be liable for any resulting loss or damage arising as a result of reliance upon this material.

The Royal Society of Chemistry is a charity, registered in England and Wales, Number 207890, and a company incorporated in England by Royal Charter (Registered No. RC000524), registered office: Burlington House, Piccadilly, London W1J 0BA, UK, Telephone: +44 (0) 20 7437 8656.

For further information see our web site at www.rsc.org

Printed in the United Kingdom by CPI Group (UK) Ltd, Croydon, CR0 4YY, UK

Foreword

The concept of *green analytical chemistry* originated in the 1990s as a nebulous idea of using less harmful solvents in sample preparation than acetonitrile, which was the customary extraction agent in analytical practice at that time. It appeared to some to be an opportunistic way for the discipline to hitch its wagon to the rising star of *green chemistry*. However, it soon became obvious, owing to the efforts of the editors of this book and other analytical chemists, including Namieśnik, Koel and Raynie, that the concept was in itself a solid science, with its own topics, problems and contents.

Analytical chemistry is much more than a set of tools supporting other branches of chemistry. Among other aims, it plays a significant role in setting limits to growth by identifying the boundaries within which technology can be applied without endangering the sustainable functioning of human society. In this role, green analytical chemistry now directly influences the greenness of analytical chemistry. As an information science, analytical chemistry requires an information carrier. One of the features by which the progress of information science is measured is the reduction of the size of the carrier. The big mainframe computers of the 1960s and 1970s have been replaced by smartphones, which are more powerful and execute many more tasks than foreseen at the beginning of the Internet era. Analytical chemistry shares this requirement of information science to reduce the bulkiness of the information carrier. While this goal still remains to be achieved, green analytical chemistry studies methods and teaches practitioners how to attain it. Therefore, a vital topic of green analytical chemistry is the miniaturization of analytical equipment and the simplification of analytical procedures, including sample collection, processing and measurement. Portability is a desirable feature that can be accomplished via miniaturization and microfluidics and modern sensor technology. In addition, the discipline advocates

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

the revival of earlier analytical methods such as colorimetry that take advantage of existing advanced technology such as smartphones. The ultimate goal of green analytical chemistry is the democratization of analytical chemistry. This idea, first proposed by de la Guardia, and the many ways of achieving it, are a central theme of this book. Democratization makes analytical measurements available to everyone who desires to understand and control their environment: the quality of food, air and water, in addition to data pertaining to a subject's health.

This book makes an important contribution to the topics and aims listed above. The editors have assembled a remarkable team of authors and experts who discuss the topics in depth. I strongly recommend this work to all practitioners of analytical chemistry, decision-makers in the fields of science and government and students and teachers of chemistry.

I congratulate the editors, Salvador Garrigues and Miguel de la Guardia, and all of the outstanding contributors for the valuable perspective on green analytical chemistry contained in this book. As it covers a wide range of challenges and successes ranging from emerging instrumental analyses to novel approaches to sample preparation, new separation techniques, and the rise of real-time sensors, this book provides insights into the evolution of the discipline over the past decade.

Mihkel Kaljurand
Tallinn, Estonia
mihkel.kaljurand@taltech.ee

Preface to the Second Edition

The publication in 2008 of our review paper 'Green Analytical Chemistry' in *Trends in Analytical Chemistry* was motivated by the fact that, at that time, many authors had rediscovered the previously called clean analytical or environmentally friendly methods. Hence, based on the international success of the green chemistry paradigm, proposed by Paul Anastas, it was necessary to redefine the objectives and practices involved in this sustainable and environmentally friendly analytical chemistry movement. The review was well accepted by the analytical community (so far it has received more than 620 citations, as indicated in Google Academics) and has attracted the attention of major publishers such as Elsevier, the Royal Society of Chemistry and John Wiley & Sons.

Our research team accepted the invitations of these prestigious publishers to produce three books on the subject of *green analytical chemistry* and in 2011 our authored book was published by Elsevier, followed also in 2011 by the first edition of this book edited by us and published by the Royal Society of Chemistry, and a further edited book published by Wiley in 2012, moving from a personal discussion of the objectives and tools of green analytical chemistry to extended contents regarding different fields of application and, most important, taking into account the points of view of other research teams in our country and others.

However, it must be acknowledged that the first book to be published on green analytical chemistry was that written by Mihkel Koel and Mihkel Kaljurand, of Tallin University of Technology, Estonia. At the time when we were working on the books for Elsevier and the Royal Society of Chemistry, we discovered that these authors were writing their own textbook. This was not at all an unexpected situation in science in the twenty-first century and there were reasons to expect that other active research teams, such as that led by Professor Jacek Namieśnik, of Gdansk University of Technology, and

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

the group of Professor Farid Chemat, at the Université d'Avignon, could also write a book on the same subject, owing to their active research in the field. Many researchers do not feel comfortable sharing their field of application and discovering that other authors have published a book in their field before them. Obviously, this was not so in our case, and we found in Mihkel and Mihkel, and also in Farid and Jacek and his co-workers, such as Marek Tobiszewski and Justyna Płotka-Wasylka, excellent friends and good collaborators in many of our editorial and research activities. Thus once again the active workers in green analytical chemistry decided to continue to work together and contribute to the extension or their research from basic and applied perspectives.

Eleven years after the publication of our review and eight years after the publication of the book by Koel and Kaljurand, a new book has been published concerning green laboratory practices by Arabinda Das, a new book edited by Justyna and Jacek has been published by Springer, the Royal Society of Chemistry has published a new edition of the Mihkels' pioneering book and Dunod published Farid Chemat's new book on green extraction, *Éco-extraction du Végétal*. All this provides clear evidence that the field of green analytical chemistry is very much alive and the dynamic of developing sustainable methods has been spread all around the world, and every year new colleagues join the research on the principles of green analytical chemistry and contribute to enlarging our knowledge of the sustainable perspectives of analytical methods.

The present book is an update of our previous edition of *Challenges in Green Analytical Chemistry*. Taking into account the advances in this field in recent years, we chose to look at a number of new topics that have emerged and incorporate new researchers' voices. Hence the reader will have the opportunity to find new authors, such as Jacek Namieśnik's group, Farid Chemat, Yukihiro Ozaki and Manel del Valle, and new subjects such as chemometrics, sensors and green solvents. Thus we have made great efforts to provide the reader with as complete as possible picture of the tools available today for greening analytical methods.

The reasons for continuing our efforts in green analytical chemistry come from the facts that this emerging field of research has been highly productive in creating new ideas and tools and that the social movement, concerning the third industrial revolution and the deleterious effects of climate change, encourages researchers to look for sustainable tools in all fields. Additionally, as Jacek suggested in one of his recent papers, green analytical chemistry is also important in providing an equitable chemistry that could widen the benefits of analytical methods to developing countries and, as evidenced by our own publications in the clinical field, move in the direction of a democratic analytical chemistry.

Finally, we would like to dedicate this new book to the memory of Jacek Namieśnik, a great scientist and close friend who contributed to enlarging the horizon of green analytical chemistry from both sides, theoretical development and the incorporation of new tools for greening laboratory

practices. We have lost a friend and one of the most prominent scientists in the field. However, we are happy that his work will be continued by the generation of analytical chemists that he encouraged to move in the green direction and thus Jacek will always remain in our minds.

Salvador Garrigues
Miguel de la Guardia

Preface to the First Edition

The general public worldwide has a poor opinion of chemistry. Almost every day the mass media broadcast bad news about environmental damage caused by uncontrolled industrial practices and accidents. Chemical elements or compounds are identified as being responsible for the pollution of air, water or soil, and also for the deaths of humans, animals and plants.

In such a doom-laden scenario it can be difficult to convince our colleagues and students of the benefits of chemistry. We believe that the chemistry community should adopt a new style of communication in order to promote the idea that chemistry is our best weapon to combat illness, and that chemical methods can solve pollution problems caused by the incorrect use of materials, or by the accumulation and transport of dangerous substances in inappropriate conditions. There is not bad chemistry and good chemistry: there are only bad and good uses of chemistry. The truth is that the advancement of chemistry is a good indicator of the progress of humanity. However, we must look for a new paradigm that can help to build bridges between the differing perspectives of chemists and the general public.

In our opinion 'green chemistry' now represents not only the right framework for developments in chemistry but also the best approach to informing the general public about advances in the subject. The term was first introduced in 1990 by Clive Cathcart (*Chemistry & Industry*, 1990, **21**, 684–687) and the concept was elaborated by Paul Anastas in his 12 principles. Briefly, green chemistry provides a way to predict the possible environmental downsides of chemical processes rather than solving them after the fact. It provides a series of recommendations for avoiding the deleterious side effects of chemical reactions, the use of chemical compounds and their transport, as well as a philosophy for improving the use of raw materials in order to ensure that our chemical development is sustainable. The principles

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

of green chemistry build on the efforts made in the past to improve chemical processes by improving the experimental conditions, but pay greater attention to the use of hazardous materials, the consumption of energy and raw materials, and the generation of residues and emissions. This is consistent with recent regulations that have come into effect in different jurisdictions relating to the registration, evaluation, authorization and restriction of chemical substances, especially the REACH norms established by the European Union.

Within the framework of green chemistry, green analytical chemistry integrates pioneering efforts to develop previously known clean methods of analysis, the search for highly efficient digestion systems for sample preparation, the minimization of analytical determinations, their automation, and the online treatment of analytical wastes. These efforts have improved the figures of merit of the methodology previously available, helped to reduce the cost of analysis and improved the speed with which analytical information can be obtained. Along with all these benefits there have been improvements in the safety of methods, both for operators and for the environment. It is therefore not surprising that green analytical chemistry is now a hot topic in the analytical literature.

Two books on green analytical chemistry have appeared in the last year: one by Mihkel Koel and Mihkel Kaljuran, published by the Royal Society of Chemistry, and one by Miguel de la Guardia and Sergio Armenta, published by Elsevier. These books help to clarify the present state of green analytical chemistry and the relationship between the relevant publications in the analytical literature. However, until now there has been no multiauthor book by specialists in the different fields of our discipline describing the various developments made in green analytical chemistry. The present book is an attempt to make such an approach to recent advances in sample preparation, miniaturization, automation and also in various analytical methods, ranging from electroanalysis to chromatography, in order to contribute to the identification of the green tools available in the literature and to disseminate the fundamentals and practices of green analytical chemistry.

We hope that this book will be useful both for readers working in the industrial field, in order to make their analytical procedures greener, and also for those who teach analytical chemistry in universities, to help them see their teaching and research activities in a new light and find ways of making our discipline more attractive to their young students.

This book has been made possible by the enthusiastic collaboration of several colleagues and good friends who have written excellent chapters on their respective fields. The editors would like to express their gratitude for the extra effort involved in this project, generously contributed by people who are continually active in the academic, entrepreneurial and research fields. During the development of this project we lost one of the authors, Professor Lucas Hernández, from the Universidad Autónoma de Madrid, an excellent scientist and a good friend. He became ill while writing his chapter and died before seeing the final version of this book. On the other hand,

Professor Lourdes Ramos, from the CSIC, became pregnant and we celebrate the arrival of her baby Lucas. So, in fact this book is also a piece of life, a human project, written by a number of analytical chemists who believe there is a better way to do their work than just thinking about the traditional figures of merit of their methods. We hope that readers will enjoy the results of our labours.

Miguel de la Guardia and Salvador Garrigues
Valencia

Contents

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia

© The Royal Society of Chemistry 2020

Published by the Royal Society of Chemistry, www.rsc.org

CHAPTER 1

Past, Present and Future of Green Analytical Chemistry

MIGUEL DE LA GUARDIA* AND SALVADOR GARRIGUES

Department of Analytical Chemistry, University of Valencia, ''Jeroni Muñoz'' Research Building, c/Dr. Moliner 50, 46100 Burjassot, Valencia, Spain
*Email: miguel.delaguardia@uv.es

1.1 Green Analytical Chemistry Data

Nobody could imagine at the end of the last century the wonderful success that green analytical chemistry (GAC) would achieve. In fact, preliminary proposals in this field spoke about environmentally friendly conscientious analytical chemistry[1] or an integrated approach of analytical methods,[2] the former being referred to in the title of the editorial in the first special issue devoted to clean analytical methods, published in the Royal Society of Chemistry journal *The Analyst* in 1995.

It could be considered that as analytical chemistry involves relatively small volumes of chemicals compared with synthetic and industrial chemical activities, the deleterious side effects of analytical methods would not be of great concern. However, the importance of analytical measurements, recognized by Paul Anastas in his books on green chemistry (GC),[3,4] and the fact that analytical chemistry methods are used extensively in both academic and application laboratories, made this subject of special relevance in everyday activities.[5] As a result, GAC has experienced tremendous growth since the end of the twentieth century. In fact, from 1995 to 2000 only 27 papers were published on this topic and the main part of those concerned only clean or

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

sustainable methods, not using the term ''green'' directly. In 2001, Jacek Namieśnik published the paper ''Green analytical chemistry – some remarks,'' including for the first time the term green analytical chemistry in the title.[6] This contribution was followed in 2002 by ''Some remarks on gas chromatographic challenges in the context of green analytical chemistry'' (Wardencki and Namieśnik)[7] and a paper by Joseph Wang entitled ''Real-time electrochemical monitoring: Toward green analytical chemistry''[8] in the electroanalytical field. Despite this, it is important to note that up to 2019 fewer than 60 papers have been published that included the complete term green analytical chemistry in the title. However, some efforts have been made in studies of the theoretical aspects of GAC and this will improve the development of green methods in the present century.

Figure 1.1 shows the evolution of the literature on green analytical methods from data obtained from the Web of Science Core Collection database considering the presence of the terms ''green analytical chemistry'', ''green analytical method'', ''clean analytical method'' or ''environmentally friendly method''. From the comparison of these data with those included in the book *Green Analytical Chemistry: Theory & Practice*,[9] published in 2011, it can be concluded that the impact of this subject on the analytical literature of this century has been substantial, especially after the publication in 2010 of the first book on GAC by Mihkel Koel and Mihkel Kaljurand entitled *Green Analytical Chemistry*.[10]

Up to 2007, only 29 review papers were published on general GAC or special topics closely related to it, such as miniaturization, sensors, less aggressive sample preparation techniques to the environment, flow analysis

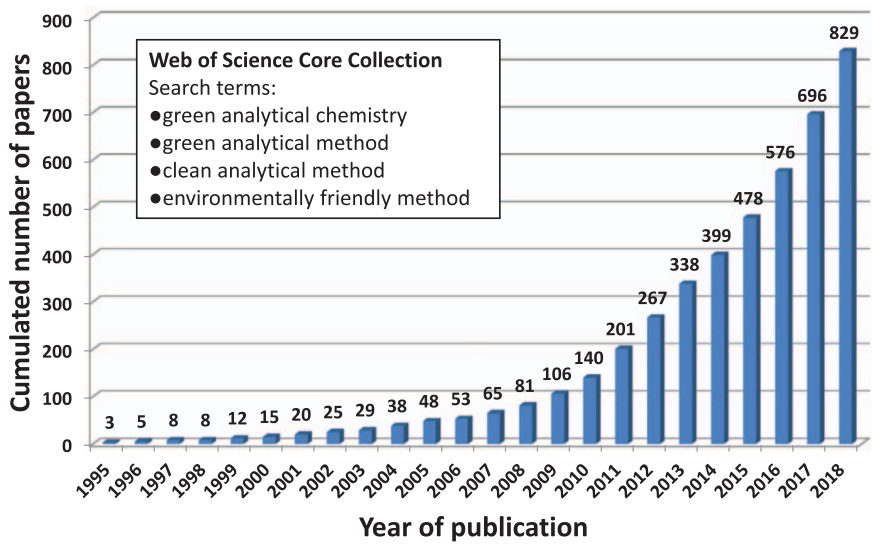


Figure 1.1 Evolution of the literature on green analytical chemistry from 1994 to 2018.

or green aspects of special application fields such as gas chromatography, electrochemical methods, spectroscopy, plasma-based techniques and ionic liquids.[9] In fact, in only 12 of the published reviews was the term ''green analytical'' included in the title. Along with this progress in publications concerning GAC, the evolution of the number of times these papers have been cited is also evident, as can be seen in Figure 1.2, indicating an exponential increase in the citations of the papers considered in Figure 1.1. The most cited papers relating to GAC are listed in Table 1.1.[5,8,11–26] As can be seen, most of them correspond to reviews published in the journal *Trends in Analytical Chemistry* (*TrAC*), including studies related to GAC fundamentals and greener metrics or applied techniques evaluated from the point of view of GAC. The importance of papers relating to sample preparation concerning the use of microextraction techniques and the large number of citations received per year (taking into consideration that the absolute number of citations per year and not the cumulated number is presented) are noticeable.

Concerning authors publishing papers on GAC, Spain, Brazil, Poland, the USA and China are the countries that have contributed the most from 1994 until now, with *Talanta*, *Trends in Analytical Chemistry*, *Journal of Chromatography*, *Analytica Chimica Acta*, *Analytical and Bioanalytical Chemistry*, *Analytical Methods* and *Microchemical Journal* being the most common journals for publication, with more than 35% of the total contributions. This clearly supports what Professor Kaljurand said about GAC: ''Authors try to be environmentally friendly, editors and journals love the term and green is easily understood by the whole of society, thus we can expect a great future for GAC'' (M. Kaljurand, personal communication).

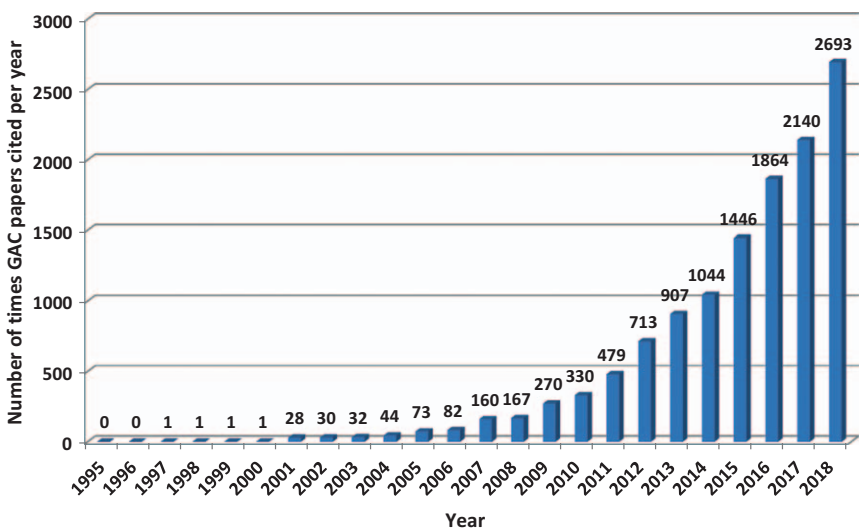


Figure 1.2 Number of times the papers relating to green analytical chemistry considered in Figure 1.1 have been cited.

Table 1.1 Most cited papers relating to green analytical chemistry.

No.	Title	Authors	Journal	Year	Total[a]	Average[a]	Ref.
1	Green analytical chemistry	Armenta, S.; Garrigues, S.; de la Guardia, M.	*TrAC, Trends Anal. Chem.*	2008	454	37.8	11
2	The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices	Gałuszka, A.; Migaszewski, Z.; Namieśnik, J.	*TrAC, Trends Anal. Chem.*	2013	331	47.3	12
3	Cloud point extraction as a procedure of separation and pre-concentration for metal determination using spectroanalytical techniques: A review	Bezerra, M. D.; Arruda, M. A. Z.; Ferreira, S. L. C.	*Appl. Spectrosc. Rev.*	2005	267	17.8	13
4	Green analytical methodologies	Keith, L. H.; Gron, L. U.; Young, J. L.	*Chem. Rev.*	2007	204	15.7	14
5	Green chemistry and the role of analytical methodology development	Anastas, P. T.	*Crit. Rev. Anal. Chem.*	1999	200	9.5	5
6	Recent advances in dispersive liquid–liquid microextraction using organic solvents lighter than water. A review	Kocurova, L.; Balogh, I. S.; Sandrejova, J.; Andruch, V.	*Microchem. J.*	2012	192	24.0	15
7	Determination and speciation of mercury in environmental and biological samples by analytical atomic spectrometry	Gao, Y.; Shi, Z.; Long, Z.; Wu, P.; Zheng, C.; Hou, X.	*Microchem. J.*	2012	153	19.1	16
8	Liquid-phase microextraction techniques within the framework of green chemistry	Pena-Pereira, F.; Lavilla, I.; Bendicho, C.	*TrAC, Trends Anal. Chem.*	2010	151	15.1	17
9	Green analytical chemistry in sample preparation for determination of trace organic pollutants	Tobiszewski, M.; Mechlinska, A.; Zygmunt, B.; Namieśnik, J.	*TrAC, Trends Anal. Chem.*	2009	148	13.5	18

10	Greening analytical chromatography	Welch, C. J.; Wu, N.; Biba, M.; Hartman, R.; Brkovic, T.; Gong, X.; Helmy, R.; Schafer, W.; Cuff, J.; Pirzada, Z.; Zhou, L.	*TrAC, Trends Anal. Chem.*	2010	147	14.7	19
11	Recent developments and future trends in solid phase microextraction techniques towards green analytical chemistry	Spietelun, A.; Marcinkowski, L.; de la Guardia, M.; Namieśnik, J.	*J. Chromatogr. A*	2013	145	20.7	20
12	Modern trends in solid phase extraction: New sorbent media	Płotka-Wasylka, J.; Szczepańska, N.; de la Guardia, M.; Namieśnik, J.	*TrAC, Trends Anal. Chem.*	2016	143	35.8	21
13	Real-time electrochemical monitoring: Toward green analytical chemistry	Wang, J.	*Acc. Chem. Res.*	2002	140	7.8	8
14	Analytical eco-scale for assessing the greenness of analytical procedures	Gałuszka, A.; Konieczka, P.; Migaszewski, Z. M.; Namieśnik, J.	*TrAC, Trends Anal. Chem.*	2012	134	16.8	22
15	Green analytical chemistry – theory and practice	Tobiszewski, M.; Mechlińska, A.; Namieśnik, J.	*Chem. Soc. Rev.*	2010	121	12.1	23
16	Green chemistry and the evolution of flow analysis. A review	Melchert, W. R.; Reis, B. F.; Rocha, F. R. P.	*Anal. Chim. Acta*	2012	116	14.5	24
17	Vibrational spectroscopy provides a green tool for multi-component analysis	Moros, J.; Garrigues, S.; de la Guardia, M.	*TrAC, Trends Anal. Chem.*	2010	116	11.6	25
18	Compressed fluids for the extraction of bioactive compounds	Herrero, M.; Castro-Puyana, M.; Mendiola, J. A.; Ibañez, E.	*TrAC, Trends Anal. Chem.*	2013	115	16.5	26
19	Miniaturized solid-phase extraction techniques	Płotka-Wasylka, J.; Szczepańska, N.; de la Guardia, M.; Namieśnik, J.	*TrAC, Trends Anal. Chem.*	2015	114	22.8	27
20	Application of ionic liquids for extraction and separation of bioactive compounds from plants	Tang, B.; Bi, W.; Tian, M.; Row, K. H.	*J. Chromatogr. B*	2012	110	13.8	28

[a]Total = number total of times cited. Average = average number of times cited per year.

Table 1.2 Books relating to green analytical chemistry.

Year	Authors/Editors	Title	Publisher	Ref.
2010	M. Koel and M. Kaljurand	*Green Analytical Chemistry*	Royal Society of Chemistry	10
2011	M. de la Guardia and S. Armenta	*Green Analytical Chemistry: Theory & Practice*	Elsevier	9
2011	M. de la Guardia and S. Garrigues (eds)	*Challenges in Green Analytical Chemistry*	Royal Society of Chemistry	29
2012	M. de la Guardia and S. Garrigues (eds)	*Handbook of Green Analytical Chemistry*	John Wiley & Sons	30
2014	Inamuddin and A. Mohammad (eds)	*Green Chromatographic Techniques: Separation and Purification of Organic and Inorganic Analytes*	Springer	31
2017	E. Ibañez and A. Cifuentes (eds)	*Green Extraction Techniques: Principles, Advances and Applications*	Elsevier	32
2019	M. Koel and M. Kaljurand	*Green Analytical Chemistry, 2nd edition*	Royal Society of Chemistry	33
2019	J. Płotka-Wasylka and J. Namieśnik (eds)	*Green Analytical Chemistry: Past, Present and Perspectives*	Springer Nature Singapore Pte Ltd.	73

In fact, one of the reasons for the success of GAC in applications laboratories, but also at the academic level, is that green methods are, in general, less expensive than classical methods. This is an added value that is welcomed by the scientific community. It explains the fact that many journals have devoted special issues to GAC that reveal the growth in this field and the permanent interest of editors and authors. In the same context, it is not astonishing that the first book devoted to GAC, as indicated previously, was published in 2010[10] and that, as can be seen in Table 1.2, nowadays there are many books available[9,10,29–33] and some of these are undergoing new editions, whereas from 1996 to 2007 books and journals devoted to green topics concerned just green chemistry.

In short, the data reported on GAC evidence today's interest in the field and the growth of the literature that has dramatically changed the mentality about the consumption of energy and reagents, modified many of the habits in laboratories and provided new activities devoted to minimizing or detoxifying analytical waste, providing a change of the paradigm from chemurgy to ecological chemistry, as was expounded by Professor Hanns Malissa in 1987.[34]

1.2 The Reasons for the Success of GAC

The attitude of the general population towards the environment, with the exception of a small number of politicians, has been modified in the last 20 years owing to the problems created by the environmental impact of human activities on climate change. The evidence for the limits of the

human and social development of our societies, the reduction of fossil fuel reserves and the new challenges created by plastic and solid residues in general, gas emissions into the atmosphere and pollution of sweet water reservoirs, including the effect of human activities on polar ice, have moved many people to have serious concerns regarding environmental protection. In such a frame, it is clear that any activity related to environmentally friendly laboratories should be welcomed, and GAC is no exception.

The concern about the deleterious side effects of many reagents, extensively used in the past in our laboratories, has moved authors, referees and editors to think seriously about the replacement of toxic compounds with innocuous materials or, at least, with less harmful products, and this also involves worries about the safety of method operators and environmental damage. Therefore, nowadays many efforts are being devoted to searching for new renewable feedstocks to be used as reagents and solvents, such as agrosolvents,[35] and to incorporate smart materials that are able to improve the sensitivity and selectivity of sample preparation methods.[36] In short, it can be concluded that GAC has provided new ideas and objectives for basic research and hence it does not concern ecological opposition to the use of chemicals. On the contrary, GAC involves the deep evaluation of new alternatives and, because of that, the new mentality has evidenced clever solutions in the face of chemical problems and put the spotlight on the resolution of problems and new challenges and not at all on a fundamental ecologism that cannot provide correct answers at the level of consumer needs.

The mixture of a pragmatic point of view and an ethical compromise with environmental sustainability has given prestige to the so-called green analytical methods which, ultimately, provides correct solutions at the required accuracy, sensitivity, selectivity and precision levels without side effects for operators and the environment.

On the other hand, the efforts made to move from off-line batch determinations in the laboratory to point-of-care and *in situ* analysis or remote sensing of target analytes provide strong reductions in the consumption of reagents and waste generation and also energy demands, and all of this reduces the method costs, thus offering cheaper alternatives that are greatly appreciated by companies and applications laboratories. Thus, as Professor Farid Chemat said: ''green analytical chemistry could also be called poor analytical chemistry'' (F. Chemat, personal communication), and this point of view is close to the common use of vanguard methodologies[37] in green analytical methods rather than costly conventional rearguard analytical systems and the extended use of screening methods suitable to provide an appropriate level of information in a short time, once again contributing to decreasing methodology costs.

Summarizing, it is not at all astonishing that GAC was developed after the popularization in the 1970s of flow methodologies, because they contributed to the adoption of method automation, taking advantage of flow injection

analysis (FIA),[38] sequential injection analysis (SIA),[39] lab-on-a-valve (LoV)[40] and lab-on-a-chip[41] and multicommutation[42] developments to reduce the consumption of samples and reagents and minimize operator manipulation and waste generation.

Automation is, basically, a very useful tool to integrate, in a single manifold, all the steps required in analytical methods and to minimize operator and environmental risks. Further, the addition of a waste treatment step after analyte determination has evidenced that chemical problems could be solved with an extra bit of chemistry[43] and, once again, this opens up new perspectives for basic and applied research.

An additional reason for the importance of GAC methods comes from the efforts to make available the advantages of analytical chemistry to isolated and less developed societies, making extra efforts to move from sophisticated methods, based on the use of high-cost instrumentation, to the modelling of signals obtained with relatively low-cost and readily available instruments. Based on the use of chemometrics and direct measurements, it is possible to develop fast and cheap methods, using a series of samples well characterized by reference methodologies as calibration standards.[44] Thus, extensive data modelling and the use of lower cost instruments and free-of-charge software available in the cloud have extended the analytical tools and the availability of these methodologies to a large number of beneficiaries and users.

All the aforementioned reasons are summarized in the scheme in Figure 1.3, which shows that reasons for moving to GAC concern knowledge advancement together with ethical and economic reasons.

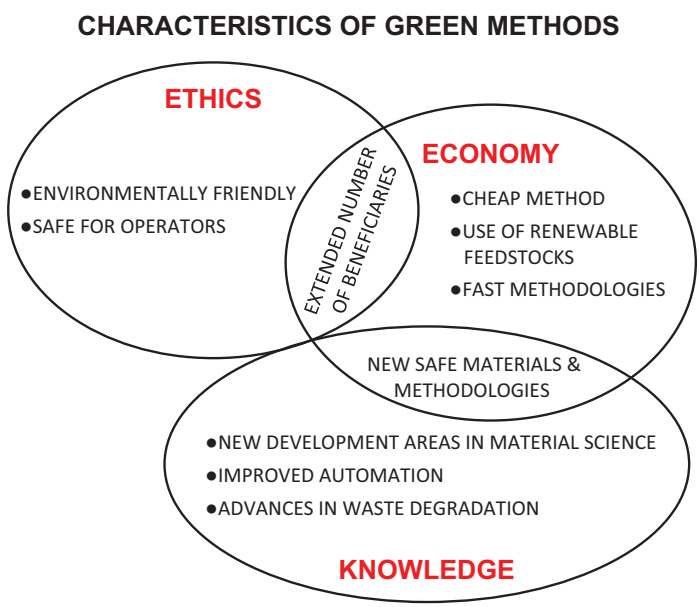


Figure 1.3 Reasons for the success of green analytical chemistry.

1.3 Theoretical Developments in GAC

At the end of the twentieth century, the ''Twelve Principles of Green Chemistry'', defined by Anastas and Warner,[4] oriented alternative research on sustainable chemistry. Of these principles, the 11th was devoted to the need for real-time analysis to prevent pollution, but many of these analyses could be directly translated to the requirements of GAC methods, hence avoiding derivatizations, if possible, is a common point between the GC and GAC principles established by Gałuszka, Migaszewski and Namieśnik in 2013[12] and also the use of renewable feedstocks and the reduction of risks, waste and energy consumption (see Table 1.3).

In fact, the so-called SIGNIFICANCE mnemonic can be considered an excellent translation to the everyday work in analytical chemistry of the main principles of Anastas, independently of the fact that GC put the stress on catalytic methods and GAC must try to be adapted to the user's needs, and well crystallized the four priorities of GAC established by Namieśnik in 2001,[6] regarding (1) elimination or reduction of reagents and solvents, (2) reduction of emissions, (3) elimination of toxic reagents and (4) reduction of labour and energy, and our own six basic strategies for greening analytical chemistry, established at that time, concerning (1) direct analysis of untreated samples, (2) alternative sample treatments, (3) miniaturization and automation, (4) on-line decontamination, (5) search for alternative reagents and (6) evaluation of energy consumption.[9]

The aforementioned theoretical approaches, together with efforts to evaluate method greenness (see Chapter 11 for additional details), have

Table 1.3 Principles of green chemistry *versus* principles of green analytical chemistry.

Green chemistry		Green analytical chemistry	
1	Prevent waste	**S**	Select direct analytical techniques
2	Design safer chemicals and products	**I**	Integrate analytical processes and operations
3	Design less hazardous chemical syntheses	**G**	Generate as little waste as possible and treat it properly
4	Use renewable feedstocks	**N**	Never waste energy
5	Use catalyst not stoichiometric reagents	**I**	Implement automation and miniaturization of methods
6	Avoid chemical derivatizations	**F**	Favour reagents obtained from renewable sources
7	Maximize atom economy	**I**	Increase safety of operator
8	Use safer solvents and reaction conditions	**C**	Carry out *in situ* measurements
9	Increase energy efficiency	**A**	Avoid derivatizations
10	Design chemicals and products to degrade after use	**N**	Note that sample number and size should be minimal
11	Analyse in real time to prevent pollution	**C**	Choose multi-analyte or multi-parameter methods
12	Minimize the potential for accidents	**E**	Eliminate or replace toxic reagents

contributed to the regularization and systematization of GAC in university courses and laboratory practice, thus creating a new generation of analytical chemists for the future.

1.4 Practical Application of GAC

One of our objectives when in 2011 we started the production of books related to GAC was to extend the ideas from our laboratory to as many research teams as possible involved all around the world on these kinds of problems and, for example, the number of authors varied from two in the 2011 Elsevier book[9] to 24 in the Royal Society of Chemistry book also published in 2011[29] and to 50 in the 2012 Wiley book.[30] In the last book mentioned there were contributors from India, Poland, Estonia, Taiwan, Argentina, Italy, Japan, Iran and Brazil, thus clearly showing that, in addition to Spanish laboratories, there was general worldwide interest in the subject.

Figure 1.4 shows the general distribution of papers published from 2007 in different fields of GAC, including spectroscopy (atomic and molecular), electroanalytical methods and separation methods, covering chromatography and electrophoresis techniques. Methods based on imaging treatment are also included.

From the reported published papers, it can be concluded that the most numerous is the group concerning fundamentals, which include papers relating to the principles of GAC, green metrics and those, mainly reviews, regarding sample preparation and the use of extraction techniques. A detailed study of the evolution of the contributions on GAC indicated that prior to this century electroanalysis, electrophoresis and especially

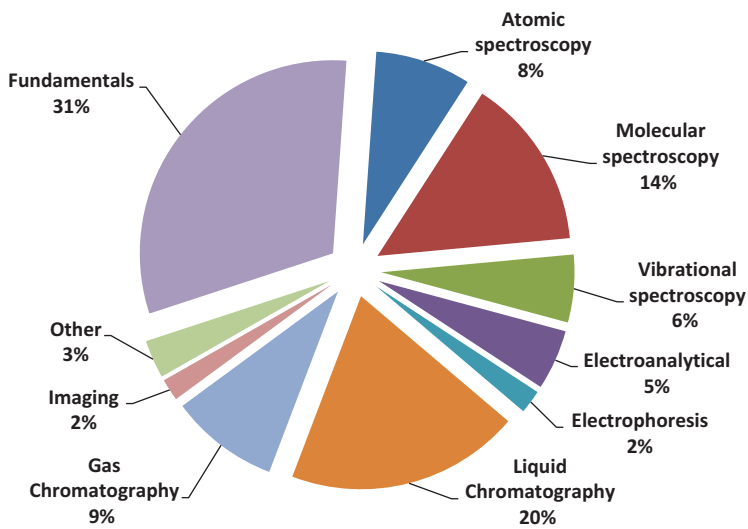


Figure 1.4 Distribution of green analytical chemistry publications as a function of the instrumental field involved in the period from 2007 to 2019.

molecular spectroscopy were the main techniques considered. Molecular spectroscopy continues to be the most common greener alternative technique. From 2010 there has been an increase in papers relating to other techniques, such as atomic spectroscopy and chromatography, particularly liquid chromatography, and also a proliferation of studies relating to sample preparation, especially focused on extraction techniques. This trend continued to grow in the following years so that currently most of the studies relating to GAC concern sample preparation and the use of chromatographic, molecular spectroscopic and electroanalytical techniques, with the progressive incorporation of applications based on image processing through the direct use of cameras and smartphones or devices coupled to them.[45]

From recently published papers, it can be also concluded that there is a general trend of authors to use remote sensing or direct methods, without involving any chemical modification of samples or analyte extraction. However, greening the analytical methods also involves reducing the consumption of reagents and energy in traditional methods, reducing and/or degrading the generated wastes and, in short, enhancing the so-called environmentally friendly characteristics of methods in addition to preserving the main features of the methods. Achieving the appropriate levels of accuracy, sensitivity and selectivity required for making decisions continues to be a basic requirement of being green.[46]

1.5 The Future: A Democratic Analytical Chemistry Paradigm?

In recent years, the conceptual advancement on GAC has moved in parallel with efforts to incorporate new screening tools and low-cost tools to solve analytical problems.

In 1975, the pioneering work on FIA[47] and the use of microwave ovens for sample digestion[48] paved the way for methodological improvements of available methods for both analyte detection and sample preparation and also evidenced the possibility of finding appropriate low-cost solutions. Thus, the use of readily available inexpensive apparatus favours the development of methods suitable for use all around the world and in spite of the level of laboratory budgets.[49]

Regarding sample preparation, the use of closed reactors showed that pressure, temperature and reagents were the main variables for assuring the complete extraction of target analytes and correct matrix removal or partial matrix decomposition. In recent years, it has been demonstrated that in addition to classical hard digestion methodologies, such as dry ashing and wet ashing, the use of microwave-assisted methods[50] and soft energy technologies such as ultrasound-assisted procedures[51] provided efficient heating systems that permitted the quantitative recovery of target analytes, with a strong reduction of matrix effects during the measurement step together

with reductions in analyte losses and contamination, also increasing the possibilities for metal speciation.[52] Additionally, these alternative digestion methods provided relatively low-cost tools in terms of both equipment and energy consumption. On the other hand, modern sample treatment alternatives were also highly compatible with the automation of systems, thus offering well-integrated methodologies.[53]

In recent years, the search for the design of point-of-care tools has taken advantage of the reduction of the power supply requirements of microwave ovens to make possible the in-field extraction of essential oils from plants,[54] and these treatments could be also adapted for in-field mineral analysis of solid samples based on closed reactor digestion and the use of colorimetric assays.

Another recent advance in sample preparation concerns the use of hard cup espresso machines to improve fast analyte extraction (of the order of less than 100 s), based on the use of relatively high temperature and pressure conditions,[55] and this is an important contribution that permits analytical problems to be solved with inexpensive and worldwide readily available instrumentation.

Hence it can be concluded that new tools for sample preparation provide low-cost and sustainable methodologies that have also reduced drastically the time, labour and costs of sample preparation. Additionally, the advantages concerning the main analytical features of selectivity and, in some cases, sensitivity that were improved by the new approaches must also be taken into consideration.

In the list of new tools to make analytical determinations easier, the use of portable devices for air quality control,[56] lateral flow analysis sensors[57] and, in general, bio(chemical) sensors[58] provides point-of-care low-cost alternatives to rearguard methods.

A good example of the changes introduced into analytical chemistry by the development of the aforementioned green tools concerns the extended use of image processing methods. Both general sample parameters[59] and specific sample characteristics[60] could be determined for natural samples without any chemical or physical damage, just based on the treatment of images obtained by using digital and smartphone cameras.[45] Additionally, image treatment methods together with simple colorimetric assays and/or paper chromatography[61,62] or gel electrophoresis[63] improve the sensitivity and selectivity of these *in situ* determinations.

The advances in chemometrics (see Chapter 10) together with the use of the available software permits a move from costly and expensive instrumentation to the use of low-cost, readily available and point-of-care methodologies, which could change the perspective of analytical chemistry[64–68] even though the use of powerful rearguard methodologies is required in most cases for a complete characterization of samples to be used for calibration purposes.

On the other hand, the Internet and the tremendous development of social networks will provide new data distribution approaches without any

time delay between data acquisition and data distribution, leading, at the same time, to great possibilities for fast decision-making but also creating problems to confirm true information.[49] Thus, as indicated in Figure 1.5, the democratic analytical chemistry (DAC) concept has moved from the extension of benefits to the main aspects of data production.

In fact, analytical chemistry throughout the world at the human scale looks very promising as an added-value side effect of GAC that will move from a main objective of being safe and sustainable to a new perspective in order to be beneficial for the whole population and all practitioners.[69]

The adoption of the term ''democratic analytical chemistry'' (DAC) to describe the new perspective[49,70] was made taking into account not only the advantages but also the risks involved in the new situation. Figure 1.6 compares some of the deleterious aspects of classical methods of analysis, described as old concepts that provided pretentious ideas about analytical science and analytical chemists, and the fact that in the new scenario created by DAC the availability of low-cost instrumentation and easy and fast measurements, free access of software in the cloud, the tremendous development of on-site and point-of-care data acquisition with speed-of-light data sharing through the Internet will create a new frame of solidarity and globalization that will provide open diffusion of data and opinions. In this respect we are absolutely convinced that it has tremendous advantages but also involves many risks.

Figure 1.7 summarizes the main risks associated with the misuse of readily available instrumentation, inexperienced data acquisition and the irresponsible generation and distribution of false analytical information. From the lack of representative or inexperienced data to the misuse of true data and distribution of false data, all these mistakes or bad attitudes can

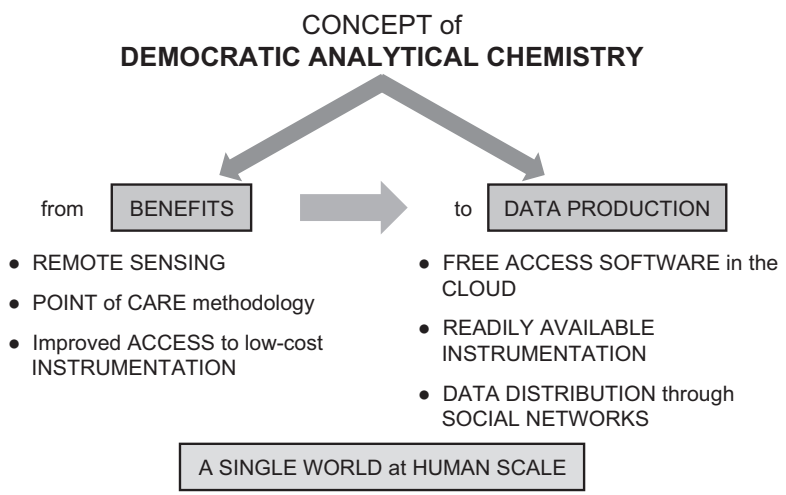


Figure 1.5 Concept of democratic analytical chemistry (DAC).

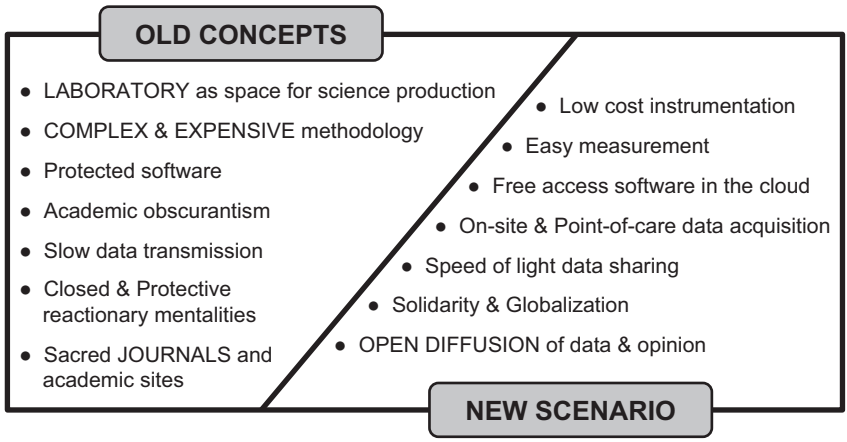


Figure 1.6 The change of scenario provided by democratic analytical chemistry compared with classical analytical chemistry.

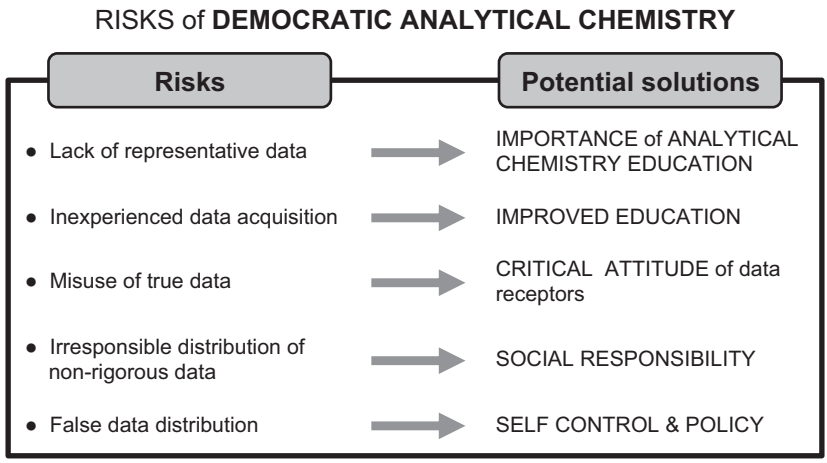


Figure 1.7 Risks and potential solutions of problems related to the advancement of democratic analytical chemistry.

dramatically affect both the trueness of analytical results and the prestige of our discipline. In view of the aforementioned risks, education and analytical chemistry education, together with self-control and social responsibility of operators[71,72] and the critical attitude of data receptors, could be the main potential solutions to the risks of democratic analytical chemistry. Additionally, the development of a simple and clear analytical chemistry language is necessary so as to be able to transmit technical analytical information to the general population, without a requirement for an adequate level of technical knowledge, in order to avoid misunderstandings that can create unjustified alarm situations.

From a social point of view, it is important to highlight the contribution that the developments based on the principles of GAC can provide for society in important matters such as clinical analysis and diagnosis or environmental control with clear implications for improving the quality of people's lives and the environment. The development and widespread use of fast, cheap and portable techniques in less developed countries, but also in those who call themselves first world but lack social and universal models of healthcare and base their policies on services provided in the health sector by private companies, may help governments to offer adequate health programmes to the entire population in a social and responsible way, avoiding the commercial interests of private companies. Portable systems for the control of parameters such as blood glucose, or smart watches that control other clinical and vital parameters, can support democratic access to the improvement of the quality of life of the population and improving their health. In the same sense, having adequate methods that allow *in situ* environmental control in a generalized and sustainable manner will allow rapid and efficient decision-making, such as establishing limitations in the traffic of cities and controlling domestic emissions and urban spills, among others.

In short, we are absolutely convinced that GAC has provided the principles for a new DAC frame in which (1) screening methodologies and fast methodologies will provide elements for preliminary decision-making, (2) the use of readily available and low-cost apparatus and instrumentation will increase the number of data acquisition personnel and multiply the eyes that look at an analytical problem and (3) the expanding social networks will improve the speed of communication of data and results. However, it seems clear that additional efforts will be required to educate well the new analytical chemistry operators and to put real data at the service of communities. This is the challenge and we must strive to provide correct answers to the multiple problems and risks in the future.

References

1. M. de la Guardia and J. Ruzicka, *Analyst*, 1995, **120**, 17N.
2. M. de la Guardia, *J. Braz. Chem. Soc.*, 1999, **10**, 429.
3. *Benign by Design; Alternative Synthetic Design for Pollution Prevention*, ed. P. T. Anastas and C. A. Farris, *ACS Symposium Series*, American Chemical Society, Washington, DC, 1994.
4. P. T. Anastas and R. Warner, *Green Chemistry, Theory and Practice*, Oxford University Press Inc, New York, 1998.
5. P. T. Anastas, *Crit. Rev. Anal. Chem.*, 1999, **29**, 167.
6. J. Namieśnik, *J. Sep. Sci.*, 2001, **24**, 151.
7. W. Wardencki and J. Namieśnik, *Pol. J. Environ. Stud.*, 2002, **11**, 185.
8. J. Wang, *Acc. Chem. Res.*, 2002, **35**, 811.
9. M. de la Guardia and S. Armenta, *Green Analytical Chemistry: Theory & Practice*, in *Comprehensive Analytical Chemistry Series*, Elsevier, Oxford, **vol. 57**, 2011.

10. M. Koel and M. Kaljurand, *Green Analytical Chemistry*, RSC Publishing, Cambridge, 2010.
11. S. Armenta, S. Garrigues and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2008, **27**, 497.
12. A. Gałuszka, Z. Migaszewski and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2013, **50**, 78.
13. M. D. Bezerra, M. A. Z. Arruda and S. L. C. Ferreira, *Appl. Spectrosc. Rev.*, 2005, **40**, 269.
14. L. H. Keith, L. U. Gron and J. L. Young, *Chem. Rev.*, 2007, **107**, 2695.
15. L. Kocurova, I. S. Balogh, J. Sandrejova and V. Andruch, *Microchem. J.*, 2012, **102**, 11.
16. Y. Gao, Z. Shi, Z. Long, P. Wu, C. Zheng and X. Hou, *Microchem. J.*, 2012, **103**, 1.
17. F. Pena-Pereira, I. Lavilla and C. Bendicho, *TrAC, Trends Anal. Chem.*, 2010, **29**, 617.
18. M. Tobiszewski, A. Mechlinska, B. Zygmunt and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2009, **28**, 943.
19. C. J. Welch, N. Wu, M. Biba, R. Hartman, T. Brkovic, X. Gong, R. Helmy, W. Schafer, J. Cuff, Z. Pirzada and L. Zhou, *TrAC, Trends Anal. Chem.*, 2010, **29**, 667.
20. A. Spietelun, L. Marcinkowski, M. de la Guardia and J. Namieśnik, *J. Chromatogr. A*, 2013, **1321**, 1.
21. J. Płotka-Wasylka, N. Szczepańska, M. de la Guardia and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2016, **77**, 23.
22. A. Gałuszka, P. Konieczka, Z. M. Migaszewski and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2012, **37**, 61.
23. M. Tobiszewski, A. Mechlinska and J. Namieśnik, *Chem. Soc. Rev.*, 2010, **39**, 2869.
24. W. R. Melchert, B. F. Reis and F. R. P. Rocha, *Anal. Chim. Acta*, 2012, **714**, 8.
25. J. Moros, S. Garrigues and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2010, **29**, 578.
26. M. Herrero, M. Castro-Puyana, J. A. Mendiola and E. Ibañez, *TrAC, Trends Anal. Chem.*, 2013, **43**, 67.
27. J. Płotka-Wasylka, N. Szczepańska, M. de la Guardia and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2015, **73**, 19.
28. B. Tang, W. Bi, M. Tian and K. H. Row, *J. Chromatogr. B*, 2012, **904**, 1.
29. *Challenges in Green Analytical Chemistry*, ed. M. de la Guardia and S. Garrigues, RSC Publishing, Cambridge, 2011.
30. *Handbook of Green Analytical Chemistry*, ed. M. de la Guardia and S. Garrigues, John Wiley & Sons, Chichester, 2012.
31. *Green Chromatographic Techniques: Separation and Purification of Organic and Inorganic Analytes*, ed. Inamuddin and A. Mohammad, Springer, Dordrecht, 2014.
32. *Green Extraction Techniques: Principles, Advances and Applications*, ed. E. Ibañez and A. Cifuentes, Elsevier, Oxford, 2017.

33. M. Koel and M. Kaljurand, *Green Analytical Chemistry*, Royal Society of Chemistry, Cambridge, 2nd edn, 2019.
34. H. Malissa in *Euroanalysis VI: Reviews on Analytical Chemistry*, ed. E. Roth, Les Editions de Physique, Les Ules, France, 1987.
35. *Alternative Solvents for Natural Products Extraction*, ed. F. Chemat and M. Abert Vian, Springer, Heidelberg, 2014.
36. *Handbook of Smart Materials in Analytical Chemistry*, ed. M. de la Guardia and F. A. Esteve-Turrillas, John Wiley & Sons Ltd, Hoboken, 2019.
37. M. Valcarcel and S. Cardenas, *TrAC, Trends Anal. Chem.*, 2005, **24**, 67.
38. F. R. P. Rocha, J. A. Nobrega and O. Fatibello, *Green Chem.*, 2001, **3**, 216.
39. A. Economou, *TrAC, Trend. Anal. Chem.*, 2005, **24**, 416.
40. M. Miro and E. H. Hansen, *Anal. Chim. Acta*, 2007, **600**, 46.
41. *Lab-on-a-Chip. Miniaturized Systems for (Bio) Chemical Analysis and Synthesis*, ed. R. E. Oosterbroek and A. van den Berg, Elsevier Science, New York, 2003.
42. M. A. Feres, P. R. Fortes, E. A. G. Zagatto, J. L. M. Santos and J. L. F. C. Lima, *Anal. Chim. Acta*, 2008, **618**, 1.
43. S. Armenta, S. Garrigues and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2010, **29**, 592.
44. D. Perez-Guaita, J. Ventura-Gayete, C. Pérez-Rambla, M. Sancho-Andreu, S. Garrigues and M. de la Guardia, *Microchem. J.*, 2013, **106**, 202.
45. K. E. McCracken and J. Y. Yoon, *Anal. Methods*, 2016, **8**, 6591.
46. M. de la Guardia and S. Garrigues, *Bioanalysis*, 2012, **4**, 1267.
47. J. Ruzicka and E. H. Hansen, *Anal. Chim. Acta*, 1975, **78**, 145.
48. A. Abu-Samra, J. S. Morris and S. R. Koirtyohann, *Anal. Chem.*, 1975, **47**, 1475.
49. M. de la Guardia and S. Garrigues, *Pharm. Sci.*, 2019, **25**, 82.
50. C. A. Bizzi, M. F. Pedrotti, J. S. Silva, J. S. Barin, J. A. Nobrega and E. M. M. Flores, *J. Anal. At. Spectrom.*, 2017, **32**, 1448.
51. F. Priego-Capote and M. D. Luque de Castro, *J. Biochem. Biophys. Methods*, 2007, **70**, 299.
52. M. B. Arain, T. G. Kazi, M. K. Jamah, N. Jalbani, H. I. Afridi and J. A. Baig, *J. Hazard. Mater.*, 2008, **154**, 998.
53. M. de la Guardia, V. Carbonell, A. Morales-Rubio and A. Salvador, *Talanta*, 1993, **40**, 1609.
54. S. Perino, E. Petitcolas, M. de la Guardia and F. Chemat, *J. Chromatogr. A*, 2013, **1315**, 200.
55. S. Armenta, M. de la Guardia and F. A. Esteve-Turrillas, *Anal. Chem.*, 2016, **88**, 6570.
56. J. Casanova-Chafer, D. Gallart-Mateu, S. Armenta and M. de la Guardia, *Microchem. J.*, 2016, **126**, 454.
57. M. Kaljurand, *Curr. Opin. Green. Sustainable Chem.*, 2019, **19**, 15.
58. P. Kassal, M. D. Steinberg and I. M. Steinberg, *Sens. Actuators, B*, 2018, **266**, 228.
59. M. Dowlati, S. S. Mohtasebi, M. Omid, S. H. Razavi, M. Jamzad and M. de la Guardia, *J. Food Eng.*, 2013, **119**, 277.

60. A. Sanaeifar, A. Bakhshipour and M. de la Guardia, *Talanta*, 2016, **148**, 54.
61. B. Coleman, C. Coarsey and W. Asghar, *Analyst*, 2019, **144**, 1935.
62. E. V. Woodburn, K. D. Long and B. T. Cunningham, *IEEE Sens. J.*, 2019, **19**, 508.
63. J. R. de Jesus, I. C. Guimaraes and M. A. Zezzi Arruda, *J. Proteomics*, 2019, **198**, 45.
64. A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, *Anal. Chem.*, 2010, **82**, 3.
65. P. Yager, G. Domingo and J. Gerdes, *Annu. Rev. Biomed. Eng.*, 2008, **10**, 107.
66. L. Shen, J. A. Hagen and I. Papautsky, *Lab Chip*, 2012, **12**, 4240.
67. S. K. Vashist, P. B. Luppa, L. Y. Yeo, A. Ozcan and J. H. T. Luong, *Trends Biotechnol.*, 2015, **33**, 692.
68. F. Figueredo, M. J. Gonzalez-Pabon and E. Corton, *Electroanalysis*, 2018, **30**, 497.
69. J. Namieśnik and M. Tobiszewski, *Curr. Opin. Green. Sustainable Chem.*, 2019, **19**, 19.
70. S. Garrigues and M. de la Guardia, *Actualidad Analítica*, 2019, **65**, 35.
71. M. Valcarcel and R. Lucena, *TrAC, Trends Anal. Chem.*, 2012, **31**, 1.
72. M. de la Guardia and S. Garrigues, *Trends Environ. Anal. Chem.*, 2014, **3–4**, 7.
73. *Green Analytical Chemistry: Past, Present and Perspectives*, ed. J. Płotka-Wasylka and J. Namieśnik, Springer Nature Singapore Pte Ltd., Singapore, 2019.

CHAPTER 2

Direct Analysis by Green Spectroscopy and Spectrometry

SALVADOR GARRIGUES* AND MIGUEL DE LA GUARDIA

Department of Analytical Chemistry, University of Valencia, ''Jeroni Muñoz'' Research Building, c/Dr. Moliner 50, 46100 Burjassot, Valencia, Spain
*Email: salvador.garrigues@uv.es

2.1 Introduction

Direct analysis methods have the tremendous advantage of obviating the need for sample treatment, thus reducing both of the main drawbacks of analyte contamination and loss and environmental side effects. They also enhance the decision-making process and reduce the cost and time required for carrying out determinations. Hence one of the main objectives of green analytical chemistry (GAC) is to obtain on-site as much information as possible about the samples without any chemical pretreatment and with no or minimal sample manipulation for a representative sampling and analysis. It also favours remote sensing and point-of-care analysis and shortens the time required to make decisions, based on the advantages offered by chemometrics and modern information technologies. Figure 2.1 summarizes the hierarchical classification of the different alternative green direct approaches that can be employed.

The physical state of samples is a starting point that limits the acquisition and use of direct spectroscopic measurements. It is evident that most analytical techniques work well in the analysis of liquids or previously dissolved

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

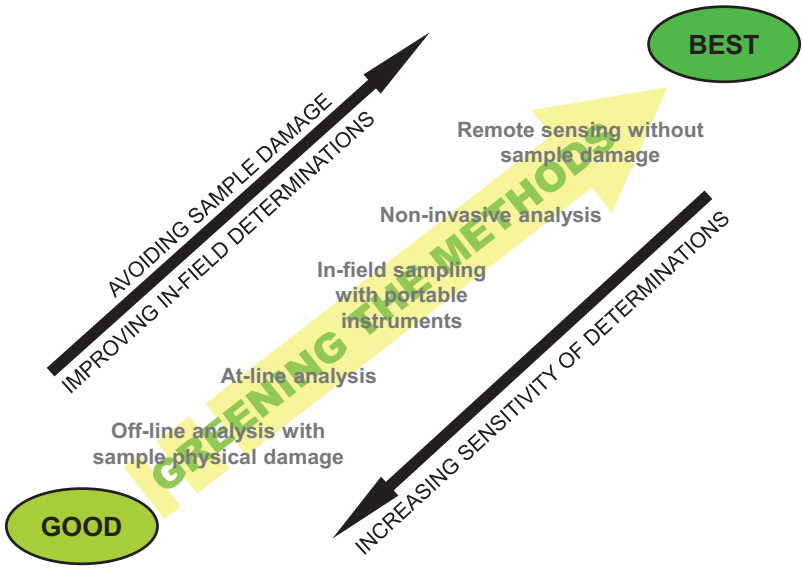


Figure 2.1 Hierarchical organization of green direct sample analysis.

samples, and just a few instruments, such as X-ray fluorescence (XRF), Raman and infrared (IR) instrumentation, in the near-infrared (NIR) and mid-infrared (MIR) ranges, are suitable for obtaining data from solid samples but with the problem of reduced sensitivity. However, highly sensitive atomic spectroscopic and mass spectrometric techniques, such as inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS), can be applied to the analysis of solid materials after laser-based treatment, and laser ablation (LA) and laser-induced breakdown spectroscopy (LIBS) can also be good alternatives for the determination of trace-level components with minimal physical damage to the samples. However, in this case, high-performance bench instruments and not easily portable instruments are required in most cases.

Table 2.1 summarizes the advantages and drawbacks of spectroscopic methods for the direct analysis of samples, and to this list must be added the main part of atomic spectroscopic methods for the determination of mineral elements in liquid samples and the use of a commercial direct mercury analyser (DMA) for the determination of Hg in solid samples by atomic absorption spectroscopy after dry-ashing digestion of samples and gold-trap preconcentration.

Throughout this chapter, the possibilities offered in the literature for the green direct determination of target analytes will be emphasized, paying special attention to X-ray fluorescence and vibrational spectroscopic techniques, mass spectrometry and image processing as green analytical tools.

Table 2.1 Advantages and drawbacks of spectroscopy for direct analysis of samples.

Technique	Type of sample	Concentration level	Advantages	Drawbacks
X-ray fluorescence	Solids Viscous liquids	%	• Direct mineral analysis	• No response for light elements • Difficult for calibration • Matrix effects • Lack of sensitivity
Visible spectroscopy (visible analysis)	Liquids Solids	Depending on chemometric treatment of measurements	• Non-invasive analysis • Low cost	• Previous chemometric modelling • Use of rearguard techniques for characterization of calibration samples
Laser treatment (LA, LIBS)	Solids	ppm	• High information level (minerals and organics)	• Physical damage at microscopy level • Appropriate interferences
Raman	Solids Liquids	%	• Organics and inorganics • Structural information	• Interference by sample fluorescence • Lack of sensitivity • Lack of precision
NIR	Solids Liquids	%	• Organics and inorganics • Reflectance and transmission measurements • Good repeatability	• Need for chemometrics
ATR-MIR	Solids Liquids	%	• Organics and inorganics • Fast analysis • Bad precision (for solids) • High qualitative information level	• Need for chemometrics for calibration

2.2 Versatility of Spectroscopic and Spectrometric Techniques

In addition to the fact that the interaction between light and samples depends on the energy or wavelength of the source and measurement radiation, each spectroscopic technique provides different ways to obtain analytical information, including transmission of reflectance, emission and fluorescence processes. Hence there are many measurement modes that can be used to obtain data on the chemical composition of samples by spectroscopy, which also depend on the physical state of the samples to be analysed.

Based on reflectance measurements, it is evident that image processing followed by XRF, NIR, MIR and Raman techniques are frequently used for the direct characterization of solids. However, liquids and dissolved samples, to a lesser extent, could also be analysed by these techniques, as indicated in Figure 2.2.

In general, solid samples create problems due to matrix effects, hence a careful calibration must be performed. For liquid samples it is easier, in general, to prepare standards. However, the effect of solvents creates transparency problems in the case of IR measurements, hence the use of dry film attenuated total reflection (ATR) measurements[1] or vapour generation strategies[2] could avoid these drawbacks and enhance the sensitivity and selectivity.

On the other hand, the recent advances in mass spectrometry instruments and measurement techniques have contributed to the direct analysis of samples in real time nowadays being a promising approach for green determinations.[3]

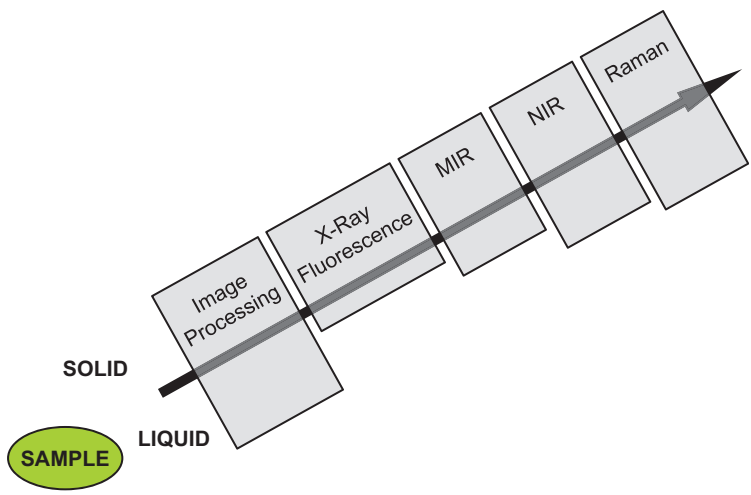


Figure 2.2 Suitability of spectroscopic techniques for the direct analysis of solid and liquid samples.

In any case, changes in the original physical state of samples reduces the green character of determinations, increasing energy consumption, time of analysis and labour, and also reducing the portability of the required systems.

2.3 Direct Analysis after Physical Treatment

In the past, arc and spark emission techniques opened the way for mineral element analysis of conductive materials without the need to use reagents or chemical reactions and to obtain data with minimal physical damage to the samples.[4] However, nowadays, these techniques are restricted to metallurgical applications and the mineral analysis of precious samples is now usually performed using LA[5,6] or LIBS.[7] These laser-based techniques provide direct and rapid signal acquisition from samples with only slight damage to their surface and without requiring reagents or solvents. However, problems arise from the difficulty in obtaining appropriate calibrations free from the effects of sample matrices and thus a previous effort must be made to compensate matrix effects and create good calibration objects. Additionally, it is possible to take aliquots of solids for the determination of their mineral composition by using atomic and ionic techniques for trace analysis (see Figure 2.3).

The following sections summarize the main aspects of direct spectroscopic and spectrometric techniques for both mineral and organic analysis.

2.3.1 Arc and Spark Optical Emission Spectrometry

For a long time in the history of spectroscopy, arc and spark excitation methods were the leading techniques for elemental analysis, but nowadays they have been replaced by inductively coupled plasmas employed as excitation sources for optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS) and by microwave-induced plasma for optical emission

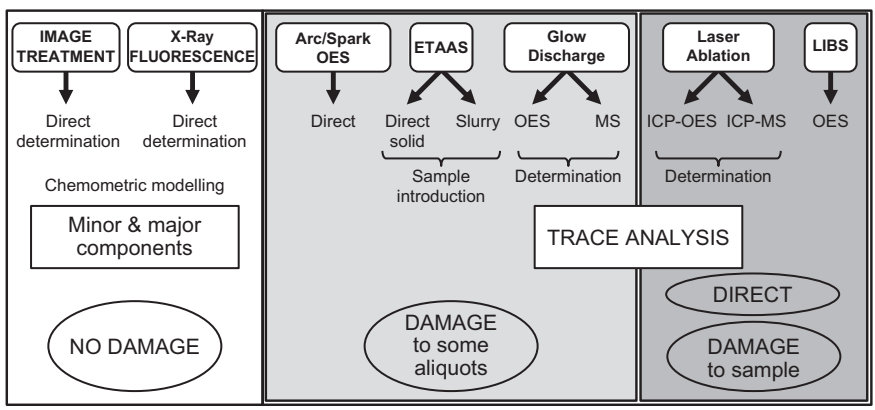


Figure 2.3 Green alternatives for direct solid sample analysis of mineral elements.

spectrometry (MIP-OES) in the gas phase. These new techniques give an almost perfect analytical performance. However, it should be pointed out that arcs and sparks provide a good excitation source for OES and still retain superior solid sampling capability in comparison with ICP or MIP techniques, which require the use of solutions or gaseous phases, respectively.[8]

Techniques based on ablation of the sample by an electric spark or arc have been used for the direct analysis of solid samples such as metals and alloys in the metallurgy industry, ores and minerals in geological prospecting and solid wastes in environmental monitoring. The principle of these techniques is based on the effect of a high potential difference applied between two electrodes (one of them being the sample) in an argon atmosphere. A repetitive unidirectional electrical discharge ablates atoms from the surface of the solid sample and produces a high-temperature plasma, in which the atoms extracted from the sample are excited and the corresponding emission can be observed by using a spectrometer.[9]

The use of new excitation sources based on an alternating current (AC) arc with low acquisition and operational costs and with the capacity to operate under atmospheric conditions without an additional inert gas and the use of a charge-coupled device (CCD) as a detector have created a new generation of low-cost and compact arc OES spectrometers. Some reasonably priced portable analysers can be obtained that may be very useful for *in situ* and rapid analyses.

2.3.2 Electrothermal Atomic Absorption Spectrometry

Electrothermal atomic absorption spectrometry (ETAAS) and, in particular, the most frequently used graphite furnace (GF-AAS) method, involve the drying and ashing of samples and atomization of the mineral residues in separate and well-controlled steps.[10] This method improves on the sensitivity of traditional flame atomic absorption spectrometry (FAAS) by three orders of magnitude. Additionally, solid samples, instead of dissolved samples, can be introduced into the atomizer, providing a fast alternative that avoids sample dissolution and makes the drying step unnecessary. However, direct solid sampling in GF-AAS has some drawbacks: (1) the difficulty of handling and introducing a small sample mass; (2) the difficulty of calibration, which in some cases requires the use of solid standards with characteristics similar to those of the samples to be analysed; (3) the limited linear working range and the difficulty of diluting solid samples; and (4) the lack of precision of the results due to the heterogeneity of the samples, a large number of repeated determinations being required when that is possible.[11]

Some of the aforementioned disadvantages can be avoided by the use of the slurry technique, but this creates new problems related to the dilution of samples provided by slurry preparation, stabilization of slurries and the need to use some reagents.[12]

Direct solid sampling GF-AAS is preferable to the use of the slurry technique for materials that cannot be easily dissolved and for the elements most susceptible to contamination. It is not affected by problems such as sedimentation or partial leaching of the analyte and particle size effects, which are less critical in direct solid analysis than in slurry sampling. On the other hand, the maximum sample mass that can be introduced into the graphite furnace for solid sampling is one to two orders of magnitude higher than that for slurry sampling.[13]

With regard to calibration in solid sampling GF-AAS, reasonable results can be obtained with standard calibration with aqueous solutions after careful optimization of the experimental conditions. It is remarkable that solid sampling GF-AAS offers a fast screening tool, essentially without any sample preparation,[14] and can also be used to determine the homogeneity and micro-heterogeneity of certified reference materials (CRMs) and other samples.[15] It is of special interest for the determination of trace elements in complex samples that are difficult to dissolve.

Modern solid sampling GF-AAS units may be equipped with a device for automatic weighing and introduction of solid samples,[16] and use a platform designed for handling large sample amounts and transverse heating of the graphite tube that provides a homogeneous temperature distribution. One of the advantages of this approach is related to the potential use of appropriate temperature programming to minimize potential matrix effects. In theory, by controlling the temperature, it is possible to separate in time the atomization of the analyte from the vaporization of the main matrix components. External calibration with aqueous standards should therefore be possible after removal of the matrix.[17]

An additional advantage of solid sampling GF-AAS is its great potential for automation; the main problem with this technique, on the other hand, is the lack of capability for multielemental determination and reduced portability.

2.3.3 Glow Discharge

Glow discharges (GDs) have been used as sources for emission and mass spectrometry. Traditionally, GD optical emission spectroscopy (GD-OES) has been a widely used technique for routine bulk analyses in material sciences and for rapid depth and profile analyses of surfaces, thin films and coatings. GD mass spectrometry (GD-MS) has been extensively used for the direct analysis of conducting solids; it is virtually unrivalled for the trace analysis of impurities in high-purity materials and for monitoring the shallow depth distribution of trace substances. For the bulk analysis of less pure samples, GD-OES competes with spark emission spectroscopic and X-ray techniques, but in many cases it exhibits fewer matrix effects or has lower detection limits than the other techniques.[18] Regarding depth profile and surface analysis, especially of thick coatings, GD-OES does not have much competition because of the low total cost of the instrumentation, the ease of sample handling and the speed of analysis. For the analysis of refractory materials, glasses, *etc.*, it is

exceeded only by XRF in terms of ease of analysis but without providing information about light elements. Auger electron spectroscopy (AES), secondary ion mass spectrometry (SIMS) and secondary neutral mass spectrometry (SNMS) may be preferred because of their possible depth resolution and the information they provide on lateral distribution and structure, but they are more costly than GD methods and require longer analysis times.[19]

GD operates in a primary vacuum and uses the bombardment of the sample surface with ions of rather random orientation and high energy for the sputtering or atomization process. The sputtered species, mostly atoms, diffuse into the negative glow area, where they are excited and/or ionized. Compared with other techniques, such as SIMS, in GD methods sputtering and ionization processes are separated in space and time, resulting in only minor variations in sensitivity and little matrix dependence, so quantification is very easy; in some cases it is possible without the absolute need for matrix-matched standards.[20]

The source commonly used in GD methods is a Grimm-type chamber, which operates in the presence of a noble gas (usually argon) under reduced pressure. This source consists of an anode tube, usually grounded, and the sample to be analysed (cathode) that is placed perpendicularly in front of this anode tube. A ceramic spacer maintains a distance of less than 0.1 mm between the flat sample surface and the anode tube and an O-ring allows this mount to be sufficiently vacuum tight. The electrical energy to ignite and maintain the plasma is fed into the plasma chamber directly through the sample. To avoid excessive heating, the samples are cooled. Sputtering is caused by bombardment of the sample surface and several particles are responsible for this process.

GD methods have been considered as a fairly rough tool able to analyse only bulk materials and rather thick coatings in the micrometre range, making it possible to characterize the elemental composition of a sequence of layers of varying thickness, ranging from nanometres to several micrometres, in a single analysis step without any sample preparation. Using a Grimm-type configuration, however, GD methods are capable of performing surface and interface analysis with a depth resolution in the nanometre range, possibly even at the atomic layer level. In Grimm-type sources, a typical anode tube has an internal diameter of 4 mm, but by working with smaller anode tubes (*e.g.* 1 mm) the lateral resolution can be improved but the sensitivity is reduced. This limits the capability of applying GD methods for micro-spot analysis compared with other techniques such as LA-ICP-MS or SEM-X-ray methods.

GD-OES has been extensively applied for the quantitative depth profile analysis of hard coatings and it is possible to analyse quantitatively a wide variety of commercial and experimental hard coatings with a single calibration if the coatings are conductive.[21]

For electrically conducting materials, the performances of direct current (DC)- and radiofrequency (RF)-powered GD sources are very similar with regard to both depth resolution and sensitivity. However, non-conductive

coatings or samples can be analysed only with RF-powered discharges. Most electrically isolating materials are also poor thermal conductors. The energy deposited at the sample surface by the sputtering process therefore cannot easily be dispersed and causes a considerable increase in the temperature of the analysed material, which can lead to sample destruction. Pulsing the RF power supply can help by reducing the average power dumped at the sample surface without reducing the instantaneous energy available for the sputtering and excitation processes.[22] Using a pulsed RF-GD approach makes possible the analysis of thermally sensitive materials such as thin coatings on glass samples.[23] Combining the capacity of RF-GD to sputter non-conductive materials with the quasi-simultaneous coverage of a large mass range, time-of-flight (TOF) mass spectrometers offer a new range of applications, not only to determine elemental information on a layered sample but also to obtain molecular information.[24] An example is the analysis of multilayer structures composed of different polymers, including polystyrene (a polymer that has not yet been successfully sputtered by cluster ion beams typically used for the TOF-SIMS analysis of polymers), the polymers being distinguished by their characteristic molecular fragments.[25]

Trends in GD spectroscopy are focused on the development of new sources and interfaces.[22] New ionization sources have been designed for the direct mass spectrometric analysis of solid materials at atmospheric pressure. These techniques allow the fast, versatile and extremely sensitive analysis of real samples with minimal or no sample preparation. In recent years, an atmospheric-pressure glow discharge (AP-GD) source has been developed for the generation of reagent ions that, coupled to a TOF-MS instrument, allows the detection of a wide variety of compounds, both polar and non-polar, on a broad range of solid substrates from glass to plastics, textiles, wood, *etc.*, with high analytical sensitivity. The analysis can be made in less than 1 min and might become an important tool for screening unknown samples.[26] Another important advance in atmospheric-pressure discharges is the reduction in size and power consumption of the discharge. These microplasmas can be used for portable, battery-operated instruments that are smaller than laboratory-scale instruments. These novel sources can be used as small and inexpensive detectors for chromatography and electrophoresis separations in combination with lab-on-a-chip systems. The advantages are that microplasmas use smaller volumes of reagents and are therefore cheaper, quicker and less hazardous to use and more environmentally friendly than conventional systems.

Regarding developments in reduced-pressure sources for GD based on Grimm-type sources, the design of new fast-flow sources has been directed to high gas flow rates, improving the efficiency of ion transport by gas convection and thus reducing diffusion losses.

2.3.4 Laser Ablation

Ablation of solids using laser pulses and subsequent transfer of the released material to the measurement device using a gas flow, usually argon, is an

attractive alternative to the nebulization of aqueous solutions for the direct analysis of samples, also providing the depth and spatial distribution of the analytes.

A laser ablation (LA) system combined with inductively coupled plasma optical emission spectrometry (ICP-OES) or mass spectrometry (ICP-MS) provides information about the elemental composition at trace and ultra-trace levels. LA-ICP-MS represents the most modern method for the direct analysis of the elemental composition of solid samples, with the ability to acquire local and depth-resolved information about their distribution, for both conducting and non-conducting samples.

A typical LA setup consists of a lens (which may be incorporated into an optical microscope so that optical and visual focusing are coincident), an ablation chamber and an adjustable platform. For LA-ICP, the sample is placed in an air-tight ablation cell flushed with an inert gas to transport the ablated material to the ICP. The volume and aerodynamics of ablation cells, and also the length and geometry of the transfer tube, affect dispersion of sample density in the ICP. It has been reported that small cell volumes may reduce the sample washout time of the cell and a high carrier gas flow rate through the cell and transfer tubes reduces the deposition of ablated material, in addition to decreasing memory effects and increasing transport efficiency.[27]

The LA of solids involves processes that include heating, melting and evaporation of sample material at extremely high temperatures and pressures. Because of the complexity of the process, ablated material may be removed from the sample in the form of atoms, molecules, vapour, droplet solid flakes, large particulates or mixtures of these and the distribution of the materials will depend on the selection of laser parameters as a function of the sample.

Three main types of lasers – ruby, Nd:YAG and excimer – have been widely reported and validated for ablation but currently the most commonly used is the Nd:YAG laser because it is relatively cheap, robust, reliable and easy to operate. Moreover, with this laser it is possible to operate at 1064 nm (fundamental wavelength) or 532, 355 and 266 nm.[28]

The laser pulse frequency and power affect the ablation rate and the particle size of the ablated material, but typical values are of the order of 1–20 $mg\,s^{-1}$ and less than 100 nm, respectively, so sample consumption is limited to a few micrograms.[29] Taking into consideration sample consumption and laser spot diameter size (from a few microns to 100 mm), LA-based techniques could be considered as almost non-destructive tools from a macro point of view,[30] and are thus especially interesting for the analysis of samples that in most cases are unique.

Compared with the use of a spark discharge, LA eliminates the restriction associated with the necessity to ensure electrical conductivity of the samples to be analysed but involves other problems and limitations, such as (1) the amount of the sample material aerosolized per unit time is significantly smaller than that which can be obtained with a spark discharge and

(2) fractional evaporation may be observed. The small area of the spot of focused laser radiation is useful, but to determine the general composition of macrosamples the area of the analysed surface has to be increased and a mechanical device for scanning the laser radiation over the sample surface is required.[31]

The major advantages of ICP-MS as a detector for LA analysis are its high sensitivity, wide dynamic range, relative simple spectra and fast scanning, which provide an excellent method for the direct simultaneous analysis of complex samples without any pretreatment or use of reagents.

Regarding calibration strategies, the best results were obtained using matrix matching for external calibration, because the ablation rate varies with the sample matrix, but this implies the use of certified reference materials (CRMs) with a composition similar to that of the sample. However, other alternatives are the use of external calibration with respect to a solid reference standard in conjunction with internal standardization or calibration using solutions when possible.[32]

LA coupled with ICP-MS has been shown to be an excellent analytical tool for the analysis of an extended number of analytes in a wide range of matrices, as can be seen in Table 2.2.

Compared with conventional dissolution techniques, LA avoids the dissolution step and the use of or exposure to potentially hazardous reagents, and also the risk of introducing contamination or losing volatile components during sample preparation. It also saves analysis time. In addition, LA can be applied to any type of solid sample and there are no sample size requirements, a chemical analysis being possible with only a few micrograms of sample. An example of the green analytical characteristics of LA is illustrated by the analysis of radioactive samples: the organic solvents or concentrated acids that are required for conventional radiochemical analysis are not necessary for LA sampling and less than 1 mg of sample is used, which reduces the risks associated with sample handling and sample contamination; moreover, elemental and isotopic analysis can be achieved entirely within a hot cell environment, further reducing the risk of environmental contamination.

2.3.5 Laser-induced Breakdown Spectroscopy

LIBS is an emission spectroscopic technique with the capability of detecting, identifying and quantifying the chemical composition of any material. This technique utilizes a pulsed laser focused on a small area to create a microplasma on the sample surface. The resulting light emission is collected optically and then resolved temporally and spectrally in order to produce an intensity *versus* wavelength spectrum containing emission lines from the atomic, ionic and molecular fragments created by the plasma.[33]

In some cases, the laser pulses reach the target sample through an optical fibre and the collection of the plasma light and transport back to the detector system can be effected using either the same fibre-optic cable or a

Table 2.2 Examples of applications of laser ablation solid sampling combined with ICP mass spectrometry. Reproduced from ref. 119 with permission from the Royal Society of Chemistry.

Field	Application	Objective
Environmental	Tree rings	Study of changes in atmospheric conditions, soil chemistry and pollution history
	Tree bark	Provide information about the degree of pollution of a certain region
	Seashells	Trace element fluctuations reflect environmental change and major pollution events
	Coral	Concentrations of trace elements in coral skeletons provide information related to changes in seawater properties
	Airborne particulates	Information for monitoring air quality and inorganic pollutants
Geology	Geochronology	U–Pb isotopic analysis for dating
	Inclusion analysis	Study of microscopic inclusions in minerals
	Isotopic analysis	Precise measurement of the isotopic composition of characteristic elements (Hf, W, Sr, U, Th, Pb, Os) at trace levels
	Bulk analysis	Measurement of rare earth elements or the platinum group elements for geological or economic importance
	In situ analysis	Spatially resolved analysis of elements
Archaeology		Authentication of precious antiques with minimal damage
Waste samples		Especially to analyse radioactive samples
Other	Fingerprinting	Forensic chemical analysis of physical evidence
	Film doping and depth profiling	Quantitative analysis of dopant dose implanted in crystalline silicon wafers or the analysis of multilayer coatings systems

second one. This method has been shown to work over distances up to 100 m, but requires the optical fibre to be positioned adjacent to the sample. Obviously, this use of fibre optics is restricted in its application and even impossible in cases where contaminated or hostile chemical or temperature environments may affect the probe and, evidently, the operators. In these instances, an open-path LIBS configuration, in which the laser beam and the returning plasma light are transmitted through the atmosphere, is available. This stand-off LIBS has been suggested for the elemental analysis of materials located in environments where physical access is impossible or

dangerous, but optical access could be envisaged. Solid samples can be analysed at distances of a few metres by open-path stand-off LIBS using nanosecond laser pulses, whereas liquid samples can be measured at distances of a few metres. The use of femtosecond laser pulses is predicted to extend the capabilities of LIBS to very long distances because the high power densities achieved with these lasers can also induce self-guided filaments in the atmosphere, which can produce LIBS excitation of a sample at kilometre ranges.[34]

For a long time, the analytical applications of laser-induced plasma spectrometry were restricted mainly to overall and qualitative determinations of elemental composition in bulk solid samples. However, the introduction of new compact and reliable solid-state lasers and technological developments in multidimensional intensified detectors have made possible new applications of LIBS for the direct sampling of any material, irrespective of its conductive status, without any sample preparation and with a sensitivity adequate for any element in different matrices.[35]

LIBS has intrinsic advantages over other analytical techniques for elemental analysis, such as X-ray microprobe or X-ray fluorescence spectrometry; it provides a rapid, spatially resolved or in-field geochemical analysis of elements of low atomic weight, being an excellent tool for mineralogical and petrological analysis, either in the laboratory or in the field using real-time field-portable instruments.[36]

In addition to being employed for mineral analysis, LIBS systems can also be combined with molecular fluorescence or Raman emission for the development of hybrid sensor systems. Figure 2.4 shows a scheme of the experimental setup for a stand-off dual Raman-LIBS mobile sensor prototype developed for the analysis of explosive materials. The system is able to measure simultaneously both the Raman spectrum and the laser-induced breakdown spectrum and this approach exploits the energy distribution profile of the laser beam to extract the vibrational fingerprinting from the non-ablated section of the interrogated target within the outer part of the laser beam jointly with the atomic information gained from the ablated mass by the inner part of the laser beam. By selecting suitable operating conditions (breakdown timing, laser power and acquisition time), molecular and multielemental spectral information, from the same sampling point and at the same laser event, can be obtained without any operator exposure.[37] On the other hand, it is important to consider the applicability of direct spectroscopic and spectrometric approaches for the determination of organic compounds (see Figure 2.5).

2.3.6 Desorption Electrospray Ionization

Desorption electrospray ionization (DESI) coupled to mass spectrometry (DESI-MS) provides a new tool for the direct analysis of solid surfaces without sample pretreatment and can give molecular information about different analytes in various solid surfaces. It is of great interest for the

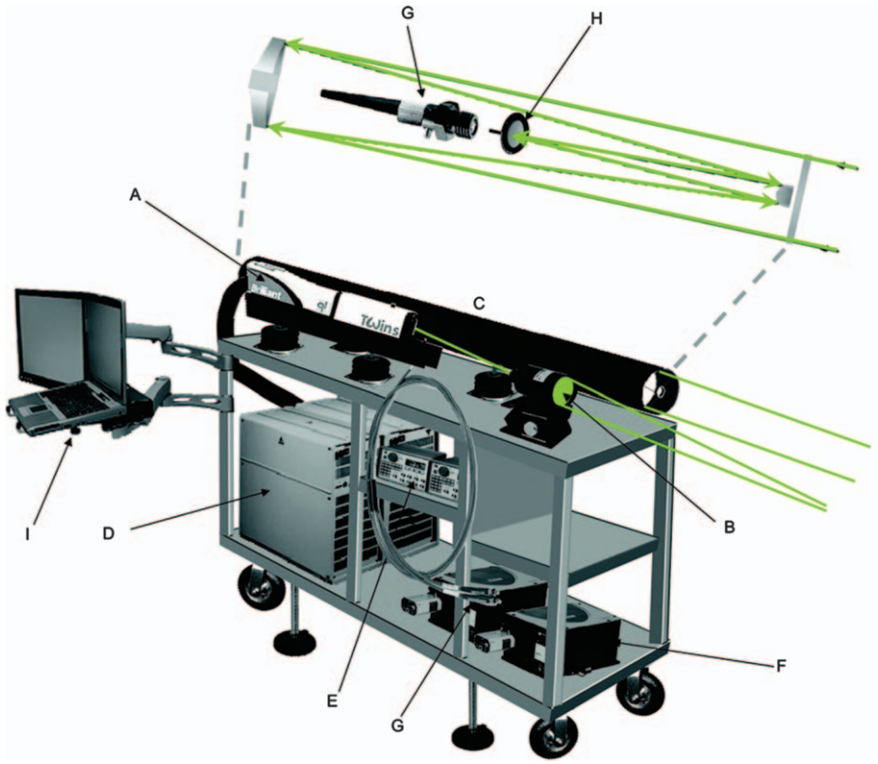


Figure 2.4 Experimental setup of the stand-off dual Raman-LIBS sensor: (A) Nd:YAG laser (532 nm); (B) beam expander; (C) telescope; (D) laser power sources; (E) pulse and delay generators; (F) spectrographs; (G) bifurcated optical fibre coupled to a collimating lens; (H) holographic SuperNotch filter; (I) personal computer. The inset shows the optical layout of the telescope. Reproduced from ref. 37 with permission from American Chemical Society, Copyright 2010.

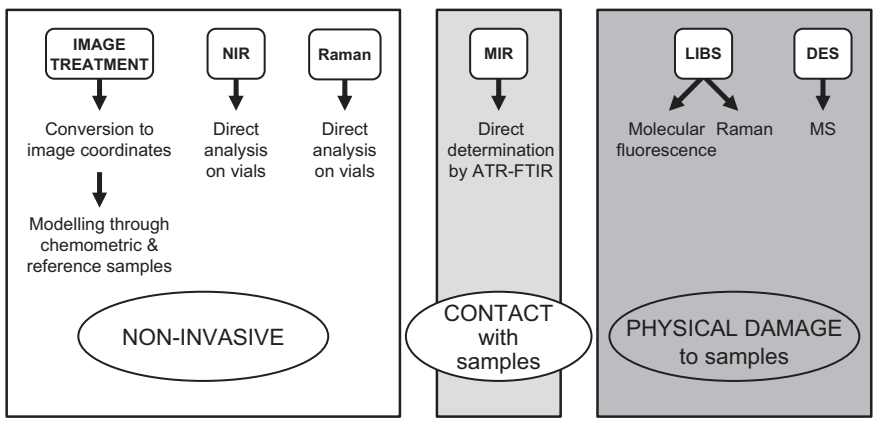


Figure 2.5 Green alternatives for direct solid sample determinations of organic compounds.

simultaneous determination of polar and non-polar compounds in liquid and solid samples.[38,39]

A DESI source, with a spray solvent and a gas nebulizer, generates a gas jet that impinges on the sample surface and creates positive and negative ions that can be sampled into a mass spectrometer. The method is fast, highly selective and sensitive; it provides absolute detection limits in the sub-nanogram to sub-picogram range and constitutes a powerful tool for *in situ* molecular analysis and also for elemental speciation.[40] Recent developments based on the use of an appropriate internal standard have improved the quantitative capabilities of this technique.[41]

2.4 Non-invasive Methods of Analysis

The use of non-invasive techniques for the direct analysis of packaged products is a very interesting approach for the development of clean analytical methodologies, especially for quality control laboratories. It requires non-destructive measurement techniques to be used through different sample containers, such as blisters, bags, vials or bottles made of various materials. However, it is possible to make this kind of measurement only if the container material is transparent or the package has a suitable window for the source radiation.

NIR and Raman spectroscopy are two techniques that have suitable characteristics for obtaining chemical and physical information from non-destructive and non-invasive measurements of packaged samples.

The use of NIR spectroscopy was proposed for determining residual moisture in lyophilized sucrose through intact glass vials.[42] Common types of glass are virtually transparent to NIR radiation and powdered samples may be measured in glass vials using the reflectance mode. This approach offers several advantages, for example (1) direct measurement without sample manipulation, (2) conservation of samples inside the vials after analysis, which means that they can be employed/consumed or stored, and (3) the lack of any deleterious effects on samples, which are not altered by the operator or the laboratory environment.

The last advantage can be very important for labile forensic samples, especially samples with legal relevance, as in the analysis of seized illicit drugs. Moros *et al.* proposed a non-destructive direct determination of heroin in seized illicit street drugs based on diffuse reflectance NIR measurements on samples contained in standard chromatographic glass vials.[43] Since neither chemicals nor time-consuming sample preparation processes are necessary, NIR spectroscopy provides an ideal analytical method for the direct and instantaneous measurement of seized drugs. The use of portable and hand-held NIR spectrometers allows the rapid checking of this type of sample in routine analysis and police checks.

NIR measurements on solid dosage forms can be performed in the diffuse reflectance mode and this technique has been applied to pharmaceutical analysis, for the determination of active principles or the

identification of pharmaceutical excipients inside USP vials[44] or through the blister pack.

The above strategy contributes to the implementation of process analytical technology (PAT),[45] which promotes strategies for the control of primary and secondary manufacturing processes and offers an excellent method for the non-destructive and direct analysis of final products stored in blister packs or other radiation-transparent containers.

NIR transmission measurements have been employed for the identity confirmation of double-blind clinical trial tablets. The correctness, shipping, packaging and labelling of the blister packs need to be checked before samples are shipped and a classification tool based on the NIR transmittance spectra has been developed to determine the different strengths of tablets using commercially available NIR instrumentation.[46]

Broad *et al.* proposed the use of NIR spectroscopy for the simultaneous determination of ethanol, propylene glycol and water contents in a pharmaceutical oral liquid formulation by direct transmission measurements through amber poly(ethylene terephthalate) (PETE) bottles.[47] These plastic containers were expected to contribute to the NIR absorption spectrum. However, the background spectrum from an empty bottle was very small and small differences between different bottle spectra were also observed, showing that for calibration samples prepared in their own individual bottles the background absorption and spectral variations will be incorporated in the calibration model and compensated for sample prediction.

NIR transmittance spectroscopy has been proposed as a technique for the direct determination of the ethanol content of alcoholic beverages, making measurements through the glass bottles.[48] This technique has been applied to commercial instruments such as the Infratec™ 1256 Beverage Analyser (Foss). This instrument, based on NIR dispersive scanning in the range 850–1050 or 570–1100 nm, includes a colour module that is suitable for the analysis of different types of alcoholic beverages and permits direct measurement of samples inside their bottles without any sample preparation. The analyser incorporates a selection of ready-to-use calibrations (regression programs) for different types of beverages, based on partial least-squares analysis and artificial neural networks. For the analysis of beer samples, the alcohol content, original extract and colour can be directly predicted and other calculated parameters are real extract, apparent extract, degree of fermentation, energy, specific gravity, original gravity, present gravity, extract gravity, spirit indication and refractive index. The complete analysis can be made in less than 45 s, making it possible to use this instrument to control production at-line.[49]

It is difficult to make NIR absorption measurements of aqueous samples in large bottles because of the inadequate energy transmission due to the long optical pathlength and the high water absorption of infrared radiation. The reproducibility of on-line measurements can be poorer as a result of the variation in pathlength resulting from the lack of reproducibility of bottle positioning. Therefore, well-validated methodologies for manual sampling

and NIR transmittance or diffuse reflectance measurements cannot be used for continuous measurements.

Raman spectroscopy is another alternative for the analysis of samples directly through glass or plastic packages. Additionally, Raman spectroscopy offers some advantages over NIR spectroscopy because it can provide a simple optical configuration that is easily interfaced for on-line measurements. In addition, the resolved details from the richer spectral features can be used to relate Raman spectra to molecular structure and composition.

Raman spectroscopy has been employed for the static analysis of the ethanol content of spirits (whisky, vodka and sugary alcoholic drinks) in 200 mL (flat) and 700 mL (round) glass bottles, using a 785 nm laser and the Raman ethanol signal at 880 cm^{-1}. The technique is applicable only to the analysis of samples in clear glass bottles because coloured bottles exhibited strong fluorescence.[48]

The quantitative *in situ* analysis of povidone (polyvinylpyrrolidone) in eyewash solutions contained in low-density polyethylene (LDPE) bottles was also performed by Raman spectroscopy.[50] In order to correct for the lack of physical and chemical homogeneity in the walls of plastic bottles and the variation in the sensitivity of the Raman response to the sample position with respect to the focal plane, Raman dispersed radiation was collected using a wide-area illumination (WAI) scheme that involves an incident laser with a large surface area (28.2 mm^2) and a long focal length (248 mm). The resulting Raman spectra are much less sensitive to morphological variations of the sample bottles and the high incident laser spot provides enhancement of reproducibility. Additionally, the use of an isobutyric anhydride external standard in front of the plastic bottles makes it possible to correct the Raman intensity and reduce laser fluctuations. This WAI scheme with the use of a synchronous external standard has the potential to allow Raman spectroscopy to be used for quality control (QC) analysis for a wide range of liquid samples contained in glass (clear or amber) or plastic containers.

Schmidt *et al.* developed a prototype hand-held Raman sensor for the *in situ* characterization of meat quality.[51] The Raman sensor head was integrated with a microsystem-based external cavity diode laser module that operated at an excitation wavelength of 671 nm and the Raman signal was guided by an optical fibre to the CCD detection unit. Raman spectra of meat were obtained with 35 mW power within 5 s or less, and for measurements of raw and packaged pork meat this Raman sensor head evidenced its capability to detect microbial spoilage on the meat surface, even through the packaging foil.

2.5 Direct Analysis of Solid and Liquid Samples Without Sample Damage

In this section, we consider the possibilities offered by several techniques, such as X-ray, nuclear magnetic resonance (NMR) and vibrational

spectroscopy, to analyse solid and liquid samples directly without any sample damage.

2.5.1 Mineral Analysis by X-ray Techniques

X-ray fluorescence (XRF) is based on the secondary X-ray emission of characteristic radiation from the internal electrons of the different atoms present in a sample, as a consequence of the interaction between the primary X-ray and the inner orbital electrons. The secondary XRF spectrum identifies the elements present in the sample by the corresponding transition peaks at characteristic wavelengths or energy positions and their intensity is proportional to the elemental concentration.

Modern XRF analysis systems, based on either wavelength-dispersive (WD-XRF) or energy-dispersive (ED-XRF) measurements, are well-established methodologies offering fast, non-destructive and clean forms of analysis that can routinely provide information about the elemental composition of samples with adequate accuracy and reproducibility. Conventional XRF systems incorporating vacuum systems can measure elements from Na to U in solids and liquids with a precision of better than 0.5% relative standard deviation in many cases, the limit of detection being typically in the low parts per million range and as low as 0.1 ppm for some elements. Almost any sample type can be analysed by XRF spectrometry: pressed powders, glasses, ceramics, metals and alloys, rock, coal, plastics, oil, *etc.* The simplicity of sample preparation, minimal manipulation and the possibility of determining some elements, such as sulfur, that are difficult to measure by other techniques have promoted XRF as a useful alternative to conventional molecular and atomic spectroscopic techniques.[52]

In recent years, the development of micro-XRF and portable XRF instruments, as a result of advances in miniaturization and semiconductor detector technology, has opened up interesting green applications. These instruments make it possible to carry out *in situ* measurements and provide green analytical tools for fast and non-destructive elemental analysis that has been used for geological studies and artwork analysis.[53]

2.5.2 Molecular Analysis by NMR Spectroscopy

The phenomenon of nuclei absorbing resonant radiofrequency energy in a static magnetic field is called nuclear magnetic resonance (NMR) and this process is always accompanied by nuclear relaxation. The resonant frequency of absorption of energy of magnetic nuclei in a magnetic field is proportional to both the strength of the field and the magnetic moment of the nucleus. The resonant NMR frequency is a fingerprint of the local electronic environment of the nucleus, but depends on the external magnetic field. An NMR spectrum is a series of peaks of various widths and shapes that are a reflection of the local molecular environment of the nuclei under observation.

Under certain conditions, the NMR intensity is proportional to the number of resonance nuclei producing the signals, thus providing interesting possibilities for quantitative analysis in addition to the traditional use of NMR signals for the structural analysis of pure compounds.

NMR spectroscopy as an analytical technique has the advantages of non-destructiveness, no need to separate the analyte from complex mixtures and avoidance of the use of toxic reagents when spectra can be obtained directly from solids. The commercialization of high-field NMR instruments and probe improvements have contributed to the development of analytical applications in the fields of natural product,[54] pharmaceutical,[55] agricultural and food and beverage analysis.[56] The main limitation is the high price of NMR instrumentation and its low sensitivity compared with other spectroscopic techniques. Additionally, in many cases, dissolution or dilution of the sample in a suitable solvent, such as deuterated water or chloroform, is required and direct analysis is limited to solid samples.

The introduction of mobile low-field NMR analysers offers an excellent method for non-destructive and fast measurements, with great potential to be used for on-line QC. Instruments such as these can be used for measurements in the near-surface volume of samples of any size. The mobile probe of the instrument is a pair of anti-parallel polarized permanent magnets joined by an iron yoke, producing a static inhomogeneous magnetic field. A surface coil is placed in the gap between the poles of the permanent magnet, generating the radiofrequency pulses. The measurement volume of the probe is about 5×5 mm in area and 2.5 mm in depth. The probe can be designed such that the first distance beneath the probe surface does not contribute to the NMR signal. As an example, this analyser has been employed for the *in vivo* determination of the fat content in salmon.[57] Similar instrumentation has been employed for the analysis and conservation of artworks.[58]

2.5.3 Molecular Analysis by Vibrational Spectroscopy

Vibrational spectroscopy is a well-established set of techniques traditionally used to obtain qualitative molecular information, especially by organic chemists. However, in recent decades developments in instrumentation and the application of chemometrics to data treatment have demonstrated that vibrational spectroscopy provides fast quantitative analytical methods that allow non-destructive analysis and permit, in a green way, the simultaneous determination of multiple components from the same sample in a single instrumental measurement without environmental side effects.[59]

Infrared spectroscopy, in both the mid- (MIR) and near-infrared (NIR) regions, and Raman spectroscopy are the main vibrational techniques employed for the direct analysis of samples.

IR spectroscopy is based on the interaction of electromagnetic radiation with a molecular system, in most cases in the form of absorption of energy from the incident beam. The absorption of IR radiation induces transitions

between the vibrational energy levels of molecular bonds. The different chemical bonds of a molecule absorb at different IR wavenumbers depending on the atoms connected, the surrounding molecules and the type of vibration that the absorbance gives rise to (stretching or bending). Most molecules have IR bands in the spectral range between 400 and 4000 cm^{-1} (MIR) and most of the intense features of any MIR spectrum can be assigned to fundamental transitions. As a consequence of the anharmonicity of the vibrational energy levels, overtone transitions appear at high wavenumbers that are multiples of fundamental transitions, but they have very weak absorption compared with fundamental bands. When two fundamental vibrational transitions absorb energy simultaneously, a combination band can appear. These overtone and combination bands are more complicated to assign than fundamental bands and provide weak signals in the NIR region between 12 800 and 4000 cm^{-1}.

On the other hand, Raman spectroscopy is an emission technique in which the sample is radiated with monochromatic visible or NIR laser radiation. This brings the vibration energy levels of the molecule into a short-lived, high-collision state, which returns to a lower energy state by emission of an energy photon. The emitted photon usually has a lower frequency than the laser radiation and in this case corresponds to the Stokes–Raman scattering emission. The difference between the frequency of the excitation laser radiation and that of the scattered photon is called the Raman shift and its units are cm^{-1}. The Raman shift corresponds to the frequency of the fundamental IR absorbance band of the bond involved in the relaxation processes.

IR spectroscopy detects vibrations during electrical dipole moment changes, whereas Raman spectroscopy measurements are based on the detection of vibrations during the electrical polarizability changes. This implies that bonds that connect two identical or practically identical parts of a molecule can be more active in Raman than in IR measurements, thus providing complementary spectral information, *e.g.* the O–H stretching vibration is very strong in IR but very weak in Raman spectra. Thus, for instance, water has high absorption in the MIR region and is practically invisible in Raman spectra.

One of the advantages of IR spectroscopy is its ability to obtain information from samples in many physical states – solid, liquid or gas – and in different measurement modes. Transmittance measurements are possible when samples are highly transparent to the radiation (*i.e.* gas) or when it is possible to obtain a suitable thick film of the sample that can let the IR radiation pass to the detector, this effect being strongly dependent on the IR energy. For example, pharmaceutical tablets can be analysed by transmittance NIR spectroscopy whereas some liquid samples have a higher absorption in the MIR region and cannot be directly measured by transmittance inclusively with a pathlength less than 0.1 mm.

On the other hand, reflection techniques are very suitable for samples that are highly absorbent or non-transparent to IR radiation. Diffuse reflection is a phenomenon observed when radiation strikes a diffuse surface and is scattered in all directions. This technique is especially interesting for the

analysis of powdered samples in the NIR region. Internal reflection or attenuated total reflectance (ATR) occurs when radiation moving through a transparent material of high reflective index impinges on the interface with a low-refractive material, such the sample, at an angle greater than the so-called critical angle. Then the radiation penetrates inside the sample, to a small depth of a fraction of the wavelength of the radiation. To obtain a good ATR spectrum, the sample must be in optical contact with the internal reflection crystal of the ATR accessory, normally ZnSe, germanium, KRS-5 (thallium bromoiodide) or, preferably, diamond owing to its high hardness and chemical resistance. Considering that the effective sample thickness is very small, from a fraction of micrometre to a few micrometres in the MIR region, the fact that internal reflection accessories normally produce multiple reflections means that the surface layer is sampled multiple times, thus increasing the intensity of the resulting spectra. Many basic ATR attachments have been developed for standard laboratory spectrometers and some of them include probes and flow cells to be used for monitoring chemical processes in the laboratory or on-line. Recent designs of ATR units for MIR spectroscopy use a composite internal reflection element, combining a diamond sample-contacting surface with a ZnSe or KRS-5 parabolic focusing element, the latter being equivalent to a beam condenser that increases the sensitivity. The strength, hardness and chemical inertness of diamond make it an exceptionally useful material for internal reflection spectroscopy. The refractive indexes of diamond (2.39), ZnSe (2.43) and KRS-5 (2.38) are very similar, so when diamond is in optical contact with either ZnSe or KRS-5 there is no significant loss of energy at the interface and the aforementioned materials provide an excellent mechanical support for the diamond. Nowadays, these ATR accessories are used extensively in the analysis of liquid, solid and paste samples by IR spectroscopy.[60]

For Raman spectroscopy, the sample must be illuminated with monochromatic radiation as brightly as possible. Then, the scattered radiation must be collected efficiently and the mixture of reflected and elastically scattered radiation must be separated from its much weaker Raman component, and this weak scattered radiation alone must be processed to obtain the Raman spectrum. Traditionally, this separation was carried out using multiple monochromators, but current Raman spectrometers incorporate a filter and a single monochromator, spectrograph or interferometer system. The expansion of Raman systems is related to their versatility for sample handling by incorporating microscopes, telescopes or fibre-optic bundles to illuminate and view the sample and then redirect the collected radiation to the detector.

Both IR and Raman spectroscopy can be applied for the direct analysis of untreated samples at the macroscopic or microscopic scale. Raman microspectroscopy has the potential for improved resolution because of the low wavelength of the radiation used. Additionally, Raman instruments offer confocality and it is possible to focus on different planes below the sample surface. Moreover, the signal-to-noise ratio is much lower in Raman than in

IR spectroscopy and if samples have natural fluorescence it may be impossible to obtain Raman spectra. This problem, also present in macro Raman spectroscopy, may be overcome if the Raman instrument is equipped with a low-energy NIR laser instead of a laser working in the visible region; this avoids the fluorescence of most molecules, but it decreases the spatial resolution. In addition, the heat generated by the laser may alter the Raman spectra and also destroy the sample during measurements. In order to avoid sample damage, a suitable laser power and measurement time may be selected.[61] Modern Raman instruments used for quantitative measurements are equipped with 1064 or 785 nm emitting lasers operating at powers of a few milliwatts to avoid sample fluorescence and thermal degradation.

As mentioned previously, one of the advantages of vibrational techniques is their ability to obtain easily the spectra of different types of samples in any physical state. From these spectra, it is possible to obtain good quality global information about the chemical or physical characteristics of samples or of individual components present in them. For this reason, IR and Raman spectroscopy have been widely employed in analyses in agricultural and food science,[62–64] pharmaceuticals[65] and petrochemicals.[66] NIR spectroscopy is widely used[67–69] because of the higher penetration depth of NIR radiation and the feasibility of sample manipulation or synergistic combination with remote fibre-optic probes. However, recent developments in mobile and portable Raman instruments offer the opportunity to make direct analyses of samples of special interest.[70]

The combined use of chemometrics with vibrational spectroscopy has contributed to the increase in analytical applications based on NIR, MIR and Raman spectroscopy in the past decades. The main features provided by vibrational spectroscopy combined with chemometrics are as follows: (1) the possibility of direct determination of an analyte without the need for a previous separation; (2) the simultaneous determination of multiple components in the same sample from a unique spectral measurement; (3) the capacity to evaluate directly several sample properties (*e.g.* physicochemical) or characteristics that do not correspond to a particular analyte or group of analytes but clearly relate to all the components, thus providing information about sample quality, *e.g.* tannins, related to long-term colour stability and astringency of wines,[71] or pH of albumen and Haugh units for testing egg quality;[72] and (4) the indirect modelling of spectra for the determination of analytes at trace levels. In this sense, the concentrations of many compounds present in the same sample can be modelled from the overlapping bands of the spectra; although there is no direct relationship between the presence of trace compounds and specific bands, vibrational modes assigned to different organic molecules, which can be modified by the presence of trace components, offer the possibility of establishing highly predictive models from a series of spectra of well-characterized samples. These indirect determinations are of special interest in the analysis of mineral elements because they offer the possibility of developing quantitative or at least screening methods suitable for obtaining information on

trace components from the NIR, MIR or Raman spectra of untreated samples, as an alternative to classical methodologies based on wet or dry-ash digestion and dissolution of samples before determination by atomic or ionic techniques. These solvent-free analytical methodologies, based on direct measurements of untreated samples, combined with chemometric techniques, offer a green alternative for studies in environmental and control analysis and for screening purposes in general. Some examples of recently developed applications are the NIR determination of mineral elements in soils,[73] sediments,[74] foods,[75] beverages[76] and forage crops.[77]

To conclude, vibrational spectroscopic techniques appear to be excellent green analytical tools because they are capable of providing high-quality spectral information on samples in a non-destructive and, in many cases, non-invasive way, avoiding the use of reagents or solvents. Their use can be extended to other exciting fields, such as clinical[78] and microbiological[79] analysis. Additionally, the development of hyperspectral imaging as a technology to integrate conventional imaging and spectroscopy, to obtain spatial and spectral information about samples, contributes to the development of new green analytical applications, especially for QC and process analytical technologies.[80,81]

2.6 Image Processing Methods

In recent decades, machine vision systems have emerged as efficient, powerful and non-invasive techniques for the determination of the global parameters,[82] particular properties[83] or composition of different types of samples.[84] The machine vision systems provide the automatic extraction of information from digital images acquired by cameras or scanners for inspection or control of processes during manufacturing or product processing. Based on this philosophy, the use of simple digital or smartphone cameras has been extended as a green approach for the direct analysis of samples without requiring sample preparation. On the other hand, the use of hyperspectral cameras, combining spectroscopy and imaging, integrates the main advantages of both techniques such as spectral and multicomponent information from a scanned sample, being sensitive in some cases to the presence of minor components.

Image treatment could be the greenest approach for analytical methods if we are able to obtain qualitative and quantitative information from images of the objects. As indicated in Figure 2.6, image treatment approaches could be based on pictures of drop reactions made in separate wells or images of surface layer chromatography, which in fact provides a complementary tool for classical or separation analytical processes. However, direct images of samples are suitable to be employed to quantify some parameters, based on a correct calibration made using well-characterized objects. Hence, as indicated in Figure 2.7, many aspects must be considered when taking and processing images. The objects (totally or partially), the illumination light, the focal distance and the use or not of a colour or black and white reference

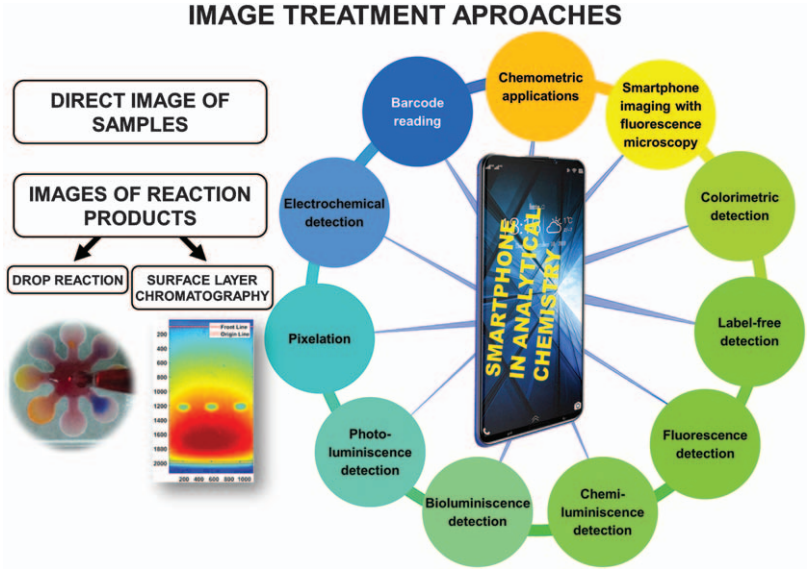


Figure 2.6 Possibilities offered by image treatment for analytical purposes.

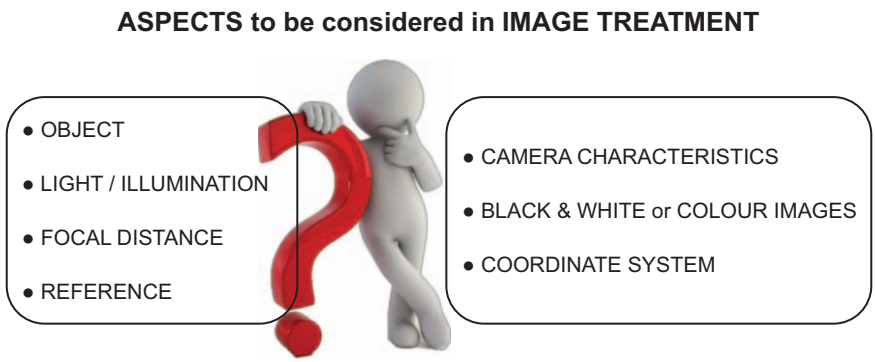


Figure 2.7 Main aspects to be considered in image treatment.

are also key aspects to be taken into account. Hence it could be summarized that nowadays the type of camera employed to obtain the images is very important and efforts must be made to transfer the calibrations obtained with a specific system to other systems, especially taking into account the rapid evolution in this field.

In recent years, a controversial aspect has been the decision to work with colour or black and white images. It is true that most published studies used colour images and their translation in RGB, HSV or CIElab systems. However, professional photographers maintain the idea that high-quality images must be based on black and white and grey-scale photographs and, in fact, many of the images for healthcare use are systematically based on black and

white. Additionally, the consideration of sample image pixels in this system provides a large number of variables and thus could contribute to improving the analytical capability of image treatment. Hence it could be expected that in the near future we could systematize correctly the best ways to extract as much information as possible from samples through their images.

Hyperspectral imaging (HSI) appears to be a powerful technique that provides information about chemical species present in a sample, their concentration and where they are distributed.[85] This technique has been employed to determine the quality, safety, contamination, authentication and adulteration of different samples types, especially in the food,[86] agricultural,[87] pharmaceutical,[88] medical,[89] environmental[90] and astronomical[91] fields.

An HSI system requires an illumination unit (which can be in the UV, visible, NIR or MIR spectral regions), a radiation dispersive device (usually a spectrometer), a camera or detector and a computer system equipped with image acquisition and processing software.[92] Hyperspectral images are generally obtained in the reflectance mode owing its feasibility; however, other measurement modes such as transmittance or interactance can also be employed. Compared with a common digital camera, which acquires spectral channels corresponding to red, green and blue primary colours, HIS systems provide a complete spectrum for each pixel resolution of images, thus providing a high level of information about sample components and/or properties.

The implementation of HSI involves three steps: (1) image acquisition and image pre-processing, (2) spectral data extraction and data treatment and (3) data modelling and image post-processing. The basic premise is the acquisition of a high-quality hyperspectral image in the selected spectral range. For this purpose, the acquisition mode, device and type of illumination, spatial resolution and the characteristics of the detector employed, such as spectral resolution, scanning velocity and sensitivity, are important. The acquisition process also requires correction of the image in the spatial and spectral domains using suitable references. Extraction of spectral data from the image can be applied to all object images or to different regions of interest. In this step, data could be pre-processed to enhance their quality (*i.e.* noise reduction, improving resolution, *etc.*). These data treatments frequently involve spectral filtering, smoothing, mean centring, normalization, baseline correction, autoscaling or corrections such as orthogonal signal correction, standard normal variate or multiplicative scatter correction. Finally, by using chemometrics and reference sample data, calibration models can be developed to correlate images and sample properties or analyte concentrations. These multivariate techniques for calibration model building include multilinear regression (MLR), partial least-squares (PLS) regression, principal component analysis (PCA), discriminant analysis (DA), artificial neural networks (ANNs) and support vector machine (SVM). More details of these data treatment and calibration process are discussed in Chapter 10.

It is clear than the development of analytical methods based on the use of hyperspectral images involves a previous chemometric modelling, and for this purpose a set of calibration samples must be analysed by rearguard methods. The prediction capability of the calibration model must be verified periodically to ensure the quality of the method. On the other hand, it must be noted that the prediction capability of the developed models cannot be higher than the quality of the reference data used to create these calibrations, so it is mandatory to have adequate reference data.

Usually, the applicability of the strategies based on HSI depends strongly on the cost of the instrumentation, the use of hyperspectral cameras nowadays being expensive. For this reason, the development of image processing methods is currently focused on the use of more easily accessible instruments such as smartphones and digital cameras.

In contrast, concerning low-cost and readily available instrumentation, the smartphone has been demonstrated to be an excellent tool for image acquisition and application in analytical chemistry. In this sense, different applications have been developed for the determination of parameters[93] or analytes[94] from photographs obtained directly from samples. New advances integrating smartphones with spectrometers are providing low-cost instrumentation with a high capability for data acquisition and processing, combining the portability of smartphone devices and the enhanced selectivity provided by the spectrometer.[95] These improvements permitted the development of a smartphone-based multispectral imager and its potential use for point-of-care testing,[96] contributing to the democratization of analytical chemistry.

2.7 Remote Sensing and Teledetection Systems

Remote sensing systems together with digital image analysis provide methods for the acquisition of data and the easy interpretation of measurements of an object without any physical contact between the measuring device and the object itself, thus enhancing the information available without any damage to the sample or the need to use preliminary analytical steps or reagents.

Remote sensing is the science of acquiring, processing and interpreting images and related data, obtained from aircraft and satellites, that record the interaction between matter and electromagnetic energy.[97] The term takes on a number of different meanings depending on the discipline involved.

Traditionally, remote sensing referred to measurements made, from a distance, of the radiation spectra reflected and emitted from the Earth's surface to acquire information without being in physical contact with the object (which in this case is the atmosphere).

In the last decade, the use of satellite remote sensing of air quality has evolved dramatically; now, thanks to the increasing spatial resolution afforded by modern instrumentation,[98] global observations are available for a

wide range of species, including aerosols, tropospheric O_3, tropospheric NO_2, CO, HCHO and SO_2. The role of remote sensing is therefore under scrutiny, given its potential capacity for systematic observations at scales ranging from local to global and the availability of data archives extending back over several decades.

Three major applications of retrieved trace gases and aerosols by satellite remote sensing are available: forecasting of events that affect air quality, interference of surface air quality itself (particulate matter, NO_2, O_3 and CO) and estimates of surface emissions [NO_x, volatile organic compounds (VOCs), CO and aerosol sources].

The availability of remote sensing technology also contributed to the decision of the Kyoto Protocol of the United Nations Framework Convention on Climate Change (UNFCCC) to limit or reduce greenhouse gas emissions to 1990 levels. Five major areas have been suggested where remote sensing technology could be applied to support the implementation of the Kyoto Protocol:[99] (1) provision of systematic observations of relevant land cover (in accordance with Articles 5 and 10); (2) support to the establishment of a 1990 carbon stock baseline (Article 3); (3) detection and spatial quantification of changes in land cover (regarding Articles 3 and 12); (4) quantification of above-ground vegetation biomass stocks and associated changes therein (also Articles 3 and 12); and (5) mapping and monitoring of certain sources of anthropogenic CH_4 (in accordance with Articles 3, 5 and 10).

The first application of satellite remote sensing of aerosols was based on the use of an advanced very high-resolution radiometer (AVHRR) to observe Sahara dust particles over the ocean[100] and later for monitoring volcanic sulfate.[101] The total ozone mapping spectrometer (TOMS) was the first instrument designed for satellite remote sensing of tropospheric trace gases. Initially it was aimed at determining the global knowledge of stratospheric O_3, but it also yields information about volcanic SO_2[102] and ultraviolet-absorbing aerosols.[103] These instruments have been very effective. The last TOMS was deactivated in 2007, but a new generation of ozone monitoring instruments (OMIs) has satisfactorily replaced them.

Satellite remote sensing is not limited to surface air quality. It can also be used for ecological applications including land cover classification, integrated ecosystem measurements and change detection, such as climate change or habitat loss.[104] This is a very important tool for ecologists and conservation biologists, as it offers new ways to approach their research in order to provide scientific responses to environmental changes. Additionally, remote sensing has been used for environmental and natural resource mapping and for data acquisition about hydrological sources and soil water and drought monitoring for early warning applications.[105] The use of remote sensing permits the monitoring of soil salinity caused by natural or human-induced processes,[106] the study of groundwater[107] and the quantitative study of soil properties.[108]

Developments in optical remote sensing related to spatial resolution provide powerful tools in precision agriculture, which has the ability to

evaluate rapidly the maturation of fruits or cultivars for optimal harvesting or possible infection with diseases.[109] It also permits the assessment of water use by crops and on-farm productivity monitoring – the latter though measuring methane emissions – thus making it possible to increase the efficiency of water use.[110] Specific properties of vegetation, *e.g.* healthy or diseased, can be related to the amount and quality of radiation reflected or emitted from the leaves and canopies of plants. Remote sensing can therefore be applied to study plant pathology.[111,112]

From the instrumentation point of view, remote chemical sensing is a group of techniques. Basically, we can be distinguish between remote electrochemical sensors and remote spectroscopic monitoring systems.[113] In addition, geoelectric techniques, such as DC-resistivity sounding, magnetotellurics, ground-penetrating radar, fixed-frequency electromagnetic (FEM) and transient electromagnetic (TEM) methods, have been used for the remote monitoring of groundwater pollution and for the estimation of the hydraulic properties of aquifers and sediments.[114]

Chemical and biochemical sensors are based on a combination of a recognition layer and a physical transducer and their use has been proposed for the *in situ* remote monitoring of organic and inorganic pollutants (see Figure 2.8).[115] The introduction of modified electrodes and ultramicroelectrodes, the design of complex biological and chemical recognition layers, molecular devices and sensor arrays and developments in micro- and nanofabrication and also in the technology of flow detectors and compact, low-powered and user-friendly instruments, have contributed to the development of electrochemical sensor devices for the real-time monitoring of a wide range of molecules and contaminants.

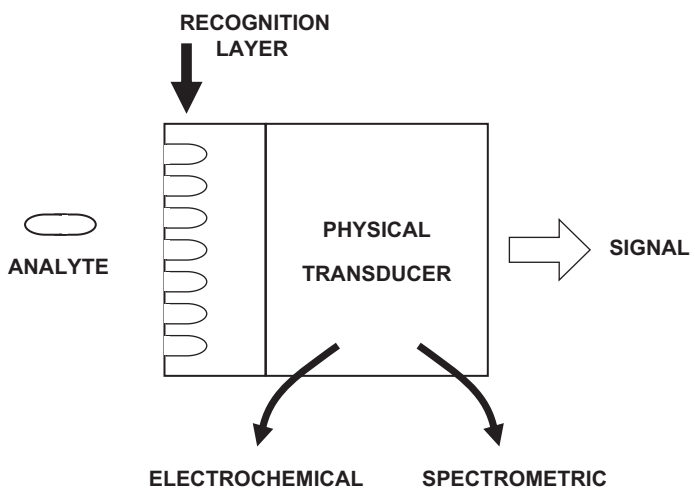


Figure 2.8 Schematic representation of a chemical sensor.
Reproduced from ref. 119 with permission from the Royal Society of Chemistry.

On the other hand, two approaches can be distinguished for spectroscopic remote sensing: (1) direct, when both the electromagnetic radiation and the signal measured are used along an open path (*i.e.* the atmosphere); and (2) indirect, where radiation or a signal is directed through fibre optics (see the scheme in Figure 2.9). Direct remote systems can be further classified as active, if the instrument contains its own source of radiation, or passive, for external emission sources, such as the Sun. Active systems based on infrared or ultraviolet radiation sources are currently used and can utilize Raman scattering, fluorescence or light absorption as their measuring principle.[116] However, there are other classification criteria for chemical sensor discrimination (see Figure 2.10) and, with reference to the position of the radiation source (transmitter) and the sensor (receiver), optical remote sensing instruments can be classified as (1) monostatic, when the transmitter and the receiver are located in a single fixed position and either a topographic target (building wall, ground, vegetation) or atmospheric aerosols and molecules or a retroreflector may be used to reflect the transmitted radiation back to the receiver, or (2) bistatic, in which the radiation source is in one location and the sensor in another, the distance between them being the open optical pathlength.

Remote active sensing systems can be grouped into monochromatic instruments and instruments with a broadband source of radiation, subdivided into non-dispersive and dispersive. Monochromatic instruments are equipped with laser sources that provide spectral lines at microwave, infrared or ultraviolet frequencies, allowing the identification and measurement of air pollutants. Non-dispersive analysers have been designed basically for specific constituents of gases. Dispersive instruments make it possible to obtain detailed information about the spectra of molecules and species present in a sample, but their sensitivity can be limited in comparison with that of monochromatic sources.

One technique used for direct optical remote sensing using monochromatic radiation is the differential adsorption laser (DAL), in which two laser beams of different wavelengths are passed through the sample, one being coincident with the absorption maximum of the target analyte and the other being a non-absorbing wavelength. The difference between the two beams is proportional to the amount of absorbing molecules. Laser photoacoustic spectrometry (PAS) and light detection and ranging (LIDAR), which use a pulsed laser system, provide systems like radar where the time required to return the reflected radiation is measured and used to determine the distance of the reflecting material. The principles of differential optical absorption spectroscopy (DOAS) and simultaneous correlation spectroscopy (COSPEC) can be employed for optical absorption measurements of gaseous constituents in the atmosphere in the ultraviolet and near-infrared ranges, using the radiance of the sky as a distributed light source.[117]

For indirect optical remote sensing, the development of fibre optics has been revolutionary. New materials, increased flexibility of fibres, long-range transmission capability, small size, broad bandwidth and imaging capability

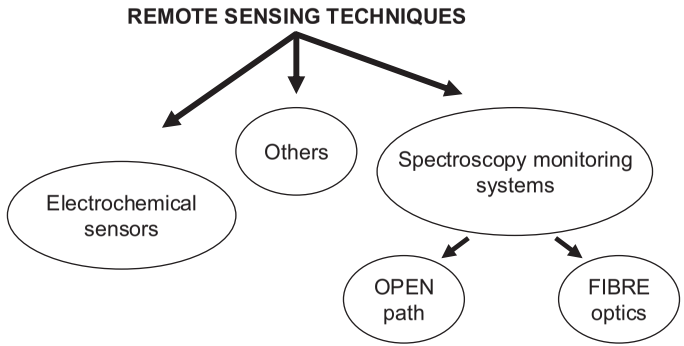


Figure 2.9 Classification of remote sensing techniques.
Reproduced from ref. 119 with permission from the Royal Society of Chemistry.

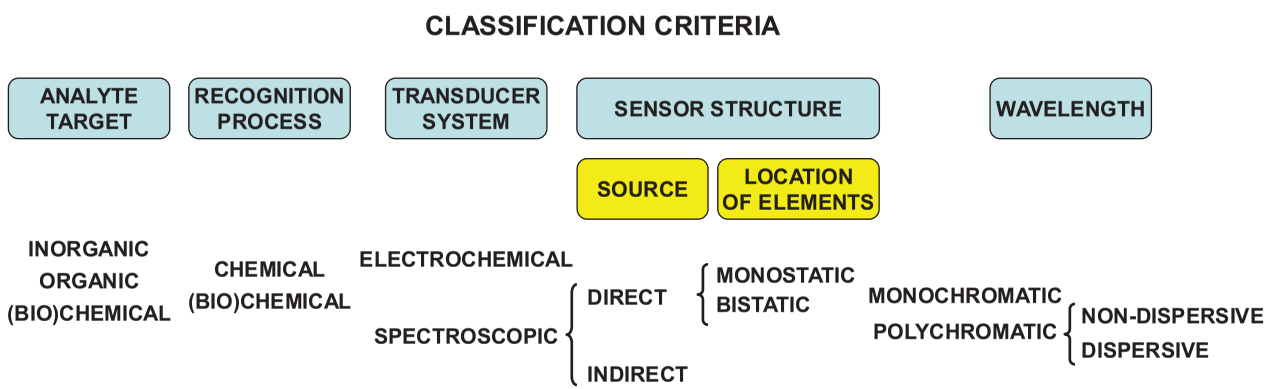


Figure 2.10 Different criteria to classify chemical sensors.
Reproduced from ref. 119 with permission from the Royal Society of Chemistry.

have made possible a variety of design options. These advances have provided fibre-optic devices that can be used over long distances or as non-invasive techniques for clinical or medical applications. Fibre-optic sensors in combination with laser-induced plasma spectroscopy can be employed for the determination of elemental sample composition, laser-induced fluorescence spectroscopy provides information about native fluorophores or fluorescently labelled molecules and Raman spectroscopy is useful for obtaining inorganic and organic vibrational structure information. Laser photofragmentation, which measures the luminescence from sample fragments, photothermal spectroscopy, which provides inorganic and organic electronic and vibrational structure data, and ultraviolet, visible and infrared absorption spectroscopy, which are suitable for obtaining data about inorganic and organic electronic and vibrational structure, are some of the tools available for remote sensing measurements that have been described in the literature.[118]

References

1. V. de la Asuncion-Nadal, S. Armenta, S. Garrigues and M. de la Guardia, *Talanta*, 2017, **167**, 344.
2. S. Armenta, S. Garrigues and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2008, **27**, 15.
3. *Direct Analysis in Real Time Mass Spectrometry. Principles and Practices of DART-MS*, ed. Y. Dong, Wiley-VCH, Weinheim, Germany, 2018.
4. Z. D. Zhou, K. Z. Zhou, X. D. Hou and H. Luo, *Appl. Spectrosc. Rev.*, 2005, **40**, 165.
5. R. E. Russo, X. L. Mao, H. C. Liu, J. Gonzalez and S. S. Mao, *Talanta*, 2002, **57**, 425.
6. A. Limbeck, P. Galler, M. Bonta, G. Bauer, W. Nischkauer and F. Vanhaecke, *Anal. Bioanal. Chem.*, 2015, **407**, 6593.
7. V. K. Unnikrishnan, R. Nayak, K. Aithal, V. B. Kartha, C. Santhosh, G. P. Gupta and B. M. Suri, *Anal. Methods*, 2013, **5**, 1294.
8. Z. Zhou, K. Zhou and X. Hou, *Appl. Spectrosc. Rev.*, 2005, **40**, 165.
9. V. B. E. Thomsen, *Modern Spectrochemical Analysis of Metals: An Introduction for Users of Arc-Spark Instrumentation*, ASTM International, Materials Park, OH, 1996.
10. M. Resano, F. Vanhaecke and M. T. C. de Loos-Vollebregt, *J. Anal. At. Spectrom.*, 2008, **23**, 1450.
11. M. G. R. Vale, N. Oleszczuk and W. N. L. dos Santos, *Appl. Spectrosc. Rev.*, 2006, **41**, 377.
12. M. J. Cal-Prieto, M. Felipe-Sotelo, A. Carlosena, J. M. Andrade, P. Lopez-Mahia, S. Muniategui and D. Prada, *Talanta*, 2002, **56**, 1.
13. M. Hornung and V. Krivan, *Spectrochim. Acta, Part B*, 1999, **54**, 1177.
14. M. A. Belarra, M. Resano and J. R. Castillo, *J. Anal. At. Spectrom.*, 1999, **14**, 547.

15. C. S. Nomura, C. S. Silva, A. R. A. Nogueira and P. V. Oliveira, *Spectrochim. Acta, Part B*, 2005, **60**, 673.
16. K. C. Friese and V. Krivan, *Spectrochim. Acta, Part B*, 1998, **53**, 1069.
17. M. A. Belarra, M. Resano, F. Vanhaecke and L. Monees, *TrAC, Trends Anal. Chem.*, 2002, **21**, 828.
18. T. Nelis and J. Pallosi, *Appl. Spectrosc. Rev.*, 2006, **41**, 227.
19. N. Jakubowski, R. Dorka, E. Steers and A. Tempez, *J. Anal. At. Spectrom.*, 2007, **22**, 722.
20. V. Hoffmann, M. Kasik, P. K. Robinson and C. Venzago, *Anal. Bioanal. Chem.*, 2005, **381**, 173.
21. R. Payling, M. Aeberhard and D. Delfosse, *J. Anal. At. Spectrom.*, 2001, **16**, 50.
22. P. Belenguer, M. Ganciu, P. Guillot and T. Nelis, *Spectrochim. Acta, Part B*, 2009, **64**, 623.
23. A. C. Muñiz, J. Pisonero, L. Lobo, C. Gonzalez, N. Bordel, R. Pereiro, A. Tempez, P. Chapon, N. Tuccitto, A. Licciardello and A. Sanz-Medel, *J. Anal. At. Spectrom.*, 2008, **23**, 1239.
24. M. Hohl, A. Kanzari, J. Michler, T. Nelis, K. Fuhrer and M. Gonin, *Surf. Interface Anal.*, 2006, **38**, 292.
25. N. Tuccitto, L. Lobo, A. Tempez, I. Delfanti, S. Canolescu, N. Bordel, P. Chapon, J. Michler and A. Licciardello, *Rap. Commun. Mass Spectrom.*, 2009, **23**, 549.
26. F. J. Andrade, J. T. Shelley, W. C. Wetzel, M. R. Webb, G. Gamez, S. J. Ray and G. M. Hieftje, *Anal. Chem.*, 2008, **80**, 2654.
27. D. Günther, I. Horn and B. Hattendorf, *Fresenius' J. Anal. Chem.*, 2000, **368**, 4.
28. N. S. Mokgalaka and J. L. Gardea-Torresdey, *Appl. Spectrosc. Rev.*, 2006, **41**, 131.
29. A. Kehden, J. Flock, W. Vogel and J. A. C. Broekaert, *Appl. Spectrosc.*, 2001, **55**, 1291.
30. F. C. Alvira, F. R. Rozzi and G. M. Bilmes, *Appl. Spectrosc.*, 2010, **64**, 313.
31. N. N. Gavrilyukov, V. N. Samoplyas and V. V. Mandrygin, *Inorg. Mater.*, 2008, **44**, 1547.
32. R. E. Russo, X. Mao, H. L. Liu, J. Gonzalez and S. S. Mao, *Talanta*, 2002, **57**, 425.
33. R. S. Harman, F. C. DeLucia, C. E. McManus, N. J. McMillan, T. F. Jenkins, M. E. Walsh and A. Miziolek, *Appl. Geochem.*, 2006, **21**, 730.
34. B. Salle, P. Mauchien and S. Maurice, *Spectrochim. Acta, Part B*, 2007, **62**, 739.
35. J. M. Vadillo and J. J. Laserna, *Spectrochim. Acta, Part B*, 2004, **59**, 147.
36. C. Fabre, M. C. Boiron, J. Dubessy, A. Chabiron, B. Charov and T. M. Crespo, *Geochim. Cosmochim. Acta*, 2002, **66**, 1401.
37. J. Moros, J. A. Lorenzo, P. Lucena, L. Miguel Torabia and J. J. Laserna, *Anal. Chem.*, 2010, **82**, 1389.

38. Z. Takats, J. M. Wiseman, B. Gologan and R. G. Cooks, *Science*, 2004, **306**, 471.
39. J. F. García-Reyes, A. U. Jackson, A. Molina-Díaz and R. G. Cooks, *Anal. Chem.*, 2009, **81**, 820.
40. Z. Lin, M. Zhao, S. Zhang, C. Yang and X. Zhang, *Analyst*, 2010, **135**, 1268.
41. J. H. Kennedy and J. M. Wiseman, *Rapid Commun. Mass Spectrom.*, 2010, **24**, 309.
42. M. S. Karmat, R. A. Lodder and P. P. Deluca, *Pharm. Res.*, 1989, **6**, 961.
43. J. Moros, N. Galipienso, R. Vilches, S. Garrigues and M. de la Guardia, *Anal. Chem.*, 2008, **80**, 7257–7265.
44. H. R. H. Ali, H. G. M. Edwards and I. J. Scowen, *Spectrochim. Acta, Part A*, 2009, **72**, 890.
45. J. Workman, M. Koch and D. J. Veltkamp, *Anal. Chem.*, 2003, **75**, 2859.
46. R. De Maesschalck and T. Van den Kerkhof, *J. Pharm. Biomed. Anal.*, 2005, **37**, 109.
47. N. W. Broad, R. D. Jee, A. C. Moffat, M. J. Eaves, W. C. Mann and W. Dziki, *Analyst*, 2000, **125**, 2054.
48. A. Nordon, A. Mills, R. T. Burn, F. M. Cusik and D. Littlejohn, *Anal. Chim. Acta*, 2005, **548**, 148.
49. S. Garrigues and M. de la Guardia, in *Beer in Health and Disease Prevention*, ed. V. R. Preedy, Elsevier/Academic Press, Oxford, 2009, pp. 943–961.
50. M. Kim, H. Chung, Y. Woo and M. Kemper, *Anal. Chim. Acta*, 2007, **587**, 200.
51. H. Schmidt, K. Sowoidnich and H. D. Kronfeldt, *Appl. Spectrosc.*, 2010, **64**, 888.
52. E. Marguí, M. Hidalgo and I. Queralt, *Spectrochim. Acta, Part B*, 2005, **60**, 1363.
53. *Portable X-ray Fluorescence Spectrometers: Capabilities for In Situ Analysis*, ed. P. J. J. Potts and M. West, Royal Society of Chemistry, Cambridge, 2008.
54. I. W. Burton, M. A. Quilliam and J. A. Walter, *Anal. Chem.*, 2005, **77**, 3123.
55. U. Holzgrabe, R. Deubner, C. Schollmayer and B. Waibel, *J. Pharm. Biomed. Anal.*, 2005, **38**, 806.
56. L. I. Nord, P. Vaag and J. O. Duus, *Anal. Chem.*, 2004, **76**, 4790.
57. E. Veliyulin, C. van der Zwaag, W. Burk and U. Erikson, *J. Sci. Food Agric.*, 2005, **85**, 1299.
58. E. Del Federico, S. A. Centeno, C. Kehlet, P. Currier, D. Stockman and A. Jerschow, *Anal. Bioanal. Chem.*, 2010, **396**, 213.
59. J. Moros, S. Garrigues and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2010, **29**, 578.
60. J. Fitzpatrick and J. A. Reffner, in *Handbook of Vibrational Spectroscopy*, ed. J. M. Chalmers and P. R. Griffths, John Wiley & Sons, Chichester, 2002.

61. L. G. Thygesen, M. M. Lokke, E. Micklander and S. B. Engelsen, *Trends Food Sci. Technol.*, 2003, **14**, 50.
62. *Applications of Vibrational Spectroscopy in Food Science*, ed. E. C. Y. Li-Chan, P. R. Griffth and J. M. Chalmers, John Wiley & Sons, Chichester, 2010.
63. M. M. Mossoba, V. Milosevic, M. Milosevic, J. K. Kramer and H. Azizian, *Anal. Bioanal. Chem.*, 2007, **389**, 87.
64. N. Viereck, T. Salomonsen, F. Van den Berg and S. B. Engelsen, in *Raman Spectroscopy for Soft Matter Applications*, ed. M. S. Amer, John Wiley & Sons, Chichester, 2009.
65. T. Van Keirsbilck, A. Vercauteren, W. Baeyens, G. Van der Weken, F. Verpoot, G. Vergote and J. P. Remon, *TrAC, Trends Anal. Chem.*, 2002, **21**, 869.
66. M. T. Bona and J. M. Andrés, *Anal. Chim. Acta*, 2008, **624**, 68.
67. T. Woodcock, G. Downey and C. P. O'Donnell, *J. Near Infrared Spectrosc.*, 2008, **16**, 1.
68. Y. Roggo, P. Chalus, L. Maurer, C. Lema-Martínez, A. Edmond and N. Jent, *J. Pharm. Biomed. Anal.*, 2007, **44**, 683.
69. H. Chung, *Appl. Spectrosc. Rev.*, 2007, **42**, 251.
70. P. Vandenabeele, K. Castro, H. Hargreaves, L. Moens, J. M. Madariaga and H. G. M. Edward, *Anal. Chim. Acta*, 2007, **588**, 108.
71. K. Fernández and E. Agosín, *J. Agric. Food Chem.*, 2007, **55**, 7294.
72. N. Abded-Nour, M. Ngadi, S. Prasher and Y. Karimi, *Int. J. Poult. Sci.*, 2009, **8**, 170.
73. G. Siebelec, G. W. McCarty, T. I. Stuczynski and J. B. Reeves III, *J. Environ. Qual.*, 2004, **33**, 2056.
74. J. Moros, M. C. Barciela-Alonso, P. Pazos-Capeans, P. Bermejo-Barrera, E. Peña-Vázquez, S. Garrigues and M. de la Guardia, *Anal. Chim. Acta*, 2008, **624**, 113.
75. D. Cozzolino and A. Morón, *Anim. Feed Sci. Technol.*, 2004, **111**, 161.
76. D. Cozzolino, M. J. Kwiatkwski, R. G. Daunbergs, W. U. Cynkar, L. J. Janik, G. Skouroumounis and M. Gishen, *Talanta*, 2008, **74**, 711.
77. J. L. Halgerso, C. C. Sheaffer, N. P. Martin, P. R. Peterson and S. J. Weston, *Agron. J.*, 2004, **96**, 344.
78. G. Hosafci, O. Klein and G. Oremek, *Anal. Bioanal. Chem.*, 2007, **387**, 1815–1822.
79. M. Harz, P. Rösch and J. Popp, *Cytometry, Part A*, 2009, **75**, 104.
80. C. P. O'Donnell, P. J. Cullen, G. Downell and J. M. Frias, *Trends Food Sci. Technol.*, 2007, **18**, 590.
81. *Hyperspectral Imaging for Food Quality and Control*, ed. D. W. Sun, Academic Press, New York, 2010.
82. M. Dowlati, S. S. Mohtasebi, M. Omid, S. H. Razavi, M. Jamzad and M. de la Guardia, *J. Food Eng.*, 2013, **119**, 277.
83. A. Sanaeifar, A. Bakhshipour and M. de la Guardia, *Talanta*, 2016, **148**, 54.

84. W. H. Zhou, J. J. Zhang, M. M. Zou, X. Q. Liu, X. L. Du, Q. Wang, Y. Y. Liu, Y. Liu and J. L. Li, *Environ. Sci. Pollut. Res.*, 2019, **26**, 1848.
85. A. Signoroni, M. Savardi, A. Boronio and S. Benini, *J. Imaging*, 2019, **5**, 52.
86. J. Ma, D. W. Sun, H. Pu, J. H. Cheng and Q. Wei, *Annu. Rev. Food Sci. Technol.*, 2019, **10**, 197.
87. T. B. Hank, K. Berger, H. Bach, J. G. P. W. Clevers, A. Gitelson, P. Zarco-Tejada and W. Mauser, *Surv. Geophys.*, 2019, **40**, 515.
88. S. J. Mazivila and A. C. Olivieri, *TrAC, Trends Anal. Chem.*, 2018, **108**, 74.
89. B. Jansen-Winkeln, M. Maktabi, J. P. Takoh, S. M. Rabe, M. Barberio, H. Kohler, T. Neumuth, A. Melzer, C. Chalopin and I. Gockel, *Chirurg*, 2018, **89**, 717.
90. M. B. Stuart, A. J. S. McGonigle and J. R. Willmott, *Sensors*, 2019, **19**, 3071.
91. M. Fickus, M. E. Lewis, D. G. Mixon and J. Peterson, *IEEE Signal Process. Lett.*, 2015, **22**, 1829.
92. G. M. ElMasry and S. Nakauchi, *Biosyst. Eng.*, 2016, **142**, 53.
93. M. Cruz-Fernandez, M. J. Luque-Cobija, M. L. Cervera, A. Morales-Rubio and M. de la Guardia, *Microchem. J.*, 2017, **132**, 8.
94. J. P. G. Rigon, S. Capuani, D. M. Fernandes and T. M. Guimaraes, *Photosynthetica*, 2016, **54**, 559.
95. A. J. S. McGonigle, T. C. Wilkes, T. D. Pering, J. R. Willmott, J. M. Cook, F. M. Mims and A. V. Parisi, *Sensors*, 2018, **18**, 223.
96. H. Ding, C. Chen, H. C. Zhao, Y. Yue and C. Y. Han, *Analyst*, 2019, **144**, 4380.
97. F. F. Sabins, *Remote Sensing. Principles and Interpretation*, 3rd edn, W. H. Freeman, New York, 1997.
98. R. V. Martin, *Atmos. Environ.*, 2008, **42**, 7823.
99. A. Rosenqvist, A. Milne, R. Lucas, M. Imhoff and C. Dobson, *Environ. Sci. Policy*, 2003, **6**, 441.
100. R. S. Fraser, *Appl. Opt.*, 1976, **15**, 2471.
101. L. L. Stowe, A. M. Ignator and R. R. Singh, *Geophys. Res. Lett.*, 1992, **19**, 159.
102. A. J. Krueger, *Science*, 1983, **220**, 1377.
103. J. R. Herman, P. K. Bhartia, O. Torres, C. Hsu, C. Seftor and C. Celarier, *J. Geophys. Res.*, 1997, **102**, 16911.
104. J. T. Kerr and M. Ostrovsky, *Trends Ecol. Evol.*, 2003, **18**, 299.
105. A. M. Melesse, Q. Weng, P. S. Thenkabail and G. B. Senay, *Sensors*, 2007, **7**, 3209.
106. G. I. Metternicht and J. A. Zinck, *Remote Sens. Environ.*, 2003, **85**, 1.
107. M. W. Becker, *Ground Water*, 2006, **44**, 306.
108. E. Ben-Dor, *Adv. Agron.*, 2002, **75**, 173.
109. A. Hall, D. W. Lamb, B. Holzapfel and J. Louis, *Aust. J. Grape Wine Res.*, 2002, **8**, 36.
110. T. G. van Niel and T. R. McVicar, *Aust. J. Agric. Res.*, 2003, **85**, 1.

111. A. K. Mahlein, E. C. Oerke, U. Steiner and H. W. Dehne, *Eur. J. Plant Pathol.*, 2012, **133**, 197.
112. R. H. J. Heim, A. J. Carnegie and P. J. Zarco-Tejada, *Trop. Plant Pathol.*, 2019, **44**, 398.
113. J. Namieśnik, *Crit. Rev. Anal. Chem.*, 2000, **30**, 221.
114. T. Mills, P. Moekstra, M. Blohm and L. Evans, *Ground Water*, 1988, **26**, 771.
115. M. Calcerrada, C. Garcia-Ruiz and M. Gonzalez-Herraez, *Laser Photonics Rev.*, 2015, **9**, 604.
116. D. W. Lamb, D. A. Schneider and J. N. Stanley, *Precis. Agric.*, 2014, **15**, 523.
117. J. Barrancos, J. I. Rosello, D. Calvo, E. Padron, G. Melian, P. A. Hernandez, N. M. Perez, M. Millan and B. Galle, *Pure Appl. Geophys.*, 2008, **165**, 115.
118. V. Panne, *TrAC, Trends Anal. Chem.*, 1998, **17**, 491.
119. *Challenges in Green Analytical Chemistry*, ed. M. de la Guardia and S. Garrigues, RSC Publishing, 2011.

CHAPTER 3

Sensors as Green Tools

MANEL DEL VALLE

Sensors and Biosensors Group, Department of Chemistry, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
Email: manel.delvalle@uab.es

3.1 Chemical Analysis Performed with Sensors

This chapter is intended to discuss the implications of chemical analysis performed with chemical sensors or biosensors with respect to sustainability or ecological aspects, which has been referred to as green chemistry. While analytical chemistry searches for information on material systems, the greening of this information search involves reducing the amount of sample, reducing the number and amounts of reagents and performing analyses in shorter times, with less effort, with less trained people and in less well equipped laboratories; further, it may involve performing the analysis on-site, *i.e.* outside the laboratory.[1] Among the different strategies considered for improving analytical methods, many have involved automation, miniaturization and direct and multianalyte analysis; in almost all cases reductions in the amounts of solvents, reagents and waste or the replacement of reagents with less harmful alternatives have always been kept in mind.[2] Obviously, the use of sensors is also one of these strategies, which suits perfectly well the above requirements.

Kaljurand and Koel reported on various aspects of instrumental analysis in relation to green chemistry,[3] considering a variety of analytical instruments ranging from heavy laboratory equipment in use in chemical laboratories to mobile sensors, performing measurements in real time. These analyses are performed with diverse instrumental techniques and they can

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

be presented in the form of a multilevel pyramid according to their complexity and analytical requirements, as illustrated in Figure 3.1.[4] The final thought is that there is a great need for simple indicator-type sensors with sufficient specificity and accuracy, which will not require trained personnel, generate little or no waste and consume minimal amounts of energy or none at all. This level forms the base of the pyramid and these sensors are used to obtain chemical information from the point-of-care, including on-site process analysis for the widest use.

From the above simplification, analytical instrumentation is the most expensive and energy- and material-intensive part of analytical chemistry and is also the most wasteful from a green chemistry perspective, especially when the footprint from the production of instruments is considered; therefore, the use of sensors is intended to provide a significant reduction in all these aspects, especially if they have been microfabricated, which promotes their implementation whenever possible. What kind of information is actually required? Sometimes a qualitative or semiquantitative result may be sufficient and a simple naked-eye visual test may be adequate. It should be remembered that analytical chemistry, as an information science, should be re-conceptualized with the aim of not utilizing more resources than are absolutely necessary in order to obtain information with the required quality level.

Let us define with precision what we imply when we talk about chemical sensors. A chemical sensor is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal.[5] This definition, intendedly too generic, deserves a clearer, pragmatic description, and a

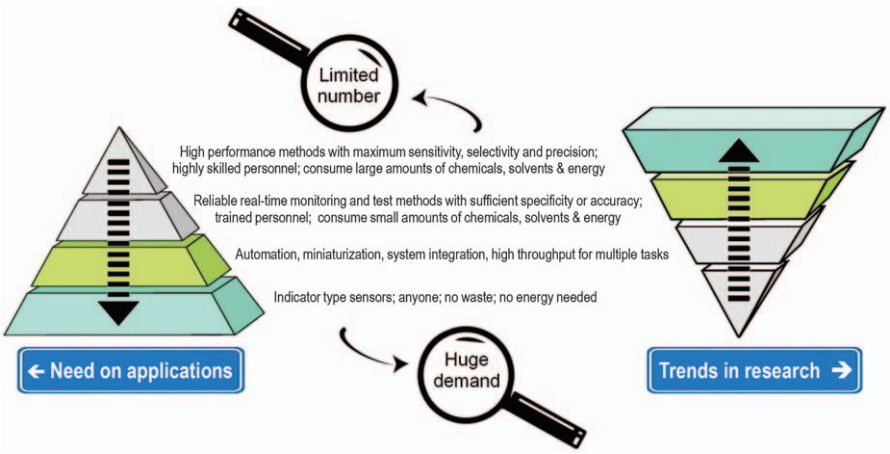


Figure 3.1 Requirements of instrumental methods according to application needs and present research trends and the situation for chemical sensors. Reproduced from ref. 4 with permission from the Royal Society of Chemistry.

useful one, for example, is that given by Wolfbeis, when he wrote: *Chemical sensors are small-sized devices comprising a recognition element, a transduction element and a signal processor capable of continuously and reversibly reporting a chemical concentration*,[6] a definition that derives in the generic scheme of a sensor, illustrated in Figure 3.2.

This definition suggests the interaction between the recognition element and the analyte to generate a chemical signal, and introduces the ideas of reduced dimensions and the possibility of real-time measurement. Chemical and biochemical sensors are thus based on the combination of a recognition layer and a physical transducer and their use is perfectly suited for the *in situ* remote monitoring of organic and inorganic pollutants. The definition of a sensor involves intrinsically a cheap, small and easy to use device, which means that its portability is of paramount importance; this, accompanied by minimal reagent and solvent use, also implies minimal waste production. To achieve these advantages, an underlying characteristic of the sensor is its integration, in the sense of joining different stages of the analytical process into one; this implies simplicity of operation, and the integration of different parts to form the device, which in turn involves aspects of microfabrication, immobilization of reagents or, more recently, the introduction of nanotechnology principles. On the other hand, operation of a sensor should be of high efficiency, normally thanks to the performance accomplished by the recognition element ant the selectivity features that it provides. The efficiency is further connected with the speed of operation, its disposable use, its achievement *in situ* and with the provision of results in real time. Even more, now that we are in the era of networks, sensor devices can be deployed

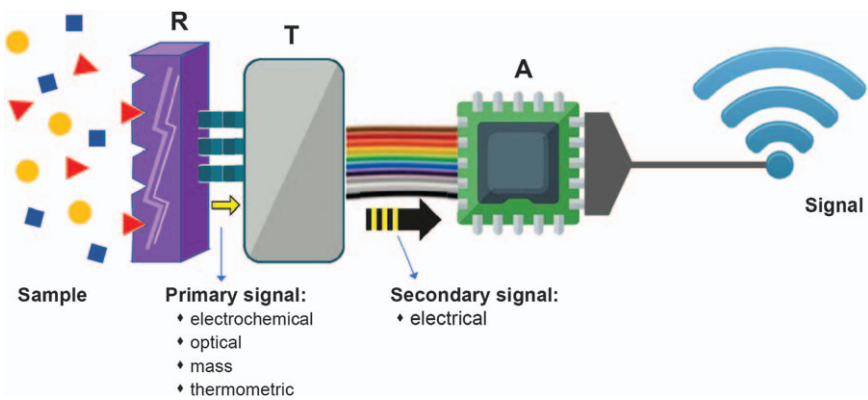


Figure 3.2 Sketch of the operation of a chemical sensor. Only one sample component, the analyte, is recognized by the recognition element (R). The primary signal generated in the recognition process is converted into an electrical signal by the transducer (T). This signal is next amplified, conditioned, processed and presented as measured data (A). When the recognition element is of a biological nature, the device is termed a biosensor.

in a distant region and, through communication links, provide temporal or spatial mapping of analytes.

A further attribute of a sensor, its reversibility, is considered important by many authors. Reversibility means that the sensor signals should not be stationary but should respond dynamically to changes in sample concentration in the course of measurement. The following characteristics of chemical sensors are generally accepted: chemical sensors should (1) transform chemical quantities into electrical signals, (2) respond rapidly, (3) maintain their activity over a long period of time and (4) be specific, *i.e.* respond exclusively to one analyte or at least to a particular group of analytes. This list could be extended with, *e.g.*, the requirement for a *low detection limit* or *high sensitivity*. This means that it would be desirable to detect low concentrations of the species sought.

Sensors can be classified, according to IUPAC recommendations, following the principles of *signal transduction*,[5] that is, according to the type of primary signal appearing after interaction of the analyte with the recognition element, as shown in Table 3.1, where the most important groups in practice are the electrochemical and optical types.

The concept of a biosensor, closely involved in the above, should be properly clarified. The biosensor concept corresponds to the definition of the responsible IUPAC Commission:[5] *biosensors are chemical sensors in which the recognition system utilizes a biochemical mechanism*; this biological mechanism is normally provided by a biological element, which can be integrated with the transducer in many ways, such as tissue (a thin slice of banana), individual cells (neuron cells) or a molecular assembly (enzyme reaction complex). The implications of green aspects for the biological element are interesting. If microbial species are the recognition elements to be used, for example, in a microbial fuel cell device, minimal impact is evident. Enzymes can be of complex use, especially when considering that they may need to be extracted, isolated and purified from special organisms,

Table 3.1 Classification of (bio)sensors according to the nature of the primary signal generated after interaction with the analyte.

Type	Example	Preferred sample type
Electric	Metal-oxide semiconductor	Gas
Electrochemical	Potentiometric (ISE, ISFET) Voltammetric	Liquid (Gas)
Optical	Absorbance Reflectance Fluorescence	Liquid Gas Solid
Mass	Piezoelectric	Gas (Liquid)
Other	Magnetic, thermometric	—

although there are cases where the great demand and knowhow permit the use of genetically engineered material. The use of antibodies is similar, when the procedure for their isolation involves infection and/or sacrifice of an animal; the impact, in this respect, can be highly significant, but again, in cases where the antibody is well known and with a high demand, the genetically engineered option can be the choice. In any case, the antibody will be the element with maximum attainable specificity, which may compensate for the disadvantages. Concerning DNA oligonucleotides, used as probes to devise genosensors or used as aptamers, they can be obtained by convenient solid-phase automated synthesis systems, which will imply minimal use of reagents, minimal waste and minimal effort. Finally, the potential use of molecularly imprinted polymers may represent the best compromise of requirements,[7] as highly selective material will be tailor-synthesized in order to obtain an extremely cheap recognition material with high performance.

From the generic motto in green chemistry ''Reduce, reutilize and recycle'',[4] it is clear that chemical sensors and biosensors are perfect prime examples for satisfying these requirements. Sensors obviously reduce the amounts of materials and resources used and also minimize the requirements on sample size or time of analysis. Therefore, mainly thanks to their small dimensions and to the simple instrumentation involved, it can be said they produce a minimum impact on the environment. Also, the simpler the method, the less intervention is required, a situation that is frequent with the simplest methods but not attainable with the more complex biosensing schemes (*e.g.* in immunoassays), where perhaps a number of stages may be required to complete the assay. The maximum simplicity in use can be achieved with software-intensive sensor systems, a situation where practically no consumables and no manual intervention will be needed. Concerning reuse, most chemical sensors can be used a repeated number of times, making possible the monitoring of processes or the environment. In this respect, biosensors can present more limited features, normally because they have a more sophisticated recognition mechanism. On the one hand, the recognition elements may have a limited duration (*e.g.* enzymes) or the recognition process can be irreversible, as usually happens with antibodies. This being the case, devices may be made disposable, which is a reasonable condition, especially in clinical diagnosis situations. With respect to recycling, the high level of integration that occurs with (bio)sensors, together with the small amounts of materials used, poses important difficulties for this goal. Evidently, there might be specific circumstances where recycling of certain materials, such as noble metal nanoparticles, lanthanide elements, *etc.*, may still be a matter of interest.

A widely known list of recommendations is the ''Twelve Principles of Green Chemistry'';[8] this list provide a framework for scientists and engineers to use when designing new materials, products, processes and systems. Many, but not all, of these principles apply to green analytical chemistry. Those that are most relevant to, or most commonly encountered in

analytical chemistry can be summarized as reducing waste, eliminating the use of solvents or using safer ones, looking for the highest efficiency, applying mild conditions of pressure and temperature in the processes, reducing derivatizations and intermediates, application to real-time analysis for efficiency and reduced pollution issues. There is great concern regarding the consumption of hazardous solvents in analytical chemistry, mostly in situations associated with chromatography. In the present case, sensor-related methodologies mostly do not require the use of solvents, except perhaps small amounts in certain preparative steps of the device (*e.g.* preparation of membranes or immobilization of reagents).

Principle 11 in the above-mentioned list specifically mentions the requirement for analytical methodologies to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances. Sensor applications play an important role, specifically in the demands for monitoring of processes or the environment. In the first instance, this has led to the field of process analytical technology (PAT), an interesting success case that emerged from collaborative efforts of plant engineers and sensor chemists. Also, whatever case example is selected, important benefits on efficiency, safety, use of resources and generation of waste can be quickly found. An important area of research towards the wider implementation of Principle 11 lies in the further development of sensors, such as electrochemical sensing (conductimetric, potentiometric or voltammetric), that offer qualitative or quantitative measurements of isolated species or analytes within complex matrices. Electrochemical sensing offers particular versatility since it relies on the possibility of detecting any species with electroactive (redox) properties. Optical (colorimetric and fluorimetric) sensors for a specific compound or broader applications have also seen a recent surge in their development, including research towards greener syntheses. Principle 11 has also been extended beyond process chemistry to encompass the environmental impacts of analytical methods more generally.

In summary, this chapter will highlight recent advances aimed at developing sensor systems following the principles of green analytical chemistry, especially those that can be made small, easy to use and connected to a ''smart'' concept. A number of paradigmatic examples, stressing the sustainability features that sensors can bring to chemical analysis, have been selected and are presented in detail. These are the use of nanoparticles obtained with green methodologies to be integrated in sensors, the development of systems based on colour changes that are measured with a smartphone camera, biosensing schemes that can be developed with use of the ubiquitous personal glucose monitor, the biofuel cell used as a sensor and finally the use of arrays of sensors plus computer data treatment, which have been named electronic noses and electronic tongues. These can be the features able to tackle the problems associated with the application of sensors in complex matrices or when the latter may be changing in nature.

3.2 Use of Nanoparticles for Sensing

Curiously, one surprise found when first doing database literature searches with the keywords ''green'' and ''sensor'' was the abundance of late entries related to the synthesis and/or use of nanoparticles obtained by methodologies claimed to be related to green chemistry technologies. These normally referred to the use of mild reagents or the use of precursors obtained from natural sources, able to produce the desired nanoparticle or nanocomponent later used in a sensing scheme. This fact, together with the potential interest of dedicating some attention to the use of nanotechnology components in analytical procedures/sensing, made this author consider adding this separate section.

In this literature search, the numerous articles connected to the green reaction conditions for the production of certain metal nanoparticles could be divided in two very different subgroups. The first was centred on metal nanoparticles and metal oxide nanoparticles and the second, much more recent and surprisingly very active, was centred on carbon dots, which have specific fluorescence-related properties and provide the proper conditions to develop direct fluorescence or fluorescence quenching sensing schemes. These two variants are covered in separate sections.

3.2.1 Use of Metal and Metal Oxide Nanoparticles

As noted, in the literature on sensing utilizing green chemistry methods there is a group of research articles relating to the use of nanoparticles (NPs). In fact, the highlight on the green aspects of the devices is not related to the simplified or (bio)sensing procedures, but to the methods used in the preparation of the NPs.[9] Researchers have essentially been studying sustainable and eco-friendly techniques for the production of reproducible, homogeneous and well-characterized NPs. Among the preferred methods, the production of metal NPs using natural source reagents, especially plants or plant extracts, stands out. Among these, plants with particular properties, *e.g.* medicinal vegetable products, seem to be the best candidates as they are suitable for the large-scale biosynthesis of NPs. Among other advantages, NPs obtained from plants can be more stable and the rate of synthesis can be faster than when using, for example, microorganisms or other living sources.

The first example to be considered is the widely used gold NPs (AuNPs); although most methods relate to organic reducing agents (*e.g.* citrate), one can find fairly frequently green methodologies for which reducing conditions have been developed, for example with *Aloe vera*, *Avena sativa*, black tea leaves, coriander, alfalfa leaves, tamarind and almond fruit. Gnanaprakasam *et al.*[10] used *Abelmoschus esculentus* (okra) vegetable extract as the reducing agent for the preparation of AuNPs on graphene, acting in two steps: (1) reduction of the starting material graphene oxide to reduced graphene and provision of adequate surface functionalization

and (2) controlled reduction of Au^{3+} ions to form AuNPs on the graphene surface. Electrodes modified with the prepared hybrid nanocomposite were employed using the square-wave anodic stripping voltammetry technique, which, employing a 40 s enrichment time, allowed for the quantification of heavy metals (Cd^{2+}, Pb^{2+}, Cu^{2+} and Hg^{2+}) at nanomolar concentrations. In another Au material-related study, β-lactoglobulin, a protein by-product in the cheese industry, was used to prepare Au nanoclusters, *i.e.* ultrasmall Au nanoparticles smaller than 1 nm, that were stable thanks to the template effect offered by the protein.[11] Because of this very small size, these nanoclusters possess fascinating molecular-like characteristics, such as discrete electronic states, size-dependent fluorescent properties and biomedical properties that are absent in larger metal nanoparticles. The Au nanoclusters, with notable fluorescence properties, were used as sensor elements for the detection and quantification of Hg^{2+} through its quenching effect, with a detection limit of 20 nM.

As an illustration of the use of metal nanoparticles other than Au, Manoj *et al.* prepared a CuNP composite with multiwalled carbon nanotubes[12] to develop a nitrite voltammetric sensor. In this case, the synthesis of the metal NP used a green route involving sodium carboxymethylcellulose as reducing agent.

The other group of examples that can be considered are related to the synthesis of metal oxide NPs to be used in different sensor configurations. NPs of WO_x were synthesized by a hydrothermal method and showed peroxidase-like activity.[13] With this idea, they were used in a colorimetric sensor configuration to determine H_2O_2 or glucose, when in combination with glucose oxidase enzyme.

Mixed $CoFe_2O_4$ NPs were used to modify a carbon paste electrode for the trace determination of Cu^{2+} using differential pulse anodic stripping voltammetry.[14a] The NPs were synthesized from a mixed solution of the metal nitrate salts, after a hydrothermal process of 14 h duration. The detection limit achieved was 0.085 ng mL^{-1} of Cu^{2+} when the enrichment time was 5 min. Now considering an example with a natural plant reagent, Mn_3O_4 NPs were prepared using an *Azadirachta indica* (neem) leaf extract.[14b] The green synthesized Mn_3O_4 NPs were employed for the modification of a glassy carbon electrode, which was further used for the voltammetric determination of 2-butanone.

In a different sensor application, Manjula *et al.* reported a green approach for the controlled synthesis of porous SnO_2 nanospheres with a high surface-to-volume ratio.[15] Glucose was used as a stabilizer and structure-directing agent, permitting the synthesis of monodisperse nanospheres with high surface area and excellent gas sensing capabilities towards hydrogen. The surface modification of the nanospheres with Pd transformed this sensing material into a highly sensitive and selective room-temperature semiconducting hydrogen gas sensor.

3.2.2 Use of Carbon Dots

Carbon dots (CDs) are a recent form of carbon nanomaterial,[16] accidentally discovered in 2004 when characterizing in detail single-walled carbon nanotubes obtained by arc discharge methods. From this moment, the simplicity of their synthesis, which matches perfectly the requirements of green chemistry, and their singular fluorescence properties resulted in an explosion of publications employing different kinds of precursors and synthetic methods.[17] These can be classified as top-down and bottom-up approaches – the top-down methods produce carbon NPs by breaking down larger pieces of materials into the desired nanostructures (by arc discharge, laser ablation, chemical or electrochemical oxidation) and the bottom-up approaches form the CDs by assembling molecular precursors (such as citrate, carbohydrates, biomaterials, *etc.*) under diverse reaction conditions (including combustion, hydrothermal, solvothermal, microwave digestion, *etc.*). The most prevalent sensing variant using CDs is the detection of metals ions using fluorescence transduction, although the tentative uses of these materials to modify electrochemical sensors has also been studied.

The first simple, high-yield (80% yield) synthesis of CDs involved a hydrothermal method with citric acid and ethylenediamine solution in a microwave oven.[18] These fluorescent NPs were used for the determination of ferric ions with a detection limit of 18 μM, through the exerted quenching effect.[19] In fact, this analytical case is the most widely reported application with the use of fluorescent CDs and led to a variety of green synthesis methods where the departure materials used were, for example, coriander leaves,[20] papaya powder,[21] blueberries[22] and *Syringa obtata* Lindl (lilac) flower.[23] When wild cherry (*Prunus avium*) fruit extract was used as the synthetic source,[24] N-doped carbon particles were obtained that showed an enhanced fluorescence yield, permitting the detection of Fe^{3+} ions at the 0.96 μM level.

Another recurrent application is similar to the above sensing of Hg^{2+}, also by fluorescence quenching of CDs obtained by different procedures. Sun's group in China reported a simple, economical and green synthesis method to produce water-soluble, fluorescent carbon NPs by a hydrothermal process using of pomelo peel waste.[25] The prepared CDs could be used as an efficient sensor to detect Hg^{2+} with a detection limit of 0.23 nM. The excellent sensing performance is attributed to the strong affinity between the metal ion and the carboxylic groups on the surface of the CDs. Similarly to the case with Fe^{3+}, different researchers prepared a variety of CDs using different synthetic sources and methods and applied them for mercury detection through fluorescence quenching phenomena; examples are the use of tamarind[26] and gum olibanum resin (frankincense).[27] A recently published procedure reported by Tabaraki and Sadeghinejad,[28] departing from a mixture of citric acid, urea and thiourea, permitted N/S co-doped CDs to be obtained, which showed interesting properties for the fluorescence quenching determination of Hg^{2+} and through its recovery the

determination of iodide with a detection limit of 72 nM. Bhatt *et al.*[29] recently reported a strategy for the synthesis of CDs using tulsi (holy basil) leaves and their potential application in the fluorescence sensing of Cr(VI), in this case through the inner filter effect.

In a study of a different nature, Xiao *et al.*[30] performed a singular synthesis of CDs in which poly(ethylene glycol) (PEG400) as the carbon source was carbonized in a strongly alkaline solution (10 M NaOH), obtaining the nanomaterial as a faint yellow powder. This nanomaterial showed a fluorescence enhancement in the presence of Fe^{2+} ions, which permitted its determination with a detection limit of 60 nM. Liu *et al.*[31] described the synthesis of N-doped CDs, in this case by an alternative process consisting of an anhydrous solvothermal treatment of the C/N source. In this process, high-temperature combustion of urea + sucrose added as a fine powder to boiling edible oil was performed, which produced CDs with specific pH-dependent fluorescence properties that permitted a pH-sensing procedure in the linear responding range 6.75–11.0 to be established.

Concerning the use of CDs for the analysis of organic compounds, practically the same trends can be identified. The most common techniques reported were related to fluorescence (or its quenching) and different mild protocols were used to obtain the transducer elements. For example, hydrothermal synthesis from citric acid + ethylenediamine solution was employed to obtain CDs that were first reacted with haemoglobin, which quenched their intrinsic fluorescence. Next, the complex could be displaced with cholesterol, recovering the initial fluorescence, permitting a cholesterol biosensor with a detection limit of 56 μM to be assembled.[32] In a direct fluorescence transduction study,[33] poly(vinyl alcohol) was the carbon source, which was carbonized in an alkaline medium at high temperature to produce fluorescent NPs, which were used as sensing elements for the detection of vitamin B_2 on the basis of spontaneous fluorescence resonance energy transfer from the CD to the organic compound. The use of natural sources for the synthesis of the CDs is also a trend, an example being the use of spinach leaves in a one-pot hydrothermal stage of 24 h duration,[34] where the CDs obtained allowed the sensing of 4-nitrophenol and/or 2-nitrophenol through fluorescence quenching. In two further examples, CDs, in this case N-doped, were employed for the sensing of anticancer drugs by fluorescence quenching, namely the protein kinase inhibitor sunitinib from the pyrolysis of citric acid + tris(hydroxymethyl)aminomethane[35] and the bisphosphonate derivative zoledronic acid from the hydrothermal degradation of palm tree date kernels.[36]

Although practically all uses of CDs and related materials in sensors are based on fluorescence, CDs have also been used in the preparation of electrochemical (bio)sensors, normally of the voltammetric type. In a first example, CDs obtained from electrolysis of a graphite rod[37] were used to modify a glassy carbon electrode together with glucose oxidase enzyme, allowing the biosensing of glucose by direct electron transfer at the very low potential of −0.5 V when using an N_2 saturated solution. In a second

example, boron nitride NPs, as CD analogues, were obtained by a combined mechanical and chemical process: high-energy ball-milling of boron nitride powder dispersed in ethanol, followed by a solvothermal process in an autoclave of the resulting particles in suspension after the first step.[38] The material obtained was first characterized with respect to its fluorescence properties and later used to modify an Au electrode, subsequently employed for the voltammetric determination of vitamin C. The novelty resides in the remarkable decrease in the oxidation peak potential for vitamin C (*ca.* +0.37 V) on the hybrid boron nitride quantum dot–Au electrode in comparison with a gold screen-printed electrode (0.25 V decrease), demonstrating catalytic activity through the BN nanocrystals on the electrode.

3.3 Colorimetric Sensing with a Smartphone Camera

A clear phenomenon that has become evident in the biosensing field is the production of application-specific portable and compact analytical instruments, which have made possible the general testing for certain analytes at any moment and by anyone, the paradigmatic example being the personal glucose meter. However, this example is not unique and many other portable devices have been developed, especially for clinical and veterinary practitioners. With these devices, the rapid diagnostic assessment of the status of an illness has been decoupled from a central laboratory, and they have permitted timely independent use and more accurate control and have made possible closer and friendly relations between healthcare staff and patients. Further examples different from the diabetes-related glucose meter include the blood oxymeter, the cholesterol meter, the blood coagulation [sintrom (acenocoumarol)] instrument and some other specific devices related to pathogen detection. The generic name of these devices is the point-of-care test analysis instrument, as they are specially designed for medical/veterinary use.

However, one criticism to be made to these instruments is that they can detect only a single target, which represents somewhat limited application. If a specific point-of-care analyser had to be developed for each individual target analyte of clinical interest, it would be very inconvenient and costly. Therefore, it has become a trend to exploit, apply and extend existing mature technologies (*i.e.* mobile phones or portable point-of-care analysers, such as the personal glucose meter, as will be described in Section 3.4) in the various fields of analysis. This trend in fact is simplifying to a great extent the requirements of analytical instrumentation needed to perform a planned analysis, which justifies its connection to sustainability and green analytical chemistry issues. To counterbalance the trend, the need for the highly specific information required is fulfilled by the use of specific chemical sensors or biosensors aimed at the target metabolite or pathogen to be screened. In this way, the use of mobile phones and similar devices for biosensing has recently become a very active field of research, especially the use of their integrated camera, which has helped to develop significant applications in the clinical field.[39]

Derived in most cases from the chemistries used in optical sensing (even from the chemistries in spot test analysis), the novelty is how a complete instrumental transduction, accompanied by an accurate spectrophotometric measurement, is then accomplished by standard appliances at our disposal, such as image scanners, digital cameras and even smartphone cameras, as the most popular device among the general population.[40]

It has become evident how the coupling of these two technologies, colour change reaction schemes and the consumer electronic image readers, is enabling analytical tests to be performed as a portable and user-friendly application. Any user can now perform quick, robust and easy (bio)assays themselves, at any moment and anywhere remote from a laboratory facility. Again, although the most popular application field is health diagnostics testing, the safety and security field and environmental monitoring are also attracting considerable attention.

A second fact, connected to the one above, is that the chemistry to be performed has to be executed with certain support in order to show the colour changes and provide image acquisition. When thinking about how to do this, together with what would be the most suitable way to dose any necessary reagent, very soon the paper platform come into consideration and many old possibilities developed within the spot test specialty were revisited. These included the possibility of performing microfluidic operation using channels defined in sorbent paper, with storage of reagents in certain receptacles used later when the liquid sample is placed in contact with the paper strip, and this become a way to perform many different tests, from a single-step reaction to multiple-step sequence procedures.[41,42]

A first model example to describe in detail as an example of this smartphone–paper support colorimetric sensing device is that produced for the simultaneous analysis of nitrite and pH.[43] The system combines a colorimetric reaction, with pH indicator change, and a low-cost paper-based microfluidic device. The application was devised with seven sensing areas, which contain the corresponding immobilized reagents that are solubilized and produce selective colour changes when a sample solution is placed in the sampling area. The device is shown schematically in Figure 3.3. The chambers contained two pH indicators (Phenol Red and Chlorophenol Red) in duplicate spots, three replicate chambers with nitrite reagents (sulfanilamide and *N*-1-naphthylethylenediamine), plus a control chamber for white correction. Under controlled conditions of light, using the flash of the smartphone as a light source, the image captured with the built-in camera is processed using a customized algorithm for multidetection of the coloured sensing areas. The developed image processing allows a reduction of the influence of the light source and the positioning of the microfluidic device in the picture. Then, the H (hue) and S (saturation) coordinates of the HSV colour space are extracted and related to pH and nitrite concentration, respectively. The device permitted the measurement of pH between 4.0 and 9.0 with a resolution of 0.04 pH units. The features for nitrite were a detection limit of 0.52 $mg\,L^{-1}$ and a linear response range between 4.0 and

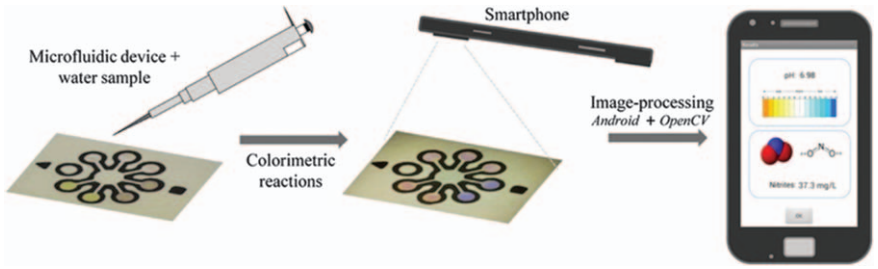


Figure 3.3 Microfluidic device with eight reagent sensing spots and the measurement using an Android smartphone camera after reaction with the liquid sample. The RGB measure of the colour intensity on each spot permitted the estimation of pH and nitrite content in the sample.
Reproduced from ref. 43 with permission from American Chemical Society, Copyright 2014.

100 mg L^{-1}. The application was tested in a second smartphone, demonstrating adequate robustness towards the different light sources used and the positioning of the device for taking the image, thanks to the software correction routines applied. Multiple zone analysis, permitting multiple analyte detection, multiple range adaptation and redundancy, is also easily accomplished with such this type of device.

From an instrumentation point of view, quantitative colorimetric detection of analytes using these paper supports is achievable using reflectance detection whenever the condition is met that the intensity of the colour formed in the test zones must be a function of their concentration. A reflectance measurement uses light reflected from the surface of the test zone and, for it to be practical, the test zone must also be illuminated with some standardized light. Then, a desktop scanner, a digital camera or a smartphone camera can capture the reflected light. The intensity of the colour in the reactive spot on the digital image can be estimated with graphics processing software (a popular software used is the open source program ImageJ[44]), although the relation between reflected light at a certain wavelength and concentration is not necessarily linear, as it occurs in solution. Reflectance detection is well suited for use with these reactive supports, although fluorescence can also be used if the platform is irradiated with suitable UV light; chemiluminescence can also be an option of choice, this being more suitable with enzyme-converting labels.

Another interesting example worth mentioning for green analysis is a 2012 paper by Vaher and Kaljurand,[45] who developed a multianalyte paper multisensing device for application in quality control in the wine field. The device used three tests in parallel, one for polyphenolic compounds using the Folin–Ciocalteu reagent, a second for flavonoids (closely related to antioxidant properties of the wine) and a third for anthocyanins (the compounds responsible for the red wine colour). In addition to permitting the quantification of the three groups of substances with sufficient sensitivity and detection capabilities, the system performance was also extended to

multivariate application possibilities, where from the three parameters evaluated and with simple principal component analysis (PCA) transformation it was possible to discriminate wine made from grapes of the Cabernet Sauvignon variety from those elaborated from other grape varieties.

One of the areas that has attracted attention is the security and forensic analysis field. In a study by Paixão and co-workers in Brazil,[46] the design of a paper platform with microzones equipped with three colorimetric reagents, iodide, creatinine and aniline, permitted the discrimination of five explosive compounds, triacetone triperoxide (TATP), hexamethylene triperoxide diamine (HMTD), 4-amino-2-nitrophenol (4A2NP), nitrobenzene (NB) and picric acid (PA). Detection was achieved at the $\mu g\,mL^{-1}$ level and, through multivariate treatment of the instrumental colorimetric data, the discrimination of the five compounds was possible. In a more recent study by the same group,[47] an office paper-based colorimetric device was proposed for the forensic field for sensing phenacetin, a chemical frequently used as a cutting agent in illicit drugs of abuse, *e.g.* cocaine. The colorimetric reaction employed involved 1,2-naphthoquinone-4-sulfonate in cetyltrimethylammonium bromide medium. To provide better quantitative measurements, the authors used an iPhone 4S smartphone with an 8 megapixel camera as the colorimetric detector, inserted in a special support built with black plastic material to control the light conditions and focal distance. The detection limit achieved was as low as $3.5\ \mu g\,mL^{-1}$ and its performance matched that achievable with high-performance liquid chromatography (HPLC) methods. A third example in this application field is a recent study by Narang *et al.* to detect ketamine,[48] a drug related to date rape attacks. A suspect drink can be easily checked with the paper device prepared, furnished with a reagent zone with deposited Bromocresol Green. The complex reaction between the pH indicator and ketamine produced an orange colour, which was measured with the smartphone camera. A specific application program was also provided that could identify the different situations, compensating for the background colour of the drink (for example, if it was cola based or contained whisky or rum) and provided a final diagnostic: safe to drink or refrain from drinking. Demonstrating advanced performance, given the communication abilities of the device, in the case of a positive test the smartphone also sends a text message to preprogrammed recipients, completing the circle of the security means planned.

This potential of sensing and integrated communication together in the same device will probably continue to maintain this device and its possible chemical applications as a top contender for a long time. The exceptional capabilities that this coupling of functionalities gives to (bio)sensing using smartphone devices were already predicted in the first studies in the field by Whitesides' group. They made a strong forecast regarding the development of inexpensive paper-based microfluidic platforms, with easy-to-use smartphone camera detection and established communications infrastructure, capable of transferring the analysis results from the assay site, which could be anywhere, to a trained medical professional, who could then send the

final diagnostics back to the field.[49] This could also provide an opportunity to improve health conditions, offering an inexpensive monitoring device, making possible a real-time telemedicine network and relieving the physician of the need to travel to remote or dangerous locations.

Taking example cases from Whitesides' laboratory, we can start to comment on biosensing applications developed using a paper microfluidic platform plus smartphone detection. Whitesides and co-workers described a clinically relevant urine biparametric test with two redundant spots for glucose detection and two additional spots for protein detection.[49] Glucose was determined in the range 0.1–300 mg dL^{-1} (100 mg dL^{-1} is the reference level for a healthy person). This was achieved using the glucose oxidase enzyme-catalysed reaction, which produced gluconic acid and H_2O_2. In order to provide a coloured product (brown), the first reaction was coupled to a second redox reaction in which H_2O_2 oxidized iodide to iodine. On the other hand, protein present in urine was deduced from a protein error colorimetric assay, in this case using Tetrabromophenol Blue, first in its acidic yellow form, which after interaction with the protein changed to blue. The range of protein concentration covered 1–30 μM.

By considering the type of biological receptor used, there are different variants in the area of smartphone biosensing,[50] and the use of enzymes, antibodies, DNA, aptamers and so on in order to provide a complete range of utilization must be considered.

Glucose analysis being the most frequent enzymatic analysis, a second additional glucose quantification system will be considered.[51] This approach used fluorescence quenching of semiconducting CdSe/ZnS core/shell quantum dots (QDs), the quenching being modulated through the presence of NADH cofactor, needed for the enzyme chain of the reaction used. The first step, using hexokinase, converted glucose into glucose-6-phosphate and the second oxidized glucose-6-phosphate to gluconic acid-6-phosphate, a reaction that involved the reduction of the cofactor NAD^+ to NADH. The degradation of glucose then involved the appearance of NADH, which, absorbing the UV light used to irradiate the QDs, in turn caused the global fluorescence value to diminish. The QDs were used immobilized in a plastic membrane, which made possible non-contact utilization and their easy reuse. The UV light was provided by a 365 nm UV LED source. The phenomenon was finally observed by the smartphone camera, permitting the quantification of glucose at concentrations between 0.1 and 450 mg dL^{-1}. The non-contact nature of the assay prevented any degradation of the QDs, which yielded an efficient, waste-free, cost-effective, portable and sustainable biosensing scheme.

In another selected example case, reaction micro spots were designed on paper by a wax printing technique[52] for bacterial quantitative analysis. The use of a specific substrate culture with a colour indicator permitted the presence of three food-borne pathogens to be estimated, again by quantification of the specific colour intensity with the use of a scanner device. The system was devised to detect *Escherichia coli* O157:H7, *Salmonella*

typhimurium and *Listeria monocytogenes* in food samples. Detection was achieved by measuring the colour change when an enzyme associated with the pathogen of interest reacted with a chromogenic substrate (*e.g.* β-galactosidase with Chlorophenol Red β-galactopyranoside for *E. coli* determination), changing from yellow to red–violet in colour, a proven chemistry derived from specific media culture tests. When combined with enrichment procedures, the method allowed the detection of 10 colony-forming units (CFU) cm^{-2} for an enrichment time (by culture) of 12 h.

In another related application,[53] the presence of specific bacteria, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus mirabilis* and *E. coli*, was deduced from colour changes or immobilized pH indicators in polymeric thin films, that responded with induced pH changes after interaction with volatile organic compounds (VOCs) emitted by bacteria. This provided a multivariate signal from five immobilized pH indicators, subsequently used to perform pattern recognition with 100% success in the identification of single bacterial strains. The method could form the basis for bacterial control, for example in the food industry.

In a final example connected with enzymes, inhibition was used to determine neurotoxic (or pesticide) compounds.[54] The system used a gelatin matrix with acetylcholinesterase (AChE) enzyme embedded together with Phenol Red pH indicator. The hydrolysis of acetylcholine by AChE produces acetic acid, which, being an acidic species, shifted the pH to lower values, causing a spectral change of the indicator. This simple scheme, accompanied by smartphone camera measurement, allowed tacrine and galantamine inhibitors to be determined at concentrations of 1 nM and 1.3 μM, respectively, results that were validated against the standard Ellman method.

The highly selective biosensing assays achievable with the use of antibodies have obviously been translated into biosensing technology that employs paper microfluidic supports and optical measurement using a smartphone camera. The first example is a method developed to measure the stress hormone cortisol in saliva.[55] The biosensing scheme involves a direct competitive immunoassay using a peroxidase–cortisol conjugate, detected by adding the chemiluminescent substrates luminol and hydrogen peroxide. The catalytic activity provided by the peroxidase-labelled enzyme will produce a higher chemiluminescence reading if low amounts of cortisol are present in the saliva sample (as most of the added labelled conjugate will be the one finally retained in the spot). In order to observe chemiluminescence without stray light interference by use of the smartphone, a specific adaptor was prepared by 3D printing technology. The method provided quantitative analysis of cortisol in the range 0.3–60 ng mL^{-1}.

In another interesting example,[56] a smartphone readout system was devised to monitor digoxigenin, a drug used in certain cardiac diseases. The system used a lateral flow assay, with gold NP-labelled antibody with affinity for this drug and which was subsequently retained in the sorbent strip by a line of immobilized bovine serum albumin–digoxigenin conjugate.

In essence, the assay adopted a competitive format, where the presence of digoxigenin in the blood sample reduced the amount of labelled antigen later fixed in the test zone of the assay (inverse relation). The scheme used a darkbox setup for acquiring the image, which after normalization permitted the detection of digoxigenin in the range 0.5–2.5 ng mL^{-1}. In a closely related example,[57] immunoassay was the variant used to detect food-borne pathogens (*Salmonella* spp. and *E. coli*), with the use of fluorescently labelled antibodies, at levels of 10^5 CFU mL^{-1}, also with a properly devised dark chamber for fluorescence readout using a smartphone camera. Viruses have been also detected with such principles, helping to diagnose specific forms of infective outbreaks.[58] A system to detect avian influenza (AI) viruses with high sensitivity and to establish their geographical transmission was formulated using a smartphone-based fluorescent diagnostic device.[58] A test strip was coated with anti-influenza A nucleocapsid (NP) antibody. Latex nanoparticles coated with anti-influenza A virus and fluorescent dendrimer coverage were used as a flowing and complexing agent. In the presence of the pathogenic virus, a sandwich complex was formed, which was captured in the test line. After developing the assay, the fluorescence intensity was measured with a smartphone camera, with the precaution of using a stand device for constant focus, controlled illumination for excitation and an optical filter for recording. After finishing the measurements, the test result was displayed on the smartphone and also transmitted *via* a text message to the central database. The response to virus subtypes H5N3, H7N1 and H9N2 was found positive and validation was completed against human specimens containing the H5N1 highly infective virus form. By dissemination of this kind of cheap and available platform, using a smartphone for detection and communication, it is possible to obtain immediate diagnostic results on-site and at the same time build an international real-time surveillance network for emerging public health threats with geographically distributed diagnostic tools.

The final biomolecule that might be used as a recognition element is DNA, either as a gene probe to detect gene analytes or as an aptamer to detect third substances. Although the initial database search did not provide results, indicating there have been few studies or they are hard to find, a more exhaustive search revealed examples of the two variants.

The first portable DNA microarray assay using smartphone detection was designed for Kaposi's sarcoma herpes virus,[59] an infectious cancer that became widely known during the early years of the AIDS epidemic. The gene sequence specific to this human herpesvirus8 (HHV-8) is quantified at concentrations down to 1 nM by hybridization against a complementary DNA sequence conjugated to gold NPs thanks to the superior absorbance properties of these nanotechnology labels.

In the second example, involving a gene marker of inherited disease, a portable fluorescence microarray-based imaging system connected to a smartphone for detecting breast cancer gene expression (BRCA-1 gene) was described.[60] The assay involved the Watson–Crick base pairing of

complementary DNA as the capture probe that binds the DNA analyte, which in this case was labelled with a Cy3 fluorescent tag. For the real application of this test, a competitive scheme with non-labelled BRCA-1 gene in the sample would be a possible choice. The assay, which was carried out in a microfluidic paper support, demonstrated functional hybridization and the validity of the approach. The imaging principles used a 3D-printed darkbox and two optical filters, one suited to excitation of the fluorescent dye and the other to capture the fluorescence emission.

With respect to a system using a DNA aptamer, a recent paper described the detection of residues of the antibiotic streptomycin.[61] The procedure used a displacement strategy, in which the 79 bases long streptomycin aptamer preferentially recognized the antibiotic in competition with its complementary sequence; in the absence (or defect) of the antibiotic, the aptamer hybridized with the complementary DNA to form a double-stranded gene sequence. Next, the intercalating dye SYB Green I combined with available dsDNA, emitting green fluorescence. The fluorescence intensity decreased with increase in streptomycin concentration, as the proportion of dsDNA was diminished. The detection limit achieved with the specified protocol was 94 nM of streptomycin, and satisfactory recoveries in chicken and milk samples were achieved.

Less frequent in this specific biosensing area is the coupling of electrochemical detection with a smartphone, essentially because specific electronics for the measuring device, both for potentiometry, for voltammetry and also for impedance transduction, need to be developed. The transduction principle can be found in a recent review.[62]

3.4 Electrochemical Biosensors Using a Portable Glucometer

An interesting example of biosensing using instrumentation of maximum simplicity is the design of different protocols for detecting different species, in this case with the use of a portable glucometer (or a personal glucose meter) designed in principle to perform the analysis of glucose in blood by diabetic patients at home. This protocol helps patients check their metabolic conditions at any time and to receive treatment at the same instant. In the short time that has elapsed since the first use of the portable glucometer to perform assays other than glucose, its general operation has been extended to the analysis of ions, the analysis of organic molecules (drugs, vitamins or toxins), the analysis of proteins (disease or tumour markers) and also disease-causing agents, such as viruses and pathogenic bacteria. A summary of these applications is provided in Table 3.2.

The development and general use of the personal glucose meter, as a routine means to control the level of blood glucose in diabetic patients with injection of insulin, was a revolutionary advance, allowing patients to evolve from a reduced life expectancy to a practically normal life. More recently, it

Table 3.2 Different variants and examples of analytes found after making the compilation of biosensing schemes using the personal glucose meter.

Sensed species	Recognition element	Analyte[a]	Sample matrix	Detection limit	Ref.
Inorganic ion	DNAzyme	UO^{2+}	Water	10 nM	63
	DNAzyme	Pb^{2+}	Wastewater	1.0 pM	64
Organic molecule	Secondary response	Vitamin C	Fruits	0.1 mg mL^{-1}	65
	Antibody	Ochratoxin A		6.8 ng mL^{-1}	66
	Aptamer	Cocaine	Blood	5 μM	63
	Aptamer	Adenosine	—	20 μM	63
Protein	Antibody	PSA		0.4 ng mL^{-1}	66
	Antibody	α-Fetoprotein		0.2 ng mL^{-1}	67
	Specific peptide	PSA		30 pg mL^{-1}	68
	Aptamer	Interferon-γ	Serum	3 nM	63
Virus	Specific cDNA	Hepatitis B		40 pM	69
	Specific cDNA	Ebola	Sputum	20 copies μL^{-1}	70
Bacteria	Antibody	*Salmonella*	Milk	10 CFU mL^{-1}	71
Enzyme activity	ALP Enzyme	GALT	Serum	0.008 U mL^{-1}	72

[a]PSA, prostate-specific antigen; ALP, alkaline phosphatase; GALT, galactose-1-phosphate uridyltransferase.

has been recognized that the personal glucose meter is a complete instrument, a pocket amperimeter/potentiostat, and that its ubiquity, low price and simplicity of operation make possible its generic use as (bio)sensor at many other levels. The sensing scheme just has to be adapted to a final reading of glucose, which can be the result of recognition of any other analyte through a proper transduction scheme. Thus, devices for measuring glucose and sucrose, as trivial starting cases, to more advanced biosensing schemes using enzymes, DNA genes, aptamers or antibodies have been described. A recent review described thoroughly most of the variants reported in the literature,[73] hence in this section we present just a few examples covering each transduction subcase.

For the adaption of the personal glucose monitor for generic biosensing, a given assay must be reformulated into a scheme producing glucose as the measured species, as many other assays have been adapted, for example, to measure a fluorescent marker as a result of the action of an enzyme as label. An easy way to do this is to use invertase, *i.e.* the enzyme that hydrolyses sucrose disaccharide (table sugar) into its glucose- and fructose-forming elements, as a label in the biosensing operation. This idea is illustrated in Figure 3.4, which depicts the scheme of a DNA biosensing assay with a sandwich protocol. Beads are used with a DNA probe to recognize a DNA analyte in a sample, and it is also designed to attach a signalling DNA probe modified with the invertase enzyme. When the unreacted species have been

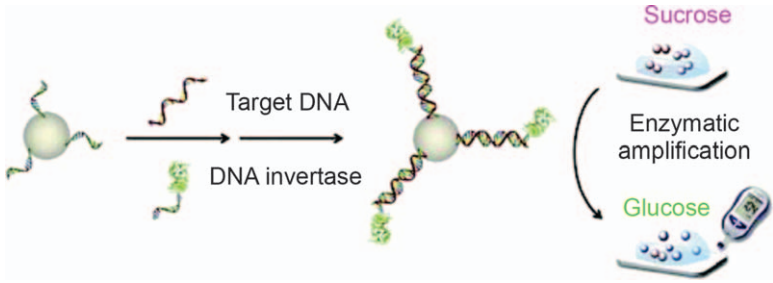


Figure 3.4 Mechanism of target DNA detection by a personal glucose monitor *via* a sandwich hybridization assay using magnetic beads coated with the capture DNA (MBs–DNA) with the DNA analyte and a signalling DNA conjugated with invertase enzyme.
Reproduced from ref. 69 with permission from American Chemical Society, Copyright 2012.

washed away, the addition of sucrose as the developer substrate will produce hydrolysis and the appearance of abundant glucose driven by amplification conditions, and this glucose is finally measured with the personal glucose monitor. In summary, a positive reading of glucose will correspond to the presence of the sought DNA gene, this being an illness-related diagnostic, a genetically modified organism test or confirmation of the adulteration of food.

The most common portable blood glucose meter is the enzymatic electrode-type meter, introduced commercially in the 1980s after intensive research to establish the proper performance features. It employs disposable enzyme-modified screen-printed electrodes as the sensor and, after the introduction of a drop of sample blood through capillary action, glucose is oxidized with the catalytic action of glucose oxidase enzyme (there are also variants using glucose dehydrogenase). Electrons generated during oxidation are measured through the interaction with redox mediators under constant voltage conditions, producing a higher current with higher glucose content. The device measures glucose in blood in the approximate range 0–33 mM (0–600 mg dL^{-1}) and it is actually factory precalibrated, hence it is of direct immediate use.

The first proposal to extend the use of personal glucose meters to analytes other than glucose was made in the pioneering paper by Xiang and Lu of the University of Illinois at Urbana-Champaign (USA) in *Nature Chemistry* in 2011.[63] They adapted different aptamer recognition elements, conjugating them with the enzyme invertase, and showed how it was possible to adapt it to determine small organic molecules, a protein and an inorganic ion, in this case through the use of a DNAzyme biomolecule.

The model example used an aptamer for cocaine with recognized detection properties in combination with a DNA fragment coupled with the enzyme invertase. The recognition is based on the conformation changes suffered by the aptamer, a phenomenon that in turn displaces the labelled DNA probe, liberating it into the solution, with invertase present in the

supernatant. After separation of all the compounds conjugated to the magnetic particles used, the net effect is the activity of the displaced invertase hydrolizing sucrose into fructose and glucose, the latter detected using the meter.

This scheme allowed the detection of cocaine in blood and blood serum at levels down to 3.4 μM. Potential endogenous glucose in blood samples had to be determined and compensated with a blank without the use of an enzyme label. The times involved in the biosensor array were 5 min for the aptamer stage and 8 min for the sucrose enzymatic stage, with a total time of analysis less than 15 min. An equivalent scheme but with an aptamer selective to adenosine was also demonstrated, allowing the determination of this organic compound at the 18 μM level.

The same work was also extended to the determination of a specific protein, in this case interferon-γ, which is related to different infectious diseases, including tuberculosis. The design of the interferon-γ aptamer sensor was similar to that of the cocaine and adenosine biosensors above. A specific aptamer was conjugated to a magnetic bead, in such a way that the recognition event displaced a DNA fragment conjugated to the invertase. The presence of the target protein caused enrichment of the solution with invertase, which was later contacted with the sucrose generated, allowing for amplification of the amount of glucose in relation to the initial analyte. This procedure allowed the detection of interferon-γ concentrations as low as 2.6 nM in diluted human serum, while the personal glucose meter remained in the mM glucose range.

Finally, in the same work, the authors extended their methodology from aptamers to DNAzymes for the on-site detection of toxic metal ions such as uranium (UO_2^+). For this, they conjugated a fragment of DNA carrying the invertase enzyme with a DNAzyme capable of interacting with the UO_2^+ ion. The presence of UO_2^+ activated the enzyme action, cleaving the aptamer at its specific cleavage point and liberating the invertase into the solution. As before, the quantification of the glucose generated from the solution after few minutes of reaction allowed the detection of UO_2^+ concentrations as low as 9.1 nM, a value which is below US and European regulations, which makes the method interesting for drinking water monitoring. The selectivity of the method was verified against other metal ions such as Pb^{2+}, Cd^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, VO^+ and Th^{4+}.

In 2012, Xiang and Lu reported an equivalent assay for a DNA fragment.[69] In this case they used a conjugated DNA probe in the quantitative detection of a specific hepatitis B virus DNA 35-nucleotide gene fragment, a DNA biosensing scheme by gene hybridization. The quantification was based on the capture of the target DNA with a magnetic bead immobilized 13-mer capture probe and the conditioned binding of a cDNA–invertase conjugate with the analyte DNA. In this case, the separation of the magnetic beads and the interrogation of their sucrose degrading activity enabled it to be established that the analyte DNA was present. In this way, the concentration of DNA in the sample was translated into glucose through the invertase-

catalysed hydrolysis of sucrose. This simple scheme made it possible to detect 40 pM DNA using the personal glucose meter. The selectivity of the method was also checked, and showed a satisfactory differentiation towards single nucleotide mismatches, a notable feature considering that the capture probe was made of just 13 DNA bases. Figure 3.4 illustrates the described protocol.

Shortly afterwards, Xiang and Lu extended the previous examples that used essentially DNA (as aptamers or capture probes) to the use of antibodies and invertase labelling. They first proposed a portable, low-cost method for the antibody-based determination of prostate-specific antigen (PSA), a prostate cancer marker.[66] To accomplish this, they used magnetic beads modified with PSA antibodies, which first bound to the target analytes in a capture scheme and subsequently a second antibody conjugated with invertase was bound to the aforementioned assembly to form a sandwich complex. The invertase in the sandwich then hydrolysed sucrose to glucose, which was detected using the portable glucose meter. The proposed scheme allowed a detection limit of 0.4 ng mL^{-1}. The same study also included an immunoassay to detect the food toxin ochratoxin A, in this case utilizing an antibody–aptamer sandwich scheme. In this protocol, the labelling invertase enzyme was incorporated into the analyte complex through an additional step of hybridization with a third signalling DNA molecule, for the occasion linked to invertase and allowing for the increasing generation of glucose after the positive assay, in an overall scheme that allowed the detection of 6.8 ng mL^{-1} of the toxin.

Other uses of antibodies as recognition elements with the use of a personal glucose meter as the final reader of the assay include the sandwich determination of α-fetoprotein.[67] In this example, the primary antibody was first immobilized on a screen-printed gold electrode, a recognition element used first to capture the protein marker and to make possible a sandwich scheme with a second antibody, this one marked with the invertase enzyme. After addition of the substrate sucrose, the generation of glucose and its detection allowed a detection limit as low as 0.18 ng mL^{-1}; the adopted design permitted the regeneration of the device with a simple pH buffer and its repeated use.

An interesting assay example was that developed by Gu *et al.*,[74a] who reported a detection method for circulating microRNA-21, a sequence of non-coding DNA, but closely related to the expression and typification of tumours of different types. For detection, an isothermal amplification strategy was designed using rolling circle amplification and a cleaving DNAzyme, leading to the release of invertase enzyme after hybridization with the target RNA. After a certain time of sucrose conversion to glucose, the additional amplification allowed the detection of 0.7 fM of the target microRNA, in a procedure that has been called liquid biopsy.

When considering the different variants, it is worth mentioning the procedure to detect the Zaire Ebolavirus that also involves a final reading with a personal glucose meter.[70] In the proposed assay, a sputum or saliva sample

was treated for isothermal amplification, which displaced a DNA fragment conjugated to invertase, thus releasing into the solution the enzyme if the DNA virus was present. After addition of sucrose substrate, their hydrolysis and glucose detection with the personal glucose meter allowed the detection of the virus at levels as low as 20 gene copies μL^{-1} (equivalent to the attomolar range). Detection of bacteria microorganisms also has been carried out, such as the detection of *Salmonella* in milk.[74b] For this purpose, specific antibodies for their capture were immobilized onto magnetic beads in order to accomplish a sandwich assay. This, using a secondary antibody linked to the invertase enzyme, produced high level of glucose after catalytic reaction with the sucrose substrate, which permitted the detection of *Salmonella* using a simple blood glucose meter at a level as low as 10 colony-forming units (CFU) mL^{-1}.

In a search for exploiting to the maximum the possibilities of the personal glucose meter, a variant to determine vitamin C in vegetables and fruits can also be mentioned. In this case, authors utilized as the analytical signal the interference produced in the glucose biosensor by the presence of antioxidant vitamin C, once the precautions taken by the commercial device to avoid this interference error had been removed.[65] For this purpose, the commercial biosensor strips were pretreated with methanol, which denatured the glucose oxidase enzyme, and dried. The inactivation of the enzyme resulted in the loss of the response properties of the device towards glucose, but still presented an adequate response to other redox species, in this case vitamin C. The performance of the modified device allowed the determination of vitamin C concentrations as low as 0.1 mg mL^{-1}, without any interference from antioxidants of the vitamin B type.

3.5 The Biofuel Cell Used for Sensing

Another paradigmatic example of biosensing with minimal use of instrumentation and resources is the biofuel cell, which in essence is a self-powered, self-standing device. The biofuel cell is normally employed as a source of energy, extracting electric power from an oxidizable substrate, *e.g.* glucose, acting as fuel. However, it can also be utilized in an alternative way, adopting the (bio)sensing principle, where it is employed, examining the electric current generated, to deduce the presence of a substrate (*e.g.* an organic load) in a sample and even how the latter is affected by a toxicant, in the inhibition of the expected performance.[75]

As shown in Figure 3.5, a microbial fuel cell generally consists of anodic and cathodic chambers separated by a proton exchange membrane, although single-chamber microbial fuel cells also exist. Microorganisms immobilized in the anodic compartment oxidize efficiently the organic or inorganic material present, promoting the circulation of protons towards the anode, plus the production of protons [eqn (3.1), written for the example case of glucose as fuel]. Normally, the counter reaction taking place in the cathode [eqn (3.2)] consists in the reduction of oxygen to form water. The

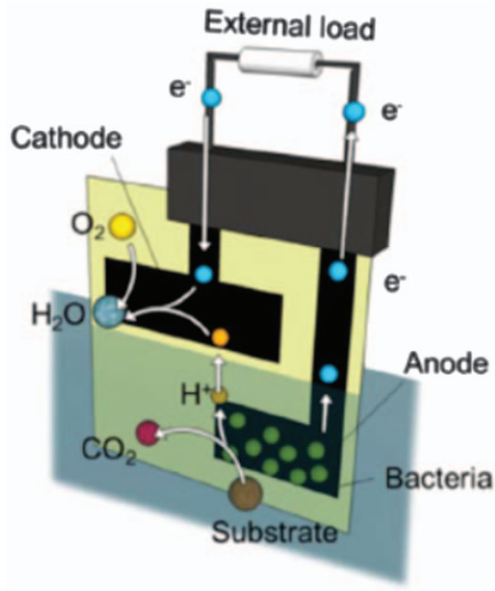


Figure 3.5 Schematic of the paper-based micro fuel cell and electrical connections, together with the details of operation.
Reproduced from ref. 81, https://doi.org/10.1016/j.bios.2017.11.018, under the terms of the CC BY 4.0 licence, https://creativecommons.org/licenses/by/4.0/.

proton exchange membrane isolates the two half cells, which also has the role of preventing the direct oxidation of the substrate by O_2, which is undesirable as it would consume the fuel without generating electricity. The described device is the microbial fuel cell, but there is also the variant of the enzymatic fuel cell, where in the anodic chamber it uses immobilized enzymes to catalyse the oxidation of the substrate.

$$C_6H_{12}O_6 + 3O_2 \rightarrow 6CO_2 + 12H^+ + 12e^- \quad (3.1)$$

$$3O_2 + 12e^- + 12H^+ \rightarrow 6H_2O \quad (3.2)$$

The overall reaction, involving the circulation of 12 electrons in the corresponding time across the interposed load, is shown in eqn (3.3).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (3.3)$$

A standard microbial fuel cell applied for estimating the biochemical oxygen demand (BOD) of a waste, that is, its degradable organic load expressed in terms of the amount needed by microorganisms to accomplish this degradation, works typically in a dynamic range of 10–200 mg L^{-1} O_2. Obviously, to obtain a faithful estimation of the organic load, a stable degradation rate must be established, a process that needs a certain amount of time after the sample is entered, with typical values in the range 5–30 min,

highly dependent on the cell design. In this type of application, typical microorganisms used in the microbial fuel cell are obtained from the activated sludge process in a wastewater treatment plant; for this reason, they are especially suited for the monitoring of digestion processes in these facilities. Certain authors see them as so powerful and cost-effective that they consider the microbial fuel cells to be the next-generation biosensing technology for environmental monitoring.[76] Additional applications of the microbial fuel cell include, for example, the detection in groundwater in shallow wells organic matter contamination, which might be of faecal origin or of a chemical nature.[77]

Toxicity is an important parameter for water quality inspection, as the toxic compounds may have dramatic effects on the population receiving this water, either humans, animals or even other live organisms. Traditionally, one can recall the use of the rainbow trout in drinking water plants, resting in fish tanks with on-line circulation of the entering water, where this was at the time a standard procedure for non-specific detection of a toxicant. Later, unicellular algae, with human or automated observation of their motility, replaced the use of this fish. Toxicity detection is indispensable in wastewater treatment, drinking water processing and water environmental monitoring and any indication of toxicity will trigger an exhaustive analytical inspection of the treated water, with the functionality of an early warning system.[78] The most recent device that may be used for toxicity detection is the microbial fuel cell, as any sudden reduction of its expected performance may indicate the presence of a toxicant.[79] Normally, to employ a microbial fuel cell as a toxicity biosensor, one will use the anodic chamber as the detection part, and this may be operated on-line, if the monitored water is recirculated continuously for continuous monitoring. Examples of toxicity detection effected with this technology variant include heavy metals (*e.g.* copper, chromium, zinc) and organic compounds (*e.g.* formaldehyde, phenols, surfactants, antibiotics). If the microorganism used in the biofuel cell is properly chosen from a selected bacterial strain, an important degree of selectivity can be conferred on the device. A clear limitation of microbial fuel cells used as biosensors of toxicants is that their detection capabilities are normally above the actual pollution levels for which they are required, hence the improvement of their sensitivity is of paramount importance for their practical application in monitoring.

Another important challenge for the improvement of the performance of a microbial fuel cell as a biosensor is its miniaturization, as this can be fundamental for increasing the speed of response and sample throughput. For this purpose, there have been attempts to design microfabricated microbial fuel cells in silicon[80] or with later trends also microfabricated microbial fuel cells on paper.[81] In any case, there is no argument that microbial fuel cells represent the best example of sustainable and cost-effective biosensors for environmental and industrial monitoring, allowing for *in situ*, on-line, high-throughput, selective and sensitive operation. Their concept with integrated sensing and function is exemplary in green chemistry, in which microorganisms (or their metabolic enzyme systems) directly generate a readable

electric signal without the need for a transducer or any other instrumentation, even the use of external power.

3.6 Smart Systems: Electronic Noses and Electronic Tongues

A final paradigmatic example uses sensor arrays as assemblies of multiple sensors. If all sensors in the array are similar and respond to a single analyte, the array is useful for parallel analysis of multiple samples, each sample being applied to one of the sensors; this just represents a multicomponent analysis accomplished with multiple sensors. If each sensor is selective to a particular analyte, it acts independently of the others and provides a particular analyte concentration. Even though it would suit our desire, this situation is too idealistic as it requires maximum selectivity features, something which can be achieved only with recognition elements with maximum recognition abilities. A more general approach proposed some years ago was to use arrays formed by poorly selective (cross-selective) sensors and computer processing to develop the analysis. In this case, each sensor responds to more than one sample component and the response of each sensor is a summation of the effects exerted by a series of components. These sensor arrays allow for multiplexing the sample analysis and, in fact, they are bioinspired in animal senses; it is known that the diversity that animals (and humans) achieve in recognizing aromas or tastes does not originate from having an enormous number of selective receptors, one per detected substance. On the contrary, combinatorial principles are employed, together with partial selectivity, *i.e.* different receptors all respond to different substances, but in a different manner. Curiously, these principles not only form the physiology of animals, but very recently have started to form the detection elements of highly advanced technological gadgets: the most recent advertised smartphone (2019) is furnished with five charge-coupled device (CCD) cameras and the final delivered picture is a computer-formed image from the five elements, with a final performance that it is impossible to attain with single sensor elements of this technology. The message to recall is that what cannot be obtained with a single sensor perhaps can be obtained with a sensor array and advanced computer processing.

This technology started with arrays of sensors for gaseous species, in a seminal paper in 1982 by Persaud and Dodd,[82] that initiated the electronic nose concept. In their initial work, they managed to detect different volatile compounds by simulating the different stages of the human olfactory system, including sampling and filtering, using semiconductor gas sensors with a generic response to different volatiles. The signal responses obtained were processed with artificial intelligence data processing tools, in this case with artificial neural networks (ANNs), accomplishing the identification of volatile compounds in a sample. The principles used in the approach are illustrated in Figure 3.6. The sensor array takes the simile of the olfactory

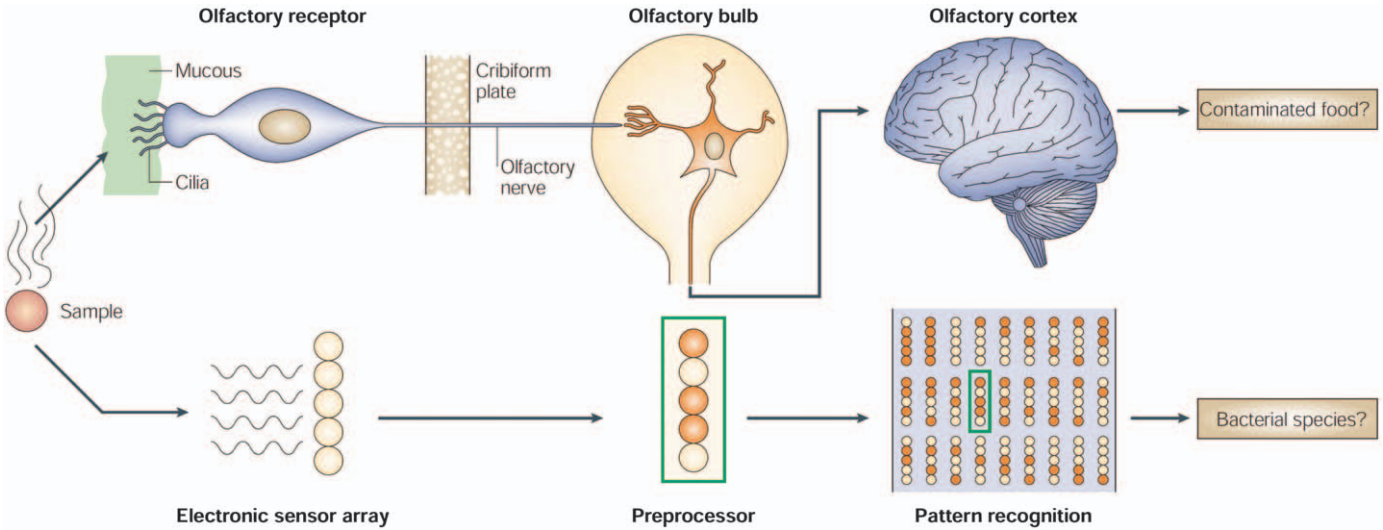


Figure 3.6 Electronic nose devices are sensor systems bioinspired by the human olfactory system. The electronic modules simulate the different stages of the human olfactory system, resulting in recognition of volatile odours, which can be used, for example, to recognize spoiled food or to discriminate types of bacterial infections.
Reproduced from ref. 89 with permission from Springer Nature, Copyright 2004.

receptors, whose signals are first pre-processed and then identified by comparison with patterns stored in the brain; in the sensor variant, the electrical signal from the sensors is first pre-processed to extract their informative component and then processed with artificial intelligence tools to perform the identification. An overview of the data processing methods that can be used for data processing in artificial nose applications can be found in the literature.[83,84]

As a selected application example, the work of Valdez and Gutierrez, who constructed an artificial olfaction system for the identification of tablet chocolates,[85] can be considered. The system was made of eight commercial metal oxide semiconductor gas sensors, devices that are of general use as specialized electronic components for smoke detectors, gas leak detectors or breath alcoholmeters. The system was first trained with the different chocolate types (note the terminology used: the system learns, then it is trained, in place of being calibrated, which is the terminology for an instrumental analysis). After this machine learning process, the system was able, through pattern recognition algorithms, to differentiate between dark black, soft black and milk chocolates or between different chocolates with added fruit varieties.

Apart from such exemplary cases, it is clear that this type of smart analysis system for detection, especially of VOCs, is an emerging research area. The final application can be customized to industrial demands, animal farm activities, food characterization, detection of explosives or drugs, forensic applications, *etc*.[86] Concerning sustainability aspects, the following application can be considered, where again maximum simplicity, now in the sensor design, is the highlighted idea.[87] The authors selected a paper platform as the substrate to deposit functionalized carbon nanotubes, to act as chemiresistive gas sensors and to form an electronic nose system. As the authors emphasized, the procedure allowed the fabrication of functional gas sensors from commercially available starting materials in less than 15 min. The chemical response diversity, in this case, was generated through simple mixing of different carbon nanoforms (graphite, single- and multiwalled carbon nanotubes) mechanically mixed in a ball-mill with small molecules capable of inducing a certain degree of selectivity [among others, titania, trifluoroacetate, polyfluoronaphthalene, aminopyrene, poly(fluorobenzyl borate), isobutylcalixarene, cyclodextrins]. The composite powder was next compressed in a pencil lead form, which was subsequently used to form a dry deposit of a sensing line by simple abrasion over weighting paper with gold electrodes previously defined for making the contacts. This very simple procedure allowed the authors to detect and identify VOCs such as acetone, tetrahydrofuran and dimethoxymethyl phosphonate at the parts per million (ppm) level. The transduction mechanism, the change in resistance after exposure to chemicals to be sensed, was selected because of its simplicity. Figure 3.7 shows the multicomponent response, visualized after the principal component analysis transformation, distinguishing different VOCs, plus water and ammonia vapour, in this case from the responses of a five chemiresistive sensor array draft on paper.

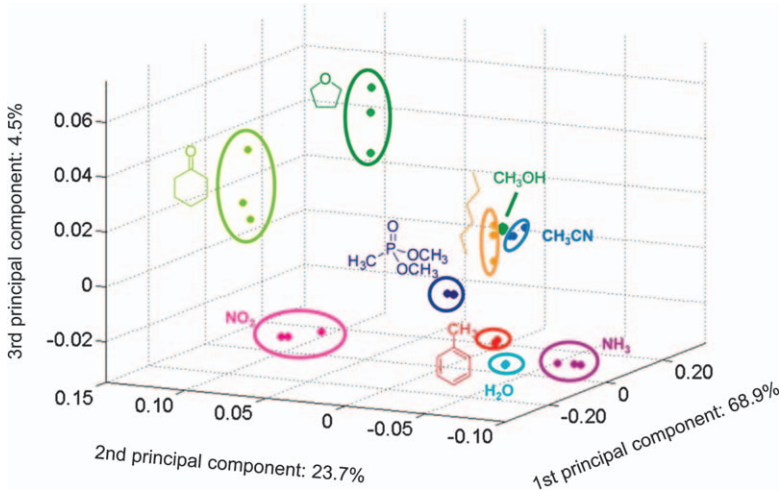


Figure 3.7 Visualization of the clustering of the different substances assayed with the cross-reactive carbon nanoforms sensor array on paper using principal component analysis. Concentrations tested at the 40–500 ppm (v/v) level. Reproduced from ref. 87 with permission from the National Academy of Sciences, Copyright 2013.

The application field for excellence of artificial olfaction systems is the food field, where it tries to complement or alternate with a human sensory panel.[88] It is obvious that a human sensory panel is a vital tool for the food industry, and also in the beverage and cosmetic areas, used in qualification of the acceptability/suitability of a food batch or food variant. However, it is also evident how difficult it is to form a panel and to get it operating; apart the difficulty of adequate training, their availability will be partial, as once in operation they may become tired or saturated, or even they may not be used because of toxicity or danger in the exposed situation. It is in these circumstances where artificial olfaction (and artificial taste) can find applications of evident interest, as the expertise of the sensory panel can be the exact information learnt to reproduce later from the signals provided by the sensors, and this system will be able to work 24 h per day, 7 days per week, without becoming tired or without saturation or blocking. Evaluation of food freshness, authentication studies or multiple aspects of food quality control comprise the main applications of electronic nose/artificial olfaction systems in the food and beverage field. In medicine, another of the ''hot'' application fields, the artificial nose can be useful for non-invasive diagnostic by the assay of volatile compound mixtures in breath, urine or sweat or even in wounds.[89]

Although the majority of electronic noses described in the literature are based on resistive or electrochemical readout, there have also been reports of optical sensing arrays based on responsive spots with immobilized dyes and pigments,[90] used to probe certain chemical reactivities of the sample analytes, rather than their specific physical properties. This general response

system, coupled with the use of pattern recognition algorithms, is able to provide high-dimensional data from the colour change or the fluorescence change of these spots and recognize or quantify chemical species, down to the parts per billion level. As before, most of the applications gathered are centred on VOCs and the industrial field, on the one hand, and to food and beverage analysis, on the other.

Arrays of cross-sensitive sensors can be operated in the electronic tongue mode,[91] as this is the term given to the above concept when applied to liquid samples. The application can be more related to mimicking the human sense of taste, in an artificial taste application, or it can be a more general analysis application, receiving the electronic tongue qualifier. With the use of arrays of cross-sensitive sensors, accurate and reliable results can be obtained compared with sensors dedicated to a particular analyte, *e.g.* think of the possibilities of counterbalancing the presence of any interfering species – instead of performing classical wet chemistry approaches to remove the offensive species, with the electronic nose/tongue principles a new approach can be started: we can measure all components present and compensate in a response model for the interfering compounds. This idea represents a maximum level of simplicity in the chemical part, where the maximum amount of information possible is obtained from direct read sensing and a complex model is built to consider all effects. In this approach, maximum simplicity is placed on the wet part and all the hard work is shifted to the computing aspects. Who could consider that such an approach is not an emblem of the reduction of chemical effort and of sustainable operation of sensors?

Rather than detecting specific components, artificial taste works intrinsically in a multivariate mode, assigning to each sample a characteristic fingerprint that depends on its overall chemical composition. In this way, it is possible to perform sample classification, identification of varieties and also quantification of components, just by selecting the appropriate chemometric treatment. As occurs with electronic noses, there is a broad range of applications of the electronic tongue in various fields.[92] The quality and organoleptic properties of foodstuffs and beverages and also their ageing or adulteration can be assessed by the assay of a characteristic assembly of chemical components and thus tackled in a multivariate approach with the electronic tongue system. There are various recognition and transduction methods with the electronic tongue, although electrochemical methods are predominant.[93] Of these, two main types of electronic tongues have been developed, the potentiometric electronic tongue, which uses an array formed of potentiometric sensors or ion-selective electrodes, and the voltammetric electronic tongue, which uses an array formed of different types of voltammetric sensors. For this purpose, different metal electrodes can be used or, alternatively, electrodes modified with different catalysts or electrochemical-providing properties can form the array. There are also systems formed by sensors of different natures, denoted the hybrid electronic tongue. The data treatment strategies for electronic tongues are the same as for electronic

noses, as the requirements of departure information or results sought are equivalent.

An interesting environmental application was described with the use of an array of potentiometric sensors in a double case study. In the first, the quantification of polluting ammonium ions in the Rio Salado (Mexico) was accomplished with an array formed of sensors for ammonium, potassium and sodium, plus a pH sensor and two different sensors with generic response to alkali metal ions, all of them based on poly(vinyl chloride) (PVC) membranes. The problem solved was the resolution of ammonium clean of the interference of sodium and potassium ions, a known problem when using nonactin-based ion-selective sensors for the measurement of ammonium ions. As an interesting example of monitoring with attention to sustainability, the system consisted of a base station formed by the sensors, their digital conversion, plus a radio data link. Numerical readings were then transmitted and processed with a sophisticated neural network response model, which yielded the concentrations of the three species considered. In this example, the measuring part was extremely simple, just the immersion of the sensors at the monitoring point, and the complexity was redirected to a central station concerned with the data treatment. The second case involved the assessment of heavy metals in polluted waters, where Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} were quantified using a sensor array formed of 11 sensors employing different commercially formulated PVC membranes for heavy metals. Figure 3.8 summarizes the strategy followed, where the electronic tongue alternative was demonstrated to be a valid option for monitoring several analytes on-site, with added advantages of simplicity, low cost of both the system and the analysis, speed of response, versatility, simple measuring setup, *etc.* Furthermore, the radio transmission presented an easy

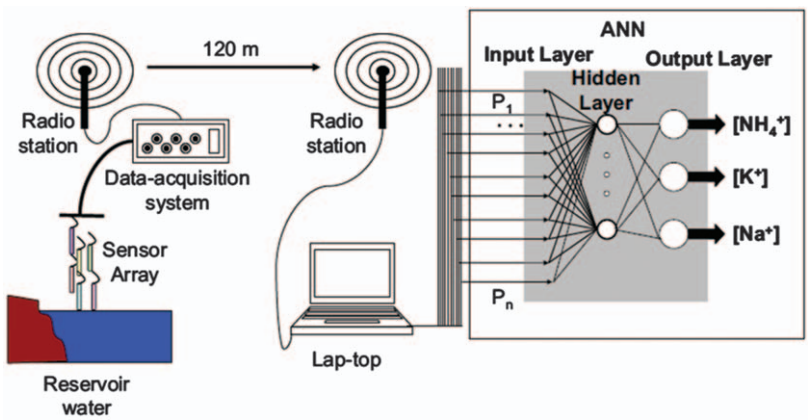


Figure 3.8 Block diagram of a potentiometric electronic tongue system with remote communication for the monitoring of the concentrations of ammonium, potassium and sodium ions in the Rio Salado (Mexico).
Reproduced from ref. 99 with permission from Elsevier, Copyright 2010.

and robust communication link, validating the feasibility of automated remote monitoring applications.

Concerning materials and their sustainability, a recent report introduced a sensor array deployed on paper-based potentiometric sensors.[94] In this work from the Institute of Chemistry, Unicamp, Brazil, an integrated potentiometric sensor array was implemented on a paper substrate using conventional PVC membranes for chloride, nitrate, sodium + potassium and calcium + magnesium; the device also incorporated a pseudo Ag/AgCl reference electrode to complete the measuring cell. The application developed was intended to differentiate natural waters from commercial mineral waters by use of principal component analysis for visualization and the K nearest neighbours (KNN) algorithm for identification of sample type.

Ant example showing the application of a voltammetric electronic tongue employed an array of six carbon composite voltammetric sensors for the discrimination of coffee varieties.[95] The sensors incorporated a mixture of graphite and different modifiers to induce a differentiated response, including Pt and Au nanoparticles, cobalt(II) phthalocyanine and conducting polymers (polypyrrole and polyaniline). The system could be trained to identify the type of cultivation (organic practices or altitude of crop) or, alternatively, to identify the geographical origin of samples. The artificial intelligence tools used for identification were linear discrimination analysis (LDA) with more limited results and support vector machines (SVM) with clearly superior performance. The procedure permitted the assessment of selected coffee features with a very simple and fast methodology without any sample treatment. Additionally, authentication applications could be developed, with limited cost, dedicated time and satisfactory accuracy.

In an advanced study at the Universitat Autònoma de Barcelona, a similar voltammetric electronic tongue was successfully applied to reproduce the results provided by a trained sensory panel, in this case in the prediction of wine sensory descriptors.[96] The sensor array combined Au and Pt metallic electrodes plus four carbon composite electrodes, an unmodified graphite one, and three of them modified with Cu nanoparticles, Pt nanoparticles and a cobalt(II) phthalocyanine. The tasks attempted were to identify the protected designation of origin (the regulated production region) for a given grape variety and to predict the wine global score assigned by a standardized sensory panel. Given the complexity and high dimensionality of the departure data, the model comprised a Fourier pretreatment intended for data compression, feeding an ANN for the final score prediction. The system was trained with a large number of wines (71), providing a final performance with an external test set of predictive slope 1.01 (1.00 is the ideal value) with a correlation coefficient of 0.830 ($n = 20$). It should be noted how remarkable this result is, given the individual nature of the expertise reproduced by the sensor-based intelligent system, as the taste perception of a sensory panel is being reproduced.

To finish this section, it is important to note how these systems can also be prepared for more elaborate applications with the use of biosensors. In this case, the biosensor elements being capable of high selectivity operation,

the types of applications are more centred on specific groups of substances, allowing results to be obtained that are comparable to those furnished by sophisticated analytical instrumentation. In a first example, a bioelectronic tongue (that is, an electronic tongue comprised of biosensors) was designed to resolve and quantify the types of phenolic compounds present in wine.[97] The biosensor array was formed of a set of epoxy–graphite biosensors, bulk modified with different redox enzymes (tyrosinase and laccase) and copper nanoparticles, aimed at the simultaneous determination of different polyphenols. The tool used to identify and predict the concentrations of the trained phenols was an ANN. After the model response building, the system was used to resolve the presence of major phenolics in wine, namely catechol, caffeic acid and catechin in the 0–200 μM range, in an application comparable to more complex analytical techniques such as HPLC.

A second interesting example, also with participation by the Barcelona laboratory, was the development of an inhibition bioelectronic tongue, in which different pesticide inhibitors were discriminated from the different degrees of inhibition offered to biosensors prepared with different acetylcholinesterases.[98] The pesticides discriminated were dichlorvos and methylparaoxon at levels down to 1 nM. For this purpose, the system used a three-biosensor array, formed by three different acetylcholinesterase enzymes: the wild type from electric eel and two different genetically modified enzymes, B1 and B394 mutants, from *Drosophila melanogaster*. The inhibition pattern described by the three responses was used to feed an ANN model, again with exceptional performance features, resembling those of a hyphenated HPLC analytical instrument.

Acknowledgements

Financial support for this work was provided by the Spanish Ministry of Economy and Innovation, MINECO (Madrid), through project CTQ2016-80170-P. M. del Valle acknowledges the support of the programme ICREA Academia.

References

1. M. Kaljurand and M. Koel, *Bioanalysis*, 2012, **4**, 1271.
2. M. de la Guardia and S. Garrigues, *Handbook of Green Analytical Chemistry*, Wiley-VCH, Weinheim, 2012.
3. M. Kaljurand and M. Koel, *Green Analytical Chemistry*, Royal Society of Chemistry, Cambridge, 2010.
4. M. Koel, *Green Chem.*, 2016, **18**, 923.
5. IUPAC, *Pure Appl. Chem.*, 1999, **71**, 2333.
6. O. S. Wolfbeis, *Fresenius' J. Anal. Chem.*, 1990, **337**, 522.
7. R. Viveiros, S. Rebocho and T. Casimiro, *Polymers*, 2018, **10**, 306.
8. P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, New York, 1998.

9. S. Iravani, *Green Chem.*, 2011, **13**, 2638.
10. P. Gnanaprakasam, S. E. Jeena, D. Premnath and T. Selvaraju, *Electroanalysis*, 2016, **28**, 1885.
11. J. Zang, C. Li, K. Zhou, H. Dong, B. Chen, F. Wang and G. Zhao, *Anal. Chem.*, 2016, **88**, 10275.
12. D. Manoj, R. Saravanan, J. Santhanalakshmi, S. Agarwal, V. K. Gupta and R. Boukherroub, *Sens. Actuators, B*, 2018, **266**, 873.
13. H. Peng, D. Lin, P. Liu, Y. Wu, S. Li, Y. Lei, W. Chen, Y. Chen, X. Lin, X. Xia and A. Liu, *Anal. Chim. Acta*, 2017, **992**, 128.
14. (a) M. A. Mohamed, F. M. El-Badawy, H. S. El-Desoky and M. M. Ghoneim, *New J. Chem.*, 2017, **41**, 11138; (b) J. K. Sharma, P. Srivastava, S. Ameen, M. S. Akhtar, G. Singh, S. Yadava and G. Zhao, *J. Colloid Interface Sci.*, 2016, **472**, 220.
15. P. Manjula, R. Boppella and S. V. Manorama, *ACS Appl. Mater. Interfaces*, 2012, **4**, 6252.
16. J. C. G. Esteves da Silva and H. M. R. Gonçalves, *TrAC, Trends Anal. Chem.*, 2011, **30**, 1327.
17. X. Sun and Y. Lei, *TrAC, Trends Anal. Chem.*, 2017, **89**, 163.
18. H. Zhu, X. Wang, Y. Li, Z. Wang, F. Yang and X. YanG, *Chem. Commun.*, 2009, 5118.
19. S. Zhu, Q. Meng, L. Wang, Z. Zhang, S. Song, J. Jin, Z. Zhang, S. Sun, W. Wang and Y. Yang, *Angew. Chem., Int. Ed.*, 2013, **125**, 4045.
20. A. Sachdev and P. Gopinath, *Analyst*, 2015, **140**, 4260.
21. N. Wang, Y. Wang, T. Guo, T. Yang, M. Chen and J. Wang, *Biosens. Bioelectron.*, 2016, **85**, 68.
22. A. M. Aslandas, N. Balcı, M. Arık, H. Sakiroglu, Y. Onganer and K. Meral, *Appl. Surf. Sci.*, 2015, **356**, 747.
23. H. Diao, T. Li, R. Zhang, Y. Kang, W. Liu, Y. Cui, S. Wei, N. Wang, L. Li, H. Wang, W. Niu and T. Sun, *Spectrochim. Acta, Part A*, 2018, **200**, 226.
24. T. N. J. I. Edison, R. Atchudan, J. J. Shim, S. Kalimuthu, B. C. Ahn and Y. R. Lee, *J. Photochem. Photobiol., B*, 2016, **158**, 235.
25. W. Lu, X. Qin, S. Liu, G. Chang, Y. Zhang, Y. Luo, A. M. Asiri, A. O. Al-Youbi and X. Sun, *Anal. Chem.*, 2012, **84**, 5351.
26. D. Bano, V. Kumar, V. K. Singh and S. H. Hasan, *New J. Chem.*, 2018, **42**, 5814.
27. R. R. Gaddam, S. Mukherjee, N. Punugupati, D. Vasudevan, C. R. Patra, R. Narayan and R. Vsn Kothapalli, *Mater. Sci. Eng., C*, 2017, **73**, 643.
28. R. Tabaraki and N. Sadeghinejad, *Ecotoxicol. Environ. Saf.*, 2018, **153**, 101.
29. S. Bhatt, M. Bhatt, A. Kumar, G. Vyas, T. Gajaria and P. Paul, *Colloids Surf., B*, 2018, **167**, 126.
30. S. J. Xiao, Z. J. Chu, J. Zuo, X. J. Zhao, C. Z. Huang and L. Zhang, *J. Nanopart. Res.*, 2017, **19**, 84.
31. X. Liu, J. Liu, B. Zheng, L. Yan, J. Dai, Z. Zhuang, J. Du, Y. Guo and D. Xiao, *New J. Chem.*, 2017, **41**, 10607.
32. T. T. Bui and S.-Y. Park, *Green Chem.*, 2016, **18**, 4245.

33. A. Kundu, S. Nandi, P. Das and A. K. Nandi, *J. Colloid Interface Sci.*, 2016, **468**, 276.
34. G. Ren, M. Tang, F. Chai and H. Wu, *Eur. J. Inorg. Chem.*, 2018, **2018**, 153.
35. H. M. Kashani, T. Madrakian and A. Afkhami, *New J. Chem.*, 2017, **41**, 6875.
36. N. Amin, A. Afkhami, L. Hosseinzadeh and T. Madrakian, *Anal. Chim. Acta*, 2018, **1030**, 183.
37. H. Yin, Y. Zhou, X. Meng, K. Shang and S. Ai, *Biosens. Bioelectron.*, 2011, **30**, 112.
38. S. Angizi, A. Hatamie, H. Ghanbari and A. Simchi, *ACS Appl. Mater. Interfaces*, 2018, **10**, 28819.
39. D. Bueno Hernández, J. L. Marty and R. Muñoz Guerrero, Smartphone as a Portable Detector, Analytical Device or Instrument Interface, in *Smartphones from an Applied Research Perspective*, ed. N. Mohamudally, IntechOpen, Rijeka, **ch. 4**, 2017, pp. 73.
40. D. Quesada-González and A. Merkoçi, *Biosens. Bioelectron.*, 2017, **92**, 549.
41. A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, *Anal. Chem.*, 2010, **82**, 3.
42. M. Dou, S. T. Sanjay, M. Benhabib, F. Xu and X. Li, *Talanta*, 2015, **145**, 43.
43. N. Lopez-Ruiz, V. F. Curto, M. M. Erenas, F. Benito-Lopez, D. Diamond, A. J. Palma and L. F. Capitan-Vallvey, *Anal. Chem.*, 2014, **86**, 9554.
44. C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat. Methods*, 2012, **9**, 671.
45. M. Vaher and M. Kaljurand, *Anal. Bioanal. Chem.*, 2012, **404**, 627.
46. M. O. Salles, G. N. Meloni, W. R. de Araujo and T. R. L. C. Paixão, *Anal. Methods*, 2014, **6**, 2047.
47. G. O. da Silva, W. R. de Araujo and T. R. L. C. Paixão, *Talanta*, 2018, **176**, 674.
48. J. Narang, C. Singhal, A. Mathur, A. K. Dubey, A. Krishna Pn, A. Anil and C. S. Pundir, *Vacuum*, 2018, **153**, 300.
49. A. W. Martinez, S. T. Phillips, E. Carrilho, S. W. Thomas, 3rd, H. Sindi and G. M. Whitesides, *Anal. Chem.*, 2008, **80**, 3699.
50. X. Huang, D. Xu, J. Chen, J. Liu, Y. Li, J. Song, X. Ma and J. Guo, *Analyst*, 2018, **143**, 5339.
51. S. A. Khan, G. T. Smith, F. Seo and A. K. Ellerbee, *Biosens. Bioelectron.*, 2015, **64**, 30.
52. J. C. Jokerst, J. A. Adkins, B. Bisha, M. M. Mentele, L. D. Goodridge and C. S. Henry, *Anal. Chem.*, 2012, **84**, 2900.
53. L. Bueno, A. Cottell, S. M. Reddy and T. R. L. C. Paixão, *RSC Adv.*, 2015, **5**, 97962.
54. A. Kostelnik, A. Cegan and M. Pohanka, *Sensors*, **16**, 2016.
55. M. Zangheri, L. Cevenini, L. Anfossi, C. Baggiani, P. Simoni, F. Di Nardo and A. Roda, *Biosens. Bioelectron.*, 2015, **64**, 63.
56. C. Ruppert, N. Phogat, S. Laufer, M. Kohl and H.-P. Deigner, *Microchim. Acta*, 2019, **186**, 119.

57. V. K. Rajendran, P. Bakthavathsalam and B. M. Jaffar Ali, *Microchim. Acta*, 2014, **181**, 1815.
58. S.-J. Yeo, K. Choi, B. T. Cuc, N. N. Hong, D. T. Bao, N. M. Ngoc, M. Q. Le, N. L. K. Hang, N. C. Thach, S. K. Mallik, H. S. Kim, C.-K. Chong, H. S. Choi, H. W. Sung, K. Yu and H. Park, *Theranostics*, 2016, **6**, 231.
59. M. Mancuso, E. Cesarman and D. Erickson, *Lab Chip*, 2014, **14**, 3809.
60. A. Prasad, S. M. A. Hasan, S. Grouchy and M. R. Gartia, *Analyst*, 2019, **144**, 197.
61. B. Lin, Y. Yu, Y. Cao, M. Guo, D. Zhu, J. Dai and M. Zheng, *Biosens. Bioelectron.*, 2018, **100**, 482.
62. A. C. Sun and D. A. Hall, *Electroanalysis*, 2019, **31**, 2.
63. Y. Xiang and Y. Lu, *Nat. Chem.*, 2011, **3**, 697.
64. J. Zhang, Y. Tang, L. Teng, M. Lu and D. Tang, *Biosens. Bioelectron.*, 2015, **68**, 232.
65. W. Wu, Z. Sun and W. Zhang, *Food Anal. Methods*, 2016, **9**, 3187.
66. Y. Xiang and D. Lu, *Anal. Chem.*, 2012, **84**, 4174.
67. X. Zhu, H. Zheng, H. Xu, R. Lin, Y. Han, G. Yang, Z. Lin, L. Guo, B. Qiu and G. Chen, *Anal. Methods*, 2014, **6**, 5264.
68. X. Hun, Y. Xu and X. Luo, *Microchim. Acta*, 2015, **182**, 1669.
69. Y. Xiang and Y. Lu, *Anal. Chem.*, 2012, **84**, 1975.
70. Y. Du, R. Hughes, S. Bhadra, Y. Jiang, A. Ellington and B. Li, *Sci. Rep.*, 2014, **5**, 11039.
71. J. Joo, D. Kwon, H. Shin, K. Park, H. Cha and S. Jeon, *Sens. Actuators, B*, 2013, **188**, 1250.
72. J. Zhang, Y. Xiang, D. E. Novak, G. E. Hoganson, J. Zhu and Y. Lu, *Chem.–Asian J.*, 2015, **10**, 2221.
73. L. Zhang, C. Gu, H. Ma, L. Zhu, J. Wen, H. Xu, H. Liu and L. Li, *Anal. Bioanal. Chem.*, 2019, **411**, 21.
74. (a) Y. Gu, T. Zhang, Z. Huang, S. Hu, W. Zhao and J. Xu, *Chem. Sci.*, 2018, **9**, 3517; (b) J. Joo, D. Kwon, H. Shin, K. Park, H. Cha and S. Jeon, *Sens. Actuators B*, 2013, **188**, 1250.
75. F. Ivars-Barceló, A. Zuliani, M. Fallah, M. Mashkour, M. Rahimnejad and R. Luque, *Appl. Sci.*, 2018, **8**, 1184.
76. J.-Z. Sun, G. Peter Kingori, R.-W. Si, D.-D. Zhai, Z.-H. Liao, D.-Z. Sun, T. Zheng and Y.-C. Yong, *Water Sci. Technol.*, 2015, **71**, 801.
77. S. B. Velasquez-Orta, D. Werner, J. C. Varia and S. Mgana, *Water Res.*, 2017, **117**, 9.
78. Y. Jiang, X. Yang, P. Liang, P. Liu and X. Huang, *Renewable Sustainable Energy Rev.*, 2018, **81**, 292.
79. T. Zhou, H. Han, P. Liu, J. Xiong, F. Tian and X. Li, *Sensors*, 2017, **17**, 2230.
80. D. Davila, J. P. Esquivel, N. Sabate and J. Mas, *Biosens. Bioelectron.*, 2011, **26**, 2426.
81. J. Chouler, A. Cruz-Izquierdo, S. Rengaraj, J. L. Scott and M. Di Lorenzo, *Biosens. Bioelectron.*, 2018, **102**, 49.
82. K. Persaud and G. Dodd, *Nature*, 1982, **299**, 352.

83. S. M. Scott, D. James and Z. Ali, *Microchim. Acta*, 2006, **156**, 183.
84. P. Oliveri, M. C. Casolino and M. Forina, *Adv. Food Nutr. Res.*, 2010, **61**, 57.
85. L. F. Valdez and J. M. Gutiérrez, *Sensors*, **16**, 2016.
86. S. K. Jha, R. D. S. Yadava, K. Hayashi and N. Patel, *Chemom. Intell. Lab. Syst.*, 2019, **185**, 18.
87. K. A. Mirica, J. M. Azzarelli, J. G. Weis, J. M. Schnorr and T. M. Swager, *Proc. Natl. Acad. Sci.*, 2013, **110**, E3265.
88. M. Peris and L. Escuder-Gilabert, *Anal. Chim. Acta*, 2009, **638**, 1.
89. A. P. F. Turner and N. Magan, *Nat. Rev. Microbiol.*, 2004, **2**, 161.
90. Z. Li, J. R. Askim and K. S. Suslick, *Chem. Rev.*, 2019, **119**, 231.
91. N. Savage, *Nature*, 2012, **486**, S18.
92. Y. Tahara and K. Toko, *IEEE Sens. J.*, 2013, **13**, 3001.
93. M. del Valle, *Electroanalysis*, 2010, **22**, 1539.
94. E. Witkowska-Nery, J. A. Guimarães and L. T. Kubota, *Electroanalysis*, 2015, **27**, 2357.
95. R. B. Domínguez, L. Moreno-Barón, R. Muñoz and J. M. Gutiérrez, *Sensors*, 2014, **14**, 17770.
96. X. Cetó, A. González-Calabuig, J. Capdevila, A. Puig-Pujol and M. del Valle, *Sens. Actuators, B*, 2015, **207**, 1053.
97. X. Cetó, F. Céspedes, M. I. Pividori, J. M. Gutiérrez and M. del Valle, *Analyst*, 2012, **137**, 349.
98. G. Valdés-Ramírez, M. Gutiérrez, M. del Valle, M. T. Ramírez-Silva, D. Fournier and J. L. Marty, *Biosens. Bioelectron.*, 2009, **24**, 1103.
99. A. Mimendia, J. M. Gutiérrez, L. Leija, P. R. Hernández, L. Favari, R. Muñoz and M. del Valle, *Environ. Modell. Softw.*, 2010, **25**, 1023.

CHAPTER 4

Innocuous and Less Hazardous Reagents

DOUGLAS E. RAYNIE

Department of Chemistry and Biochemistry, South Dakota State University, Brookings, SD 57007, USA
Email: douglas.raynie@sdstate.edu

This chapter touches on two components of analytical chemistry that can have a significant impact on the greenness of analysis, but that are often overlooked: solvents and reagents. Both are integral parts of sample preparation and analysis. In the ''Twelve Principles of Green Chemistry'' outlined by Anastas and Warner,[1] prevention of waste, atom economy, safer solvents and reagents, energy efficiency, renewability, reducing derivatives, real-time analysis and inherently safer chemistry for accident prevention can be linked to the solvents and reagents chosen for the analytical technique. Green analytical chemistry is influenced by these principles,[2,3] although not all of them may apply in every situation. The goal is to strive for improved greenness and continual improvement.

Several approaches have attempted to bring a level of greenness to the field of analytical chemistry. One review of environmentally friendly extractions described reduced-solvent approaches such as supercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction, solid-phase microextraction and more.[4] Solid-phase microextraction and similar approaches do not use a traditional extraction solvent – rather, a stationary phase is used to adsorb the analyte from the sample solution. These sorbent-based methods are not discussed in this chapter and life-cycle analysis or other green assessments have not been conducted for these

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

methods. A trend towards solvent minimization, beyond the microscale approach, has involved the modification of existing techniques or the creation of new analytical approaches. For example, in 2009, the journal *Spectroscopy Letters* published a series of articles outlining this approach, with techniques such as flow-through solid-phase spectroscopy,[5] direct analysis,[6] tungsten-coil atomic spectroscopy, long-pathlength spectrophotometry, flow-based methodology and surfactant-mediated extractions[7] and miniaturization, reagent replacement, on-line analysis and spectroscopy.[8] Of course, complete elimination of any solvent would be the ideal situation and as a result the use of reflectance spectroscopy is becoming increasingly popular. However, many analytical methods still rely on the use of solvents and reagents and reducing their impact on human health and the environment rather than the specific analytical techniques is the focus of this chapter.

4.1 Green Solvents and Reagents: What This Means

A few research studies have qualitatively and quantitatively measured the greenness of analytical techniques. In one approach, the greenness of analytical methods was considered with respect to four criteria: the use of persistent, bioaccumulative and toxic (PBT) chemicals, the use of hazardous chemicals, corrosiveness based on pH during the analysis and the amount of waste generated from the analysis.[3] The study compared the greenness of over 500 environmental testing methods in the National Environmental Methods Index (NEMI), an on-line database, based on a scale of pass/fail for each specifically defined criterion. More specifically, a method is considered ''less green'' according to the following criteria:[3]

- *PBT:* A chemical used in the method is listed as PBT as defined by the Toxic Release Inventory (TRI) of the United States Environmental Protection Agency (USEPA).
- *Hazardous:* A chemical used in the method is listed on the TRI or one of the US Resource Conservation and Recovery Act (RCRA)'s D, F, P or U hazardous waste lists.
- *Corrosive:* The pH during the analysis is <2 or >12.
- *Waste:* The amount of waste generated is >50 g.

Among the findings,[3] two-thirds of the methods in the NEMI failed the waste criterion, which means that the methods generated more than 50 g of waste. In many of those instances, the large quantities of waste were a result of solvent extractions to isolate the analyte prior to analysis. It should be noted that the study did not account for the amount of solvent used as a mobile phase, such as in high-performance liquid chromatography (HPLC), which would further add to the amount of waste generated in analyses that use carrier fluids or mobile phases. Also adding to waste generation are the strong mineral acids used for the preservation or digestion of samples.

Half of the methods in the NEMI failed the hazardous criterion, primarily because of the solvents and reagents used in the method.[3] Again, those solvents were mainly used for extractions and the reagents for derivatization, digestion or preservation. A small number of methods in the NEMI (5%) failed with respect to the use of PBTs in the analysis, primarily from the use of mercury or lead in some part of the sample preparation. Overall, the greener methods in the NEMI use smaller quantities of less hazardous solvents and reagents.

Although the NEMI is a limited set of methods for analyzing environmental samples, this general methodology and the related knowledge can be applied to all types of analytical methods as a way to compare the relative greenness of methods. For example, the same criteria were applied to each of the laboratory experiments in a popular college-level analytical chemistry textbook.[9]

In another assessment of the greenness of analytical methods, the categories were expanded to include five topics: health, safety, environmental, energy and waste.[10] The criteria are based on toxicity, bioaccumulation, reactivity, waste generation, corrosivity, safety, energy consumption and related factors. Furthermore, each category receives a score on a scale of 1–3 using readily available chemical data. By expanding the categories and scoring scale, the comparison of two analytical methods can provide more information and further distinction between methods.

These are just two examples of evaluating metrics on analytical techniques that provide some measure of greenness. Clearly, it is difficult to define quantitatively how green an analytical method is, let alone the greenness of the solvents and reagents. In the remainder of this chapter, specific types of solvents and reagents will be examined further.

4.2 Greener Solvents

Solvents are vital to the analytical process. In nearly all analytical methods, analytes must be taken into solution for separation, diluted volumetrically to the appropriate analytical concentration and analyzed. When organic solvents are used, issues surrounding toxicity and flammability are often of concern; for aqueous systems, wastewater generation becomes a potential problem. In all cases, the energy of evaporation of non-volatile solvents adds to the green concerns. The largest use of solvents in analytical chemistry is in separation techniques, including extraction and mobile phases in liquid chromatography. This solvent use results in most of the waste generated in an analytical procedure, and this waste may be toxic, flammable or possess other deleterious properties.

Solvents are probably the most active area of green chemistry research[11] and several studies have addressed the topic,[12–14] usually from a focus on synthetic chemistry. Directions in green solvents have been identified as (1) replacement of hazardous solvents with those that show better environmental, health and safety properties; (2) bio-derived solvents;

(3) supercritical fluids; and (4) ionic liquids (ILs).[15] On the other hand, Jessop informally surveyed key researchers in the field, asking ''If the adoption of greener solvents over the next 20–30 years will reduce environmental damage from human activities, then the adoption of what class of solvents will be responsible for the greatest reduction in environmental damage?''[16] The research leaders answered as follows: supercritical carbon dioxide (30.2%), water (22.9%), organic solvents (18.8%), ILs (12.5%), switchable solvents (6.3%), glycerol (4.2%), solventless (3.1%) and bio-derived solvents (2.3%). This contrasts with actual publication numbers. Jessop reported that during the first 9 months of 2010, research articles in *Green Chemistry* focused on ILs (41.0%), water (28.4%), solventless methods (11.5%), carbon dioxide (7.1%), glycerol and ethers (3.3%) and all others (including alcohols, switchable solvents, liquid polymers, fluorous solvents and methyltetrahydrofuran) (8.7%). However, these solvents were primarily used as reaction media in syntheses, catalyst recovery following synthesis and biomass processing, rather than for analytical chemistry.[16]

Jessop set out the challenges that researchers should adopt to ensure that green solvents are available as replacements for any type of non-green solvent, requiring detailed characterization such as using the Kamlet–Taft solvatochromic parameters, recognizing green solvents through energetic, environmental impact or life-cycle assessment approaches, developing an easy to remove polar aprotic solvent and eliminating the need for distillation.[16] From an analytical perspective, the ''easy to distill'' criterion can be changed to ''easy to concentrate for analysis'', generally by evaporation or solvent exchange.

When selecting a solvent for an analytical procedure, criteria such as solute solubility, viscosity and compatibility with the analytical method are still the major factors. However, cost and ''greenness'' must also be of concern. As previously mentioned, we will reach a fully developed green chemistry awareness when chemists have a more instinctive knowledge of green concerns, similar to their instinctive knowledge of solute solubility. This recognition of what is a green solvent is partially developed for traditional molecular solvents, but is lacking for alternative solvents such as supercritical fluids and ILs. Additionally, the search for green solvent alternatives in a given analytical procedure can take the approach of finding a green solvent replacement or finding an approach that will make the overall analytical method greener. In the context of green solvent research, bio-derived solvents have made a minor impact in analytical chemistry, as have switchable solvents. Each of these solvents shows an intriguing potential that may come into play in the future. In this section, we specifically explore supercritical fluids, ILs, water and green organic solvents.

4.2.1 Supercritical Fluids

It is becoming widely known that substances at temperatures and pressures near or above the critical point – supercritical fluids – possess solvent

properties favorable for analytical purposes. As a general rule, these fluids have liquid-like solvating power and gas-like diffusivity. Furthermore, these properties may be varied as a function of temperature and pressure. Perhaps the most important property of supercritical fluids for separation processes is diffusion; after all, regardless of green properties, the solvent must perform. The well-developed rate theory of chromatography relates chromatographic efficiency to solute diffusion, and the hot-ball model of extraction[17] shows that solubility and diffusion are significant in obtaining quantitative extraction yields.

The most widely used fluid is carbon dioxide, with critical parameters of 31.1 °C and 73 atm (7.39 MPa). This fluid has been used extensively in chromatography [supercritical fluid chromatography (SFC)] and extraction [supercritical fluid extraction (SFE)], especially since the 1980s. Carbon dioxide is non-toxic, non-flammable, readily available and inexpensive. As a supercritical fluid, it behaves as a non-polar, or polarizable, solvent and low molar mass alcohols (co-solvents) are often added in small amounts to alter the solvent polarity. Because carbon dioxide can be depressurized to the gaseous state, the solvent is easily removed and supercritical fluid-based separation methods are easily coupled with subsequent analysis. Supercritical carbon dioxide is routinely used for analyses of lipids, essential oils and flavor and fragrance compounds in foods and natural products, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides and related compounds from environmental samples, polymer additives and, less commonly, ions and metals. A recent revival of SFC activity has been spurred by the pharmaceutical industry, looking at the technique for chiral separations, as a replacement for normal-phase liquid chromatography and in process scale-up. Key applications and the current state of the art have been surveyed.[18,19] Fluids other than carbon dioxide have been reported, but these solvents, usually organic, do not possess any distinct advantages, may be flammable and often have high critical temperatures. Some inorganic compounds such as ammonia are neurotoxic and nitrous oxide is a strong oxidizing agent.[20] Water has a very high critical temperature, but has unique properties in the subcritical region and will be considered separately.

4.2.2 Ionic Liquids

ILs have shown increased use in analytical chemistry in the past decade. These liquids are generally composed of large cations and smaller anions, such that the coordination (electrostatic attraction) between the ions is somewhat weak. Hence they are commonly defined as liquids at less than 100 °C. ILs have low volatility, somewhat tunable viscosity and miscibility and electrolyte conductivity. Their interest as green solvents stems primarily from the low volatility, as volatile organic compounds (VOCs) are generally not emitted in IL processes. However, ''green'' is a relative term and several ILs are also toxic. Several reviews have described the use of ILs in analytical chemistry.[21–27] Another review cited the most important advances such as

unique multifunctional ILs and called for an increased understanding of their chemical and physical properties as ILs.[28] An example of the development of task-specific hydrophobic ILs is for the isolation of Cd(II) in water and foods with the workup coupled directly with flame atomic absorption spectrometry.[29]

A limitation to the use of ILs is their viscosity. Thus, a suitable application of ILs in separations is as the acceptor phase in supported-liquid membrane extraction.[30] Similarly, these liquids can be used as stationary phases in chromatography or sorptive extractions. For instance, phosphonium-based liquids were immobilized on solid supports for the preparation of a novel solid-phase system.[31] A non-separation analytical technique taking advantage of the viscosity of ILs is matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, where ionic liquids can replace glycerol or other sample matrices.[32]

While investigating the role of ILs in analyte solvation, the lyotropic theory was employed to consider all ions in solution and describe ion-pairing, ion-exchange and hydrophobic interactions.[33] Similarly, pH and the presence of salts impact the solvating ability and extraction with imidazolium-based ILs.[34]

4.2.3 Water

Liquid water has several desirable (and green) solvent properties. It is non-toxic, safe, inexpensive, pure and readily available. Water can dissolve a host of polar and ionic materials and is denoted the universal solvent. On the other hand, liquid water has two decidedly non-green concerns – clean-up of the wastewater generated and the energy necessary for solvent removal. Supercritical water is an oxidizing agent that is rarely used analytically because of its extreme critical parameters of 374.1 °C and 218 atm (22 MPa).

A unique, green use of water is in hot-water extraction, also called near-critical extraction, subcritical water extraction or pressurized hot-water extraction. Water under these conditions is also being used as a chromatographic mobile phase. There are two modes of water-based separation processes. Under mild heating (perhaps with the application of pressure), the solvating ability towards slightly polar compounds increases, the kinetics are more favorable, diffusion is faster and viscosity decreases. Thus in a system such as accelerated solvent extraction (also known as pressurized solvent extraction), hot water extracts the same types of analyte as in traditional aqueous extractions, but much faster and with up to 95% less solvent use. However, when heated to above its atmospheric boiling point, but under sufficient pressure to keep it in liquid form, water begins to lose its effective polarity. For example, at room temperature water has a dielectric constant of 80, but at 250 °C and 50 bar (5 MPa) the dielectric constant decreases to 27. This relationship between temperature and the dielectric constant of water is shown in Figure 4.1.[35] Under practical conditions, the temperatures employed are generally in the range 100–200 °C, with higher temperatures

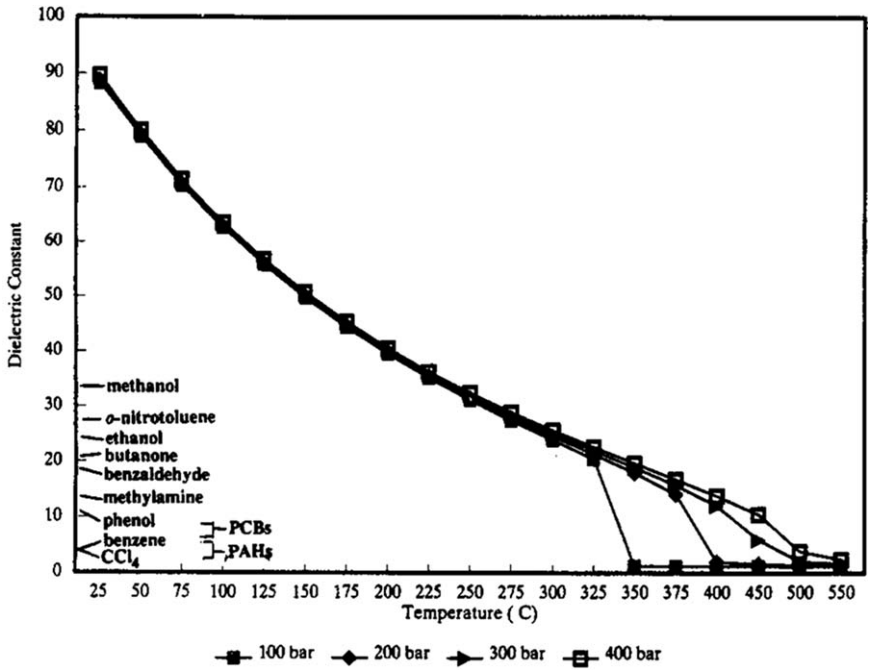


Figure 4.1 Effect of temperature on the dielectric constant of water, with comparison of selected organic compounds.
Reproduced from ref. 35 with permission from American Chemical Society, Copyright 1990.

reserved for the most non-polar analytes. The hot-water extraction approach can be used for aliphatic and aromatic hydrocarbons and other solutes of environmental interest. As the temperature increases and the effective polarity decreases, previously water-insoluble compound classes can be extracted with water. This approach was first used in the mid-1990s[35] but has been slow to catch on because of a lack of commercial instrumentation and the high temperatures required.

It has been shown that temperature is the most important consideration in these extractions and that time and flow rate are most important when the extraction is solubility limited.[36] This verifies the thermodynamic and kinetic models of hot-water extraction previously developed.[37] As a consequence, and verified by on-line monitoring of the extraction process, a static extraction step prior to dynamic extraction is important to shorten the overall extraction time.[38] However, one concern with performing extractions at these high temperatures is analyte decomposition. This has been studied during the isolation of anthocyanins from red onion at 110 °C.[39] Both extraction and degradation rates were determined and used, for example, to explain the overall extraction rate curve shown in Figure 4.2. Here, the extraction follows the previously described thermodynamic hot-ball model (sollid and dashed curves) while analyte degradation occurs simultaneously

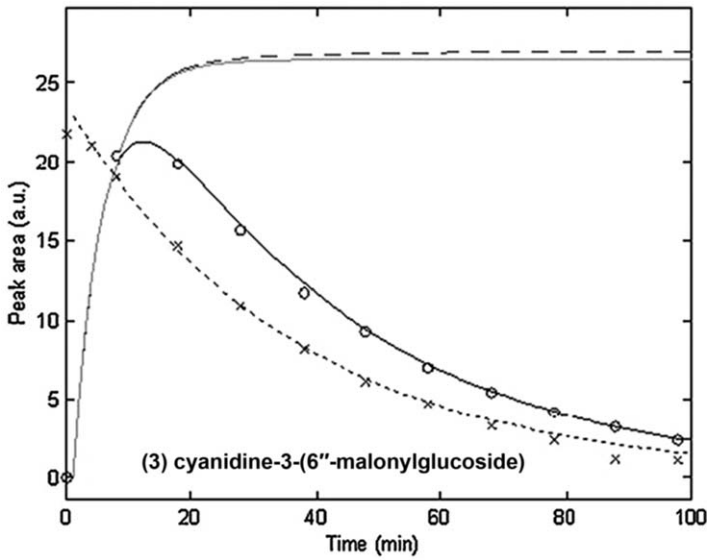


Figure 4.2 Extraction rates for the isolation of cyanidine-3-(6″-malonylglucoside) from red onion using water at 110 °C. The upper trace represents the theoretical rate of extraction and the lower curve represents the measured analyte degradation rate. The resulting curve (middle trace) is the actual observed extraction rate.
Reproduced from ref. 39 with permission from Elsevier, Copyright 2010.

(indicated by X). The middle curve (indicated by circles) represents the actual, observed extraction rate. However, since these extractions occur in a closed (air-free) system, if the water or other solvent is degassed, analyte decomposition is less of a concern.

4.2.4 Green Organic Solvents

To consider green organic solvents, we can break them down into two classes: ''traditional'' organic solvents and non-traditional, ''newer'' organic solvents. The green attributes of traditional solvents have been compared and contrasted to a greater extent in research studies, and the newer solvents have more recently been employed to address some specific aspects of green chemistry, such as renewability and inherent safety. Although many studies of greener traditional and newer solvents have been performed in other contexts, particularly in synthesis, the same conclusions can be carried over to the use of these solvents in analytical chemistry.

A comprehensive investigation of 26 solvents used an environmental health and safety (EHS) approach combined with life-cycle assessment (LCA).[15] The EHS approach examined nine categories: release potential, fire/explosion, reaction/decomposition, acute toxicity, irritation, chronic toxicity, persistence, air hazards and water hazards. The results from the EHS

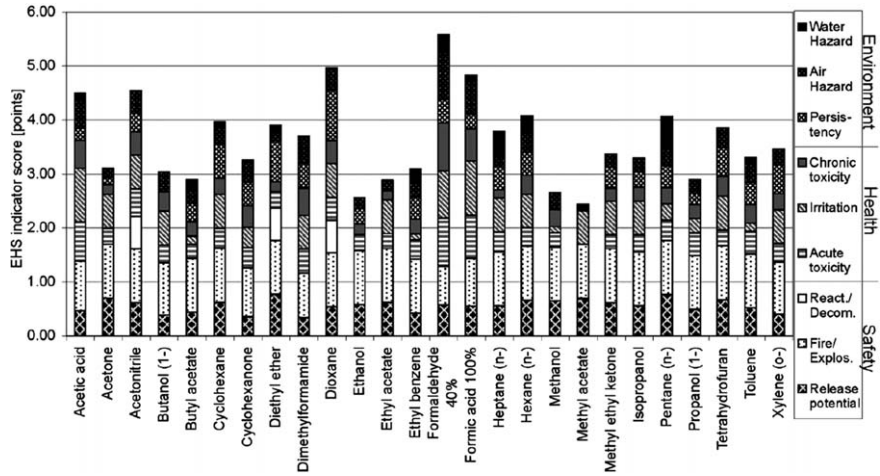


Figure 4.3 Environmental, health and safety assessment of 26 common organic solvents. The lower the indicator score, the more favorable is the green assessment of the solvent.
Reproduced from ref. 15 with permission from the Royal Society of Chemistry.

approach are summarized in Figure 4.3. The combined EHS and life-cycle results were evaluated by Pareto analysis, with the EHS-preferred conventional organic solvents being methanol, ethanol and methyl acetate, whereas the life-cycle-preferred solvents were hexane, heptane and diethyl ether. However, it should be noted that hexane has significantly greater toxicity concerns than other aliphatic hydrocarbons. Petroleum-based solvents require fewer processing steps, so their environmental impact is lower than, for example, that of tetrahydrofuran and cyclohexanone. Solvent mixtures have not been extensively examined and mixtures, such as hexane–acetone, can have an even greater toxicity than the individual components. Non-recommended solvents (from an environmental perspective) were dioxane, acetonitrile, acids, tetrahydrofuran and formaldehyde; other solvents with a high environmental impact included pentane, cyclohexane, dimethylformamide and cyclohexanone. From the LCA perspective, methanol and ethanol are preferred solvents. When the EHS and LCA methods are combined, such as displayed in Figure 4.4, compromises must be made in assessing greenness. The most preferred solvents, found in the lower left quadrant, include methanol, ethanol and a cluster of 10 assorted solvents. However, no solvent appears truly green by both methods, so compromises must be made during solvent selection. It has also been pointed out that ILs and supercritical carbon dioxide were not included in the study because of a lack of data.

A similar study examined the cumulative energy demand and environmental effects,[40] as shown in Table 4.1. The results indicated that hexane and cyclohexane were preferred from an energy perspective and ethyl acetate from an environmental perspective.

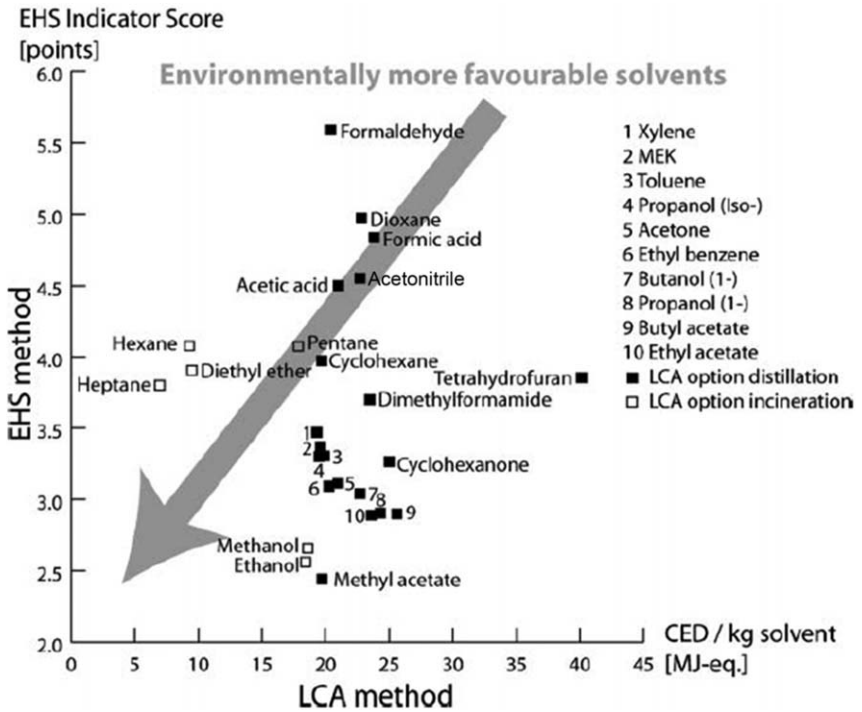


Figure 4.4 Solvent comparison *via* environmental, health and safety and life-cycle considerations. The most favorable solvents from a green perspective are found in the lower left portion of the plot.
Reproduced from ref. 15 with permission from the Royal Society of Chemistry.

Another study applied LCA to solvent selection on 47 solvents.[41] The research built on a previously reported solvent selection guide (relative ranking based on environmental, health and safety issues) to include cradle-to-grave life-cycle impacts. Among the higher scoring (greener) solvents for the life cycle were ethylene glycol, ethanol, *tert*-butanol, methanol, dimethyl carbonate, formamide, acetic acid, methyl *tert*-butyl ether and diisopropyl ether. However, it should be noted that except for ethylene glycol, all other solvents had at least one poor score in another category (environmental waste, environmental impact, health or safety). For solvents with a low (poor) life-cycle score, it was found that solvent recycling or recovery and minimization of use become even more important if the solvent is to be utilized.

Several solvent selection guides have been developed by the pharmaceutical industry and professional organizations, such as the American Chemical Society (ACS) Green Chemistry Institute (GCI) Pharmaceutical Roundtable. These solvent guides have been rigorously reviewed.[42] The impact of the European Union's Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) and other regulations has been discussed.

Table 4.1 Cumulative energy demand and environmental effects of selected solvents. Adapted from ref. 40, with permission from the Royal Society of Chemistry.

	Solvent								
Property	Water	Acetonitrile	Dichloromethane	Ethyl acetate	Benzene	Toluene	*o*-Xylene	*n*-Hexane	Cyclohexane
Cumulative energy demand (CED)									
CED for solvent supply/MJ L^{-1}	0.01	50.6	40.3	43.6	53.0	61.0	60.7	43.2	47.6
CED for heating/MJ L^{-1} [a]	9.0	8.7	Unstable	7.9	8.5	5.4	5.7	Unstable	8.5
CED for workup/MJ L^{-1}	14.0	3.9	2.7	2.5	2.5	2.5	2.6	1.7	2.2
Thermal disposal credit/MJ L^{-1}	0	− 18.0	− 7.0	− 17.1	− 27.9	− 27.7	− 33.2	− 23.7	− 27.3
Environmental effects									
Acute toxicity for humans	Low	Medium	Low–medium	Low–medium	Medium	Low–medium	Medium	Medium	Medium
Chronic toxicity for humans	Low	Low	Medium	Low	High	Low–medium	Low	Medium	Low
Acute toxicity for aquatic organisms	Low	Medium	Low	Low	High	Medium	Medium	Medium	Medium
Persistence in the environment	Low	Low–medium	Low–medium	Low	Low–medium	Low–medium	Low–medium	Low–medium	Medium
Bioaccumulation	Low	Low	Low–medium	Low	Low–medium	Low–medium	Medium	Medium	Medium

[a] $T_{\frac{1}{4}} = 80\ ^{\circ}C$, $t_{\frac{1}{4}} = 5.75$ h.

Solvent guides include those from Pfizer, GlaxoSmithKline, Sanofi, AstraZeneca, Rowan University, ACS GCI and the public–private consortium CHEM21. The methodologies used by each organization vary and may include environmental factors such as waste disposal (either biological or incineration) or recovery, combustion by-product emissions, safety, including flammability and reactivity, physical properties, including boiling point, melting point and surface tension, health and toxicity, solvent source and LCA. A chemometric approach has also been presented. As can be imagined, with these various methodologies, it is difficult to come to a consensus on the green evaluation of solvents. Figure 4.5, for example, presents a simplified evaluation of dipolar aprotic solvents by five different methodologies, and widely disparate opinions on solvent greenness can be seen. A summary of three of the more accepted solvent selection guides is displayed in Table 4.2 with a more fine-tuned solvent classification.

The purpose of showing these various solvent selection guides is not meant to be confusing, but to illustrate the complexity of the issue and also the common themes between the guides. In three of the studies presented,[15,41,42] ethanol is among the preferred solvents. Ethanol–water mixtures are becoming more common as replacements for other solvents in analytical chemistry, particularly in high-performance liquid chromatography (HPLC). Ethanol is considered a greener alternative than methanol and acetonitrile, which are common solvents for HPLC analyses.[43] Examples are the separation of sunscreens and pesticides.[44] A systematic study of two test mixtures, a series of alkylbenzenes and a mixture of compounds of different functional group classes, including caffeine and *p*-hydroxybenzoic acid, demonstrated the ability of ethanol to replace acetonitrile and methanol in HPLC despite its higher viscosity.[45] Method modifications included the solvent gradient, UV detection wavelength, flow rate and temperature. Another HPLC example utilized butyl alcohol and demonstrated comparable results to classical methods using methanol and acetonitrile.[46] In this study, several vitamins (A, E, D_3 and K_1) in food and pharmaceutical supplements were separated using a C_{18} column modified with sodium dodecyl sulfate (SDS) surfactant and elution with a solution of the surfactant in butyl alcohol.[46]

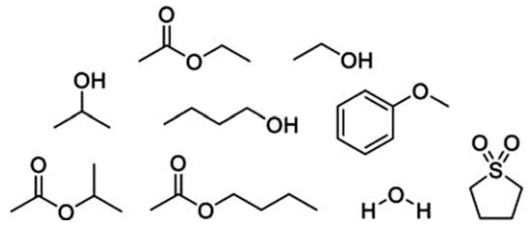
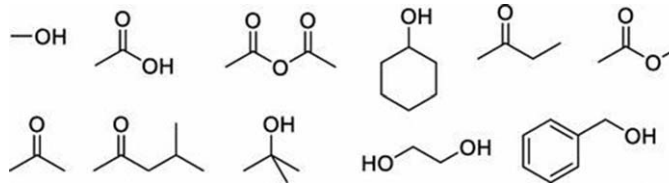
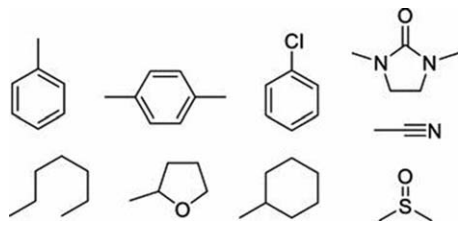
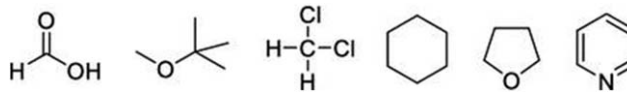
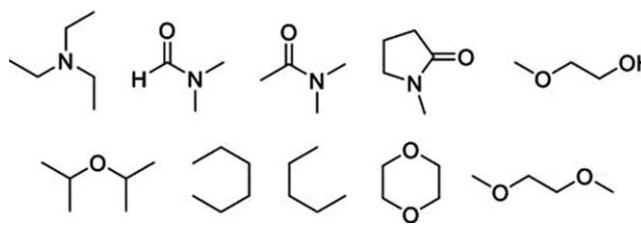
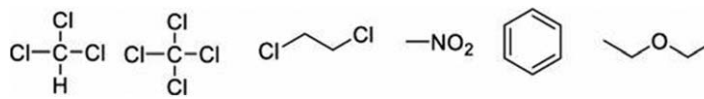
Non-traditional, ''newer'' organic solvents are being studied, in part, because of the greenness that they can offer. Whereas methanol and ethanol can be derived from renewable sources and already find many uses in analytical chemistry, other solvents from renewable sources may have applications as alternative solvents for separations and analyses. Other organic solvents may be favored because they have low volatility and/or are less hazardous.

Ethyl lactate is a renewable solvent that has low toxicity, is biodegradable and is finding industrial uses in the cleaning, pharmaceutical and paint industries.[47] Glycerol, a waste product of biodiesel production, is non-volatile, non-toxic, non-flammable and biodegradable and has been studied as a solvent[48] along with its derivatives.[49] A natural product, D-limonene, has been studied as a replacement for hexane in the extraction of lipids (fats and oils)

Solvent		S	H	E	Conclusion	Survey result
DMSO	*GSK*	R	Y	Y	Y	Problematic
	AZ	G	G	Y	G	
	Sanofi	Y	G	G	Y	
	ACS GCI	G	Y	R	Y	
	CHEM21	G	G	Y	G Y	
Sulpholane	*GSK*	G	Y	Y	G	Recommended
	AZ	G	G	Y	G	
	Sanofi	G	G	G	Y	
	ACS GCI	G	G	R	G	
	CHEM21	G	R	R	R	
Acetonitrile	*GSK*	Y	Y	R	Y	Problematic
	AZ	Y	R	R	R	
	Sanofi	Y	Y	G	G	
	ACS GCI	G	Y	Y	G	
	CHEM21	Y	G	G	G Y	
DMF	*GSK*	G	R	Y	R	Hazardous
	AZ	G	R	R	Y	
	Sanofi	G	R	G	R	
	ACS GCI	G	Y	Y	Y	
	CHEM21	G	R	Y	R	
DMAc	*GSK*	G	R	R	R	Hazardous
	AZ	G	R	R	Y	
	Sanofi	G	R	G	R	
	ACS GCI	G	Y	Y	Y	
	CHEM21	G	R	Y	R	
NMP	*GSK*	G	R	Y	R	Hazardous
	AZ	G	R	R	G	
	Sanofi	Y	R	G	R	
	ACS GCI	G	Y	Y	Y	
	CHEM21	G	R	R	R	
DMPU	*GSK*	Y	Y	R	Y	Problematic
	AZ					
	Sanofi	Y	Y	G	Y	
	ACS GCI					
	CHEM21	G	Y	R	Y	

Figure 4.5 Comparison of dipolar aprotic solvents *via* methods developed by GlaxoSmithKline (GSK), AstraZeneca (AZ), Sanofi, the ACS Green Chemistry Institute (ACS GCI) and CHEM21. S, safety; H, health; E, environment; R, red; Y, yellow; G, green. DMF, dimethylformamide; DNAc, *N*,*N*-dimethylacetamide; NMP, *N*-methylpyrrolidone; DMPU, *N*,*N*′-Dimethylpropyleneurea.
Reproduced from ref. 15 with permission from the Royal Society of Chemistry.

Table 4.2 Solvent ratings from the GSK, AstraZeneca and ACS GCI solvent selection guides. Adapted from ref. 42, https://doi.org/10.1186/s40508-016-0051-z, under the terms of the CC BY 4.0 licence, https://creativecommons.org/licenses/by/4.0/.

Category	Solvents
Recommended	
In between recommended and problematic	
Problematic	
In between problematic and hazardous	
Hazardous	
Highly hazardous	

from food using Soxhlet extraction and Clevenger distillation.[50] Another renewable solvent, 2-methyltetrahydrofuran (2-methyl-THF) has been demonstrated as a replacement for THF and other solvents,[51] although peroxide formation in non-stabilized 2-methyl-THF has been demonstrated.[52]

Some other types of solvents are not renewable but have other advantages. Cyclopentyl methyl ether (CPME) has been studied as a replacement for other ethereal solvents and shows lower peroxide formation than 2-methyl-THF and THF.[53] Poly(ethylene glycol)s with a range of molecular weights and properties have low toxicity and low volatility and are biodegradable.[54]

Another new class of solvents worth mentioning is switchable solvents.[55,56] For green analytical chemistry, the advantage of switchable solvents is in separations. These solvents are designed to react to a trigger, *e.g.* exposure to carbon dioxide or nitrogen, by switching a property such as polarity or hydrophilicity, thus causing a separation. Furthermore, the switchable solvent can be restored to its original composition and recycled in the process. Some other classes of solvents that have been applied in green chemistry, not included in this chapter, are fluorous solvents and deep eutectic solvents.

Beyond conventional solvents, whether traditional or not, other alternatives for HPLC mobile phases are sought.[57] These may include micelle-forming reagents, cyclodextrins, ILs, carbon dioxide and superheated water. The use of such alternatives may greatly reduce the organic solvent consumption in HPLC. However, these alternatives have yet to gain mainstream acceptance, but should be viewed with promise.

4.3 Greener Reagents

Reagents are used to a lesser extent in analytical chemistry. Three instances where reagents are used are for chelation, derivatization and preservation. Chelating agents permit the analysis of ions in solution. They are high molecular weight auxiliaries that bind with metal ions and ultimately become part of the waste stream. The most common, ethylenediaminetetraacetic acid (EDTA), is hazardous, cumulative and persistent. Reagents are also used to prepare derivatives of the analyte to allow characterization, and these auxiliaries also ultimately become part of the waste stream. With the advances in analytical techniques, derivatization is not as common as it once was, but nonetheless it is still a reagent-using process in analytical chemistry to be considered in relation to the greenness of the method. Lastly, preservatives represent another use of reagents in analytical techniques, particularly for maintaining the composition of aqueous and biological samples prior to analysis. The green chemistry considerations for preservatives include hazardousness and energy consumption. Greener alternatives will be discussed for chelation, derivatization and preservation.

4.3.1 Chelating Agents

Despite the prevalence of chelating agents in sequestering metals during analytical procedures, little research has been carried out on the development of greener chelating agents. Most commonly, EDTA is used both industrially and in analytical laboratories. However, since the rate at which

Table 4.3 Titration of divalent ions with EDDS as a replacement for EDTA.

Ion	Recovery with EDDS titrant relative to EDTA titrant/%
Mn^{2+}	115.3 (RSD 0.44%)
Zn^{2+}	101.1 (RSD 0.19%)
Pb^{2+} (UV–VIS detection)	110.0 (RSD 1.11%)

EDTA is discharged into the environment far exceeds the rate at which it degrades, EDTA is considered bioaccumulative. One isomer of EDTA is ethylenediaminedisuccinate (EDDS) and this, especially the *S*,*S*-isomer, is readily biodegradable. EDDS has found use industrially, for example in laundry detergents. A study by Raynie *et al.* showed that EDDS can successfully replace EDTA in the titration of aqueous divalent cations.[58] For example, using EDDS as a replacement for EDTA in USEPA Method 130.2 to determine water hardness *via* titration using Eriochrome Black T indicator, two analysts reported accuracies of 102% relative to the standard method. The use of EDDS for the titration of other divalent ions is shown in Table 4.3. A similar chelating agent, L-glutamic acid-*N*,*N*-diacetic acid (GLDA), is also suggested for similar analytical chelations. Separately, it was determined that the hydrophobicity of ILs allows their use as chelating agents,[59] but more work is needed in this area.

4.3.2 Derivatization

In the context of this chapter, derivatization refers to chemically reacting the molecule of interest, usually by adding functionality, in order to change the properties of the molecule such that it can be more easily analyzed and characterized.

One of the principles of green chemistry is avoiding the use of chemical derivatives. The reason for this is the contribution of derivatives towards poor atom economy. Atom economy is defined as[60]

$$\text{Atom economy} = \frac{\text{MW desired product}}{\sum \text{MW all reactants}} \times 100\,\%$$

Derivatization adds to the molecular weight of the reactants but is not part of the desired product, thus decreasing the atom economy. Ultimately, derivatization increases the amount of waste generated.

In the early days of chemistry, until analytical instrumentation advanced in its ability to identify chemical structures, derivatization was commonly employed for chemical analysis. In particular, if a chemical did not crystallize, a derivative was formed that would crystallize, for characterization of the chemical by melting point determination. Even today, beginner chemistry students are commonly taught this technique in secondary school and university laboratory experiments.

Derivatization today has become more sophisticated; derivatives of compounds are generated not only for crystallinity, but also for changing the solubility properties of the compound, improving the separation of similar compounds (such as isomers) and allowing detection.

The use of derivatives to change the solubility or retention in chromatography is applied for the separation of isomers and especially for chiral separations for analysis and characterization, particularly for pharmaceutical compounds and other fine chemicals. Chromatographic separations include gas chromatography, planar chromatography and capillary electrophoresis. Derivatization of compounds can also permit spectroscopic detection, using methods such as chemiluminescence, bioluminescence, phosphorescence, infrared spectroscopy and ultraviolet/light-absorbing spectroscopy.

An example where derivatization may be greener than the alternative technique is a protein analyzer technique. CEM Corporation was awarded a Presidential Green Chemistry Challenge Award in 2009 for the Sprint® Rapid Protein Analyzer, which measures protein content, such as in foods (baby formula, pet food, *etc.*) and distinguishes between melamine and protein, unlike the common Kjeldahl method.[61] The method works by tagging histidine, arginine and lysine, common amino acids found in proteins, with a tagging solution containing an acidic group to attach to the basic amino acids and also containing an extensive aromatic group for colorimetric measurement. The bound protein is removed by filtration and the remaining tagging solution is measured by colorimetry. The solution is nontoxic, non-reactive and water soluble and most samples can be run in 2–3 min in comparison with 4 h for the Kjeldahl method, with dramatic waste savings in comparison with the latter method, eliminating 5.5×10^6 lb (2.5×10^6 kg) of hazardous waste in the USA each year.[61] In this example, the use of derivatives for analysis saved tremendous amounts of hazardous waste and time in comparison with the alternative method of analysis.

The next time a procedure calls for derivatization, consider avoiding derivatives if possible. Some questions to ask are as follows:

- Is there another way to separate or analyze without involving derivatives?
- Is there a direct analysis technique available with different instrumentation, without derivatization?

The small quantities associated with analytical samples add up and derivatization increases the amount of waste. If derivatization must be used, consider the health and environmental effects of the chemicals used for the derivatization. Consider if the benefits of derivatization outweigh the consequences.

4.3.3 Preservatives

Preservation of samples before analysis is another aspect to include when considering the greenness of an analytical method. The human health

Table 4.4 Summary of preservation methods. Reproduced from ref. 62 with permission from John Wiley & Sons, Copyright © 2003, John Wiley and Sons.

Sample	Preservation method	Container type	Holding time
pH	—	—	Immediately on-site
Temperature	—	—	Immediately on-site
Inorganic ions			
Bromide, chloride, fluoride	None	Plastic or glass	28 days
Chlorine	None	Plastic or glass	Analyze immediately
Iodide	Cool to 4 °C	Plastic or glass	24 h
Nitrate, nitrite	Cool to 4 °C	Plastic or glass	48 h
Sulfide	Cool to 4 °C, add zinc acetate and NaOH to pH 9	Plastic or glass	7 days
Metals			
Dissolved	Filter on-site, acidify to pH 2 with HNO_2	Plastic	6 months
Total	Acidify to pH 2 with HNO_2	Plastic	6 months
Cr(VI)	Cool to 4 °C	Plastic	24 h
Hg	Acidify to pH 2 with HNO_2	Plastic	28 days
Organics			
Organic carbon	Cool to 4 °C, add H_2SO_4 to pH 2	Plastic or brown glass	28 days
Purgeable hydrocarbons	Cool to 4 °C, add 0.008% $Na_2S_2O_3$	Glass with Teflon septum cap	14 days
Purgeable aromatics	Cool to 4 °C, add 0.008% $Na_2S_2O_3$ and HCl to pH 2	Glass with Teflon septum cap	14 days
Polychlorinated biphenyls	Cool to 4 °C	Glass or Teflon	7 days to extraction, 40 days after
Organics in soil	Cool to 4 °C	Glass or Teflon	As soon as possible
Fish tissues	Freeze	Aluminum foil	As soon as possible
Biochemical oxygen demand	Cool to 4 °C	Plastic or glass	48 h
Chemical oxygen demand	Cool to 4 °C	Plastic or glass	28 days
DNA	Store in Tris-EDTA (pH 8) under ethanol at −20 °C; freeze at –20 or −80 °C		Years
RNA	Deionized formamide at −80 °C		Years
Solids unstable in air for surface and spectroscopic characterization	Store in argon-filled box; mixed with hydrocarbon oil		

effects of preservatives may include toxicity and irritation due to corrosivity and environmental effects may include bioaccumulation and energy usage, depending on the method of preservation.

There are a number of important reasons why certain analytical samples are preserved. Preservatives are used to prevent changes within the sample, including physical change (volatilization), chemical change or reaction (oxidation, photochemical reaction, precipitation, heat-induced reaction) and biological change (degradation, enzymatic reaction, microbial growth). Certain types of samples are more prone to these changes and require some type of preservation, especially aqueous, soil, food and biological samples. There are also instances where a preservative may be used in another part of the methodology, such as in the standard or in the extractant.

Some of the approaches taken to preserve a sample include chemical methods such as addition of an acid or base and non-chemical energy-intensive methods such as freezing, refrigeration, freeze-drying, irradiation and microwave irradiation. Preservation can also be achieved *via* the type of container used and limiting the hold time prior to analysis. A summary of approaches is presented in Table 4.4.[62]

Historically, chemical preservatives have included some hazardous chemicals, such as mercuric chloride, tributyltin, formaldehyde, chloroform and dichloromethane,[63] but now that there is greater awareness of the potential hazards (bioaccumulation, toxicity), many of these chemical preservatives are no longer used. For improving the greenness of preservation, some questions to consider are the following:

- If a hazardous chemical is used as the preservative, is there a less hazardous alternative?
- Can chemical preservation be avoided entirely through a shorter hold time and/or refrigeration?
- If freezing is recommended, is refrigeration an option? If freezing is necessary, can the hold time be minimized?
- If freeze-drying is recommended, is microwave radiation a viable alternative?

Seeking an alternative preservation method can improve the overall environmental profile of the analytical method.

References

1. P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, New York, 1998.
2. M. Koel and M. Kaljurand, *Pure Appl. Chem.*, 2006, **78**, 1993.
3. L. H. Keith, L. U. Gron and J. L. Young, *Chem. Rev.*, 2007, **107**, 2695.
4. M. Tobiszewski, A. Mechlinska, B. Zygmunt and J. Namiesnik, *TrAC, Trends Anal. Chem.*, 2009, **28**, 943.

5. J. F. Garcia-Reyes, B. Gilbert-Lopez and A. Molina-Diaz, *Spectrosc. Lett.*, 2009, **42**, 383.
6. S. Armenta and M. de la Guardia, *Spectrosc. Lett.*, 2009, **42**, 277.
7. F. R. P. Rocha, L. S. G. Teixeira and J. A. Nobrega, *Spectrosc. Lett.*, 2009, **42**, 418.
8. M. L. Cervera, M. de la Guardia, S. Dutta and A. K. Das, *Spectrosc. Lett.*, 2009, **42**, 284.
9. D. C. Harris, *Exploring Chemical Analysis: Lab Experiments*, W. H. Freeman, San Francisco, CA, 4th edn, 2008.
10. D. Raynie and J. L. Driver, *13th Green Chemistry and Engineering Conference*, Washington, DC, 2009.
11. P. Anastas and N. Eghbali, *Chem. Soc. Rev.*, 2010, **39**, 301.
12. W. Leitner, P. G. Jessop, C. J. Li, P. Wassersheid and A. Stark (eds), *Handbook of Green Chemistry—Green Solvents*, Wiley-VCH, Hoboken, NJ, 2010.
13. W. M. Nelson, *Green Solvents for Chemistry: Perspectives and Practice*, Oxford University Press, New York, 2003.
14. F. M. Kerton, *Alternative Solvents for Green Chemistry*, Royal Society of Chemistry, Cambridge, 2009.
15. C. Capello, U. Fischer and K. Hungerbuhler, *Green Chem.*, 2007, **9**, 927.
16. P. G. Jessop, *Green Chem.*, 2011, **13**, 1391.
17. K. D. Bartle, A. A. Clifford, S. B. Hawthorne, J. J. Langenfeld, D. J. Miller and R. Robinson, *J. Supercrit. Fluids*, 1990, **3**, 143.
18. M. Herrero, J. A. Mendiola, A. Cifuentes and E. Ibanez, *J. Chromatogr. A*, 2010, **1217**, 2495.
19. M. Zougagh, M. Valcarcel and A. Rios, *TrAC, Trends Anal. Chem.*, 2004, **23**, 399.
20. D. E. Raynie, *Anal. Chem.*, 1993, **65**, 3127.
21. P. Sun and D. W. Armstrong, *Anal. Chim. Acta*, 2010, **661**, 1.
22. Z. Li, J. Chang, H. Shan and J. Pan, *Rev. Anal. Chem.*, 2007, **26**, 109.
23. X. Han and D. W. Armstrong, *Acc. Chem. Res.*, 2007, **40**, 1079.
24. J. L. Anderson, D. W. Armstrong and G. Wei, *Anal. Chem.*, 2006, **78**, 2893.
25. S. Pandey, *Anal. Chim. Acta*, 2006, **556**, 38.
26. M. Koel, *Crit. Rev. Anal. Chem.*, 2005, **35**, 177.
27. J. F. Lui, G.-B. Jiang and J. A. Jonsson, *TrAC, Trends Anal. Chem.*, 2005, **24**, 20.
28. R. J. Soukup-Hein, M. M. Warnke and D. W. Armstrong, *Annu. Rev. Anal. Chem.*, 2009, **2**, 145.
29. N. Li, G. Fang, B. Liu, J. Zhang, L. Zhao and S. Wang, *Talanta*, 2010, **26**, 455.
30. M. Matsumoto, T. Ohtani and K. Kondo, *J. Membr. Sci.*, 2007, **289**, 92.
31. G. V. Myasoedova, N. P. Molochnikova, O. B. Mokhodoeva and B. F. Myasoedov, *Anal. Sci.*, 2008, **24**, 1351.
32. D. W. Armstrong, L. K. He and M. L. Gross, *Anal. Chem.*, 2001, **73**, 3679.
33. A. Berthod, M. J. Ruiz-Angel and S. Carda-Broch, *J. Chromatogr. A*, 2008, **1184**, 6.

34. J. Fan, Y. Fan, Y. Pei, K. Wu, J. Wang and M. Fan, *Sep. Purif. Technol.*, 2008, **61**, 324.
35. S. B. Hawthorne, Y. Yang and D. J. Miller, *Anal. Chem.*, 1994, **66**, 2912.
36. J. Kronjolm, K. Hartonen and M. L. Riekkola, *TrAC, Trends Anal. Chem.*, 2007, **26**, 396.
37. A. Kubatova, B. Jansen, J. F. Vaudoisot and S. B. Hawthorne, *J. Chromatogr. A*, 2002, **975**, 175.
38. S. Morales-Munoz, J. L. Luque-Garcia and M. D. Luque de Castro, *Anal. Chem.*, 2002, **74**, 4213.
39. E. V. Petersson, J. Liu, P. J. R. Sjoberg, R. Danielsson and C. Turner, *Anal. Chim. Acta*, 2010, **663**, 27.
40. D. Kralisch, A. Stark, S. Korsten, G. Kreisel and B. Ondruschka, *Green Chem.*, 2005, **7**, 301.
41. C. Jiménez-González, A. D. Curzons, D. J. C. Constable and V. L. Cunningham, *Clean Technol. Environ. Policy*, 2005, **7**, 42.
42. F. P. Byrne, S. Jim, G. Paggiola, T. H. M. Pethey, J. H. Clark, T. J. Farmer, A. J. Hunt, C. R. McElroy and J. Sherwood, *Sustainable Chem. Processes*, 2016, **4**, 7.
43. A. I. Olives, V. Gonzalez-Ruix and M. A. Martin, *ACS Sustainable Chem. Eng.*, 2017, **5**, 5618.
44. E. Destandau and E. Lesellier, *Chromatographia*, 2008, **68**, 985.
45. C. J. Welch, T. Brkovic, W. Schafer and X. Y. Gong, *Green Chem.*, 2009, **11**, 1232.
46. V. Kienen, W. F. Costa, J. V. Visentainer, N. E. Souza and C. C. Oliveira, *Talanta*, 2008, **75**, 141.
47. S. Aparicio and R. Alcalde, *Green Chem.*, 2009, **11**, 65.
48. Y. Gu and F. Jérôme, *Green Chem.*, 2010, **12**, 1127.
49. J. I. García, H. García-Marín, J. A. Mayoral and P. Pérez, *Green Chem.*, 2010, **12**, 426.
50. M. Virot, V. Tomao, C. Ginies and F. Chemat, *Chromatographia*, 2008, **68**, 311.
51. D. F. Aycock, *Org. Process Res. Dev.*, 2007, **11**, 156.
52. V. Fábos, G. Koczó, H. Mehdi, L. Boda and I. T. Horváth, *Energy Environ. Sci.*, 2009, **2**, 767.
53. K. Watanabe, N. Yamagiwa and Y. Torisawa, *Org. Process Res. Dev.*, 2007, **11**, 251.
54. J. Chen, S. K. Spear, J. G. Huddleston and R. D. Rogers, *Green Chem.*, 2005, **7**, 64.
55. P. G. Jessop, L. Phan, A. Carrier, S. Robinson, C. J. Dürr and J. R. Harjani, *Green Chem.*, 2010, **12**, 809.
56. D. Vinci, M. Donaldson, J. P. Hallett, E. A. John, P. Pollet, C. A. Thomas, J. D. Grilly, P. G. Jessop, C. L. Liotta and C. A. Eckert, *Chem. Commun.*, 2007, **14**, 1427.
57. A. I. Olives, V. Gonzalez-Ruix and M. A. Martin, *ACS Sustainable Chem. Eng.*, 2017, **5**, 5618.

58. D. E. Raynie, G. Degam, B. A. Anderson and K. J. Odegaard, *14th Green Chemistry and Engineering Conference*, Washington, DC, 2010.
59. K. Kidani, N. Hirayama and H. Imura, *Anal. Sci.*, 2008, **24**, 1251.
60. B. M. Trost, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 259.
61. *The Presidential Green Chemistry Challenge Award Recipients 1996–2010*, United States Environmental Protection Agency, 744K10003, 2010.
62. *Sample Preparation Techniques in Analytical Chemistry*, ed. S. Mitra, John Wiley & Sons, Hoboken, NJ, 2003.
63. *Handbook of Water Analysis*, ed. L. L. M. Nollet, CRC Press, Boca Raton, FL, 2nd edn, 2007.

CHAPTER 5

Greening Sample Preparation: New Solvents, New Sorbents

LOURDES RAMOS

Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain
Email: l.ramos@iqog.csic.es

5.1 Introduction

The term ''sample preparation'' refers to any type of treatment carried out on a representative subsample selected for analysis before final instrumental determination of the target compounds. The goal of the sample preparation step is to present the investigated analyte to the measurement instrument in a form and concentration that allow its unambiguous identification and the accurate determination of its concentration in the original matrix. For obvious reasons, the probability of performing such a type of determination without any previous sample treatment decreases as the sample complexity increases and as the analyte concentration decreases,[1] and becomes virtually impossible when the aim is the determination of trace organic compounds in highly complex matrices, such as most environmental and food samples. For such determinations, highly manipulative and time-consuming multistep procedures are still used for sample preparation, in particular when involving semi-solid and solid samples.[2] In most cases, these conventional (*i.e.* large-scale) analytical procedures involve relatively large amounts of reagents and solvents, which subsequently need to be evaporated and/or treated as toxic wastes. Efforts made in this field in recent decades to

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

approach the principles of green chemistry have resulted in a plethora of novel (and frequently miniaturized) techniques and analytical methodologies, some of which were discussed in the original version of this chapter[3] and in a number of revision papers published since then.[1,2,4] Despite the progress that has been made, significant differences remain in this field depending on the nature of the investigated sample. Although impressive advances have been achieved in the treatment of gaseous, liquid and viscous matrices, developments regarding (semi-)solid samples have been much more limited, most probably because of the lack of appropriate commercial instruments.[2,4] In any case, and despite the many advances, the nature of the solvent used as extractant remains a key aspect limiting the greenness of many of these methodologies.

This chapter reviews developments and innovations introduced in the area of sample preparation during the last decade in line with previous considerations. To avoid as far as possible overlapping with the first edition of this book[3] and with other recently published revision papers (*e.g.* ref. 2 and 5–7), rather than presenting a comprehensive revision of all additions to the field, this chapter aims to present the main analytical preparation techniques in use and their evolution in recent years. Special attention will be paid to current trends and possible avenues of evolution in line with the principles of green analytical sample preparation.[1] In particular, the penetration of novel solvents and sorbents in the field of sample preparation and their impact and influence on greening of the treatment step will be discussed. Attention will be focused on applications dealing with the analysis of minor (*i.e.* trace) organic components in complex matrices (*e.g.* food and environmental samples) owing to the greater difficulty typically associated with this type of determination. Nonetheless, where relevant, application examples from closely related areas will also be discussed where they involve chromatographic (or related separation) techniques for the final instrumental determination. Each section starts with a short description of the basis of the specific technique revised, then the main developments and evolution trends observed in recent years are identified and discussed through representative application studies. Examples dealing with the analysis of real samples are always given preference.

5.2 Solvent-based Extraction Techniques

Despite its recognized shortcomings, *viz.* the formation of emulsions, consumption of large volumes of organic (and frequently toxic) solvent(s) and dilution of the target compounds,[8] liquid–liquid extraction (LLE) remains the reference method of many well-established and official protocols. As for other sample preparation techniques, some of the practical limitations of LLE can be circumvented by simple scaling down of the process. The miniaturization of the LLE process results, on the one hand, in significant

reductions in the consumption of solvent(s) and reagent(s), so reducing waste generation, but also on a faster phase separation and a more favourable phase ratio, overall yielding more efficient, green and rapid analytical processes with improved analyte recoveries.[2] When miniaturized LLE is, in addition, performed with a selective or green solvent alternative to the most frequently used volatile organic solvents (VOSs), the technique results in a really advantageous, simple and cheap approach with many positive features. These considerations explain the increasing use of novel and tailored solvents, in particular ionic liquids (ILs), deep eutectic solvents (DESs) and natural deep eutectic solvents (NADESs), in combination with LLE-based techniques.[9–11] However, it should be mentioned that, when these new solvents are used in an LLE format, their high viscosity frequently makes stirring, heating[12] or shaking (in many instances by the application of ultrasound[13] or bubbling) of the LLE mixtures mandatory to speed up the analyte partition process.

Miniaturized liquid–liquid partitioning is also the basis of a plethora of solvent microextraction (SME) techniques introduced essentially during the last two to three decades and whose principles and main application fields have been discussed in a number of recent reviews[14–16] and a book.[17] The current level of acceptance of SME-based techniques and formats for general use is rather variable. Single-drop microextraction (SDME),[18] hollow fibre-protected solvent microextraction, in both its two- and three-phase formats [HF(2/3)ME],[14] and dispersive liquid–liquid microextraction (DLLME)[15,19] are among the most successful and widely accepted approaches, probably owing to the simplicity of their principles and instrumentation, the flexibility in their operational conditions and the possibility of obtaining ready-for-analysis extracts.

5.2.1 Single-drop Microextraction

In SDME, a microdrop of a water-insoluble solvent (1–8 μL for VOSs and slightly larger for more viscous solvents) suspended at the tip of a gas chromatography (GC) syringe is either immersed in the investigated aqueous sample (typically 1–10 mL) or exposed to the headspace (HS) of the vial that contains it until equilibrium is reached. The latter approach, HS-SDME, is feasible only for the analysis of volatile non-polar analytes (or volatile non-polar analyte derivatives, which can be formed *in situ*), but can be applied to gaseous, liquid or solid samples. The former approach, direct-immersion SDME (DI-SDME), has been demonstrated to be useful for the extraction of relatively non-polar and semivolatile analytes from clean aqueous samples. Its application to relatively complex samples is usually feasible only after sample filtration. In both cases, enrichment factors as large as 300 can be obtained. Once the extraction is completed, the microdrop is drawn into the syringe and the concentrated extract is directly subjected to instrumental analysis, typically by GC.[14,18] In SDME, diffusion governs the transport of the analyte from the drop surface to its inner part. Therefore, provided that the

microdrop was not dislodged or dissolved, the extraction time and efficiency can be favoured by stirring, salting out or heating (including nebulization)[20] of the sample or, alternatively, by bubbling of a certain volume of air into the droplet.[21]

SDME of polar compounds is also possible but requires a modification that results in a three-phase system involving a liquid organic intermediate phase in which the neutralized polar analytes are extracted from the aqueous sample. The neutral analytes preconcentrated in this liquid membrane are then back-extracted into an aqueous microdrop as polar compounds. This microdrop is finally drawn into a syringe for direct liquid chromatography (LC) or capillary electrophoresis (CE) analysis. This three-phase configuration is usually termed liquid–liquid–liquid microextraction (LLLME). The higher stability conferred on the extraction system by the intermediate liquid membrane allows the use of faster stirring rates of up to 1000 rpm *versus* the 300 rpm typically used in the two-phase format unless a different type of set-up was used.[22] In turn, faster stirring results in shorter preconcentration times (typically 15 min) and improved enrichment factors (up to 500) compared with the two-phase SDME systems. In practice, the intermediate organic phase also acts as an organic liquid membrane allowing the simultaneous enrichment and clean-up of the analytes. Consequently, LLLME would be more appropriate for the analysis of relatively complex samples, such as human biological fluids, than the two-phase SDME format.[14]

Although automation continues to be mentioned as one of the main limitations of SDME-based techniques, complete unattended sample preparation should be possible by using any of the modern multipurpose autosamplers that are nowadays commercially available.[23,24] In recent years, research in the field of SDME has been mainly oriented to exploring the possibilities of using extractants alternative to the conventional VOSs used in the early stages of the technique.[2] Among them, ILs,[9] supramolecular solvents[25] and, more recently, DESs[11] are the most representative examples. Selected application studies involving these and other alternative extractant systems for SDME are presented in Table 5.1. ILs are non-molecular solvents that remain as liquids at or near room temperature (in general, defined as less than 150 °C) due to poor coordination of the ions.[9,45] ILs are characterized by their negligible volatility, high thermal stability and low flammability over a wide range of temperatures. These characteristics, together with their high viscosity, the possibility of tuning their physicochemical characteristics by controlling the length and branching of the alkyl groups and/or the nature of the cation with which they are incorporated and their capability of dissolving simultaneously compounds of very different nature, have contributed to the expansion of their use as solvents in SDME in recent years. However, surprisingly owing to the constantly increasing number of ILs synthesized and commercialized, but in agreement with what is observed for other extraction techniques, imidazolium-based ILs are clearly predominant in the application studies reported so far, even although these

Table 5.1 Selected SME application studies involving the use of extractants alternative to conventional VOSs.[a]

Sample	Analyte	Technique	Extractant (ratio or μL)	Extraction time/min	Recovery (RSD)/%	Instrumental technique	Ref.
Vegetable oils	Phenolic acids	UA-LLE	ChCl–ethylene glycol (1 : 2)	15	95–113	LC–UV–VIS	13
Sesame oil	Lignans	UA-LLE	ChCl–*p*-cresol (1 : 2)	35	97–120	LC–UV–VIS	12
Soft beverages and tea	Caffeine	Automated LLE	ChCl–phenol (1 : 3)	—	101–104	LC–UV–VIS	26
Leaves of *Chamaecyparis obtuse*	Volatile active terpenoids	HS-SDME	ChCl–ethylene glycol (1 : 4)	30	79–103	GC–FID	27
Digested fish	Trimethylamine	HS-SDME	(CdSe/ZnS QDs)–$[C_6MIM][PF_6]$ (20)	2	92–106 (4)	Spectrofluorimetry	28
Ground, lake and pool water	Sulfonamides	DI-SDME	$[C_8MIM][PF_6]$ (10)	20	64–116 (4–10)	LC–UV	29
Digested dried sausage	2-Amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline	DI-SDME	$[BMIM][PF_6]$ + *o*-NC + c-CNT (4)	30	90–95 (3)	CE–DAD	30
Ground, surface and wastewater	Chlorophenols	DI-SDME	Decanoic acid (30)	60	79–106 (4–6)	LC–DAD	31
River water	BTEX	HF(2)ME	$[BMIM][PF_6]$ (8)	30	90–112 (1–5)	GC–FID	29
Rain and river water	PAHs	HF(3)ME	[BMIM]Cl (10)	15	91–110 (3–7)	LC–DAD	32
River and farm water	Sulfonamides	HF(3)ME	$[C_8MIM][PF_6]$ with 14% w/v TOPO	480	82–103 (0.2–7)	LC–UV	33
River and tap water	OPPs	SBME	$[C_8MIM][PF_6]$	60	87–104 (1–3)	LC–UV	34

Tap, lake and fountain water	Heterocyclic insecticides	DLLME	$[C_6MIM][PF_6]$ in MeOH (52 mg)	—	79–106 (4–11)	LC–DAD	35
River water and urine	PCBs, PBDEs	TC-DLLME	$[C_8MIM][PF_6]$ in MeOH (40)	3	81–127 (1–6)	LC–DAD	36
Infant formula milk powder	Sulfonamides	UA-TC-DLLME	$[C_6MIM][PF_6]$ in $[C_4MIM][BF_4]$ (70)	15	90–115 (2–8)	LC–DAD	37
Tap, river and wastewater	Aromatic amines	DLLME	[BMIM][PF_6] (50)	—	93–106 (6–10)	LC–UV	38
Red wine, fruit juices	Sudan dyes	DLLME	$[C_6MIM][PF_6]$ (50)	10	68–108 (1–6)	LC–DAD	39
Fruit juice, vegetables	Pesticides	TC-DLLME	ChCl–*p*-chlorophenol (1 : 8.5) (142)	5	56–93 (3–5)	GC–FID	40
Food supplements, herbal tea	Curcumin	VA-DLLME	ChCl–phenol (1 : 4) (400)	2	96–102 (1–6)	LC–UV–VIS	41
Tap, lake and wastewater and fruit juice	Fungicides	UA-DLLME	Tween 80 (0.05 mg)	3	86–115 (4–8)	LC–DAD/ESI–MS	42
Water samples	OPPs	VA-DLLME	Triton X-100	3	82–99 (3–8)	GC–FPD	43
Urine	Non-steroidal anti-inflammatory drugs (NSAIDs)	In-syringe DLLME	[BMIM][PF_6] in MeOH (250)	5	100–106 (3–9)	LC–UV	44

[a]Abbreviations: UA-LLE, ultrasound-assisted liquid–liquid extraction; ChCl, choline chloride; FID, flame ionization detection; $[C_6MIM][PF_6]$, 1-hexyl-3-methylimidazolium hexafluorophosphate; $[C_8MIM][PF_6]$, 1-octyl-3-methylimidazolium hexafluorophosphate; [BMIM][PF_6], 1-benzyl-3-methylimidazolium hexafluorophosphate; DAD, diode-array detection; OPPs, organophosphorus pesticides; SBME, solvent bar microextraction; PCBs, polychlorinated biphenyls; PBDEs, polybrominated diphenyl ethers; DLLME, dispersive liquid–liquid microextraction; TC-DLLME, temperature-controlled DLLME; UA-TC-DLLME, ultrasound-assisted temperature-controlled DLLME; $[C_4MIM][BF_4]$, 1-butyl-3-methylimidazolium hexafluorophosphate; VA-DLLME, vortex-assisted DLLME; ESI-MS, electrospray ionization mass spectrometry; FPD, flame photometric detection.

salts are known to absorb over the entire UV region. Fluoride-containing anions are also widely used (in particular PF_6^- and BF_4^-), although, in the presence of moisture, these anions can produce HF and cause glassware and steel corrosion.[46]

In practice, the usual extractant volumes in IL-based SDME studies are in the 4–10 μL range. However, the high viscosity of these salts (typically 2–3 times greater than those of conventional organic solvents) should allow the use of relatively large drops (up to 20 μL)[47] without the risk of dislodgement even if fast stirring rates or dynamic approaches are used.[48] In addition, the low volatility of ILs prevents solvent volatilization during extraction, making possible the use of higher extraction temperatures. Overall, this results in higher enrichment factors, better extraction efficiencies and improved detectability with shorter extraction times compared with conventional SDME procedures. On the other hand, the high viscosity of ILs makes their handling with microsyringes difficult, a problem that has been solved by attaching a tube with a wider inner diameter to the needle.[49]

Coupling IL-based SDME techniques to LC does not require any special interface or modification of the system. The short retention time of ILs in reversed-phase LC (in general, near the dead volume) favours the chromatographic separation of the IL from the target analytes and can be considered an extra advantage of this coupling. In contrast, the low volatility of ILs would prevent direct injection into GC systems. However, this problem can be solved through the installation of home-made removable GC interfaces[47] or by the modification of a commercial interface.[49] Coupling between IL-based SDME and other separation techniques (*e.g.* CE)[30] or detection techniques (*e.g.* ion-mobility spectrometry)[50] is still relatively rare in the literature.

Recent trends in IL-based DI-SDME include the use of more hydrophobic and hydrolytically stable salts than those based on the use of PF_6^- as anion to minimize drop dissolution.[51] More complex extraction mixtures have also been used in both HS- and DI-SDME to improve the extraction efficiency and selectivity when dealing with the analysis of trace components in complex matrices. In a recent illustrative example of the analytical potential of this type of approach, the feasibility of using 1-benzyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) mixed with oxidized nanofibres of cellulose (*o*-NCs) and carboxylated carbon nanotubes (c-CNTs) for the fast and selective extraction of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline from fried sausage was evaluated and found to be superior to the results observed when each component of the mixture was applied separately.[30] The method provided quantitative extraction of the target compound in 30 min with satisfactory repeatability (3%) and reproducibility (4%), adequate limit of quantitation (LOQ 0.96 $mg\,L^{-1}$) and a satisfactory linear response in the investigated range of 0.1–10 $mg\,L^{-1}$. No matrix interference was reported and the maximum interference levels tolerated for closely related compounds, such as 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline and -[4,5-*b*]pyridine, were 60 and 80%, respectively.

The DESs, also called deep eutectic ILs, low-melting mixtures or low transition temperature mixtures,[11] could be considered as second-generation ILs. The DESs are composed of two or three cheap and non-toxic components capable of self-association, mainly through hydrogen bonds.[52] The resulting eutectic fluid exhibits a melting point much lower than that of each of its individual components and, in general, remains as a liquid at temperatures below 130 °C. DESs share a number of physicochemical properties with the previously described ILs (*i.e.* low vapour pressure and flammability, chemical and thermal stability and high viscosity), which makes them feasible for the same types of applications. However, DESs are safe, biodegradable and cheap because they can easily be prepared from accessible bulk chemicals. These extra notable features have made DESs attract researchers' attention since their first introduction by Abbott *et al.* in 2001,[53] and they could also explain the increasing use of these green solvents in many application areas, in particular in the food and pharmaceutical fields.[54]

In a representative early study, Tang *et al.*[27] reported on the feasibility of choline chloride–ethylene glycol (ChCl–EG) for the HS-SDME of volatile bioactive terpenoids (linalool, α-terpineol and terpinyl acetate) from a slurry of *Chamaecyparis obtusa* leaves. A 2 μL droplet of ChCl–EG (1 : 4) was exposed to the HS of a sealed vial containing 0.30 g of the dried and powdered plant leaves dissolved in 3 mL of methanol. The slurry was heated at 100 °C and the extraction continued for 30 min before any loss of the target terpenoids was reported. The DES droplet was then withdrawn into a GC syringe and subjected to gas chromatography with flame ionization detection (GC–FID) without any extra treatment. Despite the simplicity of the proposed method, its efficiency for the preconcentration of the three terpenoids studied was superior to that obtained by alternative LLE- and ultrasonic extraction (USE)-based methods used as reference methodologies.

Coacervates based on supramolecular assemblies (*e.g.* surfactant micelles) have also been evaluated as extractants in SDME. Coacervates can be considered as multifunctional solvents able to extract a wide variety of analytes owing to the different interactions they can undergo simultaneously. As an example, the feasibility of the approach for the effective SDME of chlorophenols from water samples of different complexities was illustrated by López-Jiménez *et al.*[31] They used tetrabutylammonium hydroxide to induce the formation of vesicular coacervates containing equimolecular amounts of decanoic acid and decanoate and demonstrated that the microextraction with coacervates fitted the thermodynamics and kinetics derived for conventional organic solvents. Consequently, the experimental parameters affecting the extraction process were essentially similar in both cases and both approaches shared their main advantages and limitations. However, the possibility of direct immersion of the drop in the water sample, with consequent simplification of the extraction procedure, and the larger number of interactions possible in the case of vesicular coacervates (Figure 5.1) resulting in improved detectability were identified as specific advantages associated with the use of these types of supramolecular extractants.

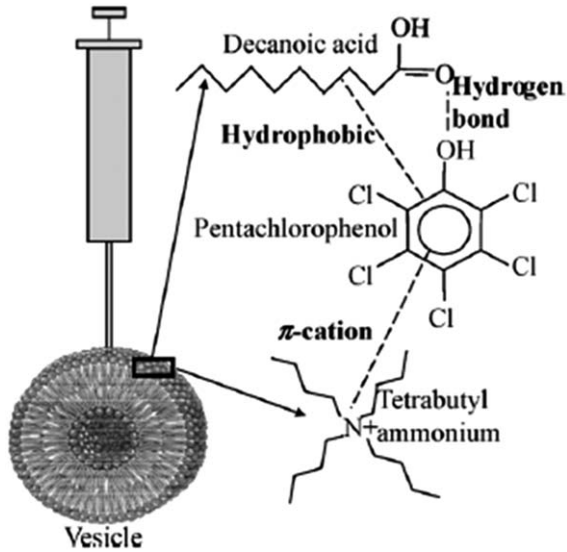


Figure 5.1 Schematic diagram of the different intermolecular forces involved in the extraction of chlorophenols by SDME using decanoic acid vesicle-based coacervates.
Reproduced from ref. 31 with permission from Elsevier, Copyright 2008.

All previously described SDME-based techniques are static in nature and, as a consequence, the main factor controlling the duration and efficiency of the extraction process is the diffusion of the extracted analytes. Although the use of less viscous solvents and higher stirring rates and temperatures can partially contribute to speeding up the diffusion of the target compounds from the surface of the drop to its inner part, constant renovation of the drop surface by using a dynamic approach is probably a more effective approach. So far, two types of dynamic SDME approaches have been described: in-syringe and in-needle SME. In the in-syringe approach, the aqueous sample or HS is repeatedly withdrawn and ejected into the syringe needle or lumen containing the receiving organic phase.[55] In contrast, in the in-needle approach, around 90% of the extraction drop is withdrawn into the syringe needle and then pushed out again repeatedly for sample exposure.[56] The in-needle approach may be feasible for the treatment of samples containing relatively high amounts of matrix components that could affect the subsequent instrumental analysis. The in-syringe approach is limited to the treatment of relatively pristine samples. Interestingly, a modification of this approach has been used for the simultaneous in-syringe derivatization and preconcentration of aliphatic amines from alkalinized aqueous samples.[24] The method, involving instrumental determination of the analyte by LC with fluorescence detection, provided limits of detection (LODs) below 19 $ng\,mL^{-1}$ and a linear response in the evaluated range of 25–500 $\mu g\,L^{-1}$.

5.2.2 Hollow Fibre-protected Two/Three-phase Solvent Microextraction

In its simplest version, the hollow fibre-protected two-phase solvent microextraction, HF(2)ME, technique involves a small-diameter microporous polypropylene tube (the hollow fibre), usually sealed at one end, containing the organic solvent used as extractant. The open end of the hollow fibre is attached to a syringe needle used to fill the fibre with 4–12 μL of the extraction solvent (*e.g.* toluene, undecane, 1-octanol or dihexyl ether). The fibre is then immersed in the investigated aqueous sample for a preselected time (*ca.* 20–60 min) to allow the (more or less) hydrophobic target analytes to migrate through the pores of the wall into the solvent. When the extraction is completed, the enriched solvent is withdrawn with a syringe and directly subjected to instrumental analysis, generally by GC. HF(2)ME can be considered a liquid–liquid membrane extraction and, consequently, it would be more suitable than the equivalent two-phase SDME technique for the treatment of ''dirty'' aqueous samples. Owing to the stability of the system, rapid stirring is possible, which, in combination with the use of relatively large extractant volumes, results in higher enrichment factors compared with SDME. However, the longer extraction times required to complete the extraction process and the fact that only a fraction of the total enriched solvent is used for final determination are usually considered shortcomings of this technique compared with SDME. Although HF(2)ME can be adapted for use with an autosampler,[56] every fibre needs to be manually sized and prepared before use, which is probably the main practical limitation of the technique.

HF(3)ME is operated in a similar way to HF(2)ME but in this case a water-immiscible organic solvent fills the pores of the hollow fibre polymer and a third phase, an aqueous acceptor phase, is used to fill the fibre lumen. Transfer of the target analytes through the three phases involved in the extraction process is controlled through pH changes and, as the final acceptor phase is aqueous, LC or CE is usually preferred for the final instrumental determination of the investigated compounds.

So far, dynamic versions of HF(2)ME has attracted only limited attention,[57,58] probably owing to the complexity of the approach. The same applies to some alternative configurations for HF(2/3)ME, such as solvent bar microextraction (SBME),[59] in which the fibre is sealed at both ends, which allows its complete immersion in the stirred solution and results in improved extraction efficiency.[60] The larger sample volume required for SBME compared with HF(2/3)ME could be a possible explanation for this observation.

HF(2/3)ME is particularly suited for the treatment of size-limited aqueous samples and, at present, one of its more active application areas is the treatment of biological fluids. The polar nature of most of the analytes investigated in this research area made it possible to improve the HF(3)ME process by the application of a potential difference between the sample and

the aqueous acceptor phase. This technique is referred to as electromembrane extraction (EME). EME is intended for charged molecules and involves electrokinetic migration of the analyte through a supported liquid membrane. The membrane prevents major matrix components from reaching the acceptor phase, so EME would be more suitable than HF(3)ME for the treatment of complex biological and environmental samples. The application of an electrical potential increases significantly the mass transfer through the membrane and typically reduces the extraction time from *ca.* 45 min in HF(3)ME to *ca.* 5 min. The basis of EME, recent innovations, including on-chip EME[61] and its hyphenation with UV and mass spectrometric (MS) detectors,[62,63] and also the most relevant application areas of this technique, have been discussed in several recent reviews.[14,24,64] Today, the commercialization of appropriate equipment (including portable instruments) and the still limited understanding of the different parameters affecting the electromigration of analytes through the supported membrane[48] remain the main limitations of this interesting, economic, selective, rapid and efficient novel technique.

In recent years, efforts have been made to improve the selectivity and efficiency of the HF(2/3)ME process by modifying the nature of the membrane by using alternative extractants. This led to increased interest in the use of ILs as solvents in HF(2/3)ME. The high affinity of polar analytes for ILs has been demonstrated to be an advantageous feature in HF(2)ME, yielding high enrichment factors and improved selectivity compared with conventional organic solvents.[29] In HF(3)ME, ILs have typically been used as an intermediate solvent owing their immiscibility in the aqueous sample and the organic acceptor phase. In this configuration, ILs appear to be particularly interesting in applications dealing with the simultaneous extraction of nonpolar and polar analytes from relatively complex aqueous matrices (Table 5.1). In this context, Tao *et al.*[33] illustrated the feasibility of HF(3)ME using 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8MIM][PF_6]$) modified with 14% w/v tri-*n*-octylphosphine oxide (TOPO) as an organic liquid membrane and alkaline water (pH 13, NaOH) as acceptor phase for quantitative extraction of sulfonamides from river and farm water at pH 4.5. The selectivity of the extraction process was demonstrated by successful analysis in the presence of up to 25 $mg\,L^{-1}$ of humic acid and up to 100 $g\,mL^{-1}$ of bovine serum albumin. The low LODs (0.1–0.4 $g\,L^{-1}$) reported for test compounds (sulfadiazine, sulfamerazine, sulfamethazine, sulfadimethoxine and sulfamethoxazole) using LC–UV and the satisfactory repeatability (RSDs better than 7%) of the procedure sharply contrasted with the 8 h required to complete the extraction process.

Carbon nanotubes (CNTs) have also been investigated as alternative extractant media with improved selectivity and efficiency.[39,62,63] Multiwalled carbon nanotubes (MWCNTs) dispersed in 1-octanol were used to fill the pores of the wall of a polypropylene hollow fibre membrane used in an HF(3)ME system employed for the extraction of caffeic acid from *Echinacea purpurea* herbal extracts.[62] The membrane remained stable between the two

aqueous phases, the sample and the acceptor buffer and the analytes were simultaneous sorbed by the nanotubes and the dispersant solvent. Extraction was completed in 25 min and enrichment factors above 2000 were reported. In a subsequent closely related study, the feasibility of this extractant mixture for the HF(2)ME of Brilliant Green from fish pond water was demonstrated.[63] In this case, the extraction was performed in the format of SBME. Quantitative recovery (120%), good repeatability (7%) and an enrichment factor of 799 were reported after 30 min of extraction and using only 5 mL of sample and 3 μL of acceptor phase. Examples of the analytical potential of SBME when using ILs[34] and magnetized ILs[65] as extraction solvents have also been reported.

5.2.3 Dispersive Liquid–Liquid Microextraction

Since its introduction in 2006,[66] DLLME has experienced a rapid evolution and today it is considered a well-accepted technique, in particular for the treatment of liquid samples and extracts.[15] DLLME was originally introduced as a modified miniaturized LLE technique in which a small volume of a water-immiscible solvent (typically 10–50 μL) was dissolved in 0.5–2 mL of a water-miscible solvent, the mixture then being rapidly injected into the investigated aqueous sample (up to 10 mL). The rapid injection of this mixture of organic solvents into the sample led the water-immiscible solvent to be dispersed in the aqueous mass as small microdrops. The promoted increase in the interfacial area between the two phases makes analyte phase transition into the organic extractant rapid and the equilibrium state to be quickly reached. The enriched organic phase is finally separated from the aqueous phase by either centrifugation or freezing (depending on its density) and then directly subjected to instrumental analysis (generally by GC). As initially proposed, the technique was suited for the enrichment of non-polar analytes from pristine aqueous samples. Its application to the analysis of polar analytes required previous water pH adjustment and/or analyte derivatization, the latter usually being performed *in situ* or, preferably, by dispersion of the derivatization agent together with the extractant.[15,19,67] The potential of DLLME for the preconcentration and/or clean-up of analytes from diluted (generally aqueous) extracts obtained from (semi-)solid matrices has also been demonstrated in a number studies.[68–70]

Whatever the goal of the analysis, DLLME can be considered a green, simple, fast and efficient extraction and preconcentration technique (with enrichment factors in the range 100–900). However, it is also a highly manipulative procedure for which even partial automation is difficult. In any case, since its introduction over a decade ago, DLLME has experienced rapid evolution and different modifications to the conventional approach have been introduced to promote further simplification of the process and/or improved efficiency and selectivity, as will be illustrated in the following paragraphs.

The dispersive solvent can decrease the partition coefficient of analytes into the extraction solvent and also complicate subsequent phase separation.[71] However, its use can be avoided by application of an appropriate emulsifier force to the extraction system, for instance, by vortex agitation or ultrasonic irradiation.[72] Vortexing promotes a mild emulsification effect that has been demonstrated to be sufficient, for example, for the quantitative extraction of chlorpyrifos and five pyrethroids from snow water (recoveries 72–102%, RSD better than 11%).[73] In this study, 30 μL of toluene were used as extraction solvent and added to 20 mL of sample. No salt was added and the mixture was vortex mixed for 1 min. A 1 μL volume of the floated solvent was used for subsequent GC with electron-capture microdetection (GC-μECD) for analyte determination. LODs of 3–10 ng L^{-1} and enrichment factors of 835–1115 were obtained. When ultrasound is applied, emulsification is achieved through cavitation, which is a more energetic process that breaks down the dispersed extractant drops and generates smaller droplets immediately after disruption.[74] The efficiency of this emulsification process and the risk of analyte degradation (especially when using ultrasonic probes) mean that short ultrasonication times (typically a few minutes) were generally applied in this type of procedure.

The range of high-density conventional organic solvents used in DLLME is essentially limited to (not really environmentally friendly) chlorinated solvents. Thereby, the interest in using alternative low-density solvents resulted in the development of new DLLME approaches and novel extraction vessels.[75] In an interesting study involving a novel type of extraction vessel, the efficiencies of high- and low-density organic solvents in the DLLME of a broad range of pharmaceuticals using ultrasound-assisted emulsification were compared.[71] Unfortunately, the results demonstrated that, under the experimental conditions proposed, high-density solvents exhibited better performance and reproducibility than low-density solvents. It was concluded that the viscosity and interfacial tension of the solvent had a profound effect on the performance of the method and that its boiling point and solubility determined the volume of extractant collected after DLLME, a variable that affected the reproducibility of the procedure. On the other hand, despite the satisfactory results obtained for the analysis of aqueous samples, the method failed in application to more complex matrices, such as urine and plasma, owing to matrix precipitation. None of the pretreatment procedures that were tried in order to reduce the matrix complexity before DLLME contributed to improving this negative finding, indicating the limitations of this technique for the direct analysis of relatively complex matrices. As indicated previously, in these cases DLLME usually performs better as a preconcentration and/or purification technique.

In recent years, DLLME has greatly benefited from the use of alternative solvents providing improved extraction efficiency and selectivity.[76] Some selected IL- and DES-based DLLME application studies are summarized in Table 5.1.

Hydrophobic ILs, dispersed[35–37] or not[38,39] in a cosolvent, have been used for the rapid, green, simple and miniaturized extraction of heterocyclic insecticides[35] and aromatic amines[38] from aqueous samples, Sudan I–IV from diluted red wine and fruit juices[39] and polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) from acidified and filtered water and urine samples.[36] Strategies to promote IL emulsification when the disperser is not used include fast reinjection of the IL–water mixture into the investigated sample,[38] heating of the mixture[36,37] and the application of auxiliary energy[37] in a manner similar to that described previously for conventional organic solvents. Despite the efficiency of these emulsification approaches, the application of the required additional energy for heating, cooling and/or shaking of the sample represents an extra treatment to be incorporated into the analytical protocol. So far, three different procedures to avoid these time-consuming steps have been described. The first was *in situ* solvent formation, which simultaneously avoided the use of a disperser and contributed to improving the efficiency of the extraction process owing to the larger contact surface between the extraction solvent and the aqueous sample.[77] The second consisted in the addition of unmodified magnetic nanoparticles (NPs) to the IL–aqueous sample to retrieve and separate the enriched IL from the mixture.[78] When the supernatant had been removed, the IL was desorbed from the nanoparticles by washing with an appropriate solvent and directly subjected to instrumental analysis. The third approach was the so-called in-syringe IL-based DLLME and required a simple plastic syringe as extraction unit. In this case, the extractant was rapidly sprayed into the aqueous sample, which was contained in a 10 mL syringe unit, to promote rapid emulsion and analyte extraction. Then, the plunger of the syringe was slowly moved to the initial point, allowing the recovery of the IL from the wall and the lower part of the syringe while the aqueous sample to be analysed was ejected from the unit. Finally, the enriched IL phase was recovered from the syringe tip and subjected to instrumental analysis. The technique was fast and simple, avoided the time-consuming centrifugation step and had potential for automation. Its main limitation was the difficulty of complete recovery of the IL (in the original application example, only 30–40% of the IL was recovered),[44] which could adversely affect the reproducibility of the process. So far, this technique has been used, for example, for the extraction of non-steroidal anti-inflammatory drugs (NSAIDs) from acidified and filtered urine,[44] benzoylurea insecticides from water and tea samples[79] and sulfonamides from serum,[80] the last application requiring the application of ultrasound for IL emulsification and subsequent cooling of the sample for IL recovery.

The feasibility of using other solvents, such as DESs,[40,41] magnetofluids[81] and supramolecular systems,[42,43,82] for DLLME has also been evaluated in a number of interesting application studies. In general, the approaches used in these investigations were similar to those described previously for DLLME with magnetic NPs and/or ILs and shared the same advantages and limitations. However, those involving DESs and supramolecular systems are

usually characterized by short extraction times (1–2 min), the possibility of extracting hydrophilic analytes over a wide range of polarities and avoidance of the use of toxic solvents.[6,11] On the other hand, centrifugation of the mixture is frequently mandatory to promote phase separation (although the possibility of freezing the mixture has also been tried).[40] As a typical example of the enhanced selectivity and improved enrichment provided by DLLME with these green solvents, Figure 5.2 shows the LC–DAD/ESI–MS (liquid chromatography with diode-array detection/electrospray ionization mass spectrometry) traces obtained for a lake water and a 1 : 1 v/v diluted apple juice before and after spiking at the 0.1 $\mu g\,L^{-1}$ level with diethofencarb and pyrimethanil and ultrasound-assisted (UA)-DLLME with 0.05 mg of Tween 80. In this case, 20 μL of carbon tetrachloride were also added to the extraction solution, which was ultrasonicated for 3 min at 25 °C. The emulsion was then disrupted by centrifugation at 350 rpm for 2 min. The organic phase was sedimented at the bottom of the conical centrifuge tube (10 ± 1 μL) and collected for instrumental analysis of the two investigated fungicides. The method provided recoveries in the range 86–115%, RSDs better than 8%, a linear response in the evaluated range of 0.05–2000 $\mu g\,L^{-1}$ and low LODs (0.01 $\mu g\,L^{-1}$). Its potential for the analysis of real matrices was illustrated by successful application to naturally contaminated waters, including tap, lake and wastewater samples.[42]

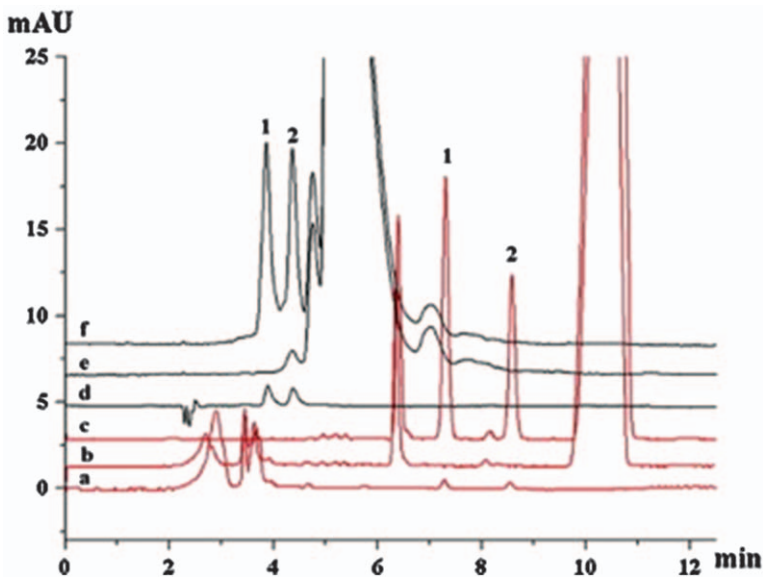


Figure 5.2 Chromatograms obtained for a spiked apple juice (a) before and (c) after UA-DLLME and for a spiked East Lake water sample (d) before and after UA-DLLME; (b) blank apple sample and (e) blank East Lake water. Peaks: (1) diethofencarb and (2) pyrimethanil.
Reproduced from ref. 42 with permission from Elsevier, Copyright 2011.

It is expected that the future development of novel (preferably green) solvents and supramolecular systems with improved extraction capabilities will contribute to expanding the application fields of DLLME in the coming years.

5.3 Sorbent-based Extraction Techniques

Most of the main miniaturized sorbent-based extraction techniques in use in laboratories, namely miniaturized solid-phase extraction (SPE), solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE), were developed years ago and, at present, they can be considered well-established and accepted procedures for the treatment of gaseous, liquid, viscous and solid samples and extracts. In recent years, conceptual additions to this field have been rather limited. However, research concerning these techniques remains active through the development of novel sorbents with improved features regarding selectivity, loading capacity and retention efficiency, with a special focus on analytes that were only slightly retained in previously available materials. Thereby, sorbent-based extraction techniques have benefited from the advances achieved during the last decade in other research areas, including the development of fine-tuned solvents, engineered materials and nanotechnology. In addition, some novel formats and configurations have been introduced. Efforts have focused on the setting up of systems that allow either hyphenation between the extraction technique and the chromatographic instrument used for final analyte determination or increased sample throughput. The feasibility of some techniques for *in situ* and *in vivo* sampling has also been evaluated.

This section reviews the most relevant advances and main achievements reported in recent years in the field of sorbent-based microextraction in the light of previous considerations.

5.3.1 Miniaturized Solid-phase Extraction

On-line SPE is a well-established technique that is routinely used in many laboratories for the preconcentration and clean-up of analytes of different natures from aqueous samples.[83] On-line SPE is typically performed by inserting a short stainless-steel precolumn (10–20 mm×1–4.6 mm i.d.) or a miniaturized SPE cartridge (10 mm×1–2 mm i.d.) packed with the appropriate sorbent in a valve system. After preconcentration and (when required) drying of the cartridge, the analytes are eluted from the sorbent with a small amount of an appropriate solvent (*ca.* 50–100 μL) and transferred directly to the instrument selected for separation and detection of the target analytes. In most applications dealing with the analysis of organic compounds, 10 mL of the investigated aqueous sample suffice for the accurate and sensitive determination of the analytes at trace levels, even for complex matrices such as wastewater. Nevertheless, depending on the sorbent and the selected detection system, volumes as small as 1 mL[84] or as large as 100 mL of sewage

water (500 mL for pure water) can be loaded without breakthrough problems.[85] The entire process takes place in a closed system, which minimizes sample and solvent consumption, avoids sample manipulation and facilitates automated and unattended work. Specific details regarding the on-line coupling of SPE with different chromatographic techniques and detectors have been discussed in more specific reviews.[5,86,87]

Many types of SPE packing materials are nowadays commercially available and many others are synthesized in-house to fulfil the demands of specific application studies. The choice of the SPE sorbent must consider the nature of both the target compounds and the investigated sample and, in the case of on-line SPE, its compatibility with the analytical column phase.

Silica and silica-bonded materials are still the most commonly used sorbents in SPE, including on-line systems. Depending on the nature of the bonded group, packing materials are classified as reversed-phase sorbents (C_8 and C_{18}), normal-phase sorbents (with, *e.g.*, NH_2 and CN groups) or ion-exchange sorbents (either cationic or anionic). Other relevant sorbents include carbon-based sorbents and high-capacity porous polymers, primarily the macroporous poly(styrene–divinylbenzene) and, in particular, its different modifications with enhanced capacity (hypercrosslinked sorbents), polarity (hydrophilic macroporous and hydrophilic hypercrosslinked sorbents) or both simultaneously (dual-phase or mixed-mode sorbents). The improved features of the last sorbents make them particularly suitable for the accurate isolation of polar and ionic species from highly complex matrices through proper pH control of the matrix and desorption solution(s).[87,88] The analyses of trace pharmaceuticals in wastewater,[85] drugs and metabolites in plasma[89] and amyloid β-peptides (early markers of Alzheimer's disease) in cerebrospinal fluid[90] are representative application examples of this type of analytical approach. Alternatively, when dealing with the treatment of very complex matrices containing large amounts of interfering species, compound-specific and class-specific sorbents based on molecular recognition, *e.g.* immunosorbents, molecularly imprinted polymer (MIP) materials and the more recently introduced aptamer-modified surfaces, should be preferred.[91–93] Compared with MIPs and immunosorbents, the preparation of aptamer-modified sorbents is rapid and relatively inexpensive, avoids the use of animals and requires only minute amounts of the analyte used as a template. Recognition can be equally specific and the approach can be applied to both large (*e.g.* proteins)[94] and small (*e.g.* ochratoxin A)[95] molecules. Despite their many positive features, the number of reported application studies involving the use of aptamers as recognition element is still relatively limited.

Immobilization of an IL on to a silica- or polymer-based support allows the sorbent to engage in multimodal-type interactions that have been exploited in a number of applications in recent years.[9,45,96] These materials showed a satisfactory loading capacity (up to 1 L) and a selectivity similar to or better than that provided by conventional sorbents. In addition, polymer-based IL-SPE materials are stable through the entire pH range (unlike silica-based

IL-SPE sorbents, which are typically limited to the pH range 2–8). Detailed descriptions of the different synthetic procedures used for their preparation, in-depth discussions of the possible mechanisms governing IL retention on the sorbent support and subsequent analyte retention in IL-based materials, and also the most appropriate activation protocols, can be found in more specific texts.[45,96]

Most of the IL-SPE sorbents synthesized so far are based on imidazolium-based salts. When applied to the analysis of ionizable and, in particular, anionic compounds, these functional groups promote π–π and anion-exchange interactions, the intensity of the latter depending on the nature of the substitution and the length of the alkyl chain.[97] Nevertheless, the nature of the anion has also been found to play a relevant role in the fine tuning of the retention mechanism. For example, different extraction efficiencies were observed during the preconcentration of acidic pharmaceuticals from environmental waters in a series of IL-SPE-based sorbents, *i.e.* VDC-DVB[MIM][BF_4], VDC-DVB[MIM][CF_3COO] and VDC-DVB[MIM][CF_3SO_3].[98] Whereas similar satisfactory results were obtained with the last two sorbents, that containing [BF_4] showed a poorer retention capability. The higher charge density in this ion compared with the other two was suggested to promote a stronger interaction with the imidazolium groups, which resulted in a reduction of the interactions with the test analytes.

Although anion exchange in combination with reversed-phase interactions appears to be the most rational mechanism for IL-based SPE,[96] these materials have also been applied to the preconcentration of analytes that did not contain any ionizable functional group. In this case, hydrophobic interactions appeared to become dominant, as illustrated for the SPE of 12 sulfonylurea herbicides from water and acidified soil extracts using a novel material obtained by chemical immobilization of a functionalized N-methylimidazolium IL on silica gel as sorbent.[99] Although essentially similar large recovery ranges were reported for both matrices (54–118 and 61–121% for water and soil extracts, respectively), the IL-based SPE procedure showed a satisfactory repeatability (RSD better than 11% in all cases) and an improved selectivity compared with commercial C_{18} SPE cartridges.

Equivalent studies reporting the use of DESs and NADESs grafted on the surface of specific sorbents to yield new materials with improved properties are still limited. In an exploratory study, Gan *et al.*[100] reported on the satisfactory performance of an anion-exchange resin grafted with ChCl–glycerol (1 : 2) for the selective trapping of a neutral aromatic diterpene, cleistanthol, from *Phyllanthus flexuosus* root extracts (recovery 82%) compared with those observed for the raw sorbent (68%) and a conventional C_{18} sorbent (72%). Apart from this enhanced retention capability, the novel sorbent showed improved selectivity for the target compounds compared with the other two materials evaluated. Similarly, the modification of the graphene surface with ChCl–ethylene glycol (1 : 1) showed a superior selectivity and a wrinkled structure that resulted in enhanced sorbent capabilities in the

determination of sulfamerazine from river water compared with the raw graphene. The high loading capacity of this novel DES-modified sorbent (18.62 $mg\,g^{-1}$) resulted in 2 mg of the material being sufficient for the intended SPE determination, which was performed in a pipette tip format.[101]

Interestingly, in a recent study dealing with the SPE of bioactive compounds from extracts obtained from *Artemisia scoparia* (a Chinese herbal medicine), the material resulting from the modification of the surface of a hybrid molecularly imprinted polymer (MIP) with ChCl–glycerol (1 : 3) provided better recoveries of the target compounds (rutin and quercetin) than when the MIP surface was modified with ILs based on 1-methylimidazole.[102] In a follow-up study, the same group observed that the use of a ternary DES mixture (methyltriphenylphosphonium bromide–chalcone–formic acid, 1 : 0.1 : 2) for the DES-based molecularly imprinted solid-phase extraction (MISPE) process[103] contributed to reducing the viscosity and melting point of the original binary DES, while providing similar (for rutin, 92%) or better (for quercetin, 94% *versus* 80%) recoveries of the investigated analytes. These results illustrate the potential of some of these novel DES-modified sorbents for the selective preconcentration of minor compounds from complex extracts and open up new perspectives regarding the development of innovative SPE materials with improved features.

Ionic surfactants can also be sorbed on the surface of active solids such as alumina, silica, titania, iron oxides and nanoparticles, as monolayers or bilayers.[104] In the monolayer structure, the hydrophobic tail of the surfactant is exposed to the sample solution. Thereby, the sorbent obtained, called a hemimicelle, would exhibit affinity towards non-polar analytes. In contrast, in the bilayer structure, called an admicelle, the ionic tail of the surfactant is exposed to the sample, making the sorbent more suitable for the preconcentration (*i.e.* adsolubilization) of polar species. In any case, one of the most interesting features of these types of sorbents is their capability to extract simultaneously analytes of divergent polarities and natures due to their amphoteric nature. Alumina modified with admicelles of sodium dodecyl sulfate (SDS) and tetrabutylammonium (TBA) was used as a mixed-mode sorbent to extract and preserve pesticides with different functionalities from river and underground water. Triazines, carbamates, phenylureas, anilides, chloroacetanilides, organophosphorus compounds and phenoxy acids were considered in the study. Despite the well-known instability of most of these analytes, no degradation was reported for a large majority of the investigated pesticides after 3 months of storage of the cartridges at $-20\ ^{\circ}C$ in the dark (recoveries in the range 70–100% and RSDs better than 8%, except for atrazine and simazine). Interestingly, up to 250 mL of sample were preconcentrated on the cartridges, while analyte elution was performed with only 1 mL of tetrahydrofuran for basic and neutral pesticides, followed by 2 mL of 0.3 M NaOH-methanol (90 : 10 v/v) for the subsequent separate elution of acidic pesticides.

Electrospun polymer nanofibres (NFs) and CNTs have also been evaluated as SPE sorbents.[83,105] The large surface areas of these nanomaterials resulted

in a very large surface-to-volume ratio, which resulted in improved retention capacities even if only a few milligrams of sorbent were used for SPE. The possibility of improving the selectivity of these nanomaterials by modifying their surface is another interesting feature that has been exploited in several recent application studies. For NFs, typical representative examples include the use of polyamide NFs for the on-line SPE of clodinafop-propargyl from water, soil and wheat samples[106] and the development of hybrid composites by immobilizing aptamers specific for the protein thrombin on a polymeric polystyrene–poly(styrene-*co*-maleic anhydride) nanofibre.[107] CNTs have been used for the analysis of non-polar analytes, for which this sorbent shows a selectivity similar to that observed for other carbon-based materials, but with improved concentration capacity (as an indication, it can be mentioned that MWCNTs are able to adsorb up to 10^{34} times more 2,3,7,8-tetrachorodibenzo-*p*-dioxin than conventional carbon[108]). CNTs can also retain highly polar and ionic compounds and their surface can be easily modified by chemical reactions to tune relevant properties of the sorbent, such as polarity, hydrophilicity or surface affinity.[109] Interestingly, several studies have reported on the possibility of modifying the surface of vinyl-functionalized MWCNTs by incorporation of MIPs synthesized *in situ*. This approach has been proved to be effective for the extraction of analytes such as chlorpyrifos[110] and erythromycin[111] from relatively complex extracts with minimal sorbent demands and solvent consumption.

5.3.2 Microextraction by Packed Sorbent

Microextraction by a packed sorbent (MEPS) is a modification of SPE in which a small amount of sorbent packing material (*ca.* 1 mg) is placed at the top of a syringe needle and used to concentrate analytes by successively withdrawing and ejecting the investigated aqueous matrix (typically 10–250 μL). Because of the small volume of solvent used for analyte desorption, the technique is suitable for hyphenation with GC, LC and CE. Sorbent materials for MEPS are similar to those used for conventional SPE, although special phases such as tailor-made materials (*e.g.* MIPs)[112,113] and carbon-based engineered nanomaterials[114,115] can be particularly suitable for this approach. Depending on the complexity of the matrix investigated and on its (possible) pretreatment before MEPS, reuse of MEPS fibres up to 100 times may be possible.[112] MEPS can be performed either manually[113] or using robotic and/or automatic platforms,[116] which contributes to increasing not only the sample throughput but also the performance and accuracy of the sample preparation process.

Since its introduction in 2004, the technique has been used for the determination of, for instance, micropollutants, personal care products and pharmaceuticals of diverse nature in environmental waters, biological matrices and foodstuffs.[113,117–119] However, as for other sorbent-based techniques, MEPS of solid samples is possible only after extraction of the target analytes from the investigated matrix. In addition, depending on the

packing sorbent used, prior solvent exchange can be mandatory. In other words, in these types of applications, MEPS is used as a fast preconcentration technique rather than as a pure extraction procedure. The high complexity of some of these extracts may also make advisable the use of highly selective packing materials that contribute to enhancing the specificity to the process, such as MIPs[113] or IL-modified sorbents.[120] To our knowledge, no study on the feasibility of using DES-modified sorbents in the MEPS format has been reported up to now.

5.3.3 Miniaturized Dispersive Solid-phase Extraction

As evidenced in previous sections, the development of new materials for SPE with improved loading capabilities has contributed to reducing significantly the amount of sorbent required in many applications. This has made possible the scaling down of the dispersive solid-phase extraction (d-SPE) process, yielding a miniaturized version of this technique. Miniaturized d-SPE relies on the improved sorption capacity provided by some of the recently introduced sorbents, in particular nanomaterials. Among them, MWCNTs are frequently preferred as dispersive sorbents because of their large active area and very high absorption capacity. However, the high affinity of this type of sorbent for compounds of very different nature has frequently made necessary the use of a co-sorbent(s) for simultaneous clean-up. This aspect becomes particularly relevant and evident when dealing with the analysis of trace analytes in relatively complex matrices, although its intensity can vary sharply depending on the nature of the target compound. Hou *et al.*[121] compared the dynamic linear ranges and scopes of solution and matrix-matched calibration lines prepared from tea extracts subjected to d-SPE with 6 mg of MWCNTs and 150 mg of PSA (primary–secondary amine) for the simultaneous extraction and purification of selected pesticides. It was concluded that, whereas matrix effects were negligible for some pesticides, for others the use of matrix-matched calibration was highly advisable. In any case, the use of PSA as co-sorbent was mandatory in all cases.

As an alternative, surface-modified materials can be prepared for improved selectivity. In this field, the use of magnetic NPs represents a particularly attractive alternative as these materials can easily retrieved from the extraction solution by applying an external magnetic field, so avoiding the laborious and time-consuming centrifugation or filtration step required for sorbent isolation after d-SPE.[122,123] As an illustration of the typical research carried out in this field and of the potential of the approach, Deng *et al.*[122] reported on the efficiency of amine-functionalized magnetic NP and MWCNT (MNP/MWCNT) composites for the rapid preconcentration and clean-up of pesticides from tea extracts. Amine-functionalized MNPs exhibited weak anion-exchange properties and, consequently, they may interact strongly with various polar organic acids. MWCNTs are able to retain large amounts of pigments and sterols. Composites with an MWCNT : MNP ratio of 3 : 7 were found to provide adequate clean-up of the tea extracts and

good pesticide recoveries (in the range 73–103%) and repeatabilities (RSDs lower than 13%), with LOQs below 0.08 mg kg^{-1} when using GC–MS for the final determination. In another application example, graphene was chemically immobilized on the surface of a silica-coated Fe_3O_4 nanocomposite and used for the enrichment of carbamates from cucumbers and pears. In this case, d-SPE was applied after minimal sample treatment (*viz.* centrifugation and filtration of the supernatant) and LODs below 0.2 ng g^{-1} were obtained using LC with UV–VIS detection.[124] Other examples involving the modification of NPs with ILs can also be found in the recent literature.[125]

Improved specificity can be achieved by the synthesis of molecularly imprinted MWCNTs. This approach was used by Zang *et al.*,[126] who reported the use of magnetic NPs of Fe_3O_4 coated with molecularly imprinted MWCNTs for the determination of bovine serum albumin (BSA) in buffered solutions and bovine serum samples. Sorbent characterization by scanning electron microscopy and Fourier transform infrared spectroscopy suggested that MIPs were successfully immobilized on the surfaces of the MWCNTs and that MIPs were located close to the sorbent surface (Figure 5.3). A small amount of this sorbent, 10 mg, sufficed for the quantitative and specific recovery (92–97%, RSD below 4%) of BSA from 10 mL of aqueous sample. The maximum capacity of the sorbent was estimated to be 52.8 mg g^{-1} and the time required to reach equilibrium at ambient temperature was 40 min. When the extraction step was completed, the sorbent was easily retrieved by the application of a magnetic field and washed with Tris–HCl buffer solution (10.0 mmol L^{-1}, pH 7.0) containing 3.0 mmol L^{-1} of NaCl to remove the nonspecifically adsorbed protein. The specifically adsorbed protein was subsequently recovered with 5.0 mmol L^{-1} NaCl.

Again, owing to the relatively recent introduction of DESs, only a few examples of the performance of DES-modified sorbents in the d-SPE format can be found in the literature. In our opinion, Ghorbanian's group proposed one of the most interesting approaches, which was evaluated for the determination of traces of nitroaromatic explosives[127] and organochlorine pesticides[128] in aqueous samples. In these studies, a colloidal gel of magnetic CNTs and DES was prepared that was used for d-SPE of the target compound. In this gel, the DES, which was compatible with GC, acted simultaneously as both carrier and stabilizer for the magnetic nanotubes, which allowed their easy and rapid dispersion in the bulk sample without any extra shaking of the mixture. The impressive enrichment factors achieved (roughly in the 250–400 range) and low LODs (in the ng L^{-1} range) demonstrated the feasibility of the optimized methodologies for the intended determination.

An alternative format for miniaturized d-SPE consisted of the loose packing of a small amount of an appropriate sorbent on a disposable pipette tip in between two frits. The liquid sample (or extract) is aspirated into the tip and the sorbent–analyte interaction is improved by air bubbling turbulence. After a preselected extraction time of typically less than 1 min, the liquid phase is ejected and, if required, the process is repeated. Then, a

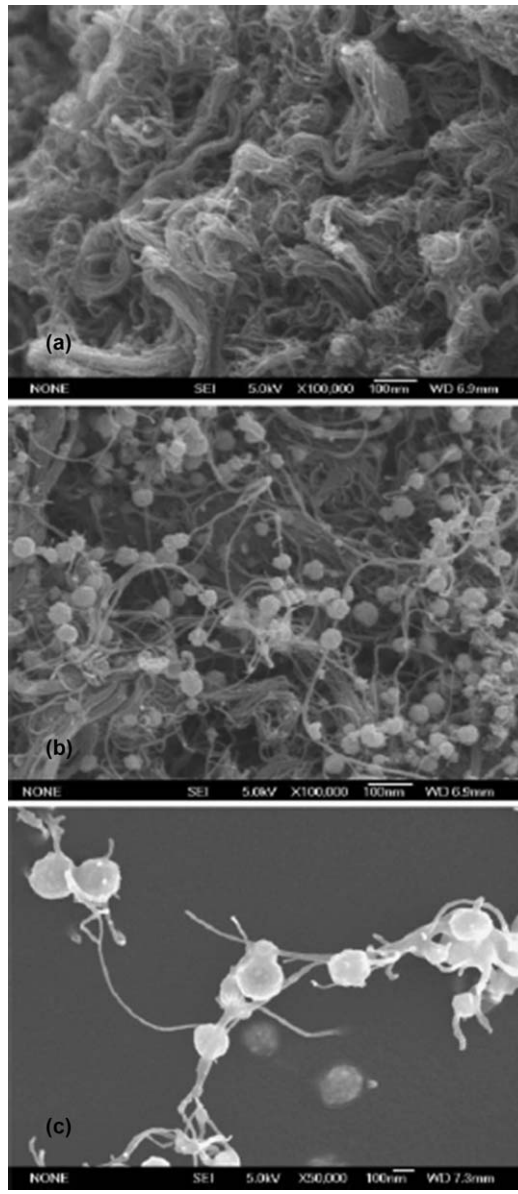


Figure 5.3 Scanning electron microscope images of (a) MWCNTs, (b) MWCNT@Fe_3O_4 and (c) MWCNT@Fe_3O_4-MIPs.
Reproduced from ref. 126 with permission from Springer Nature, Copyright 2011.

small volume of the extraction solvent is aspirated and the target analytes are eluted out of the column. The method is rapid, simple, easy to automate, fulfils the principles of green chemistry (*viz.* minimal reagent and energy

consumption and reduced waste generation) and, owing to its features, it is particularly suitable for the treatment of small-sized samples. The technique, termed disposable pipette extraction (DPX), was initially applied to the preconcentration of drugs from biological fluids[129] and of pesticides from fruit and vegetable extracts.[130] However, more recent applications include, for example, the rapid multiresidue analysis of pesticides[131] and explosives residues[132] and the purification of complex biological extracts during the analysis of PCBs[133] and antibiotics.[134]

The possibility of preparing a home-made 36-syringe d-SPE array by packing the sorbent in a syringe barrel was investigated by Zhu *et al.*[135] The system was applied to the rapid extraction of endogenous cytokinins from *Oryza sativa*. The large surface area of the mesoporous silica fibres used as the sorbent allowed the quantitative recovery of the target compounds with only 15 mg of sorbent. This sorbent format simultaneously contributed to increased solvent flow rates due to the low back-pressure inside the syringe. Efficient sorbent–sample contact and rapid mass transfer were ensured by vortexing of the mixture in the syringe. Under optimized conditions, sample extraction of the analytes from the plant extracts was completed in only 4 min and recoveries in the range 77–107% were reported. The batch-to-batch reproducibility was satisfactory, with RSDs below 13%. Overall, this demonstrates the potential of this type of sorbent for miniaturized, high-throughput and rapid sample preparation on array platforms.

5.3.4 Solid-phase Microextraction

SPME was introduced as a miniaturized (virtually) solvent-free technique for the preconcentration and/or purification of analytes from gaseous, liquid, viscous and solid samples. In its most popular, widely used format, SPME consists of a fused-silica or metal-wire support coated with an appropriate sorbent layer into which the analyte(s) is(are) adsorbed by simple exposure of the fibre for a preselected time to the headspace above the sample (HS-SPME) or alternatively by direct immersion into the investigated aqueous sample (DI-SPME). SPME is an equilibrium (*i.e.* non-exhaustive) technique and, similarly to other non-exhaustive techniques previously considered, analytical strategies such as stirring, heating or derivatization of the sample are common practices to speed up the analyte transfer from the matrix to the SPME fibre coating. Rapid acceptance of the technique as a simple, green, reproducible and miniaturized methodology that could be applied to the analysis of analytes of widely divergent natures contributed to the expansion of its application in different research areas. Today, full automation of the SPME process can be achieved through a number of systems (*e.g.* autosamplers) and different platforms allow on-line coupling of SPME with GC–MS[136] and LC, the latter primarily through the in-tube SPME configuration.[137,138] For high-throughput analysis, novel SPME multiwell plate-based formats has been introduced in recent years.[139] Improved features of the multiwell plate formats over conventional automatic fibre and

in-tube SPME include reduced solvent consumption, low cost, reusability, improved selectivity and compatibility with small-volume samples.[140] The original automated rod-based 96-well plate system[141] evolved rapidly to a thin-film configuration that it is now commercialized as the Concept 96 SPME robotic sample preparation system.[142,143] Compared with the rod-based format, the novel blade format used for SPME in thin-film microextraction (TFME) contributed to increasing the surface area of the solid support exposed to the sample. This resulted in improved mass transfer due to the more favourable extractive phase volume (*ca.* 3.5-fold compared with the original rod-based design). In addition, the blades allowed more effective sample agitation. As a consequence, the technique provided improved sensitivity without sacrificing time. The TFME format also simplified direct coupling with other instrumental techniques, in particular with MS.[136,144] On the other hand, complete immersion of the coating in the sample necessitated increasing the minimum sample volume to 0.8–1.8 mL and the same consideration applies to the amount of solvent required for analyte desorption. In other words, the practicality of the technique for handling size-limited samples becomes compromised.[140] Thereby, for this type of application, the in-tip SPME format can be considered more suitable. This configuration consists of an SPME fibre positioned inside a disposable pipette tip and kept in place with a polyethylene frit, although for enhanced extraction efficiency monolithic phases prepared *in situ* can also be used. The former approach is termed fibre-packed in-tip SPME[145] and the latter pipette-tip SPME.[146,147] In both cases, sorbent conditioning and sample preconcentration and desorption are achieved by successive aspiration and ejection cycles in a manner similar to in-tube SPME. Consequently, although adequate for the handling of small-sized samples (0.1 mL sufficed for some applications), its format prevents application to the treatment of relatively complex matrices. The technique, including its 96-well plate format, can be fully automated using conventional automation systems available in most laboratories for liquid handling, which, in principle, should facilitate its setting up and adoption. As in pipette-tip SPME, the aspiration speed does not affect the precision of the method,[145] and complete sample preparation times in the range 2–6 min have been reported for 96 samples using monolithic sorbents.[148] In-depth discussions of the principles and relative merits of these two approaches for high-throughput analysis, including *in vivo* applications and devices, in addition to emerging application fields, are available in reviews of a more specific nature.[149–152]

Similarly to what is observed for other sorbent-based techniques, in recent years SPME has increasingly turned to the refinement of the analyte retention modes through the development of new coating materials. Achievements in this field have simultaneously contributed to solving some of the most pressing shortcomings of conventional sorbents and to expanding the scope and type of application studies afforded with SPME.[153] The development of biocompatible materials[154,155] allowing *in vivo* studies through the design of a disposable device with a hypodermic needle housing

the fibre[156] and of disk thin films for *in vivo* saliva sampling[157] are considered remarkable and illustrative examples in this context.[152]

Today, apart from the originally introduced non-polar polydimethylsiloxane (PDMS), semipolar polydimethylsiloxane–divinylbenzene (PDMS–DVB), polar polyacrylate (PA), Carbowax–divinylbenzene (CW–DVB) liquid-like phases, coated porous particle phases such as polydimethylsiloxane–Carboxen (PDMS–Carboxen), poly(3-methylthiophene) and Nafion are commercially available. Conductible polymers, in particular polyaniline (PANI)-, polypyrrole (PPy)- and polythiophene (PT)-based phases, have been proved to be effective alternatives for the preconcentration of volatile, polar and ionic compounds from aqueous matrices.[158] Nevertheless, despite the (on many occasions) tailored properties of these fibres, their use remains limited. In recent years, the possibility of improving the analytical performance and thermal stability of these coatings by the incorporation of nanomaterials in their preparation has been evaluated. Mehdinia and Mousavi[159] prepared a nanostructured PANI coating for the first time in 2008. The results revealed that this SPME coating showed a higher extraction efficiency for PCBs than the microstructured PANI coating owing to its nanostructure. When this material was electrodeposited in microemulsions containing [BMIM][PF_6], a composite SPME fibre with an excellent preconcentration capability for organochlorine pesticides (OCPs) from the HS of the investigated aqueous samples was obtained.[160] The fibre was cheap and easy to prepare (the reproducibility between fibres was below 11% RSD), exhibited high thermal stability (up to 350 °C) and, according to the authors, it could be reutilized more than 250 times without any obvious decrease in the extraction efficiency. For this particular application, its analytical performance was superior to that provided by conventional PANI and PDMS SPME fibres. Similar advantageous features were observed when PANI was modified with MWCNTs.[161] In this case, the electrodeposited coating film had a porous structure with higher specific surface area and enhanced adsorption capacity compared with the PANI fibre, as illustrated for the HS-SPME of phenolic compounds from aqueous samples (recoveries in the range 87–112%, RSDs below 7%, fibre-to-fibre RSD lower than 12%). The chemical binding between the Pt substrate and the coating and the interaction of PANI with the CNTs resulted in a material with high thermal stability (up to 320 °C) and excellent reusability (each fibre could be used more than 250 times). Essentially similar conclusions were drawn from a closely related application study involving a PPy–graphene composite fibre and phenolic compounds as target analytes.[162] In this case, the coating thickness (20–30 μm) was controlled through the polymerization time. The extraction efficiency of the PPy–graphene-coated fibre was superior to those of Carboxen–PDMS, polyacrylate, PPy and PPy–graphene oxide. The results reported so far have demonstrated the potential of inexpensive non-covalent procedures for the preparation of thermally stable, homogeneous and high-capacity graphene-based SPME coatings. The improved mechanical stability conferred to the fibre by covalent bonding methods may stimulate an increase in research in this field in the future.

Siliceous nanoparticles and CNTs are attracting increased attention as SPME fibre coatings owing to their large specific surface area and high adsorption capacity. Different procedures can be used for their physical and chemical immobilization on the supporting fibre surface[163] and, although efficient extraction properties have been reported for the unmodified material, their chemical modification frequently resulted in an improved adsorption capability or selectivity.[164,165] This is particularly relevant when MIPs are immobilized on the surface of the fibre coating.[92] Liu *et al.*[166] reported on the benefits derived from combining the high selectivity associated with the use of MIPs as shape-selective recognition elements with the improved extraction efficiency achieved by applying an electrochemically controlled SPME to the extraction of ionic compounds, such as fluoroquinolone antibiotics, from urine and soil extracts. They used a molecularly imprinted polypyrrole (MIPPy)–MWCNT composite coating deposited on a Pt wire. The application of a current potential to the MIPPy–MWCNT–Pt fibre, which was used as a working electrode in a standard three-electrode system, promoted the electrophoretic transfer of fluoroquinolones to the coating surface, from which they entered shape-complementary MIP cavities by hydrogen-bonding and ion-exchange interactions. The preconcentrated analytes were subsequently desorbed by elution with 400 μL of a methanol–acetic acid (80 : 2 v/v) solution and the enriched solvent was analysed by LC–UV. Despite the satisfactory performance and selectivity of the SPME process (recoveries 85–94% for the urine samples and 90–96% for the soil extracts, average RSDs below 7% and LODs lower than 2 $\mu g\,L^{-1}$), it should be mentioned that the time required for the preconcentration step was 60 min.

As for SPE, when ILs are immobilized on to the surface of an SPME fibre, they lose their liquid nature, but provide the modified sorbent with a number of properties that have already been exploited in a number application studies and highlighted in several review papers.[9,153] Physical sorption of ILs on an SPME fibre resulted in relatively weak interactions that negatively affected the coating integrity and extraction capacity in direct immersion applications and also limited the possibility of fibre reutilization. The enhanced stability achieved by chemically bonded IL-based fibres contributed to solving these shortcomings, as demonstrated in a pioneering study by Amini *et al.*,[167] in which the performance of a newly synthesized IL, 1-methyl-3-(3-trimethoxysilylpropyl)imidazolium bis(trifluoromethylsulfonyl)imide, crosslinked to the surface of a fused-silica fibre, was compared with that of the equivalent physically coated fibre in the HS-SPME of methyl *tert*-butyl ether (MTBE) from gasoline. The chemically IL-modified fibres showed improved thermal stability (working temperature up to 220 °C *versus* 180 °C for the physically coated fibre) and reproducibility (RSD 9% *versus* 12%, $n = 6$) and could be reutilized up to 16 times, whereas for the physically coated fibre reutilization was not possible. IL-based fibres with enhanced thermal and chemical stabilities have been prepared by using sol–gel methodology.[168] Improved selectivity and stability have also been achieved by developing functionalized ILs. Although this approach can certainly complicate the

synthesis process,[169] it has been demonstrated to be a valuable alternative that can contribute to expanding the applicability of IL-based fibres.[153]

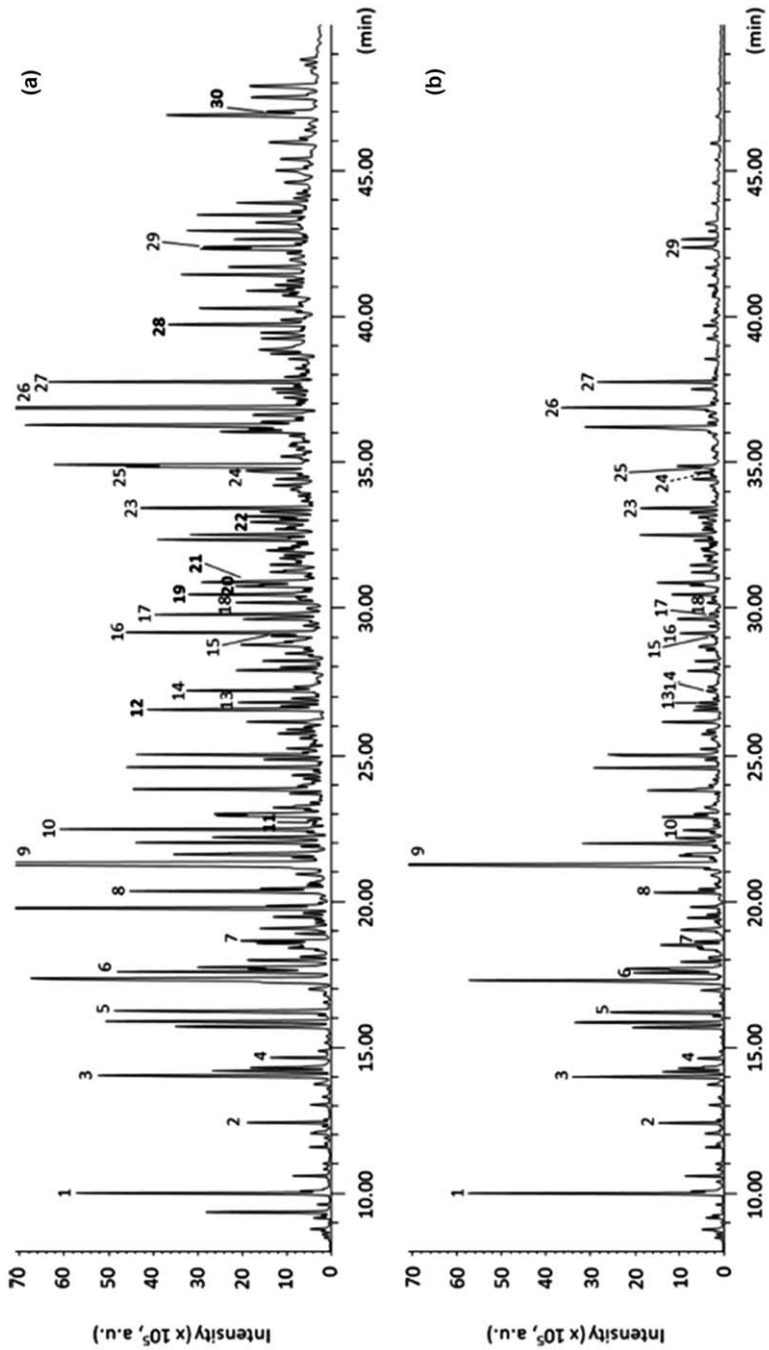
Despite the improved features of some of these materials, the remaining limitations led to the synthesis of so-called polymeric IL (PIL)-based coatings being considered at present one of the most valuable alternatives for preparing IL-based fibres with improved thermal and chemical stability and reproducible films and with lifetimes comparable to those of conventional SPME films.[9] PILs are synthesized from IL monomers and exhibit intrinsic polymer characteristics while retaining the tuneable chemical features of ILs. In general, structurally they are polyelectrolytes that are insoluble in water, which makes them a feasible proposition for direct immersion SPME. In a series of illustrative examples, Anderson's group[170] proposed the use of the PIL poly(1-vinyl-3-hexadecylimidazolium) bis[(trifluoromethyl)sulfonyl]imide {poly([VHDIM][NTf_2])} as a novel SPME fibre coating material for the DI-SPME of 18 pollutants, including polycyclic aromatic hydrocarbons (PAHs) and substituted phenols, from water samples with satisfactory results. Functionalization of the PIL with benzyl groups to yield poly(1–4-vinylbenzyl)-3-hexadecylimidazolium bis[(trifluoromethyl)sulfonyl]imide {poly([VBHDIM][NTf_2])} enhanced π–π interactions between the sorbent coating and the target analytes. This resulted in an impressive selectivity towards the extraction of particular PAHs compared with the non-functionalized PIL, poly{1-vinyl-3-hexadecylimidazolium bis[(trifluoromethyl)sulfonyl]imide} (poly[HDIM][NTf_2]), and substantially lower LODs: 0.003–0.07 $\mu g\,L^{-1}$ for poly([VBHDIM][NTf_2]) *versus* 0.2–0.6 $\mu g\,L^{-1}$ for poly([HDIM][NTf_2]) and 0.1–6 $\mu g\,L^{-1}$ for PDMS.

Replacement of the hydrophobic counteranion used in these PILs with Cl^-, as in poly(1-vinyl-3-hexylimidazolium chloride) (poly[VHIM][Cl]), reduced the coating solubility in organic solvents and improved their affinity for polar compounds.[171] Unfortunately, this simultaneously reduced the thermal stability of the fibre, so compromising the possibility of re-utilization. The preparation of chemically bonded PILs has also been investigated.[172] Despite promising results, the fabrication procedures for this type of fibre still need to be improved to increase the fibre-to-fibre reproducibility and their average lifetime.

In-depth discussions on the advantages, remaining shortcomings and future perspectives for the use of these and other sorbents can be found in recent review papers.[105,153,173]

5.3.5 Stir-bar Sorptive Extraction

Stir-bar sorptive extraction (SBSE) is another interesting, miniaturized, simple and environmentally friendly extraction technique. In its most common format, a magnetic stirring rod contained in a glass jacket covered with an appropriate sorbent is directly immersed in the investigated liquid sample or matrix extract for analyte preconcentration for a preselected (generally fairly long) time. Subsequently, the stir-bar is retrieved from the


(a)
(b)
Intensity (x 10^5, a.u.)
Retention time
(min)
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
0
10
20
30
40
50
60
70

sample and dried and the analytes are desorbed from the enriched sorbent phase either by thermal desorption in the injection port of the gas chromatograph or by elution with a (small) volume of an appropriate solvent for subsequent separation and detection. The SBSE rod can also be exposed to the HS of a vial containing a gaseous, liquid or solid sample, but this approach is much less common. The amount of phase involved in SBSE is *ca.* 100 times larger than that used in conventional SPME. Hence an improved phase ratio is achieved, which yields an improved extraction efficiency and lower LODs compared with SPME. SBSE is currently considered to be a well-established and accepted technique in application fields such as environmental and food analysis and, to a lesser extent, pharmaceutical and clinical research.[174]

Irrespective of the SBSE format, for many years the main shortcomings of the technique were the limited number of commercially available coatings (initially restricted to PDMS) and the difficulty of full automation.[175] The latter remains a handicap for the technique. However, efforts made with the former aspect resulted in the development of, primarily, dual-phase/hybrid twisters, in which the conventional PDME phase was combined with another sorbent to increase the selectivity and/or efficiency of the extraction process.[176] Later, novel coating materials with improved analytical features were introduced,[177,178] although not all of them are commercially available.[174] Alternatively, novel working modes for the simultaneous extraction of analytes with different polarities, such as multishot,[179] sequential extraction,[180] ice concentration linked with extractive stirrer (ICECLES)[181,182] and solvent-assisted SBSE,[183] and new SBSE configurations, such as dual-solvent SBSE, in which the organic phase was confined to a pair of hollow-fibre membranes fixed on a stir bar,[184] have been investigated. The feasibility of SBSE with *in situ* and in-tube derivatization, and also *in situ* deconjugation, has also been demonstrated and discussed in recent review articles.[178,185,186] Despite the improved extraction efficiency provided by some of these approaches compared with conventional SBSE [see Figure 5.4 for a typical

Figure 5.4 Comparison of total ion chromatograms obtained for roasted green tea (Houji-cha) by (a) SA-SBSE and (b) conventional SBSE followed by thermal desorption-GC–MS. Peaks: (1) 1-ethylpyrrole, (2) 2-methylpyrazine, (3) 2,5-dimethylpyrazine, (4) 2,3-dimethylpyrazine, (5) 2,3,5-trimethylpyrazine, (6) furfural, (7) 2-acetylfuran, (8) 5-methylfurfural, (9) 1-ethyl-1*H*-pyrrole-2-carboxyaldehyde, (10) furfuryl alcohol, (11) isovaleric acid, (12) hexanoic acid, (13) guaiacol, (14) benzyl alcohol, (15) maltol, (16) 2-acetylpyrrole, (17) phenol, (18) 2-formylpyrrole, (19) furaneol, (20) methylpyrrole-2-carboxylate, (21) octanoic acid, (22) nonanoic acid, (23) 4-vinylguaiacol, (24) 2,6-dimethoxyphenol, (25) 3-ethyl-4-methyl-1*H*-pyrrole-2,5-dione, (26) 4-vinylphenol, (27) indole, (28) vanillin, (29) methoxyeugenol and (30) raspberry ketone. Analytes 30–36 were not detected with conventional SBSE.

Reproduced from ref. 186, https://doi.org/10.1021/acs.jafc.8b02182, under the terms of the CC BY 4.0 licence, https://creativecommons.org/licenses/by/4.0/.

example involving solvent-assisted SBSE (SA-SBSE)],[186] final acceptance of some of these new SBSE-based working modes for general application studies appears to be rather limited.[174]

Apart from this, a variety of novel coating materials have been tried as SBSE sorbents during the last decade. Among then, monolithic materials have been demonstrated to be particularly suitable for SBSE. Apart from the simplicity and low cost of their preparation, these sorbents show high permeability, which favours mass transfer and contributes to shortening the extraction time. In a study in 2014,[187] the potential of a novel polar monolithic coating, poly(PEGMA-*co*-PETRA), obtained by copolymerization of the monovinyl monomer poly(ethylene glycol) monomethacrylate (PEGMA) with pentaerythritol triacrylate (PETRA) as crosslinking agent, was evaluated for the simultaneous extraction of a group of polar and non-polar emerging microcontaminants from synthetic water. The promising results reported should stimulate research in this field and further evaluation by application to the analysis of more complex matrices. The application of sol–gel technology to the preparation of SBSE coatings resulted in phases with improved thermal and chemical stability, low bleeding and relatively long lifetimes owing to the strong adhesion achieved between the coating and the glass surface.[185] The facility to introduce chemical groups with different functionalities during the preparation of the coating material is an additional advantage associated with this methodology. A novel IL-bonded sol–gel stir-bar coating was prepared by chemically binding an *N*-vinylimidazolium-based IL, 1-allylimidazolium tetrafluoroborate ([AIM][BF_4]), to the surface of a bare stir-bar with γ-(methacryloxypropyl)trimethoxysilane (KH-570) as bridging agent. The material showed satisfactory mechanical strength and durability and provided recoveries from the test water in the range 81–116% for NSAIDs (ketoprofen, naproxen and fenbufen) after 30 min of SBSE.[188] Application to more complex liquid or viscous matrices required prior sample pretreatment, such as dilution and filtration in the case of urine or defatting and protein precipitation for milk. For these types of application studies, coatings with more selective recognition capabilities, such as those based on biocompatible restricted access material (RAM)[189] or, even better, MIP[92,190] coatings, are to be preferred.

As already shown for other sorbent-based techniques, SBSE has also benefited from advances achieved in nanomaterials science. A representative example is the development of a poly(ethylene glycol dimethacrylate)–graphene composite as an SBSE coating.[191] This new material was used for the preconcentration of PAHs from aqueous samples. Compared with the neat polymer, the polymer–graphene composite material showed a much higher specific surface area (4.4-fold increase) and improved affinity for the test compounds. In another study,[192] an amino-modified MWCNT–polydimethylsiloxane [MWCNT–4,42032-diaminodiphenylmethane/polydimethylsiloxane (MWCNT–DDM/PDMS)] was synthesized and utilized for the SBSE of phenols from environmental water and soil samples. Under optimized conditions, the proposed method showed a linear response over three ranges of magnitude and adequate

LODs in the range 0.1–1.8 $\mu g\,L^{-1}$ and provided enrichment factors as high as 63 for some of the target compounds.

Modified magnetic NPs have also been evaluated in the preparation of SBSE coatings with improved features. In contrast to their non-magnetic homologues, magnetic NPs can move (and so be efficiently isolated) by application of an external magnetic field. In a recent study, lipophilic oleic acid-coated cobalt ferrite ($CoFe_2O_4$@oleic acid) magnetic NPs were used as a hydrophobic coating physically supported on a neodymium-core stir-bar to yield an SBSE modification that combined the principles of this technique with those of d-SPE; this novel method was named stir-bar sorptive–dispersive microextraction (SBSDE).[173,193] In a typical experiment, the coated magnetic stir-bar was immersed in a sample solution. Provided that a relatively slow stirring rate was applied, the magnetism was strong enough to retain the modified magnetic NPs attached to the bar surface. Under these conditions, the extraction was performed in a similar manner to SBSE. As the stirring rate increased, centrifugal forces increased and, at a certain point, the NPs were dispersed into the aqueous solution and extraction was afforded as in d-SPE. Once the extraction time was completed, the stirring process was finished. At this point, the strong magnetic field of the stir-bar prevailed again and the dispersed magnetic NPs were rapidly retrieved. Then, the stir-bar was collected and the preconcentrated analytes were back-extracted into an appropriate solvent for subsequent chromatographic separation and detection. The performance of this novel SBSE-based approach was illustrated by successful application to the analysis of eight common UV filters in seawater. The method showed good analytical features in terms of linearity, enrichment factors (11–148), LODs (low $\mu g\,L^{-1}$), intra- and inter-day repeatability (RSD $< 11\%$) and relative recoveries (87–120%). More importantly, it demonstrated the practical potential of this new approach that can easily be adapted for application in other areas, so contributing to expanding the potential of SBSE in the near future.

5.4 Conclusion

The progress achieved in recent decades in the field of sample preparation has yielded a number of novel and frequently miniaturized techniques that have contributed to greening this part of the analytical process. These techniques have contributed to solving, at least partially, some of the most pressing shortcomings of conventional (*i.e.* large-scale) sample treatment procedures, *viz.* consumption of large amounts of sample, reagents and energy, slow analytical response time and waste generation. Today, sample preparation can be completed with minimal sample manipulation, in a short time and, in many instances, in an unattended and/or hyphenated manner, especially in the case of aqueous matrices. Novel sample preparation techniques are also better suited for the treatment of size-limited samples and, when combined with an appropriate instrumental system, proper analyte detection and quantitation are possible even at low trace

levels. In recent years, these novel techniques have greatly benefited from the advances achieved in other research areas, especially those related to the development of new materials and nanotechnologies. Investigations on the feasibility of new safe and non-toxic extraction media with improved analytical features, with IL, DES and supramolecular solvents as representative examples, are a recognizable trend in this field that contributes effectively to the greening of the sample preparation procedures. However, as demonstrated in this chapter, despite the many positive analytical features of most of these novel analytical approaches and techniques, problems remain, so limiting the general acceptance and wider use of some of these approaches and techniques, but also stimulating further investigations in active research areas.

Acknowledgements

The author thanks MICINN, Comunidad of Madrid and European funding from FSE and FEDER for projects AGL2016-80475-R and S2018/BAA-4393 (AVANSECAL-II-CM).

References

1. S. Armenta, S. Garrigues and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2015, **71**, 2–8.
2. L. Ramos, *J. Chromatogr. A*, 2012, **1221**, 84–98.
3. M. Pena-Abaurrea and L. Ramos, in *Challenges in Green Analytical Chemistry*, ed. M. de la Guardia and S. Garrigues, RSC Green Chemistry Series, No. 13, RSC Publishing, Cambridge, (UK), 2011, pp. 107–143.
4. L. Ramos and B. Richter, *LC–GC Europe*, 2016, **29**, 558–568.
5. C. Ribeiro, A. R. Ribeiro, A. S. Maia, V. M. F. Goncalves and M. E. Tiritan, *Crit. Rev. Anal. Chem.*, 2014, **44**, 142–185.
6. A. Spietelun, L. Marcinkowski, M. de la Guardia and J. Namieśnik, *Talanta*, 2014, **119**, 34–45.
7. Q. Liu, Q. Zhou and G. Jiang, *TrAC, Trends Anal. Chem.*, 2014, **58**, 10–22.
8. A. Samsidar, S. Siddiquee and S. M. Shaarani, *Trends Food Sci. Technol.*, 2018, **71**, 188–201.
9. L. Ruiz-Aceituno, M. L. Sanz and L. Ramos, *TrAC, Trends Anal. Chem.*, 2013, **43**, 121–145.
10. M. Espino, M. D. Fernandez, F. J. V. Gomez and M. F. Silva, *TrAC, Trends Anal. Chem.*, 2016, **76**, 126–136.
11. H. Vanda, Y. T. Dai, E. G. Wilson, R. Verpoorte and Y. H. Choi, *C. R. Chim.*, 2018, **21**, 628–638.
12. W. Liu, K. D. Zhang, G. L. Yang and J. J. Yu, *Food Chem.*, 2019, **281**, 140–146.
13. T. Khezeli, A. Daneshfar and R. Sahraei, *Talanta*, 2016, **150**, 577–585.
14. Y. Yamini, M. Rezazadeh and S. Seidi, *TrAC, Trends Anal. Chem.*, 2019, **112**, 264–272.

15. N. Campillo, P. Vinas, J. Sandrejova and V. Andruch, *Appl. Spectrosc. Rev.*, 2017, **52**, 267–415.
16. H. Ebrahimzadeh, F. Mirbabaei, A. A. Asgharinezhad, N. Shekari and N. Mollazadeh, *J. Chromatogr. B*, 2014, **947–948**, 75–82.
17. J. M. Kokosa, A. Przyjazny and M. A. Jennot, *Solvent Microextraction. Theory and Practice*, Wiley, New Jersey, (USA), 2009.
18. S. Tang, T. Qi, P. D. Ansah, J. C. N. Fouemina, W. Shen, C. Basheer and H. K. Lee, *TrAC, Trends Anal. Chem.*, 2018, **108**, 306–313.
19. M. Saraji and M. K. Boroujeni, *Anal. Bioanal. Chem.*, 2014, **406**, 2027–2066.
20. L. Wang, Z. M. Wang, H. H. Zhang, X. Y. Li and H. Q. Zhang, *Anal. Chim. Acta*, 2009, **647**, 72–77.
21. D. B. G. Williams, M. J. George, R. Meyer and L. Marjanoyic, *Anal. Chem.*, 2011, **83**, 6713–6716.
22. H. Bagheri, M. Dehghan, A. Ali Es'haghi and M. Naderi, *Anal. Methods*, 2013, **5**, 4846–4851.
23. E. Ghasemi, *J. Chromatogr. A*, 2012, **1251**, 48–53.
24. Y. Yamini, S. Seidi and M. Rezazadeh, *Anal. Chim. Acta*, 2014, **814**, 1–22.
25. A. Melnyk, L. Wolska and J. Namiesnik, *J. Chromatogr. A*, 2014, **1339**, 1–12.
26. A. Shishov, N. Volodina, D. Nechaeva, S. Gagarinova and A. Bulatov, *Microchem. J.*, 2019, **144**, 469–473.
27. B. Tang, W. Bi, H. Zhang and K. H. Row, *Chromatographia*, 2014, **77**, 373–377.
28. C. Carrillo-Carrion, B. Simonet and M. Valcarcel, *Analyst*, 2012, **137**, 1152–1159.
29. X. Guo, D. Yin, J. Peng and X. Hu, *J. Sep. Sci.*, 2012, **35**, 452–458.
30. C. Ruiz-Palomero, M. L. Soriano and M. Valcárcel, *Talanta*, 2014, **125**, 72–77.
31. F. J. López-Jiménez, S. Rubio and D. Pérez-Bendito, *J. Chromatogr. A*, 2008, **1195**, 725–733.
32. L. Qian and Y. He, *J. Chromatogr. A*, 2006, **1134**, 32–37.
33. Y. Tao, J. F. Liu, X. L. Hu, H. G. Li, T. Wang and G. B. Jiang, *J. Chromatogr. A*, 2009, **1216**, 6259–6266.
34. Y. Zhang, R. Wang, P. Su and Y. Yang, *Anal. Methods*, 2013, **5**, 5074–5078.
35. Y. Liu, E. Zhao, W. Zhu, H. Gao and Z. Zhou, *J. Chromatogr. A*, 2009, **1216**, 885–891.
36. A. Zhao, X. Wang, M. Ma, W. Wang, H. Sun, Z. Yan, Z. Xu and H. Wang, *Microchim. Acta*, 2012, **177**, 229–236.
37. S. Gao, X. Yang, W. Yu, Z. Liu and H. Zhang, *Talanta*, 2012, **99**, 875–882.
38. Y. C. Fan, Z. L. Hu, M. L. Chen, C. S. Tu and Y. Zhu, *Chin. Chem. Lett.*, 2008, **19**, 985–987.
39. S. Sun, Y. Wang, W. Yu, T. Zhao, S. Gao, M. Kang, Y. Zhang, H. Zhang and Y. Yu, *J. Sep. Sci.*, 2011, **34**, 1730–1737.

40. M. A. Farajzadeh, A. S. Hojghan and M. R. A. Mogaddam, *J. Food Compost. Anal.*, 2018, **66**, 90–97.
41. F. Aydin, E. Yilmaz and M. Soylak, *Food Chem.*, 2018, **243**, 442–447.
42. J. Cheng, Y. Xia, Y. Zhou, F. Guo and G. Chen, *Anal. Chim. Acta*, 2011, **701**, 86–91.
43. Z. H. Yang, P. Wang, W. T. Zhao, Z. Q. Zhou and D. H. Liu, *J. Chromatogr. A*, 2013, **26**, 58–63.
44. M. Cruz-Vera, R. Lucena, S. Cárdenas and M. Valcárcel, *J. Chromatogr. A*, 2009, **1216**, 6459–6465.
45. K. Yavir, L. Marcinkowski, R. Marcinkowska, J. Namieśnik and A. Kloskowski, *Anal. Chim. Acta*, 2019, **1054**, 1–16.
46. J. F. Liu, G. B. Jiang, Y. G. Chi, Y. Q. Cai, Q. X. Zhou and J. T. Hu, *Anal. Chem.*, 2003, **75**, 5870–5876.
47. E. Aguilera-Herrador, R. Lucena, S. Cardenas and M. Valcarcel, *Anal. Chem.*, 2008, **80**, 793–800.
48. A. Gjelstad and S. Pedersen-Bjergaard, *Anal. Methods*, 2013, **5**, 4549–4557.
49. A. Chisvert, I. P. Roman, L. Vidal and A. Canals, *J. Chromatogr. A*, 2009, **1216**, 1290–1295.
50. E. Aguilera-Herrador, R. Lucena, S. Cardenas and M. Valcarcel, *J. Chromatogr. A*, 2009, **1216**, 5580–5587.
51. C. Yao, W. R. Pitner and J. L. Anderson, *Anal. Chem.*, 2009, **81**, 5054–5063.
52. Q. Zhang, K. D. Vigier, S. Royer and F. Jerome, *Chem. Soc. Rev.*, 2012, **41**, 7108–7146.
53. A. P. Abbott, G. Capper, D. L. Davies, H. L. Munro, R. K. Rasheed and V. Tambyrajah, *Chem. Commun.*, 2001, **19**, 2010–2011.
54. S. C. Cunha and J. O. Fernandes, *TrAC, Trends Anal. Chem.*, 2018, **105**, 225–239.
55. G. Shen and H. K. Lee, *Anal. Chem.*, 2003, **75**, 98–103.
56. G. Ouyang, W. Zhao and J. Pawliszyn, *J. Chromatogr. A*, 2007, **1138**, 47–54.
57. S. P. Huang and S. D. Huang, *J. Chromatogr. A*, 2006, **1135**, 6–11.
58. N. J. Petersen, H. Jensen, S. H. Hansen, S. T. Foss, D. Snakenborg and S. Pedersen-Bjergaard, *Microfluid. Nanofluid.*, 2010, **9**, 881–888.
59. G. Jiang and H. K. Lee, *Anal. Chem.*, 2004, **76**, 5591–5596.
60. J. A. Lopez-Lopez, C. Mendiguchia, J. J. Pinto and C. Moreno, *TrAC, Trends Anal. Chem.*, 2019, **110**, 57–65.
61. F. A. Hansen, D. Sticker, J. P. Kutter, N. J. Petersen and S. Pedersen-Bjergaard, *Anal. Chem.*, 2018, **90**, 9322–9329.
62. N. J. Petersen, J. S. Pedersen, N. N. Poulsen, H. Jensen, C. Skonberg, S. H. Hansen and S. Pedersen-Bjergaard, *Analyst*, 2012, **137**, 3321–3327.
63. N. J. Petersen, S. T. Foss, H. Jensen, S. H. Hansen, C. Skonberg, D. Snakenborg, J. P. Kutter and S. Pedersen-Bjergaard, *Anal. Chem.*, 2011, **83**, 44–51.
64. N. K. Drouin, P. S. Rudaz, S. Pedersen-Bjergaard and J. Schappler, *TrAC, Trends Anal. Chem.*, 2019, **113**, 357–363.

65. L. Y. Guan, Q. Luo, J. Y. Shi and W. Yu, *J. Sep. Sci.*, 2018, **41**, 868–876.
66. M. Rezaee, Y. Assadi, M. R. Milani-Hosseini, E. Aghaee, F. Ahmadi and S. Berijani, *J. Chromatogr. A*, 2006, **1116**, 1–9.
67. M. A. Farajzadeh, N. Nouri and P. Khorram, *TrAC, Trends Anal. Chem.*, 2014, **55**, 14–23.
68. J. Hu, L. Y. Fu, X. N. Zhao, X. J. Liu, H. L. Wang, X. D. Wang and L. Y. Dai, *Anal. Chim. Acta*, 2009, **640**, 100–105.
69. C. Yang, J. Wang and D. Li, *Anal. Chim. Acta*, 2013, **799**, 8–22.
70. C. Bosch-Ojeda and F. Sanchez-Rojas, *Chromatographia*, 2011, **74**, 651–679.
71. S. Nojavan, T. Gorji, S. S. H. Davarani and A. Morteza-Najarian, *Anal. Chim. Acta*, 2014, **838**, 51–57.
72. V. Andruch, M. Burdel, L. Kocúrová, J. Šandrejová and I. S. Balogh, *TrAC, Trends Anal. Chem.*, 2013, **49**, 1–19.
73. C. Jia, X. Zhu, J. Wang, E. Zhao, M. He, L. Chen and P. Yu, *J. Chromatogr. A*, 2010, **1217**, 5868–5871.
74. J. Regueiro, M. Llompart, C. Garcia-Jares, J. C. Garcia-Monteagudo and R. Cela, *J. Chromatogr. A*, 2008, **1190**, 27–38.
75. L. Kocúrová, I. S. Balogh, J. Šandrejová and V. Andruch, *Microchem. J.*, 2012, **102**, 11–17.
76. H. Sereshti, P. Khorram and N. Nouri, *Sep. Purif. Rev.*, 2019, **48**, 159–178.
77. B. Xu, D. Q. Song, Y. P. Wang, Y. Gao, B. C. Cao, H. Q. Zhang and Y. Sun, *J. Sep. Sci.*, 2014, **37**, 1967–1973.
78. M. Li, J. H. Zhang, Y. B. Li, B. Peng, W. F. Zhou and H. X. Gao, *Talanta*, 2013, **107**, 81–87.
79. H. Z. Wang, L. Hu, W. Z. Li, X. L. Yang, R. H. Lu, S. B. Zhang, W. F. Zhou, H. X. Gao and J. Li, *Talanta*, 2017, **162**, 625–633.
80. W. Yu, Z. Liu, S. Cui, S. Zhang, X. Yang, L. Lei, H. Zhang and A. Yu, *Anal. Methods*, 2014, **6**, 2545–2552.
81. Z. Shen, Z. He, P. Wang, Z. Zhou, M. Sun, J. Li and D. Liu, *Anal. Chim. Acta*, 2013, **793**, 37–43.
82. Y. Li, P. S. Chen and S. D. Huang, *J. Chromatogr. A*, 2013, **1300**, 51–57.
83. M. Rogeberg, H. Malerod, H. Roberg-Larsen, C. Aass and S. R. Wilson, *J. Pharm. Biomed. Anal.*, 2014, **87**, 120–129.
84. G. A. K. Khan, R. Lindberg, R. Grabic and J. Fick, *J. Pharm. Biomed. Anal.*, 2012, **66**, 24–32.
85. N. Fontanals, P. A. G. Cormack, D. C. Sherrington, R. M. Marcé and F. Borrull, *J. Chromatogr. A*, 2010, **1217**, 2855–2861.
86. E. V. S. Maciel, A. L. de Toffoli and F. M. Lancas, *Electrophoresis*, 2018, **13**, 1582–1596.
87. S. Valsecchi, S. Polesello, M. Mazzoni, M. Rusconi and M. Petrovic, *Trends Environ. Anal. Chem.*, 2015, **8**, 27–37.
88. N. Fontanals, R. M. Marce, F. Borrull and P. A. G. Cormack, *TrAC, Trends Anal. Chem.*, 2010, **29**, 765–779.
89. C. Emotte, O. Heudi, F. Deglave, A. Bonvie, L. Masson, F. Picard, A. Chaturvedi, T. Majumdar, A. Agarwal, R. Woessner and O. Kretz, *J. Chromatogr. B*, 2012, **895–896**, 1–9.

90. M. E. Lame, E. E. Chambers and M. Blatnik, *Anal. Biochem.*, 2011, **419**, 133–139.
91. F. Augusto, L. W. Hantao, N. G. S. Mogollon and S. C. G. N. Braga, *TrAC, Trends Anal. Chem.*, 2013, **43**, 14–23.
92. A. Martin-Esteban, *TrAC, Trends Anal. Chem.*, 2013, **45**, 169–181.
93. W. W. Wang, S. Q. Liu, Y. Xue, Y. Wang and C. Yan, *Chin. J. Chromatogr.*, 2017, **35**, 99–104.
94. T. S. Romig, C. Bell and D. W. Drolet, *J. Chromatogr. B*, 1999, **731**, 275–284.
95. F. Chapuis-Hugon, A. Boisbaudry, B. Madru and V. Pichon, *Anal. Bioanal. Chem.*, 2011, **400**, 1199–1207.
96. N. Fontanals, F. Borrull and R. M. Marce, *TrAC, Trends Anal. Chem.*, 2012, **41**, 15–26.
97. L. Vidal, J. Parshintsev, K. Hartonen, A. Canals and M. L. Riekkola, *J. Chromatogr. A*, 2012, **1226**, 2–10.
98. D. Bratkowska, N. Fontanals, S. Ronka, F. Borrull, A. W. Trochimczuk and R. M. Marce, *J. Sep. Sci.*, 2012, **35**, 1953–1958.
99. G. Fang, J. Chen, J. Wang, J. He and S. Wang, *J. Chromatogr. A*, 2010, **1217**, 1567–1574.
100. K. R. Gan, W. Y. Tang, T. Zhu, W. Li, H. Y. Wang and X. J. Liu, *J. Liq. Chromatogr. Relat. Technol.*, 2016, **39**, 882–888.
101. L. L. Liu, W. Y. Tang, B. K. Tang, D. D. Han, K. H. Row and T. Zhu, *J. Sep. Sci.*, 2017, **40**, 1887–1895.
102. G. Li, W. S. Ahn and K. H. Row, *J. Sep. Sci.*, 2016, **39**, 4465–4473.
103. G. Li, T. Zhu and K. H. Row, *J. Sep. Sci.*, 2017, **40**, 625–634.
104. S. Gangula, S. Y. Suen and E. D. Conte, *Microchem. J.*, 2010, **95**, 2–4.
105. L. N. Xu, X. Y. Qi, X. J. Li, Y. Bai and H. W. Liu, *Talanta*, 2016, **146**, 714–726.
106. H. Bagheri, S. Asgari and H. Piri-Moghadam, *Chromatographia*, 2014, **77**, 723–728.
107. J. H. Kim, E. T. Hwang, K. K. Kang, R. Tatavarty and M. B. Gu, *J. Mater. Chem.*, 2011, **21**, 19203–19206.
108. R. Q. Long and R. T. Yang, *J. Am. Chem. Soc.*, 2001, **123**, 2058–2059.
109. J. Tian, J. Xu, F. Zhu, T. Lu, C. Su and G. Ouyang, *J. Chromatogr. A*, 2013, **1300**, 2–16.
110. T. S. Anirudhan and S. Alexander, *J. Chem. Technol. Biotechnol.*, 2013, **88**, 1847–1858.
111. Z. Zhang, X. Yang, H. Zhang, M. Zhang, L. Luo, Y. Hu and S. Yao, *J. Chromatogr. B*, 2011, **879**, 1617–1624.
112. S. M. Daryanavard, A. Jeppsson-Dadoun, L. I. Andersson, M. Hashemi, A. Colmsjo and M. Abdel-Rehim, *Biomed. Chromatogr.*, 2013, **27**, 1481–1488.
113. W. Du, C. Lei, S. Zhang, G. Bai, H. Zhou, M. Sun, Q. Fu and C. Chang, *J. Pharm. Biomed. Anal.*, 2014, **91**, 160–168.
114. H. Bagheri, Z. Ayazi, A. Aghakhani and N. Alipour, *J. Sep. Sci.*, 2012, **35**, 114–120.

115. A. L. de Toffoli, E. V. S. Maciel, B. H. Fumes and F. M. Lancas, *J. Sep. Sci.*, 2018, **41**, 288–302.
116. H. Vlčkováa, J. Janáka, T. Gottvalda, F. Trejtnarb, P. Solicha and L. Novákova, *J. Pharm. Biomed. Anal.*, 2014, **88**, 337–344.
117. M. Abdel-Rehima, *Anal. Chim. Acta*, 2011, **701**, 119–128.
118. Y. He and M. Concheiro-Guisan, *Biomed. Chromatogr.*, 2019, **33**, e4444.
119. L. Yang, R. Said and M. Abdel-Rehim, *J. Chromatogr. B*, 2017, **1043**, 33–43.
120. K. Z. Mousavi, Y. Yamini, B. Karimi, S. Seidi, M. Khorasani, M. Ghaemmaghami and H. Vali, *Microchim. Acta*, 2019, 186.
121. X. Hou, S. R. Lei, S. T. Qiu, L. A. Guo, S. G. Yi and W. Liu, *Food Chem.*, 2014, **153**, 121–129.
122. X. Deng, Q. Guo, X. Chen, T. Xue, H. Wang and P. Yao, *Food Chem.*, 2014, **145**, 853–858.
123. X. Zhao, Y. Shi, T. Wang, Y. Cai and G. Jiang, *J. Chromatogr. A*, 2008, **1188**, 140–147.
124. M. Sun, X. X. Ma, J. T. Wang, W. N. Wang, Q. H. Wu, C. Wang and Z. Wang, *J. Sep. Sci.*, 2013, **36**, 1478–1485.
125. S. Jamshidi, M. K. Rofouei and G. Thorsen, *J. Sep. Sci.*, 2019, **42**, 698–705.
126. Z. Zhang, X. Yang, X. Chen, M. Zhang, L. Luo, M. Peng and S. Yao, *Anal. Bioanal. Chem.*, 2011, **401**, 2855–2863.
127. A. R. Zarei, M. Nedaei and S. A. Ghorbanian, *J. Mol. Liq.*, 2017, **246**, 58–65.
128. S. M. Yousefi, F. Shemirani and S. A. Ghorbanian, *Talanta*, 2017, **168**, 73–81.
129. S. T. Ellison, W. E. Brewer and S. L. Morgan, *J. Anal. Toxicol.*, 2009, **33**, 356–365.
130. H. X. Guan, W. E. Brewer, S. T. Garris, C. Craft and S. L. Morgan, *J. Chromatogr. A*, 2010, **1217**, 1867–1874.
131. P. Kaewsuya, W. E. Brewer, J. Wong and S. L. Morgan, *J. Agric. Food Chem.*, 2013, **61**, 2299–2314.
132. H. Guan and K. Stewart, *Anal. Lett.*, 2014, **47**, 1434–1447.
133. M. Pena-Abaurrea, V. S. García de la Torre and L. Ramos, *J. Chromatogr. A*, 2013, **1317**, 223–229.
134. S. J. Lehotay, K. Mastovska, A. R. Lightfield, A. Nunez, T. Dutko, C. Ng and L. Bluhm, *J. Chromatogr. A*, 2013, **1313**, 103–112.
135. G. T. Zhu, X. M. He, B. D. Cai, H. Wang, J. Ding, B. F. Yuan and Y. Q. Feng, *Analyst*, 2014, **139**, 6266–6271.
136. J. Deng, Y. Yang, X. Wang and T. Luan, *TrAC, Trends Anal. Chem.*, 2014, **55**, 55–67.
137. H. L. Lord, *J. Chromatogr. A*, 2007, **1152**, 2–13.
138. D. Vuckovic, X. Zhang, E. Cudjoe and J. Pawliszyn, *J. Chromatogr. A*, 2010, **1217**, 4041–4060.
139. D. Vuckovic, E. Cudjoe, D. Hein and J. Pawliszyn, *Anal. Chem.*, 2008, **80**, 6870.
140. D. Vuckovic, *TrAC, Trends Anal. Chem.*, 2013, **45**, 136–153.

141. J. P. Hutchinson, L. Setkova and J. Pawliszyn, *J. Chromatogr. A*, 2007, **1149**, 127–137.
142. E. Cudjoe, D. Vuckovic, D. Hein and J. Pawliszyn, *Anal. Chem.*, 2009, **81**, 4226–4232.
143. F. S. Mirnaghi, Y. Chen, L. M. Sidisky and J. Pawliszyn, *Anal. Chem.*, 2011, **83**, 6018–6025.
144. F. S. Mirnaghi, D. Hein and J. Pawliszyn, *Chromatographia*, 2013, **76**, 1215–1223.
145. W. Xie, W. M. Mullett, C. Miller-Stein and J. Pawliszyn, *J. Chromatogr. B*, 2009, **877**, 415–420.
146. W. Xie, C. Chavez-Eng, W. Fang, M. L. Constanzer, B. K. Matuszewski, W. M. Mullett and J. Pawliszyn, *J. Chromatogr. B*, 2011, **879**, 1457.
147. I. Ueta and Y. Saito, *Anal. Sci.*, 2014, **30**, 105–110.
148. J. Luckwell and A. Beal, *Bioanalysis*, 2011, **3**, 1227–1239.
149. G. A. Gomez-Rios, M. Tascon and J. Pawliszyn, *Bioanalysis*, 2018, **10**, 257–271.
150. E. A. Souza-Silva, R. F. Jiang, A. Rodriguez-Lafuente, E. Gionfriddo and J. Pawliszyn, *TrAC, Trends Anal. Chem.*, 2015, **71**, 224–235.
151. E. A. Souza-Silva, E. Gionfriddo and J. Pawliszyn, *TrAC, Trends Anal. Chem.*, 2015, **71**, 236–248.
152. E. A. Souza-Silva, N. Reyes-Garces, G. A. Gomez-Rios, E. Boyaci, B. Bojko and J. Pawliszyn, *TrAC, Trends Anal. Chem.*, 2015, **71**, 249–264.
153. H. Piri-Moghadam, M. N. Alam and J. Pawliszyn, *Anal. Chim. Acta*, 2017, **984**, 42–65.
154. F. S. Mirnaghi, M. R. N. Monton and J. Pawliszyn, *J. Chromatogr. A*, 2012, **1246**, 2–8.
155. F. S. Mirnaghi and J. Pawliszyn, *J. Chromatogr. A*, 2012, **1261**, 91–98.
156. D. Vuckovic, R. Shirey, Y. Chen, L. Sidisky, C. Aurand, K. Stenerson and J. Pawliszyn, *Anal. Chim. Acta*, 2009, **638**, 175–185.
157. R. Jiang and J. Pawliszyn, *TrAC, Trends Anal. Chem.*, 2012, **39**, 245–253.
158. H. Bagheri, Z. Ayazi and M. Naderi, *Anal. Chim. Acta*, 2013, **767**, 1–13.
159. A. Mehdinia and M. F. Mousavi, *J. Sep. Sci.*, 2008, **31**, 3565–3572.
160. Z. Gao, W. Li, B. Liu, F. Liang, H. He, S. Yang and C. Sun, *J. Chromatogr. A*, 2011, **1218**, 6285–6291.
161. W. Du, F. Zhao and B. Zeng, *J. Chromatogr. A*, 2009, **1216**, 3751–3757.
162. J. Zou, X. Song, J. Ji, W. Xu, J. Chen, Y. Jiang, Y. Wang and X. Chen, *J. Sep. Sci.*, 2011, **34**, 2765–2772.
163. A. Kabir, K. G. Furton and A. Malik, *TrAC, Trends Anal. Chem.*, 2013, **45**, 197–218.
164. H. Bagheri and A. Roostaie, *J. Chromatogr. A*, 2012, **1238**, 22–29.
165. Y. Sun, W. Y. Zhang, J. Xing and C. M. Wang, *Microchim. Acta*, 2011, **173**, 223–229.
166. X. Liu, X. Wang, F. Tan, H. Zhao, X. Quan, J. Chen and L. Li, *Anal. Chim. Acta*, 2012, **727**, 26–33.
167. R. Amini, A. Rouhollahi, M. Adibi and A. Mehdinia, *J. Chromatogr. A*, 2011, **1218**, 130–136.

168. M. Liu, X. Zhou, Y. Chen, H. L. Liu, X. Feng, G. Qiu, F. Liu and Z. Zeng, *Anal. Chim. Acta*, 2010, **683**, 96–106.
169. X. Zhou, P. F. Xie, J. Wang, B. B. Zhang, M. M. Liu, H. L. Liu and X. H. Feng, *J. Chromatogr. A*, 2011, **1218**, 3571–3580.
170. J. López-Darias, V. Pino, J. L. Anderson, C. M. Graham and A. M. Afonso, *J. Chromatogr. A*, 2010, **1217**, 1236–1243.
171. Y. Meng, V. Pino and J. L. Anderson, *Anal. Chim. Acta*, 2011, **687**, 141–149.
172. L. Pang and J. F. Liu, *J. Chromatogr. A*, 2012, **1230**, 8–14.
173. N. Reyes-Garcés, E. Gionfriddo, G. A. Gómez-Ríos, M. N. Alam, E. Boyaci, B. Bojko, V. Singh, J. Grandy and J. Pawliszyn, *Anal. Chem.*, 2018, **90**, 302–360.
174. F. David, N. Ochiai and P. Sandra, *TrAC, Trends Anal. Chem.*, 2019, **112**, 102–111.
175. C. H. Yu and B. Hu, *J. Sep. Sci.*, 2010, **33**, 2176–2183.
176. W. A. W. Ibrahim, A. S. A. Keyon, N. Prastomo and A. Matsuda, *J. Sol-Gel Sci. Technol.*, 2011, **59**, 128–134.
177. N. Gilart, R. M. Marce, F. Borrull and N. Fontanals, *TrAC, Trends Anal. Chem.*, 2014, **54**, 11–23.
178. M. He, B. Chen and B. Hu, *Anal. Bioanal. Chem.*, 2014, **406**, 2001–2026.
179. E. van Hoeck, F. Canale, C. Cordero, S. Compernolle, C. Bicchi and P. Sandra, *Anal. Bioanal. Chem.*, 2009, **393**, 907–919.
180. M. C. Sampedro, M. A. Goicolea, N. Unceta, A. Sanchez-Ortega and R. J. Barrio, *J. Sep. Sci.*, 2009, **32**, 3449–3456.
181. N. Maslamani, E. Manandhar, D. K. Geremia and B. A. Logue, *Anal. Chim. Acta*, 2016, **941**, 41–48.
182. A. H. Alluhayb and B. A. Logue, *J. Chromatogr. A*, 2017, **1518**, 8–14.
183. N. Ochiai, K. Sasamoto, F. David and P. Sandra, *J. Chromatogr. A*, 2016, **1455**, 45–56.
184. C. Yu, Q. Liu, L. Lan and B. Hu, *J. Chromatogr. A*, 2008, **1188**, 124–131.
185. M. Kawaguchi, R. Ito, H. Nakazawa and A. Takatsu, *TrAC, Trends Anal. Chem.*, 2013, **45**, 280–293.
186. N. Ochiai, K. Sasamoto, F. David and P. Sandra, *J. Agric. Food Chem.*, 2018, **66**, 7249–7255.
187. N. Gilart, R. M. Marce, P. A. G. Cormack, N. Fontanals and F. Borrull, *J. Sep. Sci.*, 2014, **37**, 2225–2232.
188. W. Fan, X. Mao, M. He, B. Chen and B. Hu, *Anal. Bioanal. Chem.*, 2014, **406**, 7261–7273.
189. J. P. Lambert, W. M. Mullett, E. Kwong and D. Lubda, *J. Chromatogr. A*, 2005, **1075**, 43–49.
190. Y. Hu, J. Pan, K. Zhang, H. Lian and G. Li, *TrAC, Trends Anal. Chem.*, 2013, **43**, 37–52.
191. Y. B. Luo, J. S. Cheng, Q. Ma, Y. Q. Feng and J. H. Li, *Anal. Methods*, 2011, **3**, 92–98.
192. C. Hu, B. B. Chen, M. He and B. Hu, *J. Chromatogr. A*, 2013, **1300**, 165–172.
193. J. L. Benedé, A. Chisvert, D. L. Giokas and A. Salvador, *J. Chromatogr. A*, 2014, **1362**, 25–33.

CHAPTER 6

Flow Analysis: A Powerful Tool for Green Analytical Chemistry

FÁBIO R. P. ROCHA,*[a] WANESSA R. MELCHERT[b] AND BOAVENTURA F. REIS[a]

[a] University of São Paulo, Centre for Nuclear Energy in Agriculture, Av. Centenário 303, P.O. Box 96, Piracicaba, SP, 13400-970, Brazil; [b] University of São Paulo, Luiz de Queiroz College of Agriculture, P.O. Box 9, Piracicaba, SP, 13418-970, Brazil
*Email: frprocha@cena.usp.br

6.1 Introduction

Flow analysis encompasses a widespread group of modalities that have been extensively exploited for automation in routine and research laboratories. The development of flow-based techniques has offered a new dimension to analytical chemistry, allowing measurements to be carried out faster and with minimum intervention from the analyst. Consequently, flow-based procedures are often characterized by high sample throughputs, improved precision and reduced risks of exposure of the analyst to toxic substances. As measurements are usually carried out in closed systems, the risks of sample contamination by external sources are also minimized. The manipulation of unstable reagents and products, including on-line generated suspensions and kinetic discrimination, have been effectively implemented in different flow configurations. Despite the several examples of flow systems for simultaneous or sequential determinations, most of the applications have been focused on the quantification of a single analyte in a large number of samples, exploiting the above-mentioned advantages.

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

The potential to develop environmentally friendly analytical procedures is also inherent in flow-based methodologies, which can be exemplified by the capability to decrease reagent consumption, reuse of immobilized reagents, waste recycling and in-line waste detoxification. The main challenge is to achieve green analytical chemistry (GAC) without affecting the reliability of the analytical results or increasing the operational costs.

New approaches in flow analysis have been pointed out as milestones in the development of GAC,[1] including flow injection analysis (FIA), sequential injection analysis (SIA), multicommutation in flow analysis (MCFA) and the lab-on-valve approach. Other potentially greener strategies adopted in flow analysis include solid-phase spectrophotometry (optosensing), photoconversions and liquid–liquid microextractions. Strategies in flow systems towards GAC have been revised, including the definition of a priority order for the development of cleaner procedures, *i.e.* chemical wastes should be avoided, minimized, reused/recycled or, as a final alternative, properly treated/disposed of.[2] Further, an overview[3] traced a parallel between the evolution of flow analysis and GAC based on the replacement of toxic reagents and the minimization of waste amounts and toxicity. Recent examples of successful greener approaches in flow analysis have also been discussed.[2,4–6]

6.2 Flow Systems

Since the early developments in the 1950s, diverse flow modalities have been devised by exploiting different approaches for handling solutions under reproducible time control. These modalities may be classified into segmented or unsegmented streams and continuous or intermittent sampling.[7] The main modalities are briefly described here and specific books[8–11] are recommended for further consultation, including those on other flow modalities not addressed in this chapter. The proposal of more environmentally friendly procedures was also highlighted as a trend in the development of flow analysis.[12,13]

6.2.1 Segmented Flow Analysis

Segmented flow analysis (SFA), which was proposed by Leonard T. Skeggs in the 1950s, was aimed at mechanizing the routine work in clinical laboratories.[14] The proposal was successful and these systems were commercialized as Technicon AutoAnalyzers®. In SFA, the sample and reagents are continuously aspirated into the manifold and segmented by air bubbles, with the aim of minimizing both axial sample dispersion and carryover effects. This approach is suitable for implementing the long sample residence times often required in analytical procedures employed in clinical analysis. As measurements are usually carried out close to the steady-state condition, sensitivity is improved; however, the sampling rate is rarely higher than 60 measurements per hour. Washing is carried out periodically by aspirating an

inert solution through the analytical path. Despite the widespread use of these systems some decades ago, they were often replaced by unsegmented flow systems.

6.2.2 Flow Injection Analysis

In FIA (Figure 6.1), samples are injected into a carrier stream and processed without air segmentation. Concentration gradients are thus formed in the sample zone because of the reproducible sample dispersion in the carrier. The extent of the dispersion depends on physicochemical properties of the solutions, such as viscosity, and system parameters, *e.g.* sample volume, dimensions and geometry of the reactor and the flow rates. Because of the concentration gradients, transient signals are obtained and quantification is usually based on peak heights. In addition, gradients can be exploited for on-line dilutions, implementation of the standard additions method and titrations, among other applications.

In FIA, samples are submitted to highly reproducible processing conditions (*e.g.* dilutions, reagent additions, matrix separation and analyte preconcentration) and timing, making measurements feasible without achieving the steady-state condition (residence times are usually <30 s). This aspect paved the way for the exploitation of kinetic aspects to improve the selectivity, for simultaneous determinations and to overcome matrix effects.

6.2.3 Sequential Injection Analysis

SIA (Figure 6.2) is a robust alternative to FIA and was developed with the aim of implementing different flow methodologies without significant modifications to the manifold.[15] SIA exploits a computer-controlled multiport

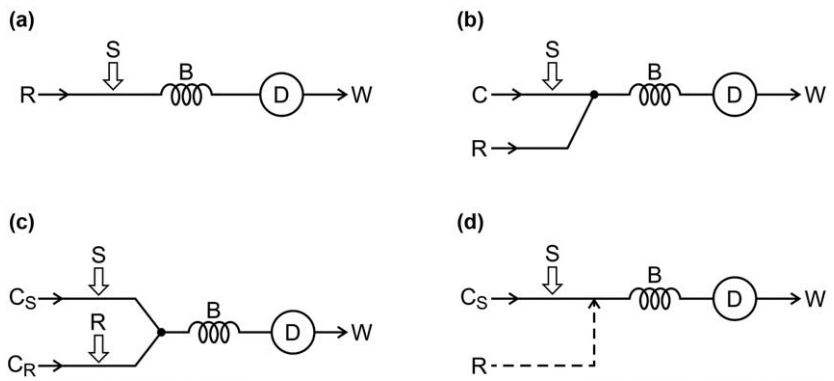


Figure 6.1 Diagram of some flow injection modalities: (a) single-line manifold; (b) confluent streams; (c) merging zones; (d) intermittent flows. R, reagent solution; S, sample; C, C_R and Cs, carrier solutions; B, reaction coil; D, detector; W, waste vessel.

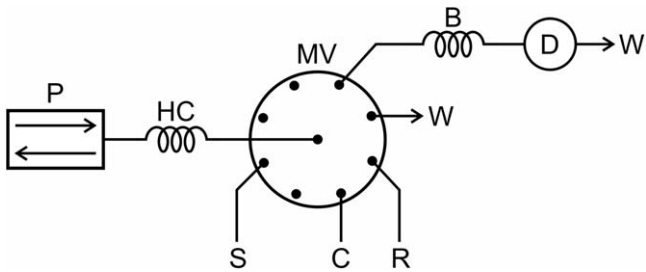


Figure 6.2 Flow diagram of an SIA system. P, bidirectional pump; HC, holding coil; MV, multiport valve; R, reagent solution; S, sample; C, carrier; B, reaction coil; D, detector; W, waste vessel.

selection valve to collect sequentially sample and reagent aliquots required for the analytical determination. Solutions are aspirated towards a holding coil usually by a syringe pump. Mixing occurs by dispersion at the interfaces and strategies such as flow reversal have been exploited to increase zone overlap. This is necessary mainly when the analytical procedure requires two or more reagent solutions and to avoid the artefacts caused by unsuitable zone overlap.[16] For medium or high sample dispersion, the system can be designed to achieve an analytical performance comparable to that attained by FIA, but with significantly lower reagent consumption.

6.2.4 Monosegmented Flow Analysis

Monosegmented flow analysis (MSFA) matches the simplicity and high sampling rate achieved by FIA with the low sample dispersion achieved by SFA. The sample aliquot is inserted in the carrier stream sandwiched by two air bubbles to constrain axial dispersion. Subsequently, the monosegment can be subjected to the long residence times required for relatively slow reactions, without impairing the washing times and sampling rate.[17] MSFA also shows potential to minimize reagent consumption, as the waste caused by its dispersion is avoided. This characteristic has been exploited by the simultaneous injection of sample and reagents[18] or using opto-switches to locate the air bubbles to ensure that the reagent addition occurs only into the sample zone.[19] MSFA was successfully applied to liquid–liquid extraction (LLE) in single[20] and two-phase[21] systems, and also for the analysis of gaseous species.[22]

6.2.5 Multicommutation and Multipumping Approaches

Flow systems based on the multicommutation approach[23] are designed with discrete computer-controlled commuting devices, such as solenoid valves (Figure 6.3a). Each commutator can be independently controlled, such that the flow manifold can be reconfigured by software. This approach greatly

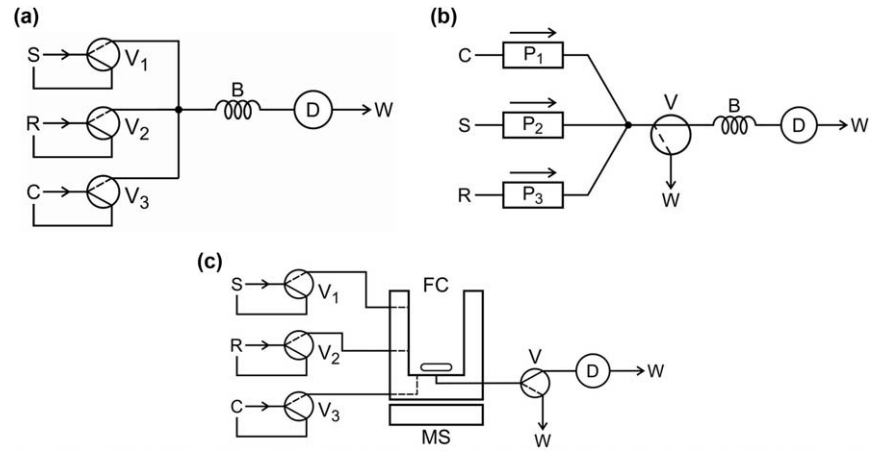


Figure 6.3 Flow diagrams of (a) multicommuted, (b) multipumping and (c) flow-batch analysers. V_i, solenoid valves; P_i, solenoid micropumps; MS, magnetic stirrer; FC, flow-batch chamber; R, reagent; S, sample; C, carrier; B, reaction coil; D, detector; W, waste vessel.

increases the system versatility, because 2^n different configurations can be established with n active devices.

Solenoid micropumps, which deliver microlitre solution volumes in a reproducible way, are employed in the design of multipumping flow systems (MPFS)[24] (Figure 6.3b). The pulsed flow inherent to these devices favours mixing and provides enhanced radial mass transport. Improvement of the heat transfer in applications involving convective heating has also been verified.[25] Other advantages include portability and low energy requirements, making the systems suitable for *in situ* (point-of-care) measurements.

In flow systems based on MCFA or MPFS, each solution is handled by an independent device aimed at intermittent reagent addition, among other advantages. Sample and reagent volumes are defined by the flow rates and the switching times of the corresponding commutators (MCFA) or, instead, by the number of pulses of the solenoid pumps (MPFS). It is then possible to introduce solutions (*e.g.* samples and reagents) simultaneously (merging zones approach) or alternately (binary sampling approach). The latter strategy makes it possible to change the volumetric fractions by software, which is useful for optimization of the procedures and for flow-based titrations.

6.2.6 Flow-batch Analysis

Flow-batch analysis (FBA) is a hybrid approach that combines the advantages of flow and batchwise sample processing.[26] Typically, solenoid valves handle the solutions to be inserted into (or removed from) a mini-chamber (with or without stirring) according to a programmed routine (Figure 6.3c). This approach is attractive when long sample residence times are required,

and also when difficult homogenization of mixtures or liquid–liquid (micro) extractions are involved.

The more recent lab-in-syringe approach combines the characteristics of SIA with FBA.[27] A multiport valve allows the selection of sample and reagents/solvents, which are directly inserted into the syringe, where chemical reactions, extractions, dilutions and other steps of sample processing occur. A reduction in reagent consumption occurs similarly to SIA. As a modality of FBA, the approach is also useful for increasing the sample residence times and to provide better interaction between the immiscible phases aiming at liquid–liquid (micro) extractions.

6.2.7 Multisyringe Approach

Multisyringe flow injection analysis (MSFIA)[28] combines features of SIA, such as robustness, versatility and ability to reduce the reagent consumption, with those of FIA, *i.e.* improved sampling rates and better analytical performance. Syringe pumps are coupled to a multiport valve or a set of solenoid valves; by the synchronized actuation of the valves and a step motor, solutions can be directed to the analytical path or delivered back to their original reservoirs.

6.3 Reduction of Waste Generation by System Design

The inception and development of flow analysis were stimulated by the high analytical demand and the need to achieve reliable analytical results in short time intervals. A high sample throughput was a key aspect in the initial development stage, but at the cost of high reagent flow rates. High effluent volumes were thus generated, even higher than in the corresponding batch procedures in some applications [*e.g.* the consumption of $Hg(SCN)_2$ was 2.5 times higher in an FIA system with confluent streams in comparison with the batchwise procedure[2]]. In the first SFA systems, for example, about 10–20 mL of waste were generated per determination.[29] New flow configurations have been proposed that focus not only on better analytical performance and on the implementation of more complex assays, but also on the minimization of both reagent consumption and waste generation. In the more recent modalities, good analytical performance was achieved with the consumption of only a few microlitres of reagents.[30,31]

A high sample throughput was also emphasized in the early development of FIA. In the single-line manifolds (Figure 6.1a), the reagent stream acted also as a sample carrier and flow rates as high as 8 mL min^{-1} were usual. Despite the simplicity, single-line systems inherently waste reagents and hinder the mixing of the sample and reagents, which occurs only by dispersion at the interfaces. Double peaks can then be generated under conditions of limited sample dispersion owing to the lack of the reagent at the centre of the sample zone, thus hindering sensitivity.

Mixing conditions were improved in flow systems with confluent streams (Figure 6.1b), in which reagent solutions are equally distributed in the whole sample zone, providing a constant volumetric fraction. The amount of reagent available to the chemical reaction can then be defined by the solution concentration and the relative flow rates of carrier and reagent streams. As an inert solution is used as carrier, the reagent flow rate can be decreased without impairing the sampling rate, reducing the reagent consumption in relation to the single-line configuration. However, reagents are consumed even when a sample is not being processed, thereby increasing waste generation.

The merging zones[32] (Figure 6.1c) and the intermittent flows[33] (Figure 6.1d) approaches are ingenious alternatives for minimizing reagent consumption in FIA. In the former, sample and reagent aliquots are simultaneously introduced into independent carrier streams, merging at the confluence point. As the volumetric fractions of sample and reagents are maintained, development of the chemical reaction and the analytical features are not affected. The potential of this approach was first demonstrated by the spectrophotometric determination of phosphate by the Molybdenum Blue method, in which the consumption of ascorbic acid was reduced to 9% of that required in the flow system with confluent streams. The intermittent flow approach was formerly implemented using a sliding-bar commutator or two independently controlled peristaltic pumps; however, modern systems exploit computer-controlled valves for this purpose. Reagent addition by confluence is synchronized with the sample injection, the solution being recycled in the sampling stage. Reagent consumption is then reduced similarly to that in the merging zones approach. Taking the spectrophotometric determination of calcium as an example, the reagent consumption per determination was reduced from 40 μg with continuous reagent addition to 0.27 μg with the intermittent flow strategy.[34] Reagent saving by intermittent addition is a common characteristic of SIA, MCFA, MPFS and MSFIA. In these approaches, only the amount of reagent required for the reaction development is consumed.

The potential to reduce reagent consumption is also inherent in flow systems exploiting reagent injection in a sample flowing stream,[35] previously named reverse flow injection analysis (r-FIA). For example, when this strategy was adopted for the sequential determination of analytes in natural waters, the reagent consumption was up to 240 and 4000 times lower in comparison with that in flow systems with continuous reagent addition and batch procedures, respectively.[35]

The efficiency of different flow approaches to minimize reagent consumption can be demonstrated by comparing the spectrophotometric procedures for the determination of total phenols,[36–40] nitrite,[30,33,41,42] phosphate,[17,29,43–46] and chloride[29–31,46,47] (Figure 6.4).

Flow analysis has also contributed to avoiding the drawbacks of other sample processing procedures, such as batch LLE. In a flow-based system, the operating time, solvent amounts and risks to the analyst are minimized. Several flow approaches have been proposed for the mechanization of LLE,

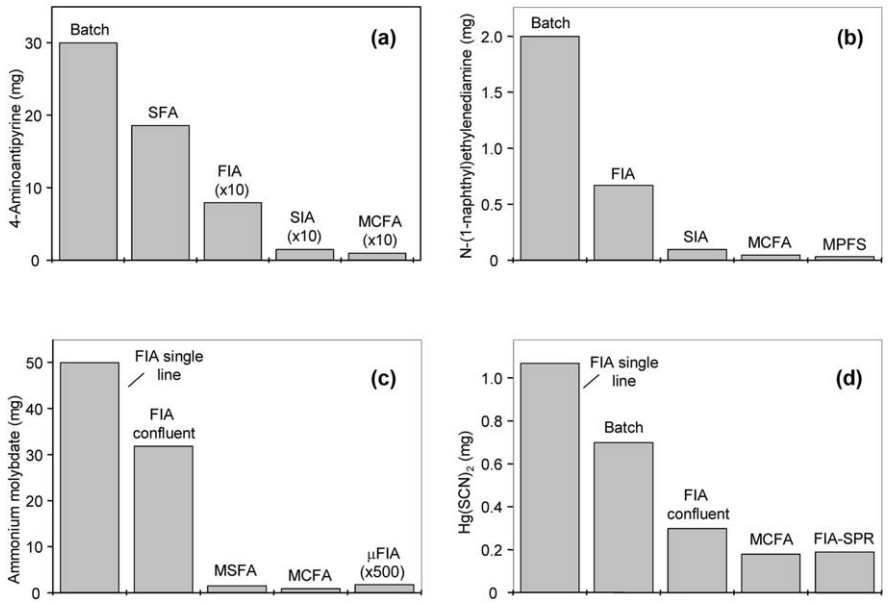


Figure 6.4 Reagent consumption per determination in spectrophotometric procedures for (a) total phenols,[36–40] (b) nitrite,[30,33,41,42] (c) phosphate,[17,29,43–46] and (d) chloride.[29–31,46,47] Numbers indicate the effluent volume per determination.

as recently reviewed.[5] The potential to minimize solvent consumption can be demonstrated by LLE in a single 1.3 μL microdrop,[48] whereas batchwise LLE usually consumes at least 10 mL of organic solvent. The tendency to reduce the volume of organic solvent is also evidenced by the applications presented in Table 6.1.[21,48,58]

6.4 Contributions of Flow-based Procedures to Green Analytical Chemistry

6.4.1 Reagentless Analytical Procedures

Reagentless analytical procedures are scarce and, as a rule, involve less complex applications. This can be illustrated by the determination of ethanol in beverages by exploiting density measurements,[59] the influence of the alcohol on the drop size[60] or the Schlieren effect.[61,62] In the first, densities were measured at the acceptor phase after analyte separation by pervaporation. The sampling rate was 15 h^{-1} and the results agreed with those for the reference method, based on the oxidation of the alcohol with potassium dichromate. Analogous procedures could be applied for the determination of other volatile analytes in relatively high concentrations. The effect of the alcohol on the solution surface tension was exploited in an FIA system for the determination of ethanol in wines.[60] Falling sample drops were

Table 6.1 Typical amounts of organic solvents in liquid–liquid extractions in flow systems.[a]

Flow approach	Solvent volume/μL	Example	Ref.
FIA	1200	Caffeine with $CHCl_3$	49
SIA-DLLME-SFOD	1000	Parabens with 1-dodecanol: MeOH	50
LOV-DLLME-MSFIA	900	Polycyclic aromatic hydrocarbons with acetonitrile	51
MCFA	225	Pb–dithizone complex with CCl_4	52
LLE-DES-SWIA	200	Procainamide with acetonitrile	53
AD-HF-LLLME	200	Organic and inorganic mercury–18-crown-6 complex in chlorobenzene	54
MSFA	100	Cd–PAN complex with $CHCl_3$	21
FIA	30	Thiamine with $CHCl_3$	55
SIA-microextraction	30	Bisphenol A with 1-octanol	56
LIS-MSA-DLLME	12	Cd, Cu, Pb, Ag–DDTP complex in xylene	57
FIA-falling drop	1.3	Sodium dodecyl sulfate with a $CHCl_3$ microdrop	48
MSFA-microextraction	0.254	Caffeine with $CHCl_3$	58

[a]AD-HF-LLLME, automatic dynamic hollow fibre-based liquid–liquid–liquid microextraction; DDTP, diethyl dithiophosphate; LIS-MSA-DLLME, lab-in-syringe magnetic stirring-assisted dispersive liquid–liquid microextraction; LLE-DES-SWIA, liquid–liquid extraction with deep eutectic solvent in a stepwise injection system; LOV-DLLME-MSFIA, lab-on-valve dispersive liquid–liquid microextraction multisyringe flow injection analysis; SIA-DLLME-SFOD, sequential injection analysis dispersive liquid–liquid microextraction based on the solidification of the organic phase.

reproducibly generated between the photometric devices and the radiation power that strikes the photodetector depends on the drop size, which is a function of the ethanol concentration. Determinations of ethanol concentrations from 1 to 30% with a sampling rate of 50 h^{-1} and a coefficient of variation (CV) of 2.5% were feasible. The reproducible refractive index gradients (Schlieren effect) formed when an alcoholic sample solution is processed in a single-line manifold (water as carrier) under limited mixing conditions were also exploited for the determination of ethanol in beverages.[61,62] A similar strategy was applied for the determination of the Brix values in soft drinks based on the Schlieren effect, caused by the incomplete mixing of sucrose in the sample with a water carrier.[63] A linear response was achieved from 3 to 47 °Bx at a sampling rate of 120 h^{-1} and the results for real samples agreed with those obtained by the refractometric reference method.

6.4.2 Replacement of Hazardous Chemicals

A significant and more realistic contribution to GAC is the minimization of waste toxicity by avoiding hazardous chemicals. Flow systems have been exploited to achieve this goal, as demonstrated by the applications in Table 6.2.[59,60,64–71]

Table 6.2 Environmentally friendly flow-based procedures achieved by replacement of hazardous chemicals.[a]

Analyte	Replaced reagents	Remarks	Ref.
Ammonium	Hg(II), phenol, nitroprusside	Conductimetric detection after separation from the sample matrix by gas diffusion	64
Cyclamate	Carcinogenic amines or Pb(II)	Replacement of toxic reagents by nitrite/iodide and waste minimization with an MPFS	65
Ethanol	$K_2Cr_2O_7$–H_2SO_4	Reagentless procedure based on detection by the solution density after analyte separation by pervaporation	59
Ethanol	$K_2Cr_2O_7$–H_2SO_4	Reagentless procedure based on photometric detection of falling drops	60
Hypochlorite	DPD	Direct detection by UV measurements before and after decomposition of the analyte to improve selectivity	66
Manganese	Organic solvents	Cloud point extraction with Triton X-114	67
Nitrate	Cd, NED, sulfanilamide	Direct UV measurements after separation of interfering species in an ion-exchange minicolumn	68
Paraquat	Dithionite	Dehydroascorbic acid as a reagent	69
Total phenols	4-Aminoantipyrine and ferricyanide	Natural source of polyphenol oxidase (sweet potato root) and spectrophotometric detection of the formed *o*-quinones	70
Urea	Urease, phenol, nitroprusside	Natural source of urease (jackbean) and conductimetric detection of ammonium	71

[a]DPD, *N*,*N*-diethyl-*p*-phenylenediamine; NED, *N*-(1-naphthyl)ethylenediamine.

The replacement of toxic reagents can be demonstrated by the flow systems proposed for the determination of nitrate and ammonium. Reliable results are obtained in a flow system by coupling a minicolumn containing copperized cadmium filings for reduction of nitrate to nitrite. The reduced form can then be quantified after a diazo-coupling (Griess) reaction.[72] The waste volume is considerably lower than that generated in the analogous batch procedure, but residues of toxic metal ions and carcinogenic amines are generated. Focusing on developing a greener procedure, the cadmium filings were replaced with a photochemical reduction step[73] or with enzymatic reduction with nitrate reductase obtained from corn leaves.[74]

Reduction efficiencies higher than 80% can be attained with these strategies under suitable working conditions. As a typical advantage of flow analysis, quantitative reduction is not required, as the same efficiency is expected for the samples and reference solutions. Analyte derivatization by the Griess method was avoided when the photochemically generated nitrite was detected by biamperometry after reaction with iodide in an acidic medium.[75] Other environmentally friendly flow-based procedures exploited direct UV measurements, avoiding the use of toxic reagents in the derivatization step. Selectivity was improved by coupling an anion-exchange minicolumn for the separation of the analyte from interfering species (*e.g.* humic substances) usually found in natural waters.[68] A dilute perchloric acid eluent solution, the consumption of which is equivalent to 18 μL of the concentrated acid per determination, was the only reagent employed.

Hazardous chemicals [mercury(II) or phenol and sodium nitroprusside] were extensively used for ammonium determinations using Nessler[76] and Berthelot[77] reactions. The waste toxicity was decreased by using salicylic acid instead of phenol in the Berthelot reaction,[78] achieving a similar analytical performance. A more recent study exploited NH_3 volatilization for separation from the sample matrix, usually by gas diffusion through a PTFE membrane. This process can be carried out reproducibly in flow systems and the low transport efficiency can be at least partially compensated for by using a high sample volume, different flow rates of the donor and acceptor streams or increasing the backpressure at the donor channel. Selective detection in the acceptor stream can be performed by spectrophotometry with an acid–base indicator or by conductimetry, which does not require additional reagents. With the latter alternative, the alkaline solution used to volatilize ammonia is the only reagent required;[64] after measurements, a clean waste was obtained by on-line neutralization of the donor stream. Similar strategies can be used for greener determinations of other volatile analytes or species that can be volatilized in a suitable medium (*e.g.* carbonate, amines, cyanide, sulfide and sulfite).

Plant tissues or vegetable extracts are greener enzymatic sources, which also have the potential to replace hazardous chemicals, as demonstrated by the determination of urea[71] and total phenols.[70] In the former application, a minicolumn filled with jackbean pieces was the source of urease used for up to 1000 urea determinations in serum.[71] Conductimetric detection after gas diffusion through a PTFE membrane was exploited to determine the ammonium ions originating from the enzymatic hydrolysis. In another application, a clean method for the spectrophotometric determination of total phenols in wastewater was based on oxidation to *o*-quinones by dissolved oxygen. A crude extract of sweet potato root, prepared in phosphate buffer, was the source of polyphenol oxidase, which acted as a reaction biocatalyst.[70] This vegetable extract was also employed in clean flow procedures for the determination of sulfite in wines[79] and L-dopa and carbidopa in pharmaceutical formulations.[80] More recently, extracts of guava (*Psidium* sp.) leaf[81] and emblica (*Phyllanthus emblica* Linn.)[82] were proposed for the

quantification of iron by exploiting the naturally occurring reducing and complexing species.

Biosorbents (*e.g.* vegetables, algae, bacteria, fungi and yeasts) have been exploited for separation, preconcentration and chemical speciation, thus avoiding synthetic sorbents, the preparation of which consumes reagents and solvents. In comparison with the analogous batchwise procedure, flow-based biosorption increases the sample throughput and allows kinetic discrimination, in addition to the application of the sorbent in several analytical cycles. As an example, peat was exploited for the separation and preconcentration of copper in an MCFA system coupled with flame atomic absorption spectrometry (FAAS).[83] An enrichment factor of 16 was achieved with a sampling rate of 100 h^{-1} and a CV of 3.3%. Other applications involving analyte preconcentration by biosorption include the use of vegetable wastes in the monitoring of toxic metals by flow injection potentiometry[84] and the aquatic macrophytes *Salvinia molesta* for the determination of Cd(II) by flow injection on-line coupled to FAAS.[85] Conversely, chemical speciation was achieved using husks of *Moringa oleifera* for the selective sorption of trivalent and hexavalent chromium at pH 7–9 and 1–2, respectively,[86] and immobilized *Saccharomyces cerevisiae* yeast for the speciation of antimony[87] and arsenic.[88]

Chemical derivatization of the analyte was avoided in a green procedure for the determination of hypochlorite in bleaching products.[66] The procedure exploited the intrinsic radiation absorption by the analyte at 292 nm, the analytical signal being based on the difference in the absorbances measured before and after the reaction of the sample constituents with solid cobalt oxide placed in a minicolumn. As the solid reagent catalyses the decomposition of hypochlorite to chloride and oxygen, the difference allows the selective determination of the analyte. The procedure requires only 20 mg of the reusable catalyst and a dilute NaOH solution.

Cloud point extraction is a green alternative for separation and preconcentration, in which toxic organic solvents are replaced with non-toxic, non-ionic surfactants. This strategy can be carried out in flow-based systems, eliminating laborious operations (*e.g.* heating, phase separation and the removal of the surfactant-rich phase).[89] The analyte extract is usually retained in a minicolumn filled with a filtration material (*e.g.* cotton) and further eluted with a suitable solution (*e.g.* dilute acid or organic solution). An alternative approach involved the direct retention of the surfactant-rich phase in the optical path of a spectrophotometric flow cell coupled to an MPFS.[90] Focusing on iron determination, the cloud point was induced by the heat released by the on-line neutralization of the acidic sample digests by sodium hydroxide, thus making an external heating device unnecessary. The consumptive index of 0.022 mL reflected the efficiency of sample utilization to achieve an enrichment factor of 8.9. Another advantage was the elution of the surfactant-rich phase by exploiting the pulsed flow of the aqueous carrier, without organic solvents. The strategies for the implementation of cloud point extraction in flow analysis and applications to environmental, agronomic and food samples have been reviewed.[89]

Photo-mediated analyte conversions (photoconversions) are powerful tools for replacing chemicals in analytical procedures. The fundamentals, characteristics and applications of this approach coupled with flow analysis have been critically reviewed.[91]

6.4.3 Reuse of Chemicals

Recycling and reuse of the wastes generated in analytical procedures are interesting approaches for GAC, also contributing to cost reduction. However, this is not usual owing to the complexity of the wastes. Some successful examples are presented in Table 6.3.[92–96] On-line solvent recycling by distillation after the analytical measurement was efficiently adopted in procedures based on LLE. A toxic organic solvent ($CHCl_3$ or CCl_4) was required for sample dissolution and as a carrier for simultaneous determinations of propyphenazone and caffeine[92] and of ketoptofen[94] in pharmaceuticals by Fourier transform infrared (FTIR) spectrometry. The flow systems would

Table 6.3 Some examples of the reuse of chemicals in flow-based systems.

Analyte	Sample	Reagents	Remarks	Ref.
Caffeine[a]	Pharmaceuticals	$CHCl_3$	On-line solvent recycling by distillation	92
Iron[b]	Natural waters	Acetate buffer and ascorbic acid	Reversible retention of the analyte in C_{18}-TAN[c] and measurements by solid-phase spectrophotometry	93
Ketoptofen[a]	Pharmaceuticals	CCl_4	On-line solvent recycling by distillation	94
Lead[a]	Gasoline	Arsenazo III	Release of the reagent by retention of the analyte in an ion-exchange minicolumn	95
Propyphenazone[a]	Pharmaceuticals	$CHCl_3$	On-line solvent recycling by distillation	92
Zinc[b]	Natural waters	Hexamine buffer	Reversible retention of the analyte in C_{18}-TAN and measurements by solid-phase spectrophotometry	96

[a]FIA system with closed-loop configuration.
[b]FIA.
[c]TAN, 1-(2-thiazolylazo)-2-naphthol.

generate about 1 mL min^{-1} of organic solvents; however, this drawback was overcome by selecting a closed-loop configuration, which incorporated distillation and cooling units. In addition to the reuse of a toxic chemical, the risks of exposure of the analyst to carcinogenic solvents were minimized.

The closed-loop configuration was also adopted for reagent reuse in the spectrophotometric determination of lead in gasoline[95] based on formation of a complex with Arsenazo III. A minicolumn filled with a cation-exchange resin was placed after the flow cell to retain the toxic metal for further treatment. The chromogenic reagent released was sent back to its vial for reuse. Reproducible analytical curves were observed without baseline drift when 50 mL of the same recirculating reagent solution were used for 7 days. Other examples of the reuse of chemicals in flow-based procedures are reagents immobilized on solid phases (*e.g.* enzymes and complexing agents), as discussed in the next section.

6.4.4 Minimization of Reagent Consumption and Waste Generation

Minimization of the amounts of reagents consumed in an analytical procedure reduces the operating costs (reagents and waste treatment) and toxicity of wastes. Waste minimization is the more general alternative for achieving GAC, because reliable analytical procedures can be implemented by consuming low amounts of chemicals. Successful examples are presented in Table 6.4[31,36,42,48,65,97–107] and are discussed in the following sections.

6.4.4.1 Immobilized Reagents

Reagent immobilization is a useful alternative for the development of environmentally friendly analytical procedures. Minicolumns filled with solid reagents are usually employed for analyte derivatization or for on-line generation of unstable reagents.[108] Exploitation of immobilized reagents often simplifies the manifolds, improves the radial mass transfer and may provide an excess of the chemical at the solid/liquid interface. Greener procedures can be developed because only the stoichiometric amounts of the solid are consumed in the reaction. However, the strategy for reagent immobilization needs to be carefully selected to minimize the risks of lixiviation and increase in backpressure.

Reagent immobilization contributes to the development of greener procedures even when toxic chemicals are employed. As an example, a minicolumn filled with a few milligrams of PbO_2 immobilized on a polyester resin was employed for the indirect determination of dypyrone (metamizole) by FAAS.[109] Measurements of the lead(II) ions released when the drug was oxidized by PbO_2 were exploited for the indirect determination of the analyte. As more than 7000 determinations were carried out with the same minicolumn, the amount of lead consumed per determination was

Table 6.4 Minimization of waste generation in flow-based systems.[a]

Analyte	Sample	Flow configuration	Reagents	Remarks	Ref.
Anionic surfactants	Natural waters	FIA-falling drop	Methylene blue and $CHCl_3$	1.3 μL of $CHCl_3$ per determination	48
Benzene	Gasoline	MCFA	Dilution in hexane	Consumption of hexane three times lower than in a flow system with continuous pumping	97
Carbamate pesticides	—	μFIA	Trimethylaniline	<300 nL of toluene per determination	98
Carbaryl	Natural waters	MPFS	PAP–periodate	1.9 μg of PAP and 5.7 μg of KIO_4 per determination/waste mineralization by persulfate–UV detection	99
Chloride	Natural waters	FIA	$Hg(SCN)_2$ immobilized on epoxy resin	1500-fold reduction in amount of Hg in the waste	31
Chloride	Natural waters	MSFIA	$Hg(SCN)_2$–iron(III)	3400-fold lower reagent consumption *versus* single-line FIA	100
Chromium(VI)	Freshwater and leachates	MSFIA with 3D device	Solid-phase extraction	49-fold reuse of disk-based solid-phase extraction device	101
Cyanide	Natural waters	MCFA	OPA and glycine	5.6 μg of OPA and 6.7 μg of glycine per determination	102
Cyclamate	Table sweeteners	MPFS	$NaNO_2$, KI, H_3PO_4	3 mg of KI, 1.3 μg of $NaNO_2$ and 125 μmol of H_3PO_4 per determination	65
Iron	—	μFIA	Nitroso-R salt	50 μL of effluent per determination	103
Mercury speciation	Fish muscle	MSFIA	Anion-exchange membrane	Reduced reagent consumption and replacement of LLE with membrane separation	104
Nitrite	Natural waters	MPFS	NED, sulfanilamide and dilute acid	55-fold lower consumption of NED in comparison with the batch procedure and in-line waste degradation	42
Phosphate	Natural waters	SIA-lab-on-valve	Molybdate–ascorbic acid	10 μL of reagent and 250 μL of waste per determination	105
Tannins	Green tea	μFBA	Urethane–acrylate resin	300-fold lower reagent consumption than in the reference method	106
Tocopherol	Olive oils	MCFA	2-Propanol	Reagent consumption four times lower; avoids using THF and acetonitrile as required in HPLC	107
Total phenols	Natural waters	MCFA	4-Aminoantipyrine and ferricyanide	200-fold lower reagent consumption by an MCFA system and increase in sensitivity by LPS avoiding LLE	36

[a]NED, *N*-(1-naphthyl)ethylenediamine; OPA, *o*-phthalaldehyde; PAP, *p*-aminophenol.

negligible. The immobilization of $Hg(SCN)_2$ in epoxy resin was also exploited in a greener procedure for the spectrophotometric determination of chloride, reducing by *ca.* 1500-fold the amount of Hg generated per determination in comparison with that of a single-line FIA manifold.[31] Another clean alternative for chloride determination exploited a minicolumn filled with silver chloranilate.[110] Formation of AgCl by reaction with the analyte released the absorbing chloranilate ions, which were quantified by spectrophotometry in a 100 cm optical path liquid-core waveguide flow cell. This resulted in a 75-fold increase in sensitivity and a reduction of the waste generated to *ca.* 100 ng of chloranilate per determination.

Analyte retention on a solid support can be exploited for direct measurements in the solid phase (optosensing), focusing on improving sensitivity, selectivity or both. Although the approach can be implemented in a batchwise format, several advantages arise when it is combined with flow systems.[111] Reversible retention of the analyte is feasible in some applications and the reuse of the reagent yields greener analytical procedures. For flow-based optosensing, the solid support is deposited in the measurement cell for detection by spectrophotometry (transmittance or reflectance mode), luminescence or infrared spectrometry. This approach was adopted to develop more environmentally friendly analytical procedures for, *e.g.*, the determination of zinc in pharmaceutical preparations[96] and iron in natural waters.[93] The solid support was C_{18}-bonded silica modified with the azo reagent 1-(2-thiazolylazo)-2-naphthol (TAN), which allowed analyte retention by formation of a coloured complex. The analyte was eluted with a few microlitres of a dilute acid solution without removing the ligand from the solid support, which was used for at least 100 measurements. The reagent consumption was less than 1 μg per determination.

6.4.4.2 Multicommuted Flow Systems

Multicommutation shows great potential for the development of environmentally friendly analytical procedures, because the reagents can be added only at the instants and in the amounts strictly required for sample processing. This aspect has been demonstrated in papers on MCFA by comparison with the amounts of reagents consumed in other flow approaches. This advantage can be achieved without hindering the analytical performance, as demonstrated by comparing the flow procedures for the determination of the pesticide carbaryl.[112] The consumption of the most toxic reagent (*p*-aminophenol) per determination was reduced from 0.14 mg in FIA with confluent streams to 5 μg using MCFA. On the other hand, the SIA procedure consumed 11 μg of *p*-aminophenol to achieve a threefold lower sensitivity.

MCFA was exploited to develop a greener spectrophotometric procedure for the determination of total phenols based on the reaction with 4-aminoantipyrine in an oxidizing alkaline medium,[36] resulting in a reagent consumption 200 times lower than that in the batch procedure.

Long-pathlength spectrophotometry yielded an 80-fold higher sensitivity relative to that in the batch procedure, thus avoiding the need for the usual liquid–liquid preconcentration, which in the batch mode would consume *ca.* 50 mL of chloroform and generate *ca.* 600 mL of waste per determination.

The spectrophotometric reference batch method for the determination of acid-dissociable cyanide in natural waters is based on the reaction with barbituric acid and pyridine. The replacement of hazardous chemicals and minimization of reagent amounts were exploited to develop an environmentally friendly procedure.[102] The MCFA system was designed to handle *o*-phthalaldehyde (OPA) and glycine; the isoindole derivative formed in the reaction was detected by fluorescence. The reagent consumption (5.6 μg of OPA and 6.7 μg of glycine per determination) was 230 times lower than that in a flow-based procedure with continuous reagent addition.

An MCFA procedure with fluorimetric detection was proposed as a greener alternative to the high-performance liquid chromatographic (HPLC) reference method for the determination of total tocopherol.[107] In the analysis of olive oils, the procedure required only sample dilution in 2-propanol and 13 mL of the solvent were required for the whole procedure. On the other hand, the HPLC procedure required a mixture of tetrahydrofuran (THF) and acetonitrile for sample preparation (10 mL) and elution (30 mL). Moreover, the sample throughput increased from 2 h^{-1} using HPLC to 40 h^{-1} in the flow-based procedure.

Furthermore, in the context of minimization of the consumption of chemicals, the amount of chloroform was reduced by 98% in the spectrophotometric determination of anionic surfactants in water.[113] In another application, MCFA made feasible the implementation of sample dilutions, external calibration and the standard additions method in the determination of benzene in gasoline by FTIR spectrometry.[97] The consumption of the organic solvent (hexane) was three times lower than that in the flow system with continuous pumping without affecting the analytical performance.

6.4.4.3 *Multipumping Flow Systems*

The potential of MPFS to minimize reagent consumption was demonstrated in the development of a greener procedure for nitrite determination in natural waters.[42] The classical method based on the Griess diazo coupling reaction was compared with a more environmentally friendly alternative, based on the formation of triiodide from nitrite and iodide in an acidic medium. The Griess method was selected owing to its better sensitivity and selectivity. The toxicity of the waste was then minimized, as the amount of the most toxic reagent, *N*-(1-naphthyl)ethylenediamine (NED), was reduced by 55- and 20-fold relative to those in the batch procedure and flow injection with continuous reagent addition, respectively. Moreover, the residue was mineralized on-line by the photo-Fenton reaction, yielding a colourless solution and reducing by 87% the total organic carbon content. In another study, waste minimization and replacement of hazardous chemicals [*e.g.* $Pb(NO_3)_2$ and NED used in previous

studies] were adopted to develop a greener procedure for the determination of cyclamate in table sweeteners.[65] The method was based on the reaction of the analyte with nitrite in an acidic medium, the excess of the reagent being determined by reaction with iodide. The procedure consumed only 3 mg of KI, 1.3 μg of $NaNO_2$ and 12.3 mg of H_3PO_4 per determination and generated only 2.0 mL of waste. The excess of the most toxic reagent (nitrite ions) was completely decomposed in the acidic medium.

Multipumping flow systems were also explored for the development of environmentally friendly analytical procedures for the determination of the pesticides paraquat[69] and carbaryl.[99] The replacement of sodium dithionite with dehydroascorbic acid and the 12-fold reduction in the amounts of reagents consumed were the strategies adopted in the paraquat assay, where the effluent volume was as low as 2.0 mL per determination. Sensitivity was increased with a 10 cm analytical path flow cell to avoid the preconcentration step required in previous work, which would result in additional waste. Carbaryl was determined in an MPFS coupled to a 100 cm optical path flow cell based on a liquid-core waveguide, reducing both the reagent consumption (1.9 μg of *p*-aminophenol and 5.7 μg of potassium metaperiodate) and the effluent volume (2.6 mL per determination). After waste degradation by UV irradiation in a medium of potassium persulfate, the total organic carbon was 94% lower than it was and the residue was non-toxic to *Vibrio fischeri* bacteria. Cloud point extraction was adopted in the clean-up step, instead of the LLE recommended by USEPA Method 8318, which consumes 90 mL of methylene chloride per determination.[114]

An MPFS with fluorimetric detection was proposed for the determination of free and total glycerol in biodiesel based on oxidation of the analyte to formaldehyde by potassium periodate and further reaction with acetylacetone.[115] The sample pretreatment was based on extraction with water (free form) and saponification of the bonded glycerol with sodium ethylate, aiming at the determination of total glycerol. Significant reductions in the consumption of the sample and reagents (up to 23-fold) were achieved in comparison with those in the analogous batch procedures.

6.4.4.4 *Sequential Injection Analysis and Multisyringe Flow Injection Systems*

An MSFIA system coupled to a multiport selection valve was proposed for the fractionation of phosphorus in soils and sediments with detection by the Molybdenum Blue method.[116] The solid sample was directly deposited into a minicolumn and the sequential extractions of labile P, P-species bonded to Fe and Al and Ca-bonded P were performed on-line with 1.0 mol L^{-1} NH_4Cl, 0.1 mol L^{-1} NaOH and 0.5 mol L^{-1} HCl according to the Hieltjes–Lijklema scheme. The proposed flow assembly has several advantages over batchwise fractionation, such as a reduction in the analysis time (from days to hours), minimization of energy expenditure and a decrease in reagent consumption by *ca.* 92%.

A flow system with two syringe pumps coupled to a cold vapour atomic fluorescence detector was used for mercury speciation after in-line sample cleanup and preconcentration.[109] The speciation was based on the separation of the inorganic form (as the tetrachloro complex) from the organic mercury on an anion-exchange membrane. The recoveries of the analyte spiked into the samples were close to 90% and the results for a fish muscle certified reference material (CRM) agreed with the certified value. The reagent consumption was decreased and the membrane separation replaced the classical LLE (with toluene and dichloromethane), thereby reducing the amount of waste and the toxicity.

MSFIA was also hyphenated to a liquid core waveguide for the determination of chloride in waters.[100] The classical analytical method based on reaction with $Hg(SCN)_2$ was improved by reducing the reagent consumption by 3400- and 600-fold in comparison with those required in a single-line flow system or with the reagent immobilized in epoxy resin,[31] respectively.

The lab-in-syringe approach has been successfully exploited to carry out liquid–liquid microextractions, including dispersive liquid–liquid microextraction[27] and cloud point extraction,[117] with reduced solvent consumption. For example, as little as 2 mg of Triton X-114 was consumed for the extraction and preconcentration of antimony as an iodide complex.[117] This approach was also exploited for the determination of trace amounts of silver in waters, involving complex formation, microextraction into the organic phase and transport of the extract to a graphite furnace for electrothermal atomic absorption spectrometric detection.[118] The procedure consumed only 120 μL of organic solvent to achieve an enrichment factor of 80 and a linear response range from 19 to 450 $ng\,L^{-1}$.

6.4.4.5 *Miniaturized Flow Systems*

Downscaling the flow manifolds is another useful approach to GAC, focusing on the implementation of analytical procedures with minimized amounts of reagents (flow rates in the order of nL to $\mu L\,min^{-1}$).[119] Microfluidics (Figure 6.5a) and microelectronics can be exploited to integrate propulsion, mixing and detection units in a single microflow injection system (μFIA), which is commonly designed for a specific application. Computerized controlled lathes, milling machines, laser engravers and low-temperature cofired ceramics technology have been exploited to build innovative (micro) platforms for flow analysis.[120] A more recent development in this field is 3D printing (Figure 6.5b), which allows the production of relatively complex manifolds based on polymers, even by ordinary users.

Recent applications of manifolds designed by 3D printing include chromium(VI)[101] and uranium(VI)[121] preconcentration. The former involved a disk-based solid-phase extraction device fabricated by stereolithography, which was reused 49 times with analyte recoveries of 95%. When coupled with an MSFIA system, the procedure was successfully applied to freshwater samples and leachates, in which as little as 1 ng of Cr(VI) can be detected.

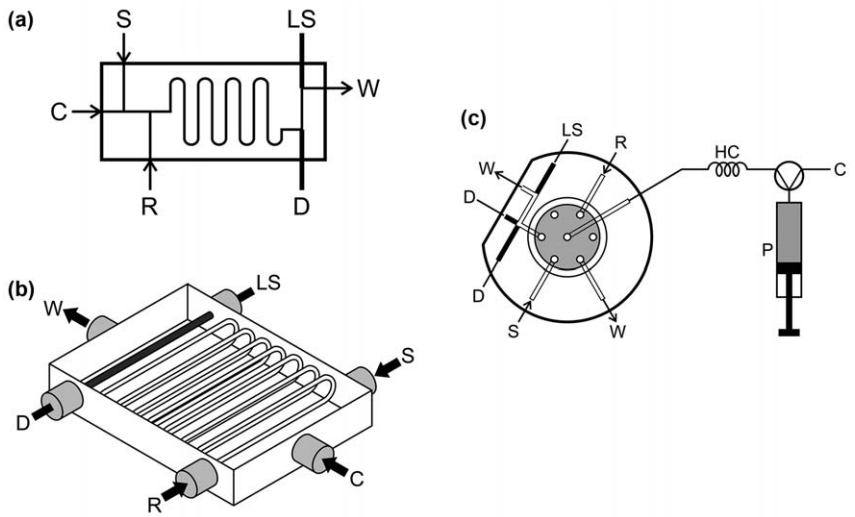


Figure 6.5 Miniaturized flow systems: (a) general scheme of a microfluidic device; (b) representation of 3D-printed manifold and (c) lab-on-valve system. P, syringe pump, HC, holding coil, R, reagent, S, sample; C, carrier, LS, light source, D, detector; W, waste vessel.

The latter application exploited a microfluidic device, in which a commercial resin was immobilized for analyte extraction. Despite the minimized dimensions, the device was used to process up to 30 mL of sample, aiming at the improvement of the enrichment factors. Both applications involved elution of the retained analytes to a detection system [UV–VIS spectrophotometer[101] or an inductively coupled plasma mass spectrometric (ICP-MS) detector[121]]; however, the integration of the detector into a chip-based analyser is sometimes feasible, as demonstrated by the determination of iodide in seawater, which incorporated fluorimetric detection.[122]

A more versatile alternative for miniaturization is the lab-on-valve approach[105,123–125] (Figure 6.5c), which exploits a microdevice integrating injection ports, microchannels and a flow cell coupled to conventional pumps and detection systems. Solutions can then be handled by conventional flow approaches, such as SIA. When the lab-on-valve approach was adopted for the spectrophotometric determination of phosphate, for example, the reagent consumption and effluent generation were reduced to 10 and 250 μL per determination, respectively.[105]

The development of inexpensive microfluidic devices with polymeric materials has been the focus of recent studies. As an example, a microflow analyser (total volume 7.0 μL) integrating a light-emitting diode (LED)-based photometer was constructed by deep UV lithography on urethane–acrylate polymer.[126] When the device was applied to the determination of chloride, the consumption of $Hg(SCN)_2$ was *ca.* 300 times lower than in the batch procedure, generating *ca.* 20 mL of effluent after an 8 h working day.

A microflow analyser was also applied to the determination of iron in water samples, based on the formation of the complex with nitroso-R salt.[103] The miniaturized system drastically reduced waste generation, which was lower than 2.0 mL h^{-1}, corresponding to 40 determinations. Another application was focused on the determination of tannins in green tea using a digital image-based microflow batch analyser.[106] The microdevice was fabricated with urethane–acrylate resin, glass slides and UV lithography; the digital images obtained from a webcam with a charge-coupled device (CCD) sensor were converted to RGB values. In addition to the low cost, the sampling rate was estimated as 190 h^{-1} and the reagent consumption was 300 times lower than in the reference method.

A more complex sample processing routine was involved in the determination of the pesticide carbaryl in a glass microchip, in which pesticide hydrolysis, diazotization and extraction of the product in toluene were implemented. The enrichment factor was estimated as 50 with a toluene volume of less than 300 nL per determination. The detection limit was estimated as 70 nmol L^{-1} with detection by thermal lens spectrometry.[98]

Another ingenious strategy for waste minimization is the analytical exploitation of falling drops.[48] Liquid drops can be reproducibly formed at the end of tubes aiming at sample processing on a removable reaction surface. The potential of this approach for GAC was demonstrated by the determination of sodium dodecyl sulfate by means of ion-pair formation with methylene blue and LLE in a 1.3 μL $CHCl_3$ microdrop. The analytical signal was measured directly in the drop surface with an LED-based photometer, achieving a detection limit of 50 μg L^{-1} and a CV of 5%.

6.4.5 Waste Treatment

In addition to the strategies for minimization of the amounts of waste and toxicity previously described, an additional step for on-line waste treatment can be efficiently coupled to flow systems. The general strategy is to introduce a suitable reagent after the flow cell to decompose or passivate the toxic species in the effluent. The detoxification of wastes can be achieved by photochemical, chemical, thermal or microbiological degradation processes. Photochemical degradation has been the most common alternative, resulting in effective degradation of dangerous organic compounds in time intervals compatible with the usual residence times in flow systems. Generally, the treatment is carried out with semiconductors (*e.g.* TiO_2 in anatase form), which, under UV irradiation, can generate electron–hole pairs and catalyse photo-assisted degradation. Thus several cleaner flow injection procedures have been developed by including the detoxification of wastes generated in the determination of carbamate pesticides[127,128] and resorcinol,[129] using a TiO_2 slurry and UV irradiation. As the semiconductor catalyst can be filtered and reused, the treatment step did not produce additional wastes. Photo-Fenton processes[42] and treatment with persulfate

under UV irradiation[99] were also used efficiently for the degradation of the wastes produced in flow-based procedures, as discussed previously.

Other efficient procedures for waste treatment, such as chemical or physical adsorption, precipitation and coprecipitation, ozonization and thermal processes have the potential to be accomplished in flow systems.[130] If longer residence times are necessary, the waste of the flow system can be directed to a batch waste treatment unit. The combined action of the minimization of reagent consumption and on-line treatment is very attractive for the development of greener analytical procedures, as discussed previously.[42] A detailed discussion of strategies for on-line decontamination of analytical wastes was presented in Chapter 10 of the first edition of this book.

6.5 Conclusions and Trends

This chapter aims to demonstrate the evolution of flow analysis towards GAC by the replacement of hazardous reagents, reuse of chemicals and waste minimization. This development has not hindered the fundamental analytical figures of merit and even better analytical performance has been achieved in some applications. For example, the improvement of sensitivity by exploiting either more effective processes or high-performance detectors allows the avoidance of preconcentration steps, which are time consuming and generate additional amounts of waste. Furthermore, the reagentless procedures, analyte extraction with minimized solvent amounts and reuse of chemicals have been made feasible by means of ingenious flow configurations. However, more general alternatives, such as MCFA, SIA, MSFIA and MPFS, seem to be more promising for achieving the GAC goals.

A clear parallel can be traced between the evolution of flow analysis and the agreement with GAC principles, encompassing especially novel flow modalities to ensure the effective use of chemical reagents without waste. The mechanization of analytical methodologies was the main aim when flow analysis was introduced, with the perspective of maximizing the sampling rate without worrying about the amount or toxicity of the waste generated. Fortunately, the focus was quickly changed to more ingenious approaches to decrease reagent consumption and waste generation without sacrificing the analytical performance. Characteristics inherent to flow analysis, such as reproducible timing and sample processing conditions, have allowed the development of innovative and environmentally friendly chemical assays involving partial extractions and incomplete reactions, for example.

System miniaturization and the development of greener approaches for sample preparation, including coupling to separation techniques, are current trends. The devices manufactured using a 3D printer without a skilled operator and which support various types of organic solvents and acidic and basic reagents stands out in the design of microflow analysers. Moreover, approaches involving innovative and greener sorbents and extraction solvents (*e.g.* surfactants and ionic liquids), liquid–liquid microextractions and analyte extractions and sample decomposition under mild experimental

conditions (*e.g.* microwave-assisted and photochemical processes) have been developed to attain this goal.

Independent of the analytical performance, methods that are not environmentally friendly tend to be unacceptable in the near future. The development of flow analysis has contributed to minimizing the impact of analytical activities, although their potential has not yet been fully exploited, thus generating a promising research field.

References

1. S. Armenta, S. Garrigues and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2008, **27**(6), 497.
2. F. R. P. Rocha, J. A. Nobrega and O. Fatibello, *Green Chem.*, 2001, **3**, 216.
3. F. R. P. Rocha, W. R. Melchert and B. F. Reis, *Anal. Chim. Acta*, 2012, **714**, 8.
4. C. Calderilla, F. Maya, L. O. Leal and V. Cerdà, *TrAC, Trends Anal. Chem.*, 2018, **108**, 370.
5. M. Alexovic, B. Horstkotte, P. Solich and J. Sabo, *Anal. Chim. Acta*, 2016, **906**, 22.
6. M. Trojanowicz and K. Kołacińska, *Analyst*, 2016, **141**(7), 2085.
7. W. E. van der Linden, *Pure Appl. Chem.*, 1994, **66**(12), 2493.
8. M. Valcarcel and M. D. Luque de Castro, *Flow Injection Analysis. Principles and Applications*, Ellis Horwood, Chichester, 1987, p. 759.
9. J. Ruzicka and E. H. Hansen, *Flow Injection Analysis*, Wiley-Interscience, New York, 1988, p. 528.
10. B. Karlberg and G. E. Pacey, *Flow Injection Analysis. A Practical Guide*, Elsevier, Amsterdam, 1989, p. 371.
11. E. A. G. Zagatto, C. C. Oliveira, A. Townshend and P. J. Worsfold, *Flow Analysis with Spectrophotometric and Luminometric Detection*, Elsevier, Amsterdam, 2012, p. 471.
12. F. R. P. Rocha, *J. Braz. Chem. Soc.*, 2018, **29**(5), 1032.
13. B. Horstkotte, M. Miró and P. Solich, *Anal. Bioanal. Chem.*, 2018, **410**(25), 6361.
14. L. T. Skeggs Jr., *Clin. Chem.*, 2000, **46**(9), 1425.
15. J. Ruzicka and G. D. Marshall, *Anal. Chim. Acta*, 1990, **237**(1), 329.
16. E. A. G. Zagatto, F. R. P. Rocha, P. B. Martelli and B. F. Reis, *Pure Appl. Chem.*, 2001, **73**(1), 45.
17. C. Pasquini and W. A. Oliveira, *Anal. Chem.*, 1985, **57**(13), 2575.
18. V. O. Brito and I. M. Raimundo Jr., *Anal. Chim. Acta*, 1998, **371**(2–3), 317.
19. I. M. Raimundo Jr. and C. Pasquini, *Analyst*, 1997, **122**(10), 1039.
20. I. Facchin, J. W. Martins, P. G. P. Zamora and C. Pasquini, *Anal. Chim. Acta*, 1994, **285**(3), 287.
21. I. Facchin and C. Pasquini, *Anal. Chim. Acta*, 1995, **308**(3), 231.
22. M. D. H. da Silva and C. Pasquini, *Anal. Chim. Acta*, 1997, **349**(1–3), 377.

23. F. R. P. Rocha, B. F. Reis, E. A. G. Zagatto, J. L. F. C. Lima, R. A. S. Lapa and J. L. M. Santos, *Anal. Chim. Acta*, 2002, **468**(1), 119.
24. J. L. F. C. Lima, J. L. M. Santos, A. C. B. Dias, M. F. T. Ribeiro and E. A. G. Zagatto, *Talanta*, 2004, **64**(5), 1091.
25. P. R. Fortes, M. A. Feres, M. K. Sasaki, E. R. Alves, E. A. G. Zagatto, J. A. V. Prior, J. L. M. Santos and J. L. F. C. Lima, *Talanta*, 2009, **79**(4), 978.
26. P. H. G. D. Diniz, L. F. Almeida, D. P. Harding and M. C. U. Araújo, *TrAC, Trends Anal. Chem.*, 2012, **35**, 39.
27. F. Maya, B. Horstkotte, J. M. Estela and V. Cerdà, *Anal. Bioanal. Chem.*, 2012, **404**(3), 909.
28. M. Miró, V. Cerdà and J. M. Estela, *TrAC, Trends Anal. Chem.*, 2002, **21**(3), 199.
29. E. H. Hansen and J. Ruzicka, *Anal. Chim. Acta*, 1976, **87**(2), 353.
30. F. R. P. Rocha, P. B. Martelli and B. F. Reis, *Anal. Chim. Acta*, 2001, **438**(1–2), 11.
31. C. R. Silva, H. J. Vieira, L. S. Canaes, J. A. Nóbrega and O. Fatibello-Filho, *Talanta*, 2005, **65**(4), 965.
32. H. Bergamin-Filho, E. A. G. Zagatto, F. J. Krug and B. F. Reis, *Anal. Chim. Acta*, 1978, **101**(1), 17.
33. E. A. G. Zagatto, A. O. Jacintho, J. Mortatti and H. Bergamin-Filho, *Anal. Chim. Acta*, 1980, **120**(1), 399.
34. F. R. P. Rocha, P. B. Martelli, R. M. Frizzarin and B. F. Reis, *Anal. Chim. Acta*, 1998, **366**(1–3), 45.
35. P. B. Martelli, F. R. P. Rocha, R. C. P. Gorga and B. F. Reis, *J. Braz. Chem. Soc.*, 2002, **13**(5), 642.
36. K. O. Lupetti, F. R. P. Rocha and O. Fatibello-Filho, *Talanta*, 2004, **62**(3), 463.
37. B. Ettinger, C. C. Kuchiioft and H. J. Lixhli, *Anal. Chem.*, 1951, **23**(12), 1783.
38. W. Frenzel, J. O. Frenzel and J. Möller, *Anal. Chim. Acta*, 1992, **261**(1–2), 253.
39. P. D. Goulden, P. Brooksbank and M. B. Day, *Anal. Chem.*, 1973, **45**(14), 2430.
40. R. A. S. Lapa, J. L. F. C. Lima and I. V. O. S. Pinto, *Analusis*, 2000, **28**, 295.
41. J. F. van Staden and T. A. van der Merwe, *Microchim. Acta*, 1998, **129**(1–2), 33.
42. W. R. Melchert, C. M. C. Infante and F. R. P. Rocha, *Microchem. J.*, 2007, **85**(2), 209.
43. J. Ruzicka and J. W. B. Stewart, *Anal. Chim. Acta*, 1975, **79**, 79.
44. R. N. Fernandes and B. F. Reis, *Talanta*, 2002, **58**(4), 729.
45. G. N. Doku and S. J. Haswell, *Anal. Chim. Acta*, 1999, **382**(1–2), 1.
46. A. D. Eaton, L. S. Clesceri, A. E. Greenberg and M. A. H. Franson, *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, 1995, p. 1325.

47. F. J. Krug, L. C. R. Pessenda, E. A. G. Zagatto, A. O. Jacintho and B. F. Reis, *Anal. Chim. Acta*, 1981, **130**(2), 409.
48. H. Liu and P. K. Dasgupta, *Anal. Chem.*, 1996, **68**(11), 1817.
49. B. Karlberg and S. Thelander, *Anal. Chim. Acta*, 1978, **98**(1), 1.
50. D. A. V. Medina, A. J. Santos-Neto, V. Cerdà and F. Maya, *Talanta*, 2018, **189**(1), 241.
51. S. Clavijo, M. Fernández, R. Forteza, M. R. Brunetto and V. Cerdà, *Anal. Methods*, 2014, **6**(10), 3335.
52. A. L. D. Comitre and B. F. Reis, *Talanta*, 2005, **65**(4), 846.
53. L. Nugbienyo, A. Shishov, S. Garmonov, L. Moskvin, V. Andruch and A. Bulatov, *Talanta*, 2017, **168**(1), 307.
54. P. Li, M. He, B. Chen and B. Hu, *J. Chromatogr. A*, 2015, **1415**, 48.
55. A. Alonso, M. J. Almendral, M. J. Porras and Y. Curto, *J. Pharm. Biomed. Anal.*, 2006, **42**(2), 171.
56. C. F. Nascimento and F. R. P. Rocha, *Microchem. J.*, 2018, **137**, 429.
57. R. Sánchez, B. Horstkotte, K. Fikarová, H. Sklenárová, S. Maestre, M. Miró and J. L. Todolí, *Anal. Chem.*, 2017, **89**(6), 3787.
58. K. Carlsson and B. Karlberg, *Anal. Chim. Acta*, 2000, **415**(1–2), 1.
59. J. González-Rodríguez, P. Pérez-Juan and M. D. Luque de Castro, *Talanta*, 2003, **59**(4), 691.
60. S. S. Borges, R. M. Frizzarin and B. F. Reis, *Anal. Bioanal. Chem.*, 2006, **385**(1), 197.
61. R. S. Costa, S. R. B. Santos, L. F. Almeida, E. C. L. Nascimento, M. J. C. Pontes, R. A. C. Lima, S. S. Simões and M. C. U. Araújo, *Microchem. J.*, 2004, **78**(1), 27.
62. D. S. Silva and B. F. Reis, *Microchem. J.*, 2016, **129**, 325.
63. T. Mantim, P. Saetear, S. Teerasong, S. Chan-Eam, K. Sereenonchai, N. Amornthammarong, N. Ratanawimarnwong, P. Wilairat, W. Meesiri, K. Uraisin and D. Nacapricha, *Pure Appl. Chem.*, 2012, **84**(10), 2015.
64. C. Pasquini and L. C. Faria, *Anal. Chim. Acta*, 1987, **193**, 19.
65. F. R. P. Rocha, E. Ródenas-Torralba, A. Morales-Rubio and M. de la Guardia, *Anal. Chim. Acta*, 2005, **547**(2), 204.
66. J. G. March and B. M. Simonet, *Talanta*, 2007, **73**(2), 232.
67. V. A. Lemos, P. X. Baliza, A. L. Carvalho, R. V. Oliveira, L. S. G. Teixeira and M. A. Bezerra, *Talanta*, 2008, **77**(1), 388.
68. W. R. Melchert and F. R. P. Rocha, *Talanta*, 2005, **65**(2), 461.
69. C. M. C. Infante, A. Morales-Rubio, M. de la Guardia and F. R. P. Rocha, *Talanta*, 2008, **75**(5), 1376.
70. I. C. Vieira and O. Fatibello-Filho, *Anal. Chim. Acta*, 1998, **366**(1–3), 111.
71. L. C. Faria, C. Pasquini and G. Oliveira-Neto, *Analyst*, 1991, **116**(4), 357.
72. M. F. Giné, H. Bergamin Filho, E. A. G. Zagatto and B. F. Reis, *Anal. Chim. Acta*, 1980, **114**(1), 191.
73. S. Motomizu and M. Sanada, *Anal. Chim. Acta*, 1995, **308**(1–3), 406.
74. C. J. Patton, A. E. Fischer, W. H. Campbell and E. R. Campbell, *Environ. Sci. Technol.*, 2002, **36**(4), 729.

75. I. G. Torró, J. V. G. Mateo and J. M. Calatayud, *Anal. Chim. Acta*, 1998, **366**(1–3), 241.
76. F. J. Krug, J. Ruzicka and E. H. Hansen, *Analyst*, 1979, **104**(1234), 47.
77. F. J. Krug, B. F. Reis, M. F. Giné, J. R. Ferreira, A. O. Jacintho and E. A. G. Zagatto, *Anal. Chim. Acta*, 1983, **151**, 39.
78. J. A. Noórega, A. A. Mozetto, R. M. Alberici and J. L. Guimarães, *J. Braz. Chem. Soc.*, 1995, **6**(4), 327.
79. O. Fatibello-Filho and I. C. Vieira, *Anal. Chim. Acta*, 1997, **354**(1–3), 51.
80. O. Fatibello-Filho and I. C. Vieira, *Analyst*, 1997, **122**(4), 345.
81. T. Settheeworrarit, S. K. Hartwell, S. Lapanatnoppakhun, J. Jakmunee, G. D. Christian and K. Grudpan, *Talanta*, 2005, **68**(2), 262.
82. N. Jaikrajang, S. Kruanetr, D. J. Harding and P. Rattanakit, *Spectrochim. Acta, Part A*, 2018, **204**, 726.
83. A. P. S. Gonzáles, M. A. Firmino, C. S. Nomura, F. R. P. Rocha, P. V. Oliveira and I. Gaubeur, *Anal. Chim. Acta*, 2009, **636**(2), 198.
84. D. Wilson, M. del Valle, S. Alegret, C. Valderrama and A. Florido, *Talanta*, 2013, **114**(1), 17.
85. F. A. S. Cajamarca, M. Z. Corazza, M. C. Prete, D. C. Dragunski, C. Rocker, J. Caetano, A. C. Goncalves Júnior and C. R. T. Tarley, *Bull. Environ. Contam. Toxicol.*, 2016, **97**(6), 863.
86. V. N. Alves and N. M. M. Coelho, *Microchem. J.*, 2013, **109**, 16.
87. A. A. Menegário, A. J. Silva, E. Pozzi, S. F. Durrant and C. H. Abreu Jr., *Spectrochim. Acta, Part B*, 2006, **61**(9), 1074.
88. J. Koh, Y. Kwon and Y. N. Pak, *Microchem. J.*, 2005, **80**(2), 195.
89. W. R. Melchert and F. R. P. Rocha, *Rev. Anal. Chem*, 2016, **35**, 41.
90. R. M. Frizzarin and F. R. P. Rocha, *Anal. Chim. Acta*, 2014, **820**, 69.
91. D. L. Rocha, M. Y. Kamogawa and F. R. P. Rocha, *Anal. Chim. Acta*, 2015, **896**, 11.
92. Z. Bouhsain, S. Garrigues and M. de la Guardia, *Analyst*, 1997, **122**(5), 441.
93. L. S. G. Teixeira and F. R. P. Rocha, *Talanta*, 2007, **71**(4), 1507.
94. M. J. Sánchez-Dasi, S. Garrigues, M. L. Cervera and M. de la Guardia, *Anal. Chim. Acta*, 1998, **361**(3), 253.
95. M. Zenki, K. Minamisawa and T. Yokoyama, *Talanta*, 2005, **68**(2), 281.
96. L. S. G. Teixeira, F. R. P. Rocha, M. Korn, B. F. Reis, S. L. C. Ferreira and A. C. S. Costa, *Anal. Chim. Acta*, 1999, **383**(3), 309.
97. E. Ródenas-Torralba, J. Ventura-Gayete, A. Morales-Rubio, S. Garrigues and M. de la Guardia, *Anal. Chim. Acta*, 2004, **512**(2), 215.
98. A. Smirnova, K. Mawatari, A. Hibara, M. A. Proskurnin and T. Kitamori, *Anal. Chim. Acta*, 2006, **558**(1–2), 69.
99. W. R. Melchert and F. R. P. Rocha, *Talanta*, 2010, **81**(1–2), 327.
100. F. Maya, J. M. Estela and V. Cerdà, *Anal. Bioanal. Chem.*, 2009, **394**(6), 1577.
101. C. Calderilla, F. Maya, V. Cerdà and L. O. Leal, *Talanta*, 2018, **184**(1), 15.
102. C. M. C. Infante, J. C. Masini and F. R. P. Rocha, *Anal. Bioanal. Chem.*, 2008, **391**(8), 2931.

103. S. Kruanetr, S. Liawruangrath and N. Youngvises, *Talanta*, 2007, **73**(1), 46.
104. A. M. Serra, J. M. Estela and V. Cerdà, *Talanta*, 2009, **78**(3), 790.
105. J. Ruzicka, *Analyst*, 2000, **125**(6), 1053.
106. M. B. Lima, S. I. E. Andrade, I. S. Barreto, L. F. Almeida and M. C. U. Araújo, *Microchem. J.*, 2013, **106**, 238.
107. F. J. Lara-Ortega, B. Gilbert-López, J. F. García-Reyes and A. Molina-Díaz, *Food Anal. Methods*, 2017, **10**(7), 2125.
108. J. M. Calatayud and J. V. G. Mateo, *TrAC, Trends Anal. Chem.*, 1993, **12**(10), 428.
109. L. L. Zamora and J. M. Calatayud, *Talanta*, 1993, **40**(7), 1067.
110. V. G. Bonifácio, L. C. Figueiredo-Filho, L. H. Marcolino Jr. and O. Fatibello-Filho, *Talanta*, 2007, **72**(2), 663.
111. F. R. P. Rocha, I. M. Raimundo Jr. and L. S. G. Teixeira, *Anal. Lett.*, 2011, **44**(1), 528.
112. B. F. Reis, A. Morales-Rubio and M. de la Guardia, *Anal. Chim. Acta*, 1999, **392**(2–3), 265.
113. E. Ródenas-Torralba, B. F. Reis, A. Morales-Rubio and M. de la Guardia, *Talanta*, 2005, **66**(3), 591.
114. http://www.epa.gov (last accessed March 2019).
115. S. G. Silva, A. Morales-Rubio, M. de la Guardia and F. R. P. Rocha, *Anal. Bioanal. Chem.*, 2011, **401**(1), 365.
116. J. Buanuam, M. Miró, E. H. Hansen, J. Shiowatana, J. M. Estela and V. Cerdà, *Talanta*, 2007, **71**(4), 1710.
117. R. M. Frizzarin, L. A. Portugal, J. M. Estela, F. R. P. Rocha and V. Cerdà, *Talanta*, 2016, **148**, 694.
118. G. Giakisikli and A. N. Anthemidis, *Talanta*, 2017, **166**(1), 364.
119. S. J. Haswell, *Analyst*, 1997, **122**(1), 1R.
120. V. Cerdà, J. Avivar and D. Moreno, *Talanta*, 2017, **166**(1), 412.
121. M. R. Ceballos, F. G. Serra, J. M. Estela, V. Cerdà and L. Ferrer, *Talanta*, 2019, **196**, 510.
122. R. M. Frizzarin, E. Aguado, L. A. Portugal, D. Moreno, J. M. Estela, F. R. P. Rocha and V. Cerdà, *Talanta*, 2015, **144**, 1155.
123. Y. Yu, Y. Jiang, M. Chen and J. Wang, *TrAC, Trends Anal. Chem.*, 2011, **30**(10), 1649.
124. S. S. M. P. Vidigal, I. V. Tóth and A. O. S. S. Rangel, *Anal. Methods*, 2013, **5**(3), 585.
125. M. Miró, *TrAC, Trends Anal. Chem.*, 2014, **62**, 154.
126. A. Fonseca, I. M. Raimundo Jr., J. J. R. Rohwedder and L. O. S. Ferreira, *Anal. Chim. Acta*, 2007, **603**(2), 159.
127. M. de la Guardia, K. D. Khalaf, V. Carbonell and A. Morales-Rubio, *Anal. Chim. Acta*, 1995, **308**(1–3), 462.
128. M. J. Escuriola, A. Morales-Rubio and M. de la Guardia, *Anal. Chim. Acta*, 1999, **390**(1–3), 147.
129. M. de la Guardia, K. D. Khalaf, B. A. Hasan, A. Morales-Rubio and V. Carbonell, *Analyst*, 1995, **120**(2), 231.
130. G. Lunn and E. B. Sansone, *Destruction of Hazardous Chemicals in the Laboratory*, Wiley-Interscience Publication, New York, 1994, p. 501.

CHAPTER 7

(Bio)electroanalysis in the Field of Greener Analytical Chemistry

PALOMA YÁÑEZ-SEDEÑO, SUSANA CAMPUZANO AND JOSÉ MANUEL PINGARRÓN*

Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, 28040 Madrid, Spain
*Email: pingarro@quim.ucm.es

7.1 Introduction

Some years ago, the use of greener solvents, reagents and electrode materials was highlighted as an outstanding trend in future electroanalysis.[1] Nowadays, it can be said that modern electroanalysis is in consonance with the principles of green chemistry.[2] Electrodes are prepared with non-toxic materials and modified with a variety of composites, alloys, polymers, biomaterials, nanomaterials or receptors synthesized by applying eco-friendly procedures using green solvents and reagents. The intrinsic nature of the electrochemical measurement, where the redox reaction takes place practically in the absence of reagents, and the high sensitivity achieved make possible the application of techniques and methodologies involving miniaturized devices and microsystems with the consequent reduction of sample size and the amounts of waste products, allowing results to be obtained in real time with a minimal impact on the environment.[3,4] Related to this, the portability of electroanalytical devices can also be highlighted as a

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

valuable property exploited for the *in situ*, rapid and sensitive determination of pesticide residues and trace amounts of metals, among other polluting substances.[5] Furthermore, the possibility of using electrochemical sensors or biosensors to obtain direct analytical responses without the need for sample treatment or derivatization, thus avoiding the addition of organic solvents and hazardous substances, falls squarely into the conceptual standards of green chemistry and sustainability.[6]

All these aspects are discussed in this chapter and illustrated with examples taken from the recent literature. In addition, with the aim of providing suitable comprehensive information, the chapter includes a table summarizing the fundamentals and characteristics of relevant electroanalytical methods involving green strategies.

7.2 Electrodes and Electrochemical Sensors

This section discusses the broad variety of strategies falling within sustainability and care of the environment. Specific materials have demonstrated their suitability as alternatives to the use of mercury and have been applied mainly in the determination of toxic metals. Other methodologies have been developed for general applications using more ''natural'' starting materials, greener protocols of synthesis and avoiding as far as possible the use of polluting reagents, decreasing the amount of wastes or reducing the number of preparation stages.

7.2.1 Alternatives to Mercury Electrodes

Since 2005, when the electroanalytical usefulness of bismuth film electrodes[7] and their applications in stripping analysis[8] were reviewed, a variety of methods, most of them used for trace metal determinations, involving electrodes constructed with materials other than mercury have been reported. In addition to bismuth electrodes, which were recently reviewed by Jovanovski *et al.*,[9] those prepared with antimony[10] and tin[11] or mixtures of these metals[12] have a large number of applications. The use of environmentally friendly mercury-free electrodes for multielement determinations at trace concentrations in waters and wastewaters was reviewed by Alves *et al.*[13] and, more recently, by Economou,[14] who discussed the use of commercial or suitably modified screen-printed electrodes (SPEs) as an attractive alternative to conventional electrodes in this field. As relevant examples, SPEs modified with antimony[15,16] or antimony and tin[17] have demonstrated their suitability for the voltammetric determination of trace metals in waters. In this context, spark discharge methods have evolved as a green alternative to electroplating for the preparation of metal-modified electrodes with much lower costs and less waste production since they do not require the use of any liquids.[18] As a recent example, sparked tin nanoparticle-modified graphite SPEs were prepared in the absence of organic solvents using a green, low-cost, rapid and extremely facile method. The modified

graphite SPEs were applied to the stripping voltammetric determination of Cd and Zn, exhibiting better analytical properties than those provided by electroplated tin electrodes.[19] These mercury-free electrodes have also been employed for the determination of organic compounds by stripping methods. Illustrative examples are those reported by Tyszczuk-Rotko *et al.*[20] using an *in situ* plated bismuth film glassy carbon electrode (GCE) to develop a method for the determination of rutin (quercetin-3-*O*-rutinoside), a glycoside combining the flavonol quercetin and the disaccharide rutinose [α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranose], by square-wave adsorptive stripping voltammetry (SWAdSV), or the use of a similar electrode for voltammetric studies on vitamin B_2 and its determination in oral, syrup and tablet samples,[21] the preparation of a bismuth nanowire-modified GCE for the selective determination of folic acid[22] and the use of sparked bismuth oxide SPEs for the determination of riboflavin at the sub-nanomolar level in non-deoxygenated solutions.[23]

Materials other than metals have also been proposed for fabricating electrodes avoiding the use of mercury. Some of them have been used in stripping analysis involving analytical protocols and devices intended for ''green chemistry'' with non-toxic materials and negligible waste. In the field of trace metal analysis, nanostructured composites and polymers have been used as eco-friendly and efficient electrode modifiers. An illustrative example is the preparation of a γ-AlOOH–carbonated bacterial cellulose (γ-AlOOH–CBC) hybrid by simple pyrolysis and hydrothermal treatments. The hybrid material was employed as a GCE modifier for the simultaneous determination of Cd(II) and Pb(II) in aqueous samples by DPASV. The intrinsic 3D nanofibrous structure decorated with chaff-like γ-AlOOH particles provided both good adsorptive and conductive properties to the synthesized material, which allowed limits of detection (LODs) of 0.17 ng mL^{-1} for Cd(II) and 0.10 ng mL^{-1} for Pb(II) to be reached. The GC-modified electrode was successfully applied to monitoring trace metals in drinking waters.[24] Figure 7.1A shows the use of an electropolymerized poly-Eriochrome Black T [poly(EBT)]-modified GCE as an environmentally friendly electrode for the detection of zinc in industrial wastewater. The modifying material extended the cathodic potential window and improved the sensitivity and repeatability of the measurements.[25] In a more recent study, a facile and green strategy was employed for the one-pot *in situ* fabrication of dandelion-like polyaniline (PANI)-coated gold nanoparticle (Au@PANI) nanocomposites, which were used as GC electrode modifiers for the square-wave anodic stripping voltammetric (SWASV) determination of Pb^{2+} and Cd^{2+} (Figure 7.1B).[26] Regarding organic analytes, a relevant example is the low-cost, easily fabricated and highly selective sodium montmorillonite-modified carbon paste electrode (Na-MMT/CPE) prepared by El-Desoky *et al.*[27] for the determination of etilefrine hydrochloride, a direct-acting sympathomimetic with β_1-agonist properties, used for the treatment of hypotensive states, by SWAdSV. The strong adsorptive properties of montmorillonite natural clay allowed a low LOD of 1.27 nmol L^{-1} in spiked human serum to be attained.

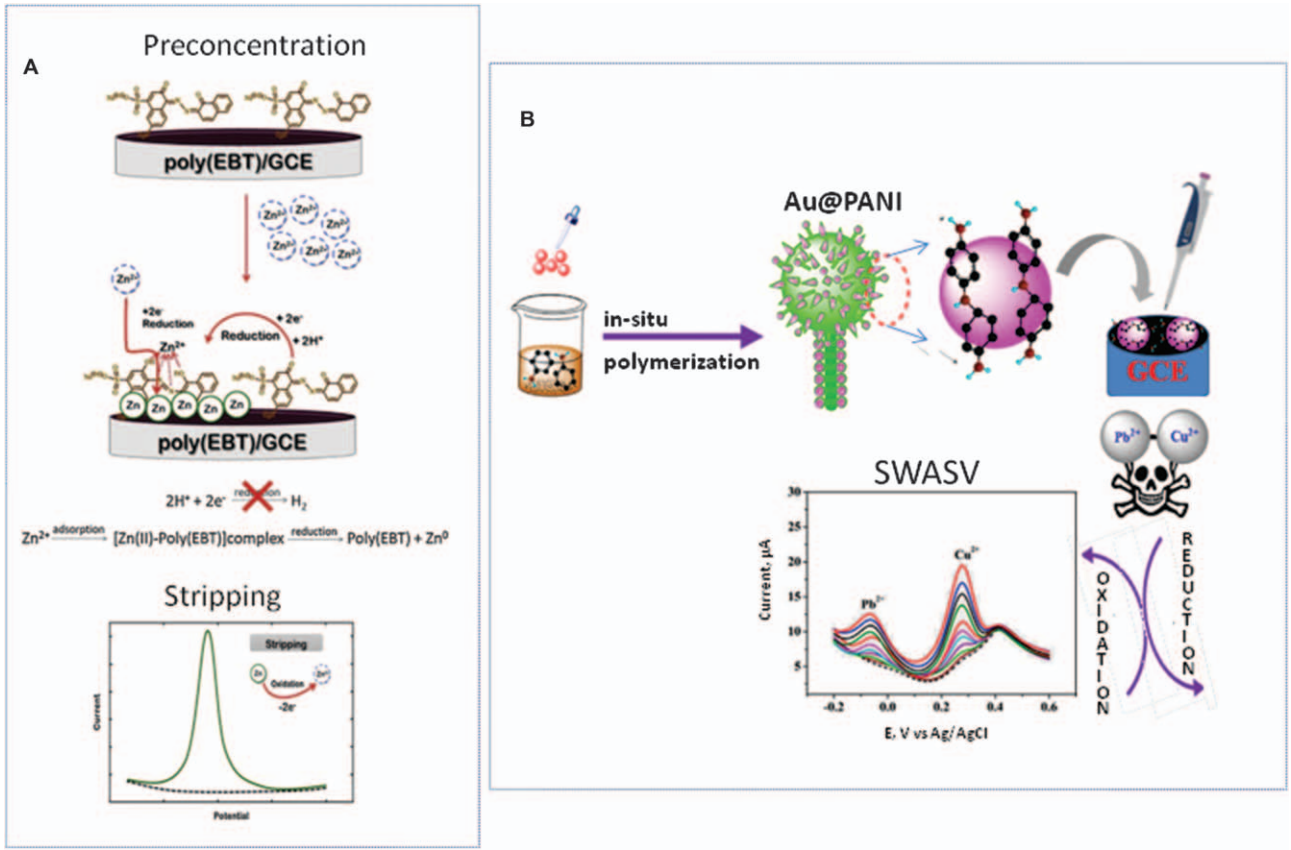


Figure 7.1 Schematic diagrams of the preconcentration and stripping method for the determination of zinc on a poly-Eriochrome Black T [poly(EBT)]-modified GCE (A) and the preparation of dandelion-like Au@PANI nanocomposites and their use a an electrode-modifying material for the determination of Pb^{2+} and Cu^{2+} by SWASV (B).
Reproduced from ref. 25 and 26 with permission from Elsevier, Copyright 2018.

In addition, electrodes fabricated from other materials have also been proposed as alternatives to mercury electrodes. A very popular electrode is the boron-doped diamond electrode (BDDE), which possesses attractive properties including a wide potential window in aqueous solutions, low background currents, high resistance to fouling and ease of chemical modification.[28] This electrode can be utilized for determining species previously analysed with mercury electrodes, using their anodic responses instead of the reduction currents. For example, a BDDE was successfully applied to determine the herbicide picloram by a voltammetric method and measurement of the peak current at +1.5 V *vs.* Ag/AgCl with high repeatability and a low LOD of 1.64 μmol L^{-1}.[29] In a more recent application, this electrode was used for the selective electrochemical detection of oxytocin and vasopressin, two nonapeptides with similar structures, based on the measurement of currents at the oxidized BDDE surface using flow injection analysis (FIA). Although electrochemical methods are not currently used for the direct detection of peptides or proteins at conventional electrodes owing to the passivation induced by the adsorption of oxidized or polymerized molecules, these drawbacks can be minimized at a BDDE.[30] The electrooxidation of the xanthine oxidase inhibitor febuxostat was also investigated on a BDDE and a green electroanalytical method was developed using square-wave voltammetry (SWV) at the unmodified electrode surface.[31]

7.2.2 Novel Eco-inspired Electrode Materials

Many green and sustainable methods for the synthesis of carbon nanomaterials and their hybrids to be used as scaffolds for (bio)electroanalytical platforms have been described in recent years. Graphene (Gr), a single sheet of sp^2-bonded carbons arranged in a honeycomb lattice, has attracted great attention because of its high surface-to-volume ratio, high electrical conductivity and flexibility.[32] Several materials related to Gr have been obtained *via* a variety of green synthesis routes, including graphite oxide, graphene oxide (GO) and reduced graphene oxide (rGO), which differ from each other in the oxidation level of carbon and the number of layers.[33] Owing to the large number of synthetic procedures, the diversity of the materials used and the variety of applications, a selection of recent relevant examples (from 2016 to 2019) are summarized in Table 7.1, where the most important characteristics of the reported methods are highlighted.[34–57] As can be seen, the most commonly used starting material for the preparation of Gr and its derivatives is GO. This is obtained from graphite by the Hummers method,[34,35,37–42,44,45,47–49,51,53–57] although this method is of concern for the environment since it involves the use of oxidants such as $KMnO_4$ dissolved in concentrated H_2SO_4.[58] Greener methods with reducing agents have been applied to synthesize rGO. Hydrothermal procedures involving environmentally friendly reagents such as ascorbic acid,[37] caffeic acid,[34] citric acid,[39] glucose[40,41] and alginate[52] have been used. Plant extracts have also been utilized to obtain nanoparticle–graphene hybrids.[35,36,42] Piperazine[47]

Table 7.1 Green methods used for the preparation of graphene-based materials and development of electrode platforms.[a]

Graphene-based material	Synthesis	Electrode	Application: analyte/sample	Technique	Analytical characteristics	Ref.
[Cu(sal-ala)(phen)]/rGO	Addition of caffeic acid to GO dispersion; heat at 95 °C; addition to [Cu(sal-ala)(phen)] prepared with 1,10-phen, salicylamine and $CuCl_2$	[Cu(sal-ala)(phen)]/rGO/GCE	Nitrite	Amperometry	LR: 0.05–1000 μmol L^{-1} LOD: 19 nmol L^{-1}	34
AgNPs/rGO	Addition of $AgNO_3$ to GO dispersion in ethanol; stirring; transfer to aqueous *Justicia adhatoda* (adulsa) leaf extract; reflux at 80 °C; wash and dry at 60 °C	AgNPs/rGO/GCE	Nitrite	Amperometry	LR: 0.01–1 and 10–1000 μmol L^{-1}	35
AuNPs/GO	$HAuCl_4$ mixed with *Bischofia javanica Blume* extract to form AuNPs; lyophilization and mixing with exfoliated GO; ultrasonication	AuNPs/GO/GCE	CAP/honey, milk, eye drops	Amperometry	LR: 1.5–2.95 μmol L^{-1} LOD: 0.25 μmol L^{-1}	36
Mb/AuNPs/pDA/Gr	GO reduction with AA by ultrasonication at 60 °C; $HAuCl_4$ and Mb added to reduced GO, stirring and addition of DA; stirring, centrifugation and wash	Mb/AuNPs/pDA/Gr/GCE	H_2O_2	Amperometry	LR: 0.6–480 μmol L^{-1} LOD: 0.2 μmol L^{-1}	37
CoNPs/amino-Gr	Addition of glycine to GO dispersion; stirring; NaOH and $Co(NO_3)_2$; heat at 100 °C in autoclave; cooling and centrifugation; water/ethanol washing	CoNPs/amino-Gr/GCE	Baicalin/capsules	SWV	LR: 0.01–0.8 μmol L^{-1} LOD: 5 nmol L^{-1}	38

Ni-TPP/rGO	Reduction of GO by citric acid at 95 °C, dry at 35 °C under vacuum; addition of Ni-TPP dissolved in DMF to the as-prepared rGO	Ni-TPP/rGO/GCE	NB/waters	DPV	LR: 5–878 μmol L^{-1} LOD: 0.14 μmol L^{-1}	39
AgNPs/GO	Addition of glucose as reducing agent to sonicated GO aqueous dispersion and mix with $Ag(NH_3)_2$ solution; wash and dry in oven at 60 °C	AgNPs/GO/GCE	TSGF/spiked human serum	DPV	LR: 0.01–50 and 50–800 μmol L^{-1} LOD: 7 nmol L^{-1}	40
AgNPs/rGO	Addition of glucose, $AgNO_3$ and NaCl to aqueous GO suspension, heat in autoclave at 160 °C; freeze at −37 °C	AgNPs/rGO/GCE	Glucose	Amperometry	LR: 0.005–3 and 3–8 mmol L^{-1}	41
Fe_3O_4/rGO	Mixing of $FeCl_3$ and $FeSO_4$ with extract of *Sapindus mukorossi* fruits and GO dispersion, aged 30 min at 50 °C	Fe_3O_4/rGO/GCE	Riboflavin	DPV	LR: 0.3–1 and 1–100 μmol L^{-1} LOD: 89 nmol L^{-1}	42
Gr	Sonicated graphite flakes in NMP; centrifuge, dry at 60 °C; addition of Nf solution and drop on SPE	GrSPE	Caffeine	DPAdSV	LR: 0.10–0.90 and 1.0–10 μmol L^{-1} LOD: 0.021 μmol L^{-1}	43
PB/rGO	Photochemical GO reduction and formation of PB nanocubes with nitroprusside as precursor	PB/rGO/GCE	AA, DA, UA	CV	LR: 283–2330 (AA), 40–2330 (DA), 40–415 (UA) μmol L^{-1} LOD: 34.7 (AA), 26.2 (DA), 8.0 (UA) μmol L^{-1}	44
MnO_2/Gr	Graphite, $KMnO_4$ and citric acid mixed and heated at 100 °C; reduction with H_2O_2 to MnO_2	Gr-MnO_2/CE	—	CV	—	45

Table 7.1 (*Continued*)

Graphene-based material	Synthesis	Electrode	Application: analyte/sample	Technique	Analytical characteristics	Ref.
ZrO_2/Gr	HOPG flakes one-step exfoliated in ethanol; heat water; graphene and $ZrOCl_2$ solution at 180 °C in an HP reactor; wash and dry at 70 °C	ZrO_2/Gr/SPCE	Acetaminophen/ tablets	DPV	LR: 10–100 μmol L^{-1} LOD: 75.5 nmol L^{-1}	46
Pip-rGO	Pip dispersion in ethanol added to sonicated GO dispersion; reflux 24 h; centrifugation, washing	Pip-rGO/GCE	Hg(II)/water	DPASV	LR: 0.4–12 μmol L^{-1} LOD: 0.2 nmol L^{-1}	47
SnO_2/rGO	Addition of $SnCl_2$ to GO dispersion; sonication; addition of lemon juice; heat at 120 °C in autoclave; dry at 60 °C; calcination at 400 °C	SnO_2/rGO/GCE	AA/tablets	DPV	LR: 400–1600 μmol L^{-1} LOD: 38.7 μmol L^{-1}	48
BiNPs/ErGO	$Bi(NO_3)_3$ addition to GO dispersion; sonication and drop cast on GCE; dry and CV reduction (0.6 to −1.7 V)	BiNPs/ErGO/GCE	Gemcitabine/ spiked urine	DPV	LR: 0.1–51.1 mmol L^{-1}	49
Gr	MW irradiation of GO for fast exfoliation	Gr/CPE	Chloroxylenol/ lotions, water	SW-AdASV	LR: 5–160 μmol L^{-1} LOD: 1.37 nmol L^{-1}	50
MIP-rGO	Reflux GO + hydrazine + NH_3 solution at 80 °C; rGO drop on GCE immersed in adrenaline/ nicotinamide solution; CV electropolymerization	MIP-rGO/GCE	Adrenaline/ spiked urine, ampoule	DPV	LR: 0.015–40 μmol L^{-1} LOD: 3 nmol L^{-1}	51

Ag-AuNPs/rGO	$AgNO_3$, alginate and $HAuCl_4$ added to rGO dispersion; heat at 90 °C for 3 h	Ag-AuNPs/rGO/GCE	H_2O_2	Amperometry	LR: 0.1–10 mmol L^{-1} LOD: 0.57–54 μmol L^{-1}	52
CuONPs/rGO	PGE immersed in GO suspension and CV (0 to −1.0 V); rGO /PGE immersed in $Cu(NO_3)_3$; CV in NaOH (0.8 to −0.3 V)	CuONPs/rGO/PGE	Glucose/serum	Amperometry	LR: 0.1–150 μmol L^{-1} LOD: 0.09 μmol L^{-1}	53
{PEI/rGO}Au@P8W48	PEI/ITO immersed in GO to give {PEI/GO} and next in reduced P8W48 and $HAuCl_4$ to give {PEI/rGO}-Au@P8W48	{PEI/rGO}Au@ P8W48/ITO	H_2O_2	Amperometry	LR: 0.15–22.15 and 22.15–137.15 mmol L^{-1} LOD: 0.54 μmol L^{-1}	54
PSS-Gr	Addition of PSS to GO dispersion; sonication; heat with AA at 90 °C; centrifugation and washing	PSS-Gr/GCE	Trp/serum	LSV	LR: 0.04–10.0 μmol L^{-1} LOD: 0.02 μmol L^{-1}	55
Eu_2O_3@rGO	Addition of $Eu(NO_3)_3$ to GO dispersion; heat in MW 3 h at 600 W	Eu_2O_3@rGO/GCE	CAP/milk, honey	Amperometry	LR: 0.02 –800.25 μmol L^{-1} LOD: 1.32 nmol L^{-1}	56
Porous graphene (PGr)	Dispersion of GO in Na citrate solution; heat at 180 °C in an HP reactor; lyophilization	CAT/PGr/GCE	H_2O_2/2,4-D/ bean sprouts	Amperometry/ FIA	LR: 0.0024–0.36 μmol L^{-1} LOD:1.5 nmol L^{-1} (2,4-D)	57

[a]Abbreviations: AA, ascorbic acid; CAT, catalase; CE, graphite electrode; CPE, carbon paste electrode; CV, cyclic voltammetry; 2,4-D, 2,4-dichlorophenoxyacetic acid; DA, dopamine; ErGO, electrochemically reduced graphene oxide; FIA, flow injection analysis; HOPG, highly oriented pyrolytic graphite; HP, high pressure; Mb, myoglobin; MW, microwave; NB, nitrobenzene; Nf, Nafion; Ni-TPP, nickel tetraphenylporphyrin; NMP, *N*-methylpyrrolidone; P8W48, $K_{28}Li_5H_7P_8W_{48}O_{184}{\cdot}92H_2O$; PB, Prussian Blue; pDA, polydopamine; PEI, polyethylenimine; 1,10-phen, 1,10-phenanthroline; Pip, piperazine; PSS, poly(sodium 4-styrenesulfonate); SPCE, screen-printed carbon electrode; Trp, tryptophan; TSGF, tumour-supplied group of factors; UA, uric acid.

and glycine[38] introduced amino groups into the graphene-based structure. The resulting products consist mainly of composites of rGO and metallic nanoparticles[35,36,38,40–42,45,46,48,49,52,53,56] with high electrocatalytic activity. Furthermore, the electrodes modified with these materials have found applications in the analysis of a variety of species and samples.

''Green electrodes'' modified with nanoparticles have been prepared using protocols that do not require the use of organic solvents or toxic reagents. One of these methods employed pure glycerol and involved ultraviolet irradiation without extra added stabilizers for the synthesis of AuNPs. This non-polluting method allowed nanoparticles of small size (<15 nm) with high stability (2 months) to be obtained, which were used to construct an AuNP–carbon paste electrode (CPE) for the detection of nitrite in water.[59] More recently, the green synthesis of nanoporous gold nanoparticles (npAuNPs) with tuneable size was reported. It involves the use of *Oryza sativa* seeds (Asian rice) extract as a reducing agent for the simultaneous bioreduction of gold(III) and silver ions to form alloy nano-precursors that led to the formation of npAuNPs. In comparison with conventional methods, no harsh reaction conditions were used during the synthesis and the size of the nanoparticles could easily be tuned by varying the concentration of the silver precursor. The as-synthesized npAuNPs exhibited electrocatalytic activity towards non-enzymatic glucose sensing with a linear range from 1 to 50 $\mu mol\,L^{-1}$.[60]

The use of plant extracts is widespread in this field. Similarly to the work commented on above, a facile, one-pot and environmentally friendly method exploiting Piper betle biomass as a reducing and stabilizing agent was proposed for the preparation of AgNPs with a face-centred cubic structure and preferred (111) orientation. An AgNP-modified GCE showed exceptional electrocatalytic activity towards nitrite oxidation, achieving an LOD of 46 $\mu mol\,L^{-1}$.[61] Other plant extracts have been employed for the synthesis of gold, silver, palladium, platinum, bimetallic[62–66] and oxide[67–69] nanoparticles, which were further used for the preparation of modified electrodes. An illustrative example is the comparison made by Goutham *et al.*[70] of two different methods involving biological and chemical protocols for the synthesis of nano-ZnO and the preparation of an electrochemical gas sensor. Figure 7.2a shows the biological method using aloe vera plant extracts, which contain reducing and stabilizing agents, to produce ZnO nanoparticles by mixing the extract with zinc nitrate and heating. Thereafter, water was evaporated and the resulting material was collected and annealed in a muffle furnace. In the case of the chemical method (Figure 7.2b), glycine was used as an organic fuel with a high heat of combustion, offering a platform for a redox reaction to occur. The resulting sensors were applied to the detection of liquefied petroleum gas (LPG) at different concentration levels (ppm) with various operating temperatures. The green synthesis route was found to be more advantageous as it was simple, eco-friendly and cost-effective. More recently, ZnO nanoparticles embedded in nitrogen-doped carbon sheets (ZnO@NDCS) were synthesized using zinc powder, aqueous

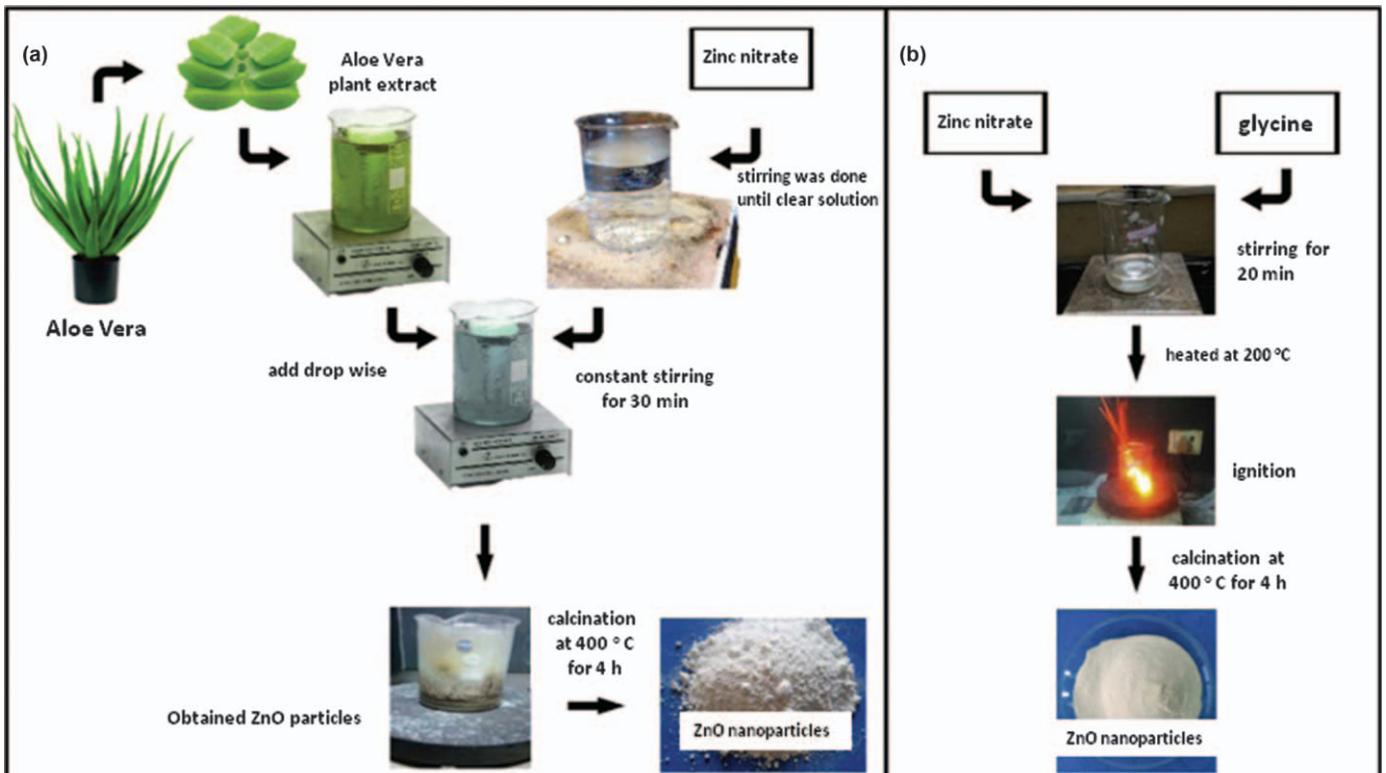


Figure 7.2 Schemes showing the preparation of ZnO nanoparticles by a green method involving aloe vera plant extract (a) and a chemical method using glycine as organic fuel (b).
Reproduced from ref. 70 with permission from Elsevier, Copyright 2017.

ammonia and peach extract as the precursor for ZnONPs, nitrogen and carbon, respectively. The further immobilization of glucose oxidase allowed the preparation of a glucose biosensor that exhibited high sensitivity with an LOD of 6.3 μM and provided good results for glucose monitoring in human blood serum.[71]

Ionic liquids (ILs) have attracted considerable attention as a class of green materials with many practical applications owing to their unique properties, including exceptional ionic conductivity, wide potential window, biocompatibility, good chemical and thermal stabilities and non-flammability. In the field of electroanalysis, ILs have been used with two main purposes: (a) as modifiers of electrodes forming part of the electrode substrate and (b) as electrolytes and solvents in a variety of applications. In this section, applications related to the first objective are considered, while the second is treated in Section 7.2.3.

Room-temperature ionic liquids (RTILs) have been widely used as conductive binders in the fabrication of carbon paste or carbon composite electrodes to improve conductivity and promote electron transfer.[72] Recent applications include the addition of one or more additives, in addition to the IL, to the electrode material to increase the electrocatalytic activity and improve the resulting analytical performance. A recent paper reported an electrochemical method for the detection of tetrabromobisphenol A (TBBPA), a flame-resistance additive, at a graphitic carbon nitride–*N*-butylpyridinium hexafluorophosphate (NBH)-doped CPE modified with poly(diallyldimethylammonium chloride).[73] Interestingly, in contrast to the poor electrical conductivity of graphitic carbon nitride, the ionic liquid NBH promoted charge transfer in TBBPA oxidation at the resulting modified electrode. TBBPA was determined by differential pulse voltammetry (DPV) with a low LOD of 0.4 nmol L^{-1}. The addition of a cyclodextrin (CD) as a modifier to IL/CPEs induced notable improvements in the sensitivity and selectivity achieved with these electrodes owing to the high molecular recognition ability of CDs. A representative example of this IL–CD coupling is the method reported by Mohd Rasdi *et al.*[74] for the determination of 2,4-dichlorophenol in environmental samples involving a β-CD–1-benzylimidazole tosylate (β-CD–BIMOT). Other additives used recently include polymers such as poly(*p*-phenylenediamine),[75] graphene[76] and nanoparticles such as Fe_3O_4 NPs[77] and $Cu(OH)_2$ NPs.[78]

Paper-based sensors constitute interesting alternatives for the fabrication of disposable inexpensive and eco-friendly analytical devices. Flexibility, lightness and porosity are important characteristics of this type of material. Porosity allows liquid transport by capillarity, which confers on paper unique exploitable properties for the preparation of electrochemical sensors. Paper electrodes (PEs) using metal nanoparticles have been used for the detection of hydrogen peroxide. An interesting example is the paper-based carbon platforms prepared through electrogenerated AuNPs described by Núñez-Bajo *et al.*[79] (Figure 7.3A), where carbon ink is deposited on a hydrophilic working area of the paper delimited with hydrophobic wax. This

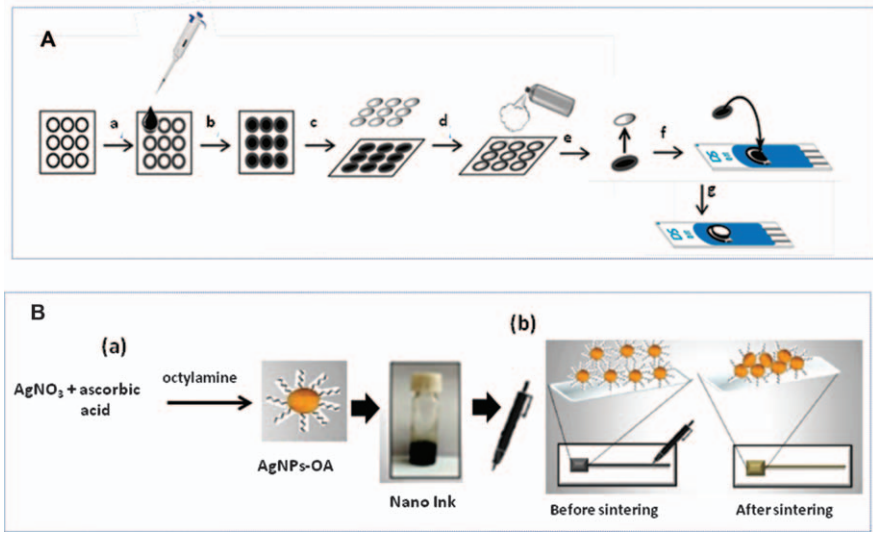


Figure 7.3 Schematic diagrams of the fabrication and coupling of paper-based carbon working electrodes (PCWEs). (A) Wax melting at 110 °C (a); carbon ink deposition (b); protection of the conductive layer with plastic covers (c); addition of spray adhesive (d); removal of the plastic cover after cutting the PCWE (e); placement of the PCWE over the SPCE (f); resulting platform composed of the PCWE with reference and counter electrodes from SPCE (g). (B) Preparation of AgNP–OA nanoink (a); direct drawing of the electrode using a roller-ball pen on photographic paper and sintering at 100 °C for 1 h (b).
Reproduced from ref. 79 and 80 with permission from American Chemical Society, Copyright 2017.

maskless procedure is fast and reduces ink waste. The connection of the working electrode to the potentiostat is ensured with the use of SPEs. The method was applied to the determination of arsenic in commercial white wines by chronoamperometric stripping of the electrodeposited As(0).

Another example is that reported by Ghosale *et al.*[80] (Figure 7.3B) using a simple, inexpensive and environmentally friendly method by direct writing on to photographic paper using a nanoink with 10 wt% AgNPs capped with octylamine (OA) and further sintered at 100 °C to make it conductive. The resulting AgNP–OA electrode exhibited a linear calibration in the range 1.7 $\mu mol\,L^{-1}$–30 $mmol\,L^{-1}$ H_2O_2 with an LOD of 0.5 $\mu mol\,L^{-1}$. Medina-Sánchez *et al.*[81] described a disposable electrochemical lateral flow paper-based sensing device for the detection of heavy metals. Owing to the filtering properties, the paper platform served as a sample pretreatment material even for turbid samples. Lead and cadmium were determined by SWASV in the concentration range 10–100 ppb in aqueous and mud samples, demonstrating the analytical usefulness of this lab-on-paper device. Furthermore, a three-dimensional origami paper-based device has recently been reported for the detection of several classes of pesticides. The method

combines different enzyme-inhibition biosensors and integrates two different office paper SPEs and multiple filter paper-based pads to load enzymes and enzymatic substrates (Figure 7.3B). Alkaline phosphatase, butyryl-cholinesterase and tyrosinase were the enzymes used for the determination of 2,4-dichlorophenoxyacetic acid (2,4-D), paraoxon and atrazine, respectively.[82]

7.2.3 Green (Bio)sensors

This section discusses the characteristics of selected green electrochemical sensors and biosensors which, according to IUPAC definitions and classifications, could provide analytical information in real time, without or with minimal sample preparation, thus making them suitable for direct analysis in concert with their sensitivity, selectivity and low cost.[83] Regarding electrochemical sensors, two classes are especially relevant in this context: ion-selective electrodes (ISEs) and sensors involving molecularly imprinted polymers (MIPs).

Potentiometric ISEs are acknowledged as eco-friendly, simple and energy-saving analytical tools that are also well suited to microfabrication. Their application as a green approach in pharmaceutical analysis is highly recommended as a key driver for moving towards methods aimed at sustainable development and preservation of the environment. Environmentally friendly ISEs have been developed for the potentiometric determination of many inorganic and organic species. For example, cinchocaine hydrochloride (CIN), a local anaesthetic agent, was determined in the presence of its degradation products and betamethasone valerate, a glucocorticoid sometimes co-formulated with CIN, using selective electrodes prepared with sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate as a cation exchanger in a PVC matrix with 2-nitrophenyl octyl ether (2-NPOE) as a plasticizer and using 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) as an ionophore. Using a conventional liquid inner contact, a linear dynamic range between 30 $\mu mol\,L^{-1}$ and 10 $mmol\,L^{-1}$, with a Nernstian slope of 54.89 mV per decade, was obtained.[84] The same group prepared similar configurations for the determination of cyclopentolate and phenylephrine hydrochlorides in their ophthalmic formulation. In this case, using solid-contact ISEs, a low LOD and discriminative ability in the presence of interfering substances were attained, which permitted the simultaneous determination of both drugs, in spite of their similar ionic characteristics, avoiding the need for any sample pretreatment or separation steps and in the presence of their degradation products.[85] Ma'mun *et al.*[86] used ISEs for the real-time tracking of the chemical and biological degradation kinetics of the skeletal muscle relaxant atracurium besylate (ATR) in serum. A selective membrane for ATR composed of PVC, 2-NPOE and tetraphenylborate in tetrahydrofuran (THF) was used for preparing both conventional ISEs and SPEs, the latter by drop coating the ion-selective membrane solution in THF directly on the electrode (Figure 7.4). In addition, other recent configurations of potentiometric sensors have been applied to the analysis of pharmaceutical samples with no need for extraction, pretreatment, filtration or derivatization steps. Recent

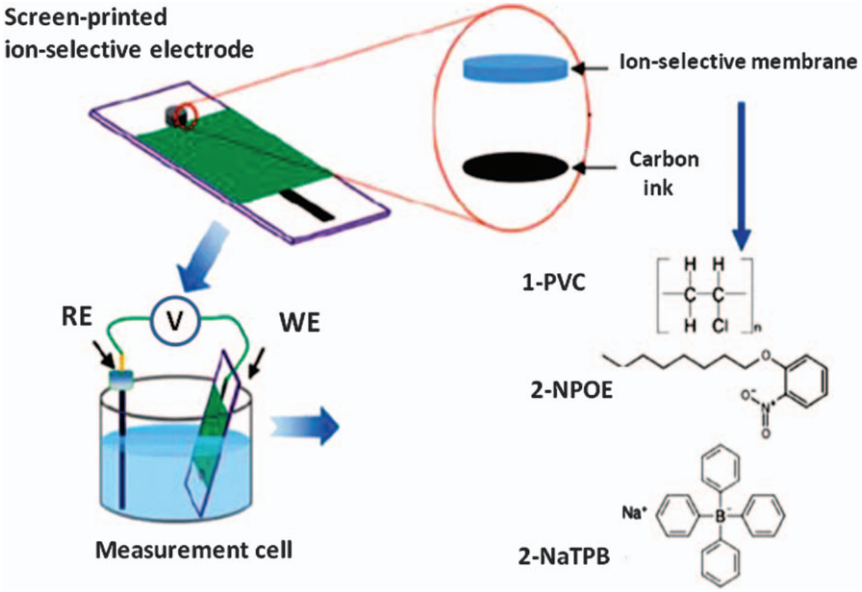


Figure 7.4 Potentiometric cell assembly with a screen-printed ion-selective membrane electrode constructed for the real-time tracking of chemical and biological ATR degradation kinetics in serum.
Reproduced from ref. 86 with permission from Elsevier, Copyright 2018.

examples include the determination of lidocaine and its metabolite 2,6-dimethylaniline[87] and the antiviral agent amantadine.[88]

Molecular imprinting allows specific recognition through the formation of sites in a highly crosslinked polymeric network grown in the presence of a template molecule that is subsequently removed, leaving behind recognition cavities. The integration of MIPs and electrochemical transducers is an attractive strategy for the development of sensors with significant advantages from the point of view of green analytical chemistry such as easy preparation, potential reusability and low cost. In addition, these advantages are extended when using non-toxic materials and protocols that avoid or minimize the consumption of reagents and solvents or waste production. Green strategies for MIP development have been reviewed by Viveiros *et al.*[89] Recently, Salajegheh *et al.*[90] used mathematical modelling for the preparation of a molecularly imprinted film involving the electrosynthesis of polyarginine, a highly conductive polymer possessing unique properties such as non-toxicity, biocompatibility and biodegradability. The modification of the electrode was performed through the electropolymerization of arginine onto a GCE coated with sodium alginate–multiwalled carbon nanotubes in the presence of theophylline as the template/analyte (Figure 7.5A). The resulting sensor allowed the determination of the alkaloid in the 0.01–60.0 μmol L^{-1} range with an LOD of 3.2 nmol L^{-1}. Water-dispersible molecularly imprinted conductive polyaniline (PANI) particles were prepared through the

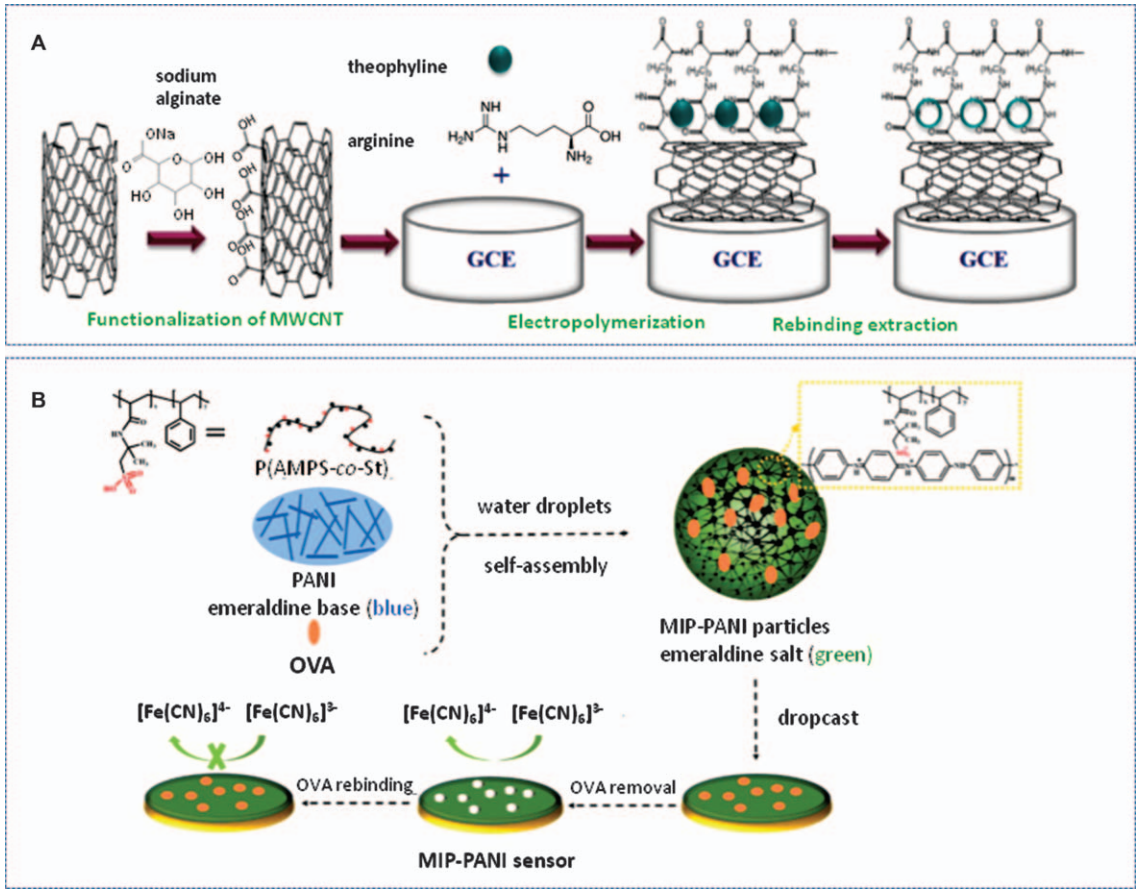


Figure 7.5 Schematic illustration of the design of the MIP/SA-MWCNTs/GCE sensor for the determination of theophylline (A) and the synthesis of MIP-PANI particles and the preparation of a MIP-PANI sensor for the determination of ovalbumin (B). Reproduced from ref. 90 (A) and 91 (B) with permission from Elsevier, Copyright 2017 and 2019.

co-assembly of PANI with an amphiphilic copolymer and applied to construct an electrochemical sensor for ovalbumin (OVA) protein.

Figure 7.5B shows the amphiphilic copolymer, P(AMPS-co-St), synthesized using 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and styrene (St), co-assembled with PANI in aqueous solution to generate PANI particles. During this process, OVA as template protein was added to form MIP-PANI particles. The electrochemical sensor was prepared by casting the MIP-PANI dispersion onto a gold electrode. The template molecule was extracted by immersing the electrode in methanol–acetic acid solution and cyclic voltammetry of $[Fe(CN)_6]^{3-/4-}$ was employed to monitor protein removal. The MIP sensor exhibited a wide linear range of 10^{-11}–10^{-6} mg mL^{-1} and a low LOD of 10^{-12} mg mL^{-1} OVA.[91]

Biosensors involving ILs also constitute interesting alternatives. ILs have been demonstrated to be suitable media for enzyme catalysis. Enzymes in ILs exhibit high conversion rates, enantioselectivity and higher stability than that shown in organic solvents. All these advantages, in combination with their green, designable properties, make ILs an attractive material for enzyme immobilization and, therefore, for the development of a new generation of enzyme bioelectrodes. RTILs have been used as modifiers by incorporation within biocomposite matrices. For instance, Zappi *et al.* reported the use of four-generation choline–Gly, –Ser, –Phe and –His RTILs as biologically friendly solvents for the preparation of enzyme biosensors using electrodes modified with graphene, AuNPs or MWCNTs and lipase, glucose oxidase or alcohol dehydrogenase as the specific enzymes for the determination of oils, glucose or alcohols.[92] Enzyme biosensors have also been fabricated using ILs in combination with conducting polymers, where the *in situ* one-step electropolymerization of monomers provides a green method for the preparation of bioelectrodes. An interesting example is the synthesis of myoglobin–gold nanoparticles–polydopamine–graphene (Mb/AuNPs/pDA/Gr) nanocomposites by an efficient one-pot green polymerization approach and their use for the immobilization and direct electrochemistry of redox proteins.[93]

Metal nanoparticles synthesized by green methods have been used to facilitate the efficient direct electron transfer (DET) between enzymes and the electrode substrates. Cellobiose dehydrogenase from *Corynascus thermophilus* (CtCDH) exhibited direct electrochemical communication with gold electrodes when the enzyme was covalently linked to green AuNPs and AgNPs modified with a dithiol self-assembled biphenyl-4,4′-dithiol (BPDT) monolayer. The green AuNPs and AgNPs were synthesized using quercetin as reducing agent at room temperature. The modified electrodes were used to develop an eco-friendly biosensor for lactose detection that exhibited excellent stability, an LOD of 3 μM and a linear range between 5 and 400 μM and was successfully applied to quantify lactose in real milk and cream samples.[94] DET was also observed for cholesterol oxidase (ChOx) immobilized on a polypyrrole/rGO composite that was obtained by a green procedure from GO through reduction with *Paederia foetide* plant extract at 60 °C. The enzyme was entrapped during the electropolymerization of pyrrole.[95]

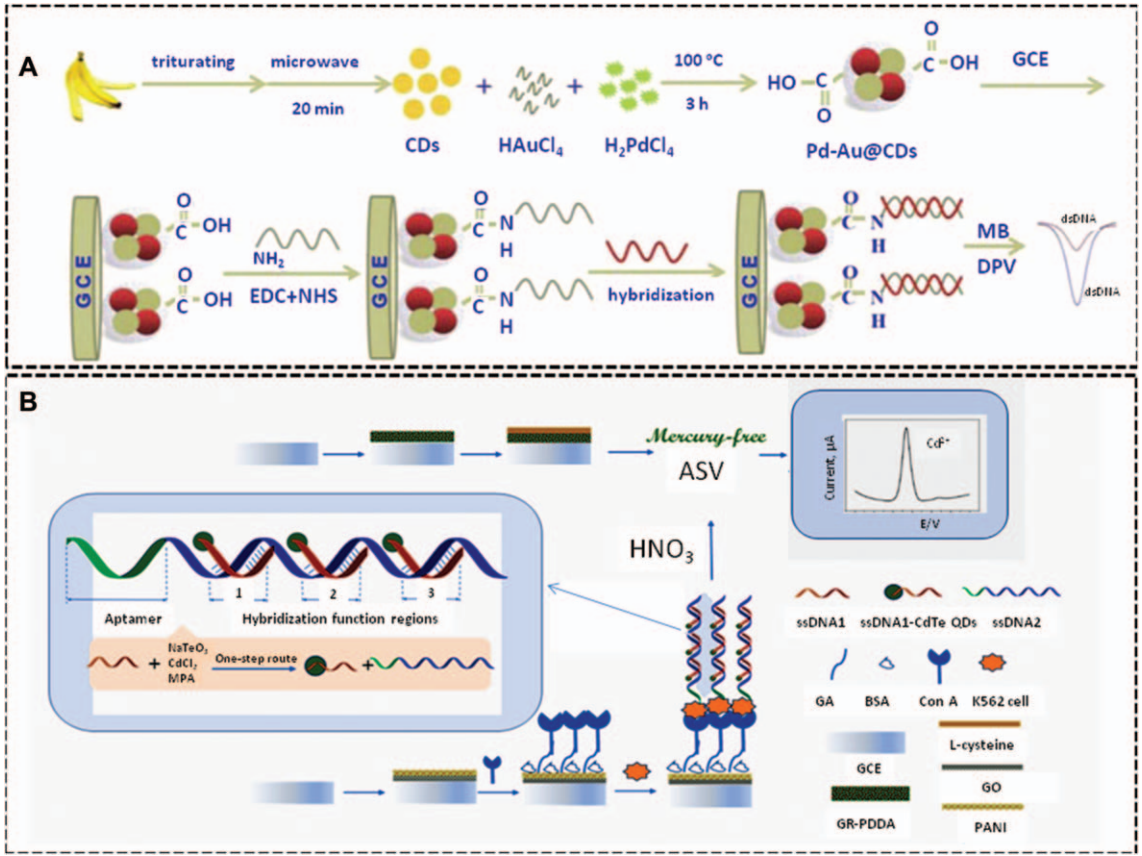


Figure 7.6 Schemes of MW-assisted one-step green synthesis of CDs from banana peel used as reductant and stabilizer for Pd–Au@CDs to detect colitoxin DNA (A) and fabrication of a supersandwich cytosensor using controlled aptamer-DNA concatamer-QDs and the detection of the released Cd^{2+} *via* mercury-free ASV (B).
Reproduced from ref. 97 (A) and 98 (B) with permission from Elsevier, Copyright 2017 and 2019.

ZnONPs encapsulated over nitrogen-doped carbon sheets (ZnO@NDCS) were synthesized by a facile hydrothermal process of a natural bio-source (peach juice) in aqueous ammonia with ZnONPs at 180 °C for 12 h and used to modify a GCE for the immobilization of glucose oxidase and glucose detection with an LOD of 6.3 μmol L^{-1}.[96]

Other nanoparticles such as carbon dots (CDs) have also been synthesized from natural sources to prepare electrochemical biosensors. An illustrative example is a green and fast method developed to synthesize high-yield CDs through a one-pot microwave treatment of banana peel (Figure 7.6A). The as-prepared CDs were used as a reducing agent and stabilizer in the synthesis of a Pd–Au@CDs nanocomposite by a simple sequential reduction strategy. A DNA biosensor was then constructed by immobilization of a single-stranded DNA probe through a carboxyl ammonia condensation reaction on the Pd–Au@CDs/GCE and applied to the detection of colitoxin DNA in human serum with an LOD of 1.82×10^{-17} mol L^{-1}.[97] An electrochemical biosensing strategy was reported for the green and ultrasensitive detection of tumour cells by combining aptamer-DNA, concatamer-CdTe quantum dots (QDs) and a signal amplification probe using mercury-free anodic stripping voltammetry. The aptamer-DNA–concatamer-CdTe QD probes were designed by DNA hybridization and covalent assembly for specific recognition of an aptamer, followed by signal amplification through the DNA concatamer-QDs. Separately, a GCE modified with GO, PANI, glutaraldehyde (GA) and concanavalin A (Con A) was used as an immobilization electrode. As Figure 7.6B shows, the detection step was performed by ASV of the cadmium released from the cytosensor after acid dissolution, at a different electrode prepared by modification of a GCE with Gr, PDDA and L-Cys. This method allowed the detection of human leukaemia K562 model cancer cells with an LOD of 60 cells mL^{-1}.[98]

7.3 Solvents

The replacement of organic solvents used in a variety of analytical methods with other, environmentally friendly solvents is an important concern in the implementation of greener electroanalysis. As has been shown in the previous sections, RTILs have demonstrated in numerous applications their excellent properties as conducting materials for the construction of electrochemical devices. However, they have also found wide use as solvents for electrochemical reactions and sample treatment. Alongside these, deep eutectic solvents (DESs) have been found to be good media in various methodologies such as the electrodeposition of polymers and the fabrication of electrochemical biosensors. Furthermore, although still scarce, electrochemistry in supercritical fluids has attracted attention in recent years.

7.3.1 Ionic Liquids

The environmentally benign behaviour of ILs derived from their low volatility and ease of recovery compared with traditional volatile organics have

made them useful solvents for various syntheses and chemical transformations for compounds with electrochemical applications. Recent progress in the use of RTILs in organic electrochemistry has been reviewed,[99] and also of ILs as green solvents and electrolytes for the development of chemical sensors.[100] The use of ILs instead of classical organic solvents as solvents in polymerization not only offers some general advantages such as low volatility and non-flammability but also markedly affects the rate and degree of polymerization. Moreover, ILs act as ideal supporting electrolytes for the electrochemical generation of conjugated polymers because of their excellent oxidative and reductive stability that expands the window of accessible potential. In the field of MIPs, conventional methods for their preparation require the use of large amounts of organic solvents. Green solvents not only can avoid that use, but also improve MIP properties such as controlled morphology and homogeneity of the binding sites.[89] In a recent and representative example, two ILs were employed to prepare an MIP with Gr as the supporter for the electrochemical sensing of 6-benzylaminopurine (6-BAP). 1-Vinyl-3-butylimidazolium tetrafluoroborate (IL1) was used as a functional monomer and 1,4-butanediyl-3,3′-bis-l-vinylimidazolium dibromide (IL2) acted as a crosslinker, enhancing the electrocatalytic activity and adsorption capacity for 6-BAP. The voltammetric response of the IL–Gr–MIP sensor was linear with respect to 6-BAP concentration in the range 0.5–50 μM with an LOD of 0.2 μM (signal-to-noise ratio = 3).[101]

Several nanoparticles used in electrochemical applications have been synthesized in IL media from a variety of precursors. Verma *et al.* recently reviewed the synthesis and stabilization of transition metal nanoparticles in ILs.[102] The properties of ILs exploited in this field are non-toxicity, high chemical and thermal stability, biodegradability and negligible vapour pressure, which make them very attractive for the replacement of toxic electrolytes.[99] Moreover, the electrochemical synthesis of nanoparticles in IL media has additional advantages to those characteristic of electrodeposition, such as the high-quality deposits with adjustable size in addition to rigid control of the film thickness, shape, uniformity and deposition rate and the possibility of deposition on substrates with a non-regular surface. One of the greatest advantages of electroplating using ILs is the absence of problems associated with the evolution of hydrogen ions.[103] An interesting example is the electrochemical preparation of platinum nanoparticles from bis(acetylacetonato)platinum(II) in aprotic amide-type ILs using cyclic voltammetry and potentiostatic reduction at a rotating disc electrode.[104] Interestingly, the average size of PtNPs depended on the kind of IL. Pd nanoparticles with an average size in the range 2.9–3.2 nm were also prepared by a simple electrochemical reduction of $[PdBr_4]^{2-}$ in bis(trifluoromethylsulfonyl)amide ($TFSA^-$) ILs composed of 1-butyl-1-methypyrrolidinium (BMP^+), 1-hexyl-1-methylpyrrolidinium (HMP^+) and 1-decyl-1-methylpyrrolidinium (DMP^+).[105] Nanoparticles of other non-noble metals have also been synthesized in ILs and used for different applications. For example, CuNPs were prepared by electrodeposition in the presence of 1-ethyl-3-methylimidazolium

ethylsulfate onto a paraffin wax-impregnated graphite electrode modified with electropolymerized L-cysteine. The use of the IL induced a rapid nucleation rate. The modified electrode was used for the enzyme-free detection of glucose and H_2O_2.[106]

Another area in which the role of ILs as cleaner solvents should be highlighted is in sample preparation for subsequent electrochemical analysis. An illustrative example is the method developed by Jahromi *et al.*[107] for the determination of haemin in biological samples using an ammonium-based task-specific IL containing salicylate anion for the extraction and preconcentration of the analyte through a dispersive liquid-phase microextraction. DPV was employed for the quantification of haemin in the IL-rich phase placed on the surface of a GCE. In this way, IL was used as both an extracting solvent due to its polarity and water immiscibility and an electrode modifier due to its ionic structure. The current measured from the electrochemical reduction of haemin allowed linear calibration in the range 0.020–2.60 $\mu mol\,L^{-1}$ with an LOD of 3.16 $nmol\,L^{-1}$. Another interesting approach involved the dispersion of ILs on support materials for solid-phase extraction (SPE) combined with the use of β-cyclodextrin-modified IL-CPEs to develop a green method for the determination of trace amounts of bisphenol A (BPA).[108] Synthesized imidazolium ILs were used to modify styrene-type macroporous resin used as an adsorbent for SPE. DPV provided a calibration plot for BPA in a linear range between 0.01 and 1.0 $\mu mol\,L^{-1}$.

Importantly, ILs have been used as media for enantiodiscrimination in electrochemical experiments. With this aim, a dialkyl-1,1′-bibenzimidazolium salt, consisting of an atropisomeric dication and an achiral counteranion, was employed as a chiral additive providing successful voltammetric enantioselection for the enantiomers of *N*,*N*′-dimethyl-1-ferrocenylethylamine selected as model chiral probes. Significant differences in their redox potentials were observed despite the low concentration (0.01 $mol\,L^{-1}$) of the chiral additive.[109] A vortex-assisted IL dispersive liquid–liquid microextraction following back-extraction and voltammetric analysis at AuNPs/SPEs was reported for the determination of mercury in urine. Mercury complexes with ammonium pyrrolidinedithiocarbamate (APDC) were directly extracted from non-digested urine in 1-hexyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide ([Hmim][NTf_2]) and back-extracted into a small volume of an acidic aqueous solution. The method was applied to spiked urine samples using a standard additions calibration plot in a range up to 15 $mg\,L^{-1}$ of mercury.[110]

In addition, the use of ILs in solvent extraction processes through aqueous solution–IL distribution for the recovery of metallic species is important for performing direct electrodeposition. In this context, the diluent characteristics and the wide electrochemical window of the IL *n*-hexyltrimethylammonium bis(trifluoromethylsulfonyl)amide ([N_{1116}][TFSA]) were exploited for the extraction of In(III) from 1,1,1-trifluoro-*N*-[(trifluoromethyl)sulfonyl]methanesulfonamide (H[TFSA]) aqueous solution using tri-*n*-butyl phosphate (TBP), followed by direct electrodeposition as indium metal from the organic phase. The method allowed the extraction of In(III)

selectively from the aqueous phase including Ni(II) and Zn(II) and the subsequent potentiostatic electrodeposition of $[In(TBP)_3]^{3+}$ to In^0 at −1.0 V.[111]

7.3.2 Deep Eutectic Solvents

Deep eutectic solvents (DESs) are a new class of green and sustainable solvents composed of at least a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), which provide a eutectic phase with low melting point, low toxicity, renewability and biodegradability.[112] DESs are highly advantageous in environmental terms to overcome the limitations of organic solvents.[113] They are often prepared by simply mixing two or several components (with or without a metal salt) to form a liquid product with melting point lower than those of the individual components.[114] Since the pioneering work in 2006,[115] DESs have been used in electroanalysis because of the benefits of their intrinsic properties, including good solubility, non-flammability and suitable electrochemical windows. The applications of DESs in electrochemical sensing have recently been reviewed by Brett.[116] As was highlighted, this type of solvent can be considered as an interesting tool in the preparation of new nanostructured materials on electrode surfaces, some of which cannot be prepared in aqueous media. Brett's group used eutectic mixtures as electrodeposition media of polymers for the modification of GCEs to construct sensing devices.[117,118] For example, polymers of 3,4-ethylenedioxythiophene (PEDOT) in eutectic mixtures composed of choline chloride (ChCl) and ethylene glycol (EG) at GCEs showed good electrocatalytic performance and morphological properties for the electrodeposited polymer. The resulting modified electrode was successfully applied to the determination of ascorbate. Other PEDOT/GCE sensors were prepared by electrodeposition of the polymer in the eutectic solvents ChCl–urea, –ethylene glycol and –glycerol. The modified electrodes exhibited advantageous sensing characteristics for ascorbic acid and acetaminophen in comparison with those provided by PEDOT-modified GCEs prepared in aqueous solution.[119]

Various ammonium- and phosphonium-based eutectic mixtures (EMs) were used for the functionalization of carbon nanotubes after pretreatment with an acidic $KMnO_4$ solution. The modified CNTs showed improved stability compared with pristine CNTs, suggesting an increase in the surface area after functionalization due to exfoliation, open-ended tubes and elimination of catalyst particles. EM-functionalized CNTs were applied to electrochemical sensing. As an example, a functionalized CNT-modified GCE was used for the determination of nitrite, showing improved catalytic activity and sensitivity in comparison with bare and pristine CNT-modified GC electrodes.[120] More recently, Lobo-Castañón's group[121] demonstrated also the potential of DESs to select in a faster way aptamers targeting poorly water-soluble species.

Natural deep eutectic solvents (NADESs) are composed of mixtures of sugars, alcohols, organic acids, amino acids and amines containing several

hydroxyl, carboxyl or amino groups that give rise to hydrogen bonding interactions leading to highly structured liquids.[122] Such liquids can, in turn, form additional hydrogen bonds with solutes, increasing their solubilization ability.[123] NADESs show biodegradability, non-toxicity, negligible volatility, liquid state even far below 0 °C, adjustable viscosity and high solubilization power for both polar and non-polar compounds, which make them sustainable and low-cost solvents suitable for use in the development of electroanalytical methods. For instance, NADESs prepared by combination of glucose or fructose and citric or lactic acid in phosphate buffer were utilized for the electrochemical determination of the antioxidant quercetin in food samples with improved sensitivity.[124]

7.3.3 Supercritical Fluids

The use of supercritical fluids (SCFs) is often highlighted as an important strategy within green chemistry to allow new, clean technologies with the replacement of volatile organic compounds (VOCs). However, owing to the high electrical resistivity of these media and their low dielectric constants, their use in electroanalysis was not feasible for electrodes of conventional size.[125] Microelectrodes with a critical surface size approaching the diffusion layer provide low currents minimizing the ohmic drop effects, and therefore they can be used in SCFs. For example, Barlett and Branch reported the voltammetric behaviour of two metallocenes in supercritical difluoromethane using platinum microdisc electrodes.[126] In the particular case of electroanalytical methods, the readily accessible supercritical conditions of CO_2, together with its abundance, non-toxicity, low cost and non-flammability, make this solvent, which is also relatively inert and easy to purify, promising for different green applications.[127] In this context, the possibility of electrochemical detection of analytes separated by means of supercritical carbon dioxide ($scCO_2$) chromatography is remarkable. For this purpose, suitable measurement cells are required, such as the flow-through column coulometric cell using carbon fibre electrodes reported by Yamamoto *et al.*[128] The same group carried out the electrochemical detection of tocopherols in vegetable oils separated by supercritical fluid chromatography (SFC).[129] The eluted α-, β-, γ- and δ-tocopherols were detected electrochemically in a mobile phase consisting of a mixture of $scCO_2$ and methanol containing 1 mol L^{-1} ammonium acetate (98 : 2 v/v), allowing linear coulometric responses in the range 5–200 μmol L^{-1}.

Interesting applications of SCFs also involve the preparation of materials employed in electrochemistry and, in particular, nanomaterials. SFCs have been used to deposit thin films of metals on a variety of surfaces and to incorporate metal nanoparticles on several organic and inorganic substrates. Different approaches for this purpose have been reported. Among them, supercritical fluid deposition, especially using $scCO_2$, is particularly attractive because no liquid wastes are generated, no solvent residues remain on the substrate and the mass transfer rates are faster. Gold nanoparticles

were uniformly distributed on graphene and CNTs using $scCO_2$. Since the Au nanoparticles were highly dispersed and tightly anchored on the carbon supports, the nanocomposites obtained exhibited an improved electro-oxidation ability towards glucose in comparison with that observed for electrodes prepared without $scCO_2$. Moreover, in the presence of an IL, a superior electrochemical performance for the Au/graphene was observed, probably due to the synergistic interactions between these materials.[130] A MIP-based disposable sensor for BPA was developed using ferrocenylmethyl methacrylate (FMMA) copolymerized with ethylene glycol dimethacrylate (EGDMA) using $scCO_2$ as a porogenic solvent.[131] The MIP on commercial carbon screen-printed electrodes, using DPV, showed a characteristic irreversible oxidation peak of BPA, which increased with increase in BPA concentration over the range 4.7–8 nmol L^{-1}.

7.4 Techniques

As has been shown in the previous sections, electrochemical techniques belong naturally to the field of green chemistry. However, one further step in the sustainability of electroanalytical methods involves the combination of electrochemical detection with continuous techniques such as flow injection and liquid chromatography because of the faster and more efficient determinations. Furthermore, miniaturization of analytical systems is a trend closely related to the Twelve Principles of Green Chemistry owing to the extremely small volumes of sample and reagents required and the possibility of performing all the analysis steps in the same device. Electrochemical detection is particularly useful in these systems because of its inherent ability for miniaturization with no decrease in sensitivity. With this in mind, we consider in this section some recent approaches within these methodologies with particularly relevant achievements.

7.4.1 Continuous Detection

FIA systems are highly compatible with green chemistry because they require less analyte than conventional methods and allow rapid detection and shorter reaction times.[132] FIA-based electrochemical methods can be considered as environmentally friendly methods owing to the low cost and minimal consumption of reagents and solvents. Recent strategies have been focused on the determination of active ingredients in pharmaceutical products. An illustrative example involves the electrochemical oxidation of the cephalosporin nucleus at high potential (+1.9 V *vs.* Ag/AgCl) using a bare BDDE and a laboratory-made injection cell. The method developed for the determination of the antibiotic cefalexin exhibited the advantage, compared with voltammetric detection, of the absence of fouling or passivation of the electrode surface owing to the short time in contact with the analyte.[133] An AuNP-decorated carbon nanofibre–chitosan-modified SPCE was used as an electrochemical detector for the FIA determination of isoniazid over a wide

linear range between 1 and 10^3 μmol L^{-1}.[134] The determination of nitrite in meat samples was carried out using a flow injection system with amperometric detection at an SPCE modified with poly(vinyl alcohol), silver microcubes and poly(acrylic acid) (AgMCs-PAA/PVA/SPCE) (Figure 7.7), which exhibited a high electrocatalytic activity. PVA is a biocompatible non-toxic synthetic polymer with high chemical stability, which makes it appropriate as a supporting material for the entrapment of AgMCs-PAA. Silver microcubes stabilized by poly(acrylic acid) (AgMCs-PAA) were prepared by a simple chemical reduction method. The resulting modified electrode was able to determine nitrite within a linear range of 2.0–800.0 mmol L^{-1}.

Flow analytical systems can also overcome drawbacks related to the many steps of manual operation involved in the preparation of immunosensors and help to improve the performance of immunoassays in terms of more automation, the use of more precise and smaller amounts of chemicals and reduced time consumption.[136] An illustrative example is the use of sequential injection analysis (SIA) with amperometric detection for the development of an immunosensor for human immunoglobulin G (HIgG).

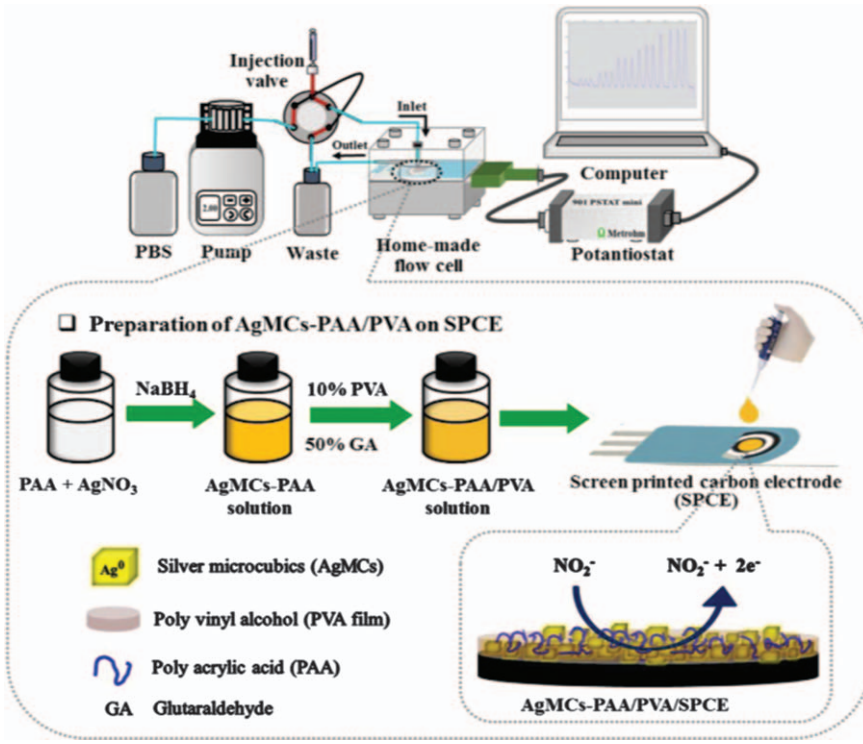


Figure 7.7 Schematic illustration of the flow injection amperometric system and the steps for preparation of AgMCs-PAA/PVA/SPCE for the determination of nitrite in meat.
Reproduced from ref. 135 with permission from Elsevier, Copyright 2017.

The immunosensor involved SPCEs modified with GO and the covalent attachment of anti-HIgG. Currents were measured before and after binding of HIgG to the electrode using $[Fe(CN)_6]^{3-/4-}$ as the redox probe and yielding a calibration graph over the range 2–100 ng mL^{-1}. The constructed biosensor was applied to the analysis of urine samples.[137] Costa García's group reported the use of the first flow cell for magnetic assays with an integrated magnet. An FIA system was used for biotin determination involving a one-step direct competitive assay between free biotin and biotin labelled with horseradish peroxidase. Once the immunocomplex was formed, 100 μL of the mixture were injected into the flow system and amperometric detection was accomplished upon injection of tetramethylbenzidine (TMB) solution.[138]

7.4.2 Microsystems

The development of new miniaturized chromatographic systems providing more sustainable methodologies is essential to make chromatographic separations more eco-friendly and closer to the Twelve Principles of Green Analytical Chemistry. They allow the reduction or elimination of organic solvents, direct detection without sample pretreatment, shortening of the analysis time and better sensitivity due to lower sample dilution. Electrochemical detection is widely used in chromatographic and microfluidic systems owing to its simplicity, high sensitivity, speed of analysis and easy integration in miniaturized systems. According to these principles, a low-cost microfluidic system for green and solvent-free chromatographic separations with electrochemical detection was integrated into cotton threads acting as the chromatographic column without the use of any mechanical pumping to transport the solutions. The developed system relied on separations based on an ion-exchange mechanism followed by detection at gold electrodes integrated into the microchannel of the system by deposition of gold in a specific portion of the cotton threads. A linear range between 0.025 and 5.0 mM was obtained for the effective separation of ascorbic acid (AA) and dopamine (DA) with LODs of 2.89 μM for AA and 4.41 μM for DA. The system was successfully applied to determine the levels of AA and DA present in tears of healthy volunteers without sample pretreatment.[139]

Various microsystems were designed with the electrochemical cell partially or totally integrated in the separation system. Multiple channel networks permit the introduction and mixing of reagents with zero dead volume connections. Among the various approaches, paper-based microfluidic devices have emerged as simple and low-cost platforms for clinical diagnostics. An example is a microfluidic cholesterol biosensor constructed with a paper-based platform in which microfluidic channels were fabricated by patterning filter-paper using photolithography and the working electrode was prepared by deposition of nickel oxide nanoparticles.[140] A three-dimensional paper-based analytical device with a hollow 3D fluid reservoir that avoids variability of the sensing area in contact with the analyte when using conventional SPEs on paper was reported recently.[141] Figure 7.8

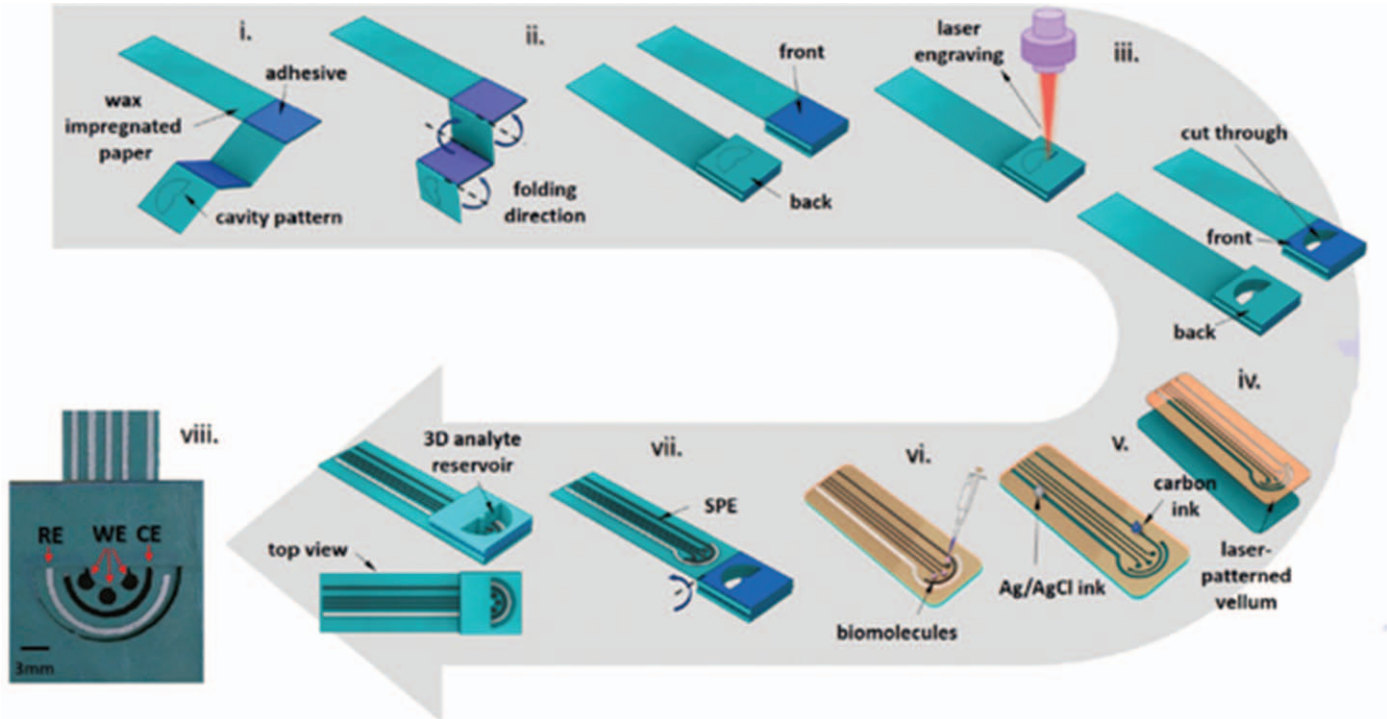


Figure 7.8 Schematic illustration of the steps involved in the fabrication of a three-dimensional paper-based electroanalytical device: wax-impregnated paper manually separated and double-sided tape adhered to the specified areas (i); device folded and laser cut leaving a hollow cavity (ii, iii); screen-printed electrodes using a patterned vellum sheet with Ag/AgCl and carbon ink (iv, v); if necessary, biomolecules are drop-cast (vi); removal of vellum sheet and folded over the hollow cavity (vii), yielding the final device (viii).
Reproduced from ref. 141 with permission from the Royal Society of Chemistry.

illustrates the fabrication of the sensors using a combination of wax printing, screen printing and simple folding. The system was shown to be versatile for performing voltammetric, amperometric and potentiometric measurements of dopamine, glucose and pH.

7.5 Conclusions and Future Trends

Great advances and dedicated efforts have been made in recent years to place modern electroanalysis more in consonance with the Twelve Principles of Green Chemistry. The intrinsic characteristics of the electrochemical transduction together with the unique features provided by electrochemical devices designed to minimize the use of reagents, organic solvents and hazardous substances, both to perform the measurement and pretreat/derivatize the sample, have been decisive in driving electrochemical (bio)-sensing to fit the demanding standards of green chemistry and sustainability. This chapter has outlined the efforts made in the use of electrodes constructed with more ''natural'' and less toxic starting materials (bismuth, antimony, tin and BDDE) to avoid mercury electrodes, of eco-friendly and efficient electrode modifiers (nanocomposites of graphene and metallic nanoparticles, polymers and ILs) and of greener protocols for the preparation of electrodes and modifiers. Regarding the last aspect, it is worth remarking on the use of spark discharge methods and electroplating using ILs instead of conventional electroplating to prepare metal-modified electrodes and noble and non-noble metal nanoparticles. Moreover, hydrothermal procedures involving environmentally friendly reagents (ascorbic acid, caffeic acid, citric acid, glucose and alginate) as reductants have been employed to prepare graphene-based materials and pure glycerol and ultraviolet irradiation or plant extracts and natural sources (banana peel) to synthesize metallic nanoparticles (AuNPs and AgNPs) and CDs, respectively.

Particular attention has been paid in recent years to the development of environmentally friendly electrochemical (bio)sensors that involve paper- or MIP-based sensors and ISEs. Moreover, the replacement of organic solvents with other, environmentally friendly, solvents (ILs, DESs and SCFs) has also played a significant role in the implementation of greener electroanalysis.

Despite the great progress made so far, illustrated through representative studies highlighted and discussed in this chapter, additional efforts must be made to develop and explore the use of novel eco-friendly solvents, reagents and materials and more biological than chemical procedures for both synthetic and modification purposes. Importantly, the use of these greener approaches should be a regular practice in the development and application of electrochemical sensors.

Special attention should be paid to translating and adapting the strategies developed so far from research laboratories to the ''real world'' and to work on the miniaturization of electrochemical devices and their coupling with continuous techniques to minimize the volumes of sample and reagents

required and to allow all of the analysis steps to be performed in the same device.

Although there is still a long way to go, the growing awareness of researchers and society at large makes the demand to use electrodes prepared using eco-friendly procedures and green solvents and reagents increasingly greater. This, together with the excellent characteristics of electroanalysis and the great possibilities of nano- and natural materials, will pave the way and ensure the development of a plethora of new greener approaches and (bio)sensing devices in the future.

References

1. P. Yáñez-Sedeño, J. M. Pingarrón and L. Hernández, Greening electroanalytical chemistry, in *Handbook of Green Analytical Chemstry*, ed. M. de la Guardia and S. Garrigues, ch. 12, Wiley, 2012.
2. P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, New York, 1998.
3. P. T. Anastas and M. M. Kirchoff, Origins, current status and future challenges of green chemistry, *Acc. Chem. Res.*, 2002, **35**, 686.
4. M. Koel and M. Kaljurand, *Pure Appl. Chem.*, 2006, **78**, 1993.
5. M. Farré, S. Pérez, C. Gonçalves, M. F. Alpendurada and D. Barceló, Green analytical chemistry in the determination of organic pollutants in the aquatic environment, *TrAC, Trends Anal. Chem.*, 2010, **29**, 1347.
6. C. M. A. Brett, Novel sensor devices and monitoring strategies for green and sustainable chemistry processes, *Pure Appl. Chem.*, 2007, **79**, 1969.
7. A. Economou, Bismuth-film electrodes: recent developments and potentialities for electroanalysis, *TrAC, Trends Anal. Chem.*, 2005, **24**, 334.
8. J. Wang, Stripping analysis at bismuth electrodes: a review, *Electroanalysis*, 2005, **17**, 1341.
9. V. Jovanovski, S. B. Hocevar and B. Ogorevc, Bismuth electrodes in contemporary electroanalysis, *Curr. Opin. Electroanal.*, 2017, **3**, 114.
10. N. Serrano, J. M. Díaz-Cruz, C. Ariño and M. Esteban, Antimony- based electrodes for analytical determinations, *TrAC, Trends Anal. Chem.*, 2016, **77**, 203.
11. E. Czop, A. Economou and A. Bobrowski, A study of *in situ* plated tin-film electrodes for the determination of trace metals by means of square-wave anodic stripping voltammetry, *Electrochim. Acta*, 2011, **56**, 2206.
12. N. Bing, W. W. Zhu, J. H. Luo and H. Q. Luo, A stannum–bismuth composite film electrode for simultaneous determination of zinc(II) and cadmium(II) using differential pulse anodic stripping voltammetry, *Analyst*, 2012, **137**, 614.
13. G. M. S. Alves, G. M. S. Rocha and L. S. Soares, HMVM: Multi-element determination of metals and metalloids in waters and wastewaters, at trace concentration level, using electroanalytical stripping methods

with environmentally friendly mercury free-electrodes: A review, *Talanta*, 2017, **175**, 53.
14. A. Economou, Screen-printed electrodes modified with ''green'' metals for electrochemical stripping analysis of toxic elements, *Sensors*, 2018, **18**, 1032.
15. C. Barceló, N. Serrano, J. M. Díaz-Cruz, C. Ariño and M. Esteban, Ex-situ antimonyscreen-printed carbon electrode for voltammetric determination of Ni(II)-ions in wastewater, *Electroanalysis*, 2016, **28**, 640.
16. C. Pérez-Ràfols, P. Trechera, N. Serrano, J. M. Díaz-Cruz, C. Ariño and M. Esteban, Determination of Pd(II) using an antimony film coated on a screen-printed electrode by adsorptive stripping voltammetry, *Talanta*, 2017, **167**, 1.
17. M. Maczuga, A. Economou, A. Bobrowski and M. I. Prodromidis, Novel screen-printed antimony and tin voltammetric sensors for anodic stripping detection of Pb(II) and Cd(II), *Electrochim. Acta*, 2013, **114**, 758.
18. D. Riman, K. Spyrou, A. E. Karantzalis, J. Hrbac and M. I. Prodromidis, Glucose sensing on graphite screen-printed electrode modified by sparking of copper nickel alloys, *Talanta*, 2017, **165**, 466.
19. M. G. Trachioti, J. Hrbac and M. I. Prodromidis, Determination of Cd and Zn with ''green'' screen-printed electrodes modified with instantly prepared sparked tin nanoparticles, *Sens. Actuators, B*, 2018, **260**, 1076.
20. K. Tyszczuk-Rotko, K. Surowiec and A. Szwagierek, Application of ecofriendly bismuth film electrode for the sensitive determination of rutin, *Curr. Pharm. Anal.*, 2018, **14**, 571.
21. É. S. Sá, P. S. Da Silva, C. L. Jost and A. Spinelli, Electrochemical sensor based on bismuth-film electrode for voltammetric studies on vitamin B2 (riboflavin), *Sens. Actuators, B*, 2015, **209**, 423.
22. A. Ananthi, S. S. Kumar and K. L. Phani, Facile one-step direct electrodeposition of bismuth nanowires on glassy carbon electrode for selective determination of folic acid, *Electrochim. Acta*, 2015, **151**, 584.
23. D. Riman, A. Avgeropoulos, J. Hrbac and M. I. Prodromidis, Sparked-bismuth oxide screen-printed electrodes for the determination of riboflavin in the sub-nanomolar range in non-deoxygenated solutions, *Electrochim. Acta*, 2015, **165**, 410.
24. D. Qin, X. Hu, Y. Dong, X. Mamat, Y. Li, T. Wågberg and G. Hu, An electrochemical sensor based on green γ-AlOOH-carbonated bacterial cellulose hybrids for simultaneous determination trace levels of Cd(II) and Pb(II) in drinking water, *J. Electrochem. Soc.*, 2018, **165**, B328.
25. A. Dueraning, P. Kanatharana, P. Thavarungkul and W. Limbutm, An environmental friendly electrode and extended cathodic potential window for anodic stripping voltammetry of zinc detection, *Electrochim. Acta*, 2016, **221**, 133.
26. Z. Lu, W. Dai, B. Liu, G. Mo, J. Zhang, J. Ye and J. Ye, One pot synthesis of dandelion-like polyaniline coated gold nanoparticles composites for electrochemical sensing applications, *J. Colloid Interface Sci.*, 2018, **525**, 86.

27. H. S. El-Desoky, M. M. Ghoneim, M. M. Abdel-Galeil and A. D. Habazy, Simple voltammetric method for nano estimation of etilefrine hydrochloride based on environmentally friendly montmorillonite natural clay, *J. Electrochem. Soc.*, 2017, **164**, H714.
28. V. A. Pedrosa, L. Codognoto, S. A. S. Machado and L. A. Avaca, Is the boron-doped diamond electrode a suitable substitute for mercury in pesticide analyses? A comparative study of 4-nitrophenol quantification in pure and natural waters, *J. Electroanal. Chem.*, 2004, **573**, 11.
29. L. Bandžuchová, L'. Švorc, M. Vojs, M. Marton, P. Michniak and J. Chýlková, Self-assembled sensor based on boron-doped diamond and its application in voltammetric analysis of picloram, *Intern. J. Environ. Anal. Chem.*, 2014, **94**, 943.
30. K. Asai, T. A. Ivandini and Y. Einaga, Continuous and selective measurement of oxytocin and vasopressin using boron-doped diamond electrodes, *Sci. Rep.*, 2016, **6**, 32429.
31. B. Nigovnic and I. Milanovic, Green electroanalytical method for fast measurement of xanthine oxidase inhibitor febuxostat, *Anal. Sci.*, 2017, **33**, 1219.
32. L. P. Yang, W. A. Yee, S. L. Phua, J. Kong, H. Ding, J. W. Cheah and X. Lu, A high throughput method for preparation of highly conductive functionalized graphene and conductive polymer nanocomposites, *RSC Adv.*, 2012, **2**, 2208.
33. S. Kochmann, T. Hirsch and O. S. Wolfbeis, Graphenes in chemical sensors and biosensors, *TrAC, Trends Anal. Chem.*, 2012, **39**, 87.
34. S. Sakthinathan, S. Kubendhiran, S.-M. Chen, F. M. A. Al-Hemaid, W. C. Liao, P. Tamizhdurai, S. Sivasanker, M. A. Ali and A. A. Hatamleh, A non-covalent interaction of Schiff base copper alanine complex with green synthesized reduced graphene oxide for highly selective electrochemical detection of nitrite, *RSC Adv.*, 2016, **6**, 107416.
35. A. Shaikh, S. Parida and S. Böhm, One step eco-friendly synthesis of Ag–reduced graphene oxide nanocomposite by phytoreduction for sensitive nitrite determination, *RSC Adv.*, 2016, **6**, 100383.
36. R. Karthik, M. Govindasamy, S.-M. Chen, V. Mani, B.-S. Lou, R. Devasenathipathy, Y.-S. Hou and A. Elangovan, Green synthesized gold nanoparticles decorated graphene oxide for sensitive determination of chloramphenicol in milk, powdered milk, honey and eye drops, *J. Colloid Interface Sci.*, 2016, **475**, 46.
37. P. Liu, F.-Q. Bai, D.-W. Lin, H.-P. Peng, Y. Hu, Y.-J. Zheng, W. Chen, A.-L. Liu and X.-H. Lin, One-pot green synthesis of mussel-inspired myoglobin–gold nanoparticles–polydopamine–graphene polymeric bionanocomposite for biosensor application, *J. Electroanal. Chem.*, 2016, **764**, 104.
38. K. Sheng, L. Wang, H. Li, L. Zou and B. Ye, Green synthesized Co nanoparticles doped amino-graphene modified electrode and its application towards determination of baicalin, *Talanta*, 2017, **164**, 249.

39. S. Kubendhiran, S. Sakthinathan, S.-M. Chen, P. Tamizhdurai, K. Shanthi and C. Karuppiah, Green reduction of reduced graphene oxide with nickel tetraphenyl porphyrin nanocomposite modified electrode for enhanced electrochemical determination of environmentally pollutant nitrobenzene, *J. Colloid Interface Sci.*, 2017, **497**, 207.
40. Y. Zhang and G. Li, A novel electrochemical sensor based on a graphene-silver platform for the sensitive determination of a tumor-supplied group of factors, *Int. J. Electrochem. Sci.*, 2017, **12**, 10095.
41. L. T. Hoa, N. T. Y. Linh, J. S. Chung and S. H. Hur, Green synthesis of silver nanoparticle-decorated porous reduced graphene oxide for antibacterial non-enzymatic glucose sensors, *Ionics*, 2017, **23**(6), 1525.
42. R. Madhuvilakku, S. Alagar, R. Mariappan and S. Piraman, Green one-pot synthesis of flowers-like Fe_3O_4/rGO hybridnanocomposites for effective electrochemical detection of riboflavinand low-cost supercapacitor applications, *Sens. Actuators, B*, 2017, **253**, 879.
43. N. Lezi, S. Economopoulos, M. Prodromidis, A. Economou and N. Tagmatarchis, Fabrication of a ''green'' and low–cost screen–printed graphene sensor and its application to the determination of caffeine by adsorptive stripping voltammetry, *Int. J. Electrochem. Sci.*, 2017, **12**, 6054.
44. P. L. dos Santos, V. Katic, K. C. F. Toledo and J. A. Bonacin, Photochemical one-pot synthesis of reduced graphene oxide/Prussian blue nanocomposite for simultaneous electrochemical detection of ascorbic acid, dopamine and uric acid, *Sens. Actuators, B*, 2018, **255**, 2437.
45. N. Arsalani, S. Mashkouri, M. G. Hosseini, A. Ramazani and H. Mostafavi, Green synthesis of water-soluble graphene nanosheets under solvent free condition and in-situ anchored with MnO_2 as supercapacitor, *J. Mater. Sci.*, 2018, **29**, 6692.
46. L. P. Lin, P. S. Khiew, W. S. Chiu and M. T. T. Tan, A disposable electrochemical sensing platform for acetaminophen based on graphene/ZrO_2 nanocomposite produced *via* a facile, green synthesis method, *IEEE Sens. J.*, 2018, **18**, 7907.
47. Y. Zuo, J. Xu, H. Xing, X. Duan, L. Lu, G. Ye, H. Jia and Y. Yu, Simple and green synthesis of piperazine grafted reduced graphene oxide and its application for the detection of Hg(II), *Nanotechnology*, 2018, **29**, 165502.
48. R. Sha and S. Badhulika, Facile green synthesis of reduced graphene oxide/tin oxide composite for highly selective and ultra-sensitive detection of ascorbic acid, *J. Electroanal. Chem.*, 2018, **816**, 30.
49. R. Tandel, N. Teradal, A. Satpati and S. Jaldappagari, Fabrication of the electrochemically reduced graphene oxide-bismuth nanoparticles composite and its analytical application for an anticancer drug gemcitabine, *Chin. Chem. Lett.*, 2017, **28**, 1429.
50. F. M. El-Badawy and H. S. El-Desoky, Quantification of chloroxylenol, a potent antimicrobial agent in various formulations and water samples: Environmental friendly electrochemical sensor based on microwave synthesis of graphene, *J. Electrochem. Soc.*, 2018, **165**, B694.

51. S. A. Zaidi, Utilization of an environmentally-friendly monomer for an efficient and sustainable adrenaline imprinted electrochemical sensor using graphene, *Electrochim. Acta*, 2018, **274**, 370.
52. L. Zhao, Y. Wang, X. Zhao, Y. Deng, Q. Li and Y. Xia, Green preparation of Ag-Au bimetallic nanoparticles supported on graphene with alginate for non-enzymatic hydrogen peroxide detection, *Nanomaterials*, 2018, **8**, 507.
53. S. Pourbeyram, J. Abdollahpour and M. Soltanpour, Green synthesis of copper oxide nanoparticles decorated reduced graphene oxide for high sensitive detection of glucose, *Mater. Sci. Eng., C*, 2019, **94**, 850.
54. X. Zhang, Y. Bao, Y. Bai, Z. Chen, J. Li and F. Feng, In situ electrochemical reduction assisted assembly of a graphene-gold nanoparticles@polyoxometalate nanocomposite film and its high response current for detection of hydrogen peroxide, *Electrochim. Acta*, 2019, **300**, 380.
55. L. Wang, R. Yang, J. Lia, L. Qua, P. de and B. Harrington, A highly selective and sensitive electrochemical sensor for tryptophan based on the excellent surface adsorption and electrochemical properties of PSS functionalized graphene, *Talanta*, 2019, **196**, 309.
56. U. Rajaji, S. Manavalan, S.-M. Chen, M. Govindasamy, T.-W. Chen and T. Maiyalagan, Microwave-assisted synthesis of europium(III) oxide decorated reduced graphene oxide nanocomposite for detection of chloramphenicol in food samples, *Composites, Part B*, 2019, **161**, 29.
57. F. Liu, Q. Xu, W. Huang, Z. Zhang, G. Xiang, C. Zhang, C. Liang, H. Lian and J. Peng, Green synthesis of porous graphene and its application for sensitive detection of hydrogen peroxide and 2,4-dichlorophenoxyacetic acid, *Electrochim. Acta*, 2019, **295**, 615.
58. G. Huang, W. Kang, Q. Geng, B. Xing, Q. Liu, J. Jia and C. Zhang, One-step green hydrothermal synthesis of few-layer graphene oxide from humic acid, *Nanomaterials*, 2018, **8**, 215.
59. D. Gobelli, N. M. Correa, M. F. Barroso, F. Moyano and P. G. Molina, ''Green electrodes'' modified with Au nanoparticles synthesized in glycerol, *Electroanalysis*, 2015, **27**, 1883.
60. N. Verma, A green synthetic approach for size tunable nanoporous gold nanoparticles and its glucose sensing application, *Appl. Surf. Sci.*, 2018, **462**, 753.
61. K. Ramachandran, D. Kalpana, Y. Sathishkumar, Y. S. Lee, K. Ravichandran and G. Gnana, kumar, A facile green synthesis of silver nanoparticles using Piper betle biomass and its catalytic activity toward sensitive and selective nitrite detection, *J. Ind. Eng. Chem.*, 2016, **35**, 29.
62. E. Turunc, R. Binzet, I. Gumus, G. Binzet and H. Arslan, Green synthesis of silver and palladium nanoparticles using *Lithodora hispidula* (Sm.) Griseb. (Boraginaceae) and application to the electrocatalytic reduction of hydrogen peroxide, *Mater. Chem. Phys.*, 2017, **202**, 310.
63. M. Baghayeri, B. Mahdavi, Z. Hosseinpor-Mohsen Abadi and S. Farhadi, Green synthesis of silver nanoparticles using water extract of Salvia

leriifolia: Antibacterial studies and applications as catalysts in the electrochemical detection of nitrite, *Appl. Organomet. Chem.*, 2018, **32**, 4057.
64. M. Sebastian, A. Aravind and B. Mathew, Green silver-nanoparticle-based dual sensor for toxic Hg(II) ions, *Nanotechnology*, 2018, **29**, 355502.
65. R. Karthik, R. Sasikumar, S.-M. Chen, M. Govindasamy, J. Vinoth Kumar and V. Muthuraj, Green synthesis of platinum nanoparticles using *Quercus Glauca* extract and its electrochemical oxidation of hydrazine in water samples, *Int. J. Electrochem. Sci.*, 2016, **11**, 8245.
66. E. E. Elemike, D. C. Onwudiwe, O. E. Fayemi and T. L. Botha, Green synthesis and electrochemistry of Ag, Au and Ag–Au bimetallic nanoparticles using golden rod (*Solidago canadensis*) leaf extract, *Appl. Phys. A: Mater. Sci. Process.*, 2019, **125**, 42.
67. N. Matinise, K. Kaviyarasu, N. Mongwaketsi, S. Khamlich, L. Kotsedi, N. Mayedwa and M. Maaza, Green synthesis of novel zinc iron oxide ($ZnFe_2O_4$) nanocomposite *via Moringa Oleifera* natural extract for electrochemical applications, *Appl. Surf. Sci.*, 2018, **446**, 66.
68. K. Karthik, S. Dhanuskodi, C. Gobinath, S. Prabukumar and S. Sivaramakrishnan, *Andrographis paniculata* extract mediated green synthesis of CdO nanoparticles and its electrochemical and antibacterial studies, *J. Mater. Sci.*, 2017, **28**, 7991.
69. D. Sharma, M. I. Sabela, S. Kanchi, K. Bisetty, A. A. Skelton and B. Honarparvar, Green synthesis, characterization and electrochemical sensing of silymarin by ZnO nanoparticles: Experimental and DFT studies, *J. Electroanal. Chem.*, 2018, **808**, 160.
70. S. Goutham, S. Kaur, K. K. Sadasivuni, J. K. Bal, N. Jayarambabu, D. S. Kumar and K. V. Rao, Nanostructured ZnO gas sensors obtained by green method and combustion technique, *Mater. Sci. Semicond. Process.*, 2017, **57**, 110.
71. N. Muthuchamy, R. Atchudan, T. N. J. I. Edison, S. Perumal and Y. R. Lee, High-performance glucose biosensor based on green synthesized zinc oxide nanoparticle embedded nitrogen-doped carbon sheet, *J. Electroanal. Chem.*, 2018, **816**, 195.
72. M. Opallo and A. Lesniewski, A review on electrodes modified with ionic liquids, *J. Electroanal. Chem.*, 2011, **656**, 2.
73. Q. Zhao, H. Zhou, W. Wu, X. Wei, S. Jiang, T. Zhou, D. Liu and Q. Lu, Sensitive electrochemical detection of tetrabromobisphenol A based on poly(diallyldimethylammonium chloride) modified graphitic carbon nitride-ionic liquid doped carbon paste electrode, *Electrochim. Acta*, 2017, **254**, 214.
74. F. L. Mohd Rasdi, S. Mohamad, N. S. Abdul Manana and H. Rashidi Nodeha, Electrochemical determination of 2,4-dichlorophenol at b-cyclodextrin functionalized ionic liquid modified chemical sensor: voltammetric and amperometric studies, *RSC Adv.*, 2016, **6**, 100186.

75. F. E. Salih, L. Oularbi, E. Halim, M. Elbasri, A. Ouarzane and M. El Rhazi, Conducting polymer/ionic liquid composite modified carbon paste electrode for the determination of carbaryl in real samples, *Electroanalysis*, 2018, **30**, 1855.
76. H. Beitollahi, K. Movlaee, M. Reza Ganjali, P. Norouzi and R. Hosseinzadeh, Application of a nanostructured sensor based on graphene- and ethyl 2-(4-ferrocenyl[1,2,3]triazol-1-yl) acetate -modified carbon paste electrode for determination of methyldopa in the presence of phenylephrine and guaifenesin, *Appl. Organomet. Chem.*, 2018, e4243.
77. F. Moreira, T. de Andrade Maranhão and A. Spinelli, Carbon paste electrode modified with Fe_3O_4 nanoparticles and BMI.PF_6 ionic liquid for determination of estrone by square-wave voltammetry, *J. Solid State Electrochem.*, 2018, **22**, 1303.
78. G. Absalan, M. Akhond, R. Karimi and A. M. Ramezani, Simultaneous determination of captopril and hydrochlorothiazide by using a carbon ionic liquid electrode modified with copper hydroxide nanoparticles, *Microchim. Acta*, 2018, **185**, 97.
79. E. Núñez-Bajo, M. C. Blanco-López, A. Costa-García and M. T. Fernández-Abedul, Electrogeneration of gold nanoparticles on porous-carbon paper-based electrodes and application to inorganic arsenic analysis in white wines by chronoamperometric stripping, *Anal. Chem.*, 2017, **89**, 6415.
80. A. Ghosale, K. Shrivas, R. Shankar and V. Ganesan, Low-cost paper electrode fabricated by direct writing with silver nanoparticle-based ink for detection of hydrogen peroxide in wastewater, *Anal. Chem.*, 2017, **89**, 776.
81. M. Medina-Sánchez, M. Cadevall, J. Ros and A. Merkoçi, Eco-friendly electrochemical lab-on-paper for heavy metal detection, *Anal. Bioanal. Chem.*, 2015, **407**, 8445.
82. F. Arduini, S. Cinti, V. Caratelli, L. Amendola, G. Palleschi and D. Moscone, Origami multiple paper-based electrochemical biosensors for pesticide detection, *Biosens. Bioelectron.*, 2019, **126**, 346.
83. M. Stoycheva and R. Zlatec, in Electrochemical Sensors for Environmental Analysis, ed. K. Ota, G. Kreysa and R. F. Savinell, *Encyclopedia of applied electrochemistry*, Springer, Verlag, Berlin, Heidelberg, 2011.
84. M. R. Rezk, A. S. Fayed, H. M. Marzouk and S. S. Abbas, Green ion selective electrode potentiometric application for the determination of cinchocaine hydrochloride in presence of its degradation products and betamethasone valerate: A comparative study of liquid and solid inner contact ion-selective electrode membranes, *J. Electrochem. Soc.*, 2017, **164**, H628.
85. M. R. Rezk, A. S. Fayed, H. M. Marzouk and S. S. Abbas, Potentiometric ion-selective electrodes for determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in their challenging ophthalmic formulation, *J. Solid State Electrochem.*, 2018, **22**, 3351.
86. A. Ma'mun, M. K. Abd El-Rahman and M. Abd El-Kawyba, Analytical real-time potentiometric sensor; an innovative tool for monitoring

hydrolysis of chemo/bio-degradable drugs in pharmaceutical sciences, *J. Pharm. Biomed. Anal.*, 2018, **154**, 166.
87. A. S. Saad, A. M. A. Al Alamein, M. M. Galal and H. E. Zaaza, Novel green potentiometric method for the determination of lidocaine hydrochloride and its metabolite 2, 6-dimethylaniline; application to pharmaceutical dosage form and milk, *Electroanalysis*, 2018, **30**, 1.
88. E. S. Elzanfaly and A. S. Saad, Green in-line ion selective electrode potentiometric method for determination of amantadine in dissolution media and in pharmaceutical formulations, *ACS Sustainable Chem. Eng.*, 2017, **5**, 4381.
89. R. Viveiros, S. Rebocho and T. Casimiro, Green strategies for molecularly imprinted polymer development, *Polymers*, 2018, **10**, 306.
90. M. Salajegheh, M. Ansari, M. M. Foroghi and M. Kazemipou, Computational design as a green approach for facile preparation ofmolecularly imprinted polyarginine-sodium alginate-multiwalledcarbon nanotubes composite film on glassy carbon electrode for theophylline sensing, *J. Pharm. Biomed. Anal.*, 2019, **162**, 215.
91. J. Luo, J. Huang, Y. Wu, J. Sun, W. Wei and X. Liu, Synthesis of hydrophilic and conductive molecularly imprinted polyaniline particles for the sensitive and selective protein detection, *Biosens. Bioelectron.*, 2017, **94**, 39.
92. D. Zappi, R. Caminiti, G. M. Ingo, C. Sadun, C. Tortolini and M. L. Antonelli, Biologically friendly room temperature ionic liquids and nanomaterials for the development of innovative enzymatic biosensors, *Talanta*, 2017, **175**, 566.
93. P. Liu, F.-Q. Bai, D.-W. Lin, H.-P. Peng, Y. Hu, Y.-J. Zheng, W. Chen, A.-L. Liu and X.-H. Lin, One-pot green synthesis of mussel-inspired myoglobin–gold nanoparticles–polydopamine–graphene polymeric bionanocomposite for biosensor application, *J. Electroanal. Chem.*, 2016, **764**, 104.
94. P. Bollella, F. Mazzei, G. Favero, G. Fusco, R. Ludwig, L. Gorton and R. Antiochia, Improved DET communication between cellobiose dehydrogenase and a gold electrode modified with a rigid self-assembled monolayer and green metal nanoparticles: The role of an ordered nanostructuration, *Biosens. Bioelectron.*, 2017, **88**, 196.
95. K. Pramanik, P. Sarkar, D. Bhattacharyay and P. Majumdar, One step electrode fabrication for direct electron transfer cholesterol biosensor based on composite of polypyrrole, green reduced graphene oxide and cholesterol oxidase, *Electroanalysis*, 2018, **30**, 2719.
96. N. Muthuchamy, R. Atchudan, T. N. J. I. Edison, S. Perumal and Y. R. Lee, High-performance glucose biosensor based on green synthesized zinc oxide nanoparticle embedded nitrogen-doped carbon sheet, *J. Electroanal. Chem.*, 2018, **816**, 195.
97. Q. Huang, X. Lin, J.-J. Zhu and Q.-X. Tong, Pd-Au@carbon dots nanocomposite: Facile synthesis and application as an ultrasensitive

electrochemical biosensor for determination of colitoxin DNA in human serum, *Biosens. Bioelectron.*, 2017, **94**, 507.
98. Y. Zheng, X. Wang, S. He, Z. Gao, Y. Di, K. Lu, K. Li and J. Wang, Aptamer-DNA concatamer-quantum dots based electrochemical biosensing strategy for green and ultrasensitive detection of tumor cells *via* mercuryfree anodic stripping voltammetry, *Biosens. Bioelectron.*, 2019, **126**, 261.
99. N. Yao, H. B. Wang and Y. L. Hu, Recent progress on electrochemical application of room-temperature ionic liquids, *Mini-Rev. Org. Chem.*, 2017, **14**, 237.
100. A. Rehman and X. Zeng, Ionic liquids as green solvents and electrolytes for robust chemical sensor development, *Acc. Chem. Res.*, 2012, **45**, 1667.
101. X. Zhu, Y. Zeng, Z. Zhang, Y. Yang, Y. Zhai, H. Wang, L. Liu, J. Hu and L. Li, A new composite of graphene and molecularly imprinted polymer based on ionic liquids as functional monomer and cross-linker for electrochemical sensing 6-benzylaminopurine, *Biosens. Bioelectron.*, 2018, **108**, 38.
102. C. Verma, E. E. Ebenso and M. A. Quraishi, Transition metal nanoparticles in ionic liquids: Synthesis and stabilization, *J. Mol. Liq.*, 2019, **276**, 826.
103. J. Łuczak, M. Paszkiewicz, A. Krukowska, A. Malankowska and A. Zaleska-Medynska, Ionic liquids for nano- and microstructures preparation. Part 2: Application in synthesis, *Adv. Colloid Interface Sci.*, 2016, **227**, 1.
104. S. Sultana, N. Tachikawa, K. Yoshii, K. Toshima, L. Magagnin and Y. Katayama, Electrochemical preparation of platinum nanoparticles from bis (acetylacetonato) platinum(II) in some aprotic amide-type ionic liquids, *Electrochim. Acta*, 2017, **249**, 263.
105. Y. Katayama, Y. Oshino, N. Ichihashi, N. Tachikawa, K. Yoshii and K. Toshima, Electrochemical preparation of palladium nanoparticles in bis (trifluoromethylsulfonyl)amide ionic liquids consisting of pyrrolidinium cations with different alkyl chain lengths, *Electrochim. Acta*, 2015, **183**, 37.
106. R. S. Babu, P. Prabhu and S. S. Narayanan, Enzyme-free selective determination of H_2O_2 and glucose using functionalized CuNP-modified graphite electrode in room temperature ionic liquid medium, *RSC Adv.*, 2014, **4**, 47497.
107. Z. Jahromi, T. Shamspur, A. Mostafavi and M. Mohamadi, Separation and preconcentration of hemin from serumsamples followed by voltammetric determination, *J. Mol. Liq.*, 2017, **242**, 91.
108. L.-L. Huang, Y. Huang, Y.-K. Chen, Y.-H. Ding, W.-F. Zhang, X.-J. Li and X.-P. Wu, Supported ionic liquids solid-phase extraction coupled to electrochemical detection for determination of trace Bisphenol A, *Chin. J. Anal. Chem.*, 2015, **43**, 313.

109. S. Rizzo, S. Arnaboldi, R. Cirilli, A. Gennaro, A. A. Isse, F. Sannicolò and P. R. Mussini, An ''inherently chiral'' 1,1′-bibenzimidazolium additive for enantioselective voltammetry in ionic liquid media, *Electrochem. Commun.*, 2018, **89**, 57.
110. E. Fernandez, L. Vidal, A. Costa-García and A. Canals, Mercury determination in urine samples by gold nanostructured screen-printed carbon electrodes after vortex-assisted ionic liquid dispersive liquide-liquid microextraction, *Anal. Chim. Acta*, 2016, **915**, 49.
111. M. Matsumiya, M. Sumi, Y. Uchino and I. Yanagi, Recovery of indium based on the combined methods of ionic liquid extraction and electrodeposition, *Sep. Purif. Technol.*, 2018, **201**, 25.
112. K. Rong, L. Huang, H. Zhang, J. Zhai, Y. Fang and S. Dong, Electrochemical fabrication of nanoporous gold electrodes in a deep eutectic solvent for electrochemical detections, *Chem. Commun.*, 2018, **54**, 8853.
113. L. I. N. Tomé, V. Baião, W. da Silva and C. M. A. Brett, Deep eutectic solvents for the production and application of new materials, *Appl. Mater. Today*, 2018, **10**, 30.
114. C. J. Clarke, W. C. Tu, O. Levers, A. Brohl and J. P. Hallett, Green and sustainable solvents in chemical processes, *Chem. Rev.*, 2018, **118**, 747.
115. A. P. Abbott and K. J. McKenzie, Application of ionic liquids to the electrodeposition of metals, *Phys. Chem. Chem. Phys.*, 2006, **8**, 4265.
116. C. M. A. Brett, Deep eutectic solvents and applications in electrochemical sensing, *Curr. Opin. Electrochem.*, 2018, **10**, 143.
117. K. P. Prathish, R. C. Carvalho and C. M. A. Brett, Electrochemical characterization of poly(3,4-ethylenedioxythiophene) film modified glassy carbon electrodesprepared in deep eutectic solvents for simultaneous sensing of biomarkers, *Electrochim. Acta*, 2016, **187**, 704.
118. O. Hosu, M. Barsan, C. Cristea, R. Sandulescu and C. M. A. Brett, Nanostructured electropolymerized poly(methylene blue) films from deep eutectic solvents. Optimization and characterization, *Electrochim. Acta*, 2017, **232**, 28.
119. O. Hosu, M. M. Barsan, C. Cristea, R. Sandulescu and C. M. A. Brett, Nanocomposites based on carbon nanotubes and redox-active polymers synthesized in a deep eutecticsolvent as a new electrochemical sensing platform, *Microchim. Acta*, 2017, **184**, 3919.
120. A. Abo-Hamad, M. Hayyan, M. A. H. AlSaadi, M. E. S. Mirghani and M. Ali Hashim, Functionalization of carbon nanotubes using eutectic mixtures: A promising route for enhanced aqueous dispersibility and electrochemical activity, *Chem. Eng. J.*, 2017, **311**, 326.
121. R. Svigelj, N. Dossi, R. Toniolo, R. Miranda-Castro, N. de-los-Santos-Álvarez and M. J. Lobo-Castañón, Selection of anti-gluten DNA aptamers in a deep eutectic solvent, *Angew. Chem.*, 2018, **130**, 13032.
122. M. Espino, M. A. Fernández, F. J. V. Gomez and M. F. Silva, Natural designer solvents for greening analytical chemistry, *TrAC, Trends Anal. Chem.*, 2016, **76**, 126.

123. Y. Dai, J. van Spronsen, G. J. Witkamp, R. Verpoorte and Y. H. Choi, Natural deep eutectic solvents as new potential media for green technology, *Anal. Chim. Acta*, 2013, **766**, 61.
124. F. J. V. Gomez, M. Espino, M. A. Fernandez, J. Raba and M. F. Silva, Enhanced electrochemical detection of quercetin by natural deep eutectic solvents, *Anal. Chim. Acta*, 2016, **936**, 91.
125. D. L. Goldfarb and H. R. Corti, Electrochemistry in supercritical trifluoromethane, *Electrochem. Commun.*, 2000, **2**, 663.
126. P. N. Barlett and J. Branch, The voltammetry of decamethylferrocene and coboltacene in supercritical difluoromethane (R32), *J. Electroanal. Chem.*, 2016, **780**, 282.
127. J. Young, J. M. Desimone and W. Tumas, Introduction, in *Green Chemistry Using Liquid and Supercritical Carbon Dioxide*, ed. J. M. Desimone and W. Tumas, Oxford University Press Inc., New York, 2003, p. xii.
128. K. Yamamoto, T. Ueki, N. Higuchi, K. Takahashi, A. Kotani and H. Hakamata, A flow-through column electrolytic cell for supercritical fluid chromatography, *J. Sep. Sci.*, 2017, **40**, 4085.
129. K. Yamamoto, A. Kotani and H. Hakamata, Electrochemical detection of tocopherols in vegetable oils by supercritical fluid chromatography equipped with carbon fiber electrodes, *Anal. Methods*, 2018, **10**, 4414.
130. J. W. Wu, C. H. Wang, Y. C. Wang and J. K. Chang, Ionic-liquid-enhanced glucose sensing ability of non-enzymatic Au/graphene electrodes fabricated using supercritical CO_2 fluid, *Biosens. Bioelectron.*, 2013, **46**, 30.
131. S. Rebocho, C. M. Cordas, R. Viveiros and T. Casimiro, Development of a ferrocenyl-based MIP in supercritical carbon dioxide: Towards an electrochemical sensor for bisphenol A, *J. Supercrit. Fluids*, 2018, **135**, 98–104.
132. S. Kurbanoglu, M. Altay Unal and S. A. Ozkan, Recent developments on electrochemical flow injection in pharmaceuticals and biologically important compounds, *Electrochim. Acta*, 2018, **287**, 135.
133. B. Feier, A. Gui, C. Cristea and R. Săndulescu, Electrochemical determination of cephalosporins using a bare boron-doped diamond electrode, *Anal. Chim. Acta*, 2017, **976**, 25.
134. S. Nellaiappan and A. S. Kumar, Electrocatalytic oxidation and flow injection analysis of isoniazid drug using a gold nanoparticles decorated carbon nanofibers-chitosan modified carbon screen printed electrode in neutral pH, *J. Electroanal. Chem.*, 2017, **801**, 171.
135. K. Promsuwan, P. Thavarungkul, P. Kanatharana and W. Limbut, Flow injection amperometric nitrite sensor based on silver microcubics-poly (acrylic acid)/poly (vinyl alcohol) modified screen printed carbon electrode, *Electrochim. Acta*, 2017, **232**, 357.
136. D. Tang, R. Niessner and D. Knopp, Flow-injection electrochemical immunosensor for the detection of human IgG based on glucose oxidase-derivated biomimetic interface, *Biosens. Bioelectron.*, 2009, **24**, 2125.

137. C. Thunkhamrak, P. Reanpang, K. Ounnunkad and J. Jakmunee, Sequential injection system with amperometric immunosensor for sensitive determination of human immunoglobulin G, *Talanta*, 2017, **171**, 53.
138. J. Biscay, M. B. González García and A. Costa García, Electrochemical biotin detection based on magnetic beads and a new magnetic flow cell for screen printed electrode, *Talanta*, 2015, **131**, 706.
139. D. Agustini, L. Fedalto, M. F. Bergamini and L. H. Marcolino-Junior, Microfluidic thread based electroanalytical system for green chromatographic separations, *Lab Chip*, 2018, **18**, 670.
140. G. Kaur, M. Tomar and V. Gupta, A simple paper based microfluidic electrochemical biosensor for point-of-care cholesterol diagnostics, *Phys. Status Solidi A*, 2017, **214**, 1700468.
141. M. Punjiya, C. H. Moon, Z. Matharu, H. Rezaei Nejad and S. Sonkusale, A three-dimensional electrochemical paper-based analytical device for low-cost diagnostics, *Analyst*, 2018, **143**, 1059.

CHAPTER 8

Green Solvents for Analytical Chemistry

ANNE-SYLVIE FABIANO-TIXIER,[a]
HARISH KARTHIKEYAN RAVI,[a] BOUTHEINA KHADHRAOUI,[a]
SANDRINE PERINO,[a] MARYLINE ABERT-VIAN,[a]
CYRILLE SANTERRE,[b] NADINE VALLET[b] AND
FARID CHEMAT*[a]

[a] Avignon University, INRA, UMR408, GREEN Extraction Team, 84000 Avignon, France; [b] Institut Supérieur International du Parfum, de la Cosmétique et de l'Aromatique Alimentaire (ISIPCA), 34–36 rue du Parc-de-Clagny, 78000 Versailles, France
*Email: farid.chemat@univ-avignon.fr

8.1 Introduction

Food and natural products are complex mixtures of primary (sugars, proteins and lipids) and secondary (aromas, pigments, antioxidants) metabolites and other organic and mineral compounds. Before such substances can be analysed, they have to be extracted from the plant matrix in order to be introduced in a liquid form into the analytical detector.[1] Different methods can be used for extraction, *e.g.* Soxhlet or Kumagawa extraction, maceration, decoction, elution and simultaneous distillation–extraction. Nevertheless, all these extraction methods are generally a form of solvent extraction, using petroleum solvents, such as hexane or mixtures of hydrocarbons. From the point of view of environmental protection and the development of green

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

chemistry, these flammable and toxic petroleum solvents will have to be replaced with alternative solvents in the future.[2]

For example, in the perfume industry, extraction of aromas was considered ''clean'' when compared with heavy chemical industries, but researchers and professional specialists found that its environmental impact is far greater than first appeared. The overall environmental impact of an industrial or analytical extraction cycle is not easy to estimate; however, it is known that it requires at least 50% of the energy of the whole industrial process. In spite of the high energy consumption and the large amounts of solvents (mainly hexane) used, often the yield is very low. For example, just 1 mL of rose absolute (used in well-known perfumes), which weighs less than 1 g, requires more than 1–2 kg of fresh roses as raw material (which becomes a chemical waste) and also large amounts of solvents (*n*-hexane, alcohol), energy (fossil) and water as cooling and cleaning agents (wastewater).[3]

This chapter gives an overview of available green solvents that could be applied in extraction as a sample preparation method prior to analysis. Examples of replacements of petroleum solvents are given to illustrate successes in using solvent-free systems, water, super- or subcritical fluids, ionic liquids, deep eutectic solvents and bio-based solvents. A survey is also presented of existing tools used as decision-making aids for the selection of one or more solvents according to the target molecules, especially HSPiP software programs (based on Hansen solubility parameters) and COSMO-RS (Conductor-like Screening Model).

8.2 Decision Support Tools for the Choice of Alternative Solvents

The family of ''green'' solvents is very heterogeneous, but overall they all have undeniable advantages. After careful examination of the extraction power and economic and environmental aspects, these solvents can be considered as a promising alternative to conventional solvents. The choice of solvent remains a crucial step in a substitution process.

In earlier times, the choice of a suitable solvent was purely empirical and was often made on the basis of experience. The effects of solvents are closely related to their chemical structures and today, several descriptors have been proposed to describe them. In the following section, we will see how two HSPiP software programs (based on Hansen solubility parameters) and COSMO-RS (Conductor-like Screening Model), which are decision-making aids for the selection of one or more solvents according to the target molecules, work.

8.2.1 Solubility Prediction Methods According to Hansen Solubility Parameters

Replacing an undesirable solvent with a green solvent is not an easy task and it is rarely possible to find a universal replacement. The use of a tool that

allows a comparison of the physicochemical properties of various alternative solvents with those of petrochemical solvents is very useful for facilitating the replacement. Determining the solubilizing power of a solvent seems to be an acceptable approach for any substitution problem.

Since 1950, this solubilizing power has been evaluated according to the Hildebrand solubility parameter:

$$\delta = \sqrt{\frac{\Delta H - RT}{V}} \tag{8.1}$$

Hildebrand's theory is based on the principle of ''the similar is the same''.[4] Thus, solvents with parameters close to those of the solute will be considered good solvents. Hansen's theory takes up this principle of ''like dissolves like''. Thus, two solvents with similar Hansen solubility parameters will be miscible.[5]

This thermodynamic approach considers three solubility parameters, δ_d, δ_p and δ_h, which account for dispersive, polar and hydrogen interactions, respectively. The parameter δ_d corresponds to London interactions, which are due to the induced dipole when two molecules approach each other. The parameter δ_p matches Keesom's interactions, which take place between two permanent dipoles. The parameter δ_h corresponds to interactions due to hydrogen bonds.

$$\delta_{total} = \sqrt{\delta_d^2 + \delta_p^2 + \delta_h^2} \tag{8.2}$$

where δ_{total} is a total solubility parameter, which is equivalent to Hildebrand's solubility parameter (HSP). The units of HSP values are $MPa^{\frac{1}{2}}$ or $(J\,cm^{-3})^{\frac{1}{2}}$. This approach provides qualitative information on whether the solvent of interest is either a ''good'' or a ''bad'' solvent. It does not allow the prediction of solubility values. Each solvent or solution is represented in a three-dimensional space according to the values of δ_d, δ_p and δ_h. The determination of the relative energy difference (RED) between two chemical entities makes it possible to describe a ''good'' or ''bad'' solvent for one or more solutes. This energy difference is calculated from the equation

$$\text{RED} = R_a/R_0 \tag{8.3}$$

where R_0 is the radius of Hansen's solubility sphere (default, $R_0 = 1$) (see Figure 8.1) and R_a is the distance between the solute and the centre of the solubility sphere, calculated using the equation

$$R_a{}^2 = 4(\delta_{d_A} - \delta_{d_B})^2 + (\delta_{p_A} - \delta_{p_B})^2 + (\delta_{h_A} - \delta_{h_B})^2 \tag{8.4}$$

where A denotes the solute and B the solvent. A low value of R_a, ≤ 1, indicates an affinity between the solvent and the solute, based on eqn (8.3). Indeed, a suitable solvent will have a RED value of ≤ 1.

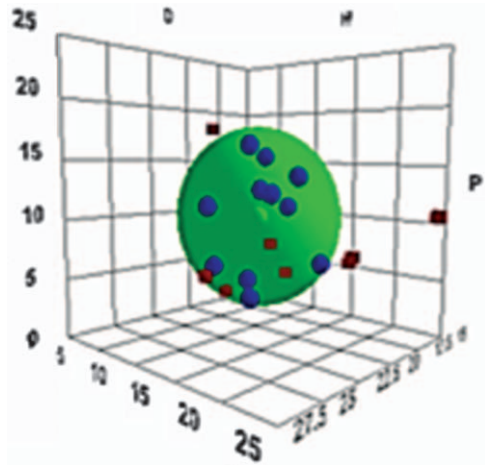


Figure 8.1 Hansen solubility sphere.

The selected solvent, here *n*-hexane, is represented by a green sphere of centre ($\delta_d = 15.2$; $\delta_p = 0.8$; $\delta_h = 2$) and radius 1. Odorous molecules are represented by a blue or red dot depending on whether they are positioned inside or outside the solubility sphere. In other words, red dots characterize solutes that cannot be miscible with the selected solvent (RED > 1) and blue dots designate miscible solutes (RED ≤ 1).

The HSPiP software presents four models of group contributions, Van Krevelen, Stefanis–Panayiotou, Hoy and Yamamoto-molecular break, better known as the Y-MB method, to determine the HSP values (δ_d, δ_p, δ_h). These methods allow the fragmentation of a chemical structure in accordance with its respective functional groups. These HSP values obtained by the Y-MB method also allow the estimation of important physicochemical parameters such as boiling point, vapour pressure, density, viscosity and environmental value.

Hansen's approach allows a quick classification of solvents. Indeed, according to the principle ''like dissolves like'', the positioning of different terpenes reveals a classification according to the chemical families of the latter. It is a rapid and visual tool that allows decision support in the context of solvent replacement. However, Hansen's approach does not consider either van der Waals bonds or acid–base effects. It remains a good qualitative approach for non-polar compounds in non-polar solvents, but it is inadequate for predicting the solubilities of polar solutes (especially when there are dipole–dipole-type forces or hydrogen bonds).

8.2.2 COSMO-RS Approach

Discovered by Klamt and Schueuermann in 1993, the COSMO (Conductor-like Screening Model) is based on solvation along a ''dielectric continuum''.[6]

In 1995, the RS (Real Solvent) extension treated complex interactions within the liquid by considering only local surface contacts (also called surface segment pairs) with charge densities σ and σ'.[7] Unlike group contribution models (UNIFAC, UNIversal Functional Activity Coefficient), where not all functional groups and interactions are considered, COSMO and COSMO-RS calculations can be applied to all types of organic solute–solvent systems.

COSMO-RS is a predictive method for thermodynamic balances of fluids and liquid mixtures that uses a statistical thermodynamic approach based on the results of quantum chemistry calculations. The COSMO-RS approach consists of two main steps (see Figure 8.2): a step of quantum chemistry calculations (COSMO) for each isolated molecule and then a step of thermodynamic calculations, with the extension (RS), which give access to electrostatic interactions between different species.

The solute molecule is immersed in a continuous medium of dielectric constant ε. This can be imagined as a perfect virtual conductor.[8,9] In such an environment, a surface is built around the molecule, which generates a large number of electrostatic charges. The structure and distribution of loads are optimized to find the minimum energy (the molecule in its most stable state) of the system through algorithm-based calculations, called DFT (density functional theory). This charge density is called the σ-surface.

Sequentially, the optimal load distribution is also segmented and reduced to a histogram, named the σ-profile. Thus, the σ-surface and σ-profile make it possible to characterize a reference molecule (minimum energy; stable molecule) as a solute in a perfect conductor. At this stage, the molecule is isolated and does not perceive the molecules in its vicinity. In order to consider a molecule as a solvent or a molecule in a solvent and to quantify the associated interaction energies, an additional step of statistical thermodynamic calculation is required.

The solvent interactions are reduced to local interactions in pairs of surface segments represented by charge densities σ and σ'. All interaction surfaces are assumed to be in close contact. These contacts can be ideal, not complementary, or highlight hydrogen bonds that generate interaction energies. The sum of these energies for a given area is the functional interaction energy.

The interactions of molecular surfaces are given by the energy of functional interaction, denoted E_{int}:

$$E_{\text{int}} = E_{\text{misfit}} + E_{\text{hb}} + E_{\text{vdw}} \tag{8.5}$$

It depends on polarities, charge densities (E_{hb}, hydrogen bond), interacting surfaces (E_{misit}, the so-called ''non-complementary'' contacts), plus a contribution of van der Waals interactions (E_{vdw}, the energy of the reference state).

Having now established the concept of interaction energies, the next step is based on statistical thermodynamic calculations in order to obtain a coherent model of molecules in solution. In this case, the σ-profile, $p_s(\sigma)$, is

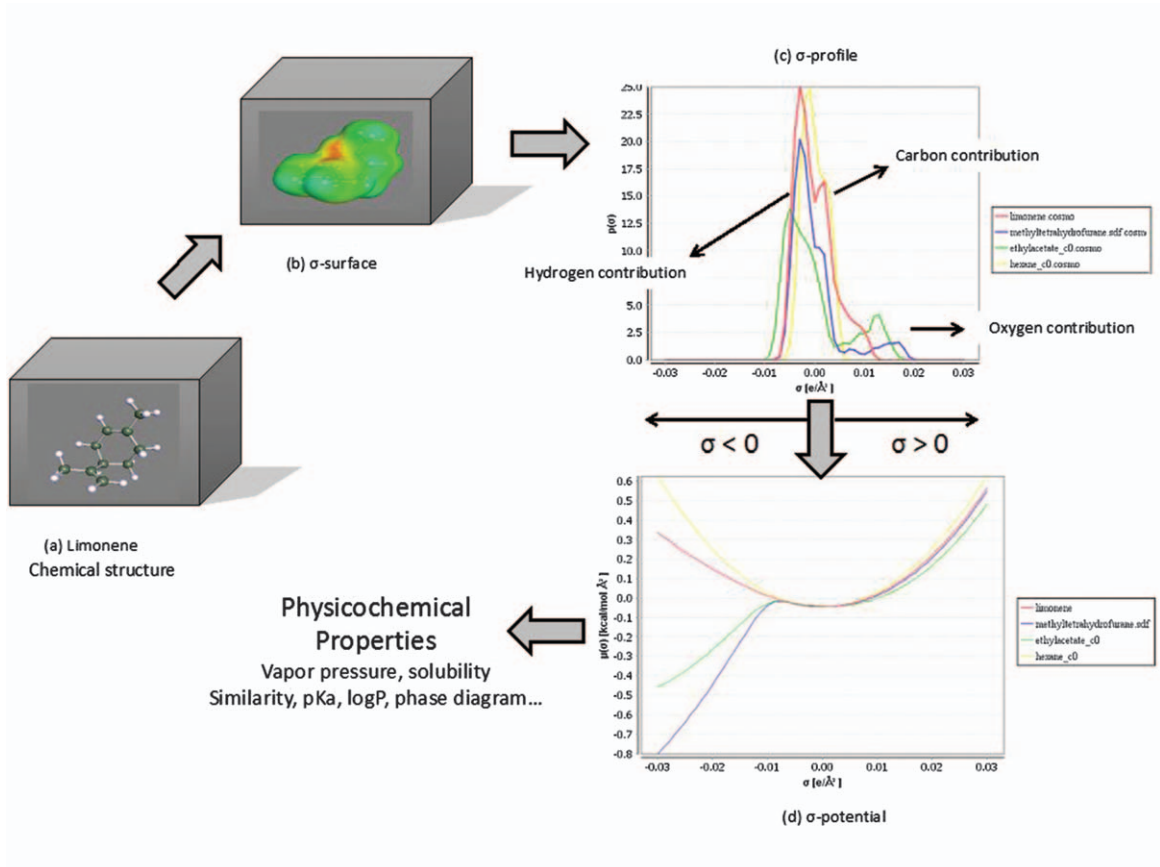


Figure 8.2 The different steps of the COSMO-RS calculation.

simply the sum of the σ-profiles of the p_{X_i} components, weighted by their molar fraction in the mixture (X_i):

$$p_s(\sigma) = \sum X_i p_{X_i}(\sigma) \tag{8.6}$$

This approach allows, on the one hand, the estimation of the affinity of a molecule as a function of the charge density σ put in contact. The molecule is then considered a solvent and this affinity is represented as a σ-potential curve $\mu^S(\sigma)$. On the other hand, the estimation of the affinity of a molecule i in a solvent S is effected by the calculation of its chemical potential μ_i^S (kcal mol^{-1} A^2).

The σ-potential is therefore calculated from the statistical thermodynamics of molecular interaction as a function of the σ-profile obtained. The chemical potential of a surface segment is perfectly described by the following expression:

$$\mu^S(\sigma) = -kT \ln \int p^S(\sigma') \exp\left[\frac{-E_{\text{int}}(\sigma, \sigma') - \mu^S(\sigma')}{kT}\right] \mathrm{d}\sigma' \tag{8.7}$$

Following all these calculation steps, the physicochemical properties can be determined.

The chemical potential μ_i^S can be used to calculate all kinds of thermodynamic properties, such as vapour pressure, heat of vaporization, free energy of solvation, log$P$,[10] and pK_a.[11] The software also offers the possibility of preparing phase diagrams[12] and working with binary or ternary mixtures.[13]

Today, various applications of the software are available in the literature. Moity *et al.*[14] applied the COSMO-RS predictive approach to conventional organic solvents and green solvents. The study provided an overview of the physicochemical properties of solvents, which facilitates comparison between conventional solvents and these green solvents. The approach *via* COSMO-RS has already been successfully applied in different areas of activity:

- in pharmaceutical formulations for the selection of an excipient;[15]
- in purification to improve the liquid–liquid extraction of monoethylene glycol;[16]
- in cosmetics as part of the solubilization of ingredients.[17]

8.3 Solvent-free Microwave Extraction (SFME)

Microwave energy as a non-contact alternative heat source is being utilized efficiently in the field of extraction. Solvent-free microwave extraction (SFME), which proved to be significantly faster, has been developed into an alternative laboratory-scale sample preparation method in the natural products analysis field in recent years. It has shown obvious advantages in terms of solvent consumption, extraction efficiency, purity and antioxidant

activity of final extracts in comparison with conventional extraction techniques.

Microwaves (MWs) are electromagnetic waves with a frequency range from 100 MHz to 3 GHz. MWs comprise electric and magnetic field components and thus act by propagating electromagnetic energy. This energy acts as non-ionizing radiation that causes molecular motions of ions and rotation of dipoles, but does not affect molecular structure. When dielectric materials containing either permanent or induced dipoles are placed in a microwave field, the rotation of the dipoles in the alternating field produces heat. More precisely, the applied microwave field causes the molecules, on average, to spend slightly more time orienting themselves in the direction of the electric field rather than in other directions. When the electric field is removed, thermal agitation returns the molecules to a disordered state in the relaxation time and thermal energy is released. Thus microwave heating results from the dissipation of the electromagnetic waves in the irradiated medium. The dissipated power in the medium depends on the complex permittivity of the material and the local time-averaged electric field strength.

SFME is a key sustainable technology in achieving the objective of green analytical chemistry. It has been rapidly developed as one of the attractive techniques for extracting and separating interesting high-added-value compounds from natural plant resources.

With the help of MWs, extraction can now be completed without solvents in minutes instead of hours with various advantages (*e.g.* high reproducibility, less energy consumption, shorter procedures and higher purity of the final product). In the case of SFME, water molecules in plant cells are stimulated to rotate under microwave irradiation, so the immediate internal change results in a subsequent pressure and temperature increase inside the plant cell, which leads to breakdown of the cell walls and release of target molecules (Figure 8.3).

The first SFME technique for extracting natural products was developed and patented by Chemat *et al.* in 2004.[18,19] This SFME process is an important laboratory-scale technique for the extraction of essential oils assisted by microwaves, without solvent and water, at atmospheric pressure. It involves a combination of microwave heating and distillation at atmospheric

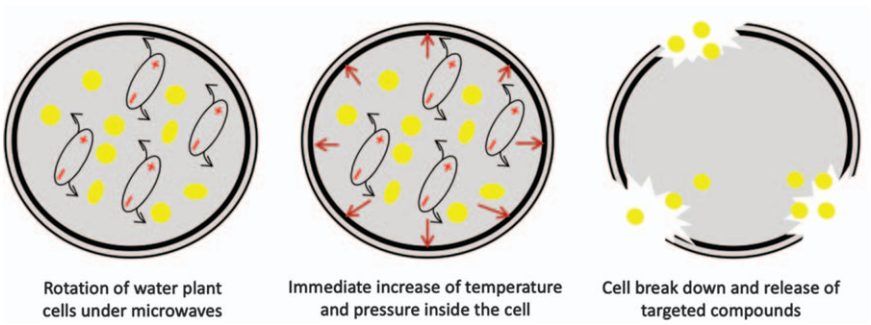


Figure 8.3 Mechanism of SFME.

pressure. The use of SFME prior to analysis has attracted growing interest in recent years because of its simplified and eco-friendly procedures, high efficiency, *etc.*

In 2008, a new and green extraction technique was designed by Chemat *et al.*[20] called microwave hydrodiffusion and gravity (MHG). This green extraction technique is an original ''upside-down'' microwave alembic combining microwave heating and the Earth's gravity at atmospheric pressure. Microwaves penetrate the water inside the plant and cause the rupture of cell walls. All possible substances, including the internal water of the plant, will be released and transferred from the inside to the outside of the plant material. This is the physical hydrodiffusion phenomenon, which allows the extracts to drop out of the microwave reactor under the effect of the Earth's gravity.

A Milestone ETHOS X microwave laboratory oven (1000 W maximum) is used for both the SFME and MHG techniques (Figure 8.4).

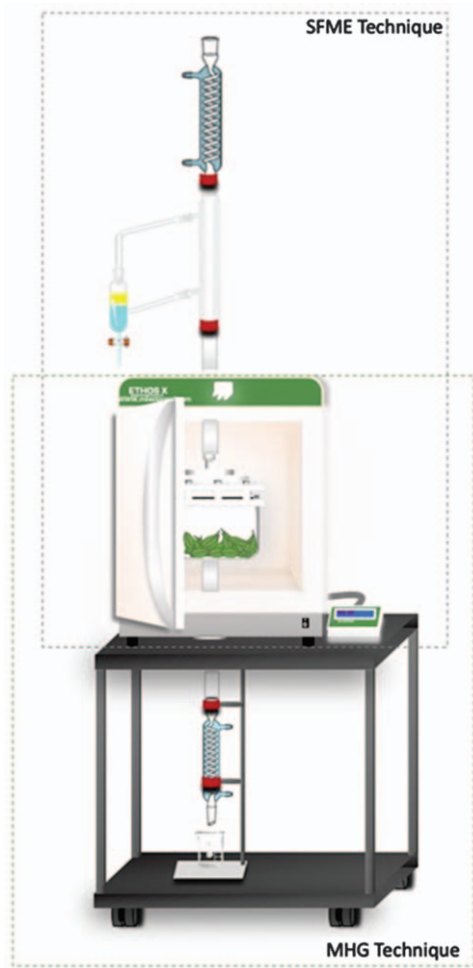


Figure 8.4 ETHOS X used for both SFME and MHG techniques.

The SFME technique has been applied to various kinds of fresh and dry plants, such as spices, aromatic herbs and citrus fruits.[21–25] The first example is *Ocimum basilicum* L.,[21] where 0.029% of essential oil was obtained by extracting 250 g of the plant at 500 W for 30 min at atmospheric pressure. In the second example, *Mentha crispa* L.[21] was heated under 500 W for 30 min at atmospheric pressure with 250 g of matrix, providing 0.095% of essential oil. *Thymus vulgaris* L.,[21] the third example, supplied 0.16% of essential oil from 250 g of matrix heated at 500 W for 30 min at atmospheric pressure. In most cases, substantially higher amounts of oxygenated monoterpenes and lower amounts of monoterpene hydrocarbons are present in the essential oil isolated by SFME in comparison with that extracted using conventional methods.[21,22]

The MHG process has been applied to many kinds of plants, such as aromatic plants, citrus, onions and fruit by-products.[10–14,26–30] The first example is the extraction of *Menthe pulegium* L.,[26] where 0.95% of essential oil was obtained by heating 500 g of matrix at 500 W for 20 min at atmospheric pressure. For *Citrus limon* L.,[27] 500 g of matrix were treated at 500 W for 15 min at atmospheric pressure and two yields of 0.7 and 1.6% of essential oil were obtained. In another example, 500 g of *Rosmarinus officinalis* L.[28] were treated at 500 W for 15 min, which provided 0.33% of essential oil. MHG has also been tested on fruits and vegetables and this innovative technique has also been applied to the extraction of antioxidant compounds.

Zill-e-Huma *et al.*[29] reported on the use of MHG for extracting flavonoids from onion. The plant tissues were strongly disrupted by microwave irradiation through the microscopic observation of extracts, so that target compounds could be efficiently extracted and detected by high-performance liquid chromatography (HPLC) and other analytical methods. MHG was also applied to sea buckthorn by-products to extract specific antioxidants, producing a slightly lower yield of flavonol in a very short time (15 min) in comparison with classical methods, but a higher content of reducing compounds was contained in the MHG extracts.[30] Extracts obtained using the MHG technique and a conventional solvent extraction (CSE) method were analysed by HPLC for quantification of flavonoids along with the evaluation of their phenolic contents by the Folin–Ciocalteu method and reducing power by the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.

Table 8.1 lists plants that have been subjected to extraction by the SFME or MHG technique.

8.4 Supercritical Fluids

A substance becomes a supercritical fluid when its temperature and pressure are simultaneously higher than the critical values. A supercritical fluid exists in a single fluid phase possessing characteristics between those of gases and liquids. Figure 8.5 illustrates a pressure–temperature diagram in the critical region of a pure compound.

Table 8.1 List of plants subjected to extraction by SFME or MHG.

Botanical species	Extraction conditions	Ref.
	SFME, 250 g, *P* (atm), 500 W, $t = 30$ min	21
Ocimum basilicum L.	$R = 0.029\%$	
Mentha crispa L.	$R = 0.095\%$	
Thymus vulgaris L.	$R = 0.160\%$	
	SFME, 250 g soaked in water for 1 h, *P* (atm), 500 W, $t = 60$ min	22
Carum ajowan L.	$R = 1.41\%$	
Cuminum cyminum L.	$R = 0.63\%$	
Illicium verum	$R = 1.38\%$	
	SFME, 150 g, *P* (atm), 850 W, $t = 10$ min	23
Nigella sativa L.	$R = 0.20\%$	
	SFME, 280 g soaked in water, P (atm), 85 W, $t = 50$ min	24
Melissa officinalis L.	$R = 0.15\%$	
Laurus nobilis L.	$R = 0.42\%$	
	SFME, 60 g, *P* (atm), 250 W, $t = 40$ min	25
Calamintha nepeta L. Savi	$R = 0.38\%$	
	MHG, 500 g, *P* (atm), 500 W, $t = 20$ min	26
Mentha pulegium L.	$R = 0.95\%$	
Mentha spicata L.	$R = 0.6\%$	
	MHG, 500 g, *P* (atm), 500 W, $t = 15$ min	27
Citrus limon L.	$R = 0.7\%$	
Citrus aurantifolia (Christm.) Swing	$R = 0.8\%$	
Citrus paradisi L.	$R = 1.0\%$	
Citrus sinensis L.	$R = 1.2\%$	
Citrus paradisi Macf.	$R = 1.2\%$	
	$R = 1.0\%$	
	MHG, 500 g, *P* (atm), 500 W, $t = 15$ min	28
Rosmarinus officinalis L.	$R = 0.33\%$	
Red, yellow, white and grelot onions (*Allium cepa*)	MHG, 500 g, *P* (atm), 300–900 W, $t = 5$–70 min	29
Sea buckthorn (*Hippophae rhamnoides*) by-products	MHG, 400 g, *P* (atm), 1 $W \cdot g^{-1}$, $t = 0$–25 min	30

The compressibility of a supercritical fluid just above the critical temperature is large compared with that of ordinary liquids. A small change in the pressure or temperature of a supercritical fluid generally causes a large change in its density. A high density generally implies a strong solvating capacity. The unique property of a supercritical fluid is that its solvating power can be tuned by changing either its temperature or pressure.

Even though the density of a supercritical fluid increases with increase in pressure and it becomes liquid-like, the viscosity and diffusivity remain between liquid-like and gas-like values. Additionally, supercritical fluids exhibit almost zero surface tension, which allows facile penetration into microporous materials. As a result of the combination of advantageous physicochemical properties, the extraction process can often be carried out more efficiently with a supercritical solvent than it can with an organic liquid

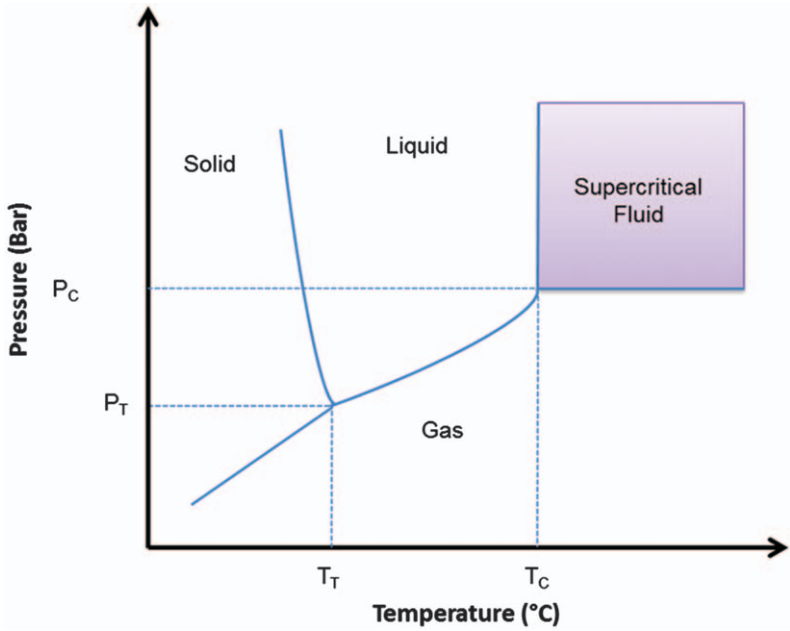


Figure 8.5 Pressure–temperature phase diagram.

Table 8.2 Selected physicochemical properties of liquids, gases and supercritical fluids.

Fluid state	Density/g cm^{-3}	Diffusivity/ cm^2 s^{-1}	Viscosity/ g cm^{-1} s^{-1}
Gas, $P = 1.01325$ bar, $T = 15$–30 °C	$(0.6–2.0)\times10^{-3}$	0.1–0.4	$(0.6–2.0)\times10^{-4}$
Liquid, $P = 1.01325$ bar, $T = 15$–30 °C	0.6–1.6	$(0.2–2.0)\times10^{-5}$	$(0.2–3.0)\times10^{-2}$
Supercritical fluid, $P = P_c$, $T \approx T_c$	0.2–0.5	0.7×10^{-3}	$(1–3)\times10^{-4}$
Supercritical fluid, $P = 4P_c$, $T \approx T_c$	0.4–0.9	0.2×10^{-3}	$(3–9)\times10^{-4}$

solvent. A comparison of selected physicochemical properties of liquids, gases and supercritical fluids is presented in Table 8.2.

Extraction can be defined as the removal of soluble material from an insoluble residue, either liquid or solid, by treatment with a liquid solvent. It is therefore a solution process and depends on mass transfer phenomena. The limiting factor for the rate of extraction is normally the rate of diffusion of the solute through the liquid boundary layer at the interface.

Supercritical fluid extraction (SFE) is the process of separating one or a mixture of components (the extractant) from another (the matrix) using a supercritical fluid as the extraction solvent. Extraction is usually from a solid matrix, but it can also be from liquids.

Carbon dioxide (CO_2) is the most commonly used supercritical fluid, sometimes modified with co-solvents such as ethanol or methanol. The extraction conditions for supercritical CO_2 are above the critical temperature of 31 °C and critical pressure of 74 bar; addition of modifiers may slightly alter this. Supercritical extraction mostly uses carbon dioxide at high pressure to extract high-value products from natural materials. Unlike other processes, the extraction process leaves no solvent residue behind. Moreover, the CO_2 has low toxicity and is non-flammable, odourless, tasteless, inert and inexpensive. Owing to its low critical temperature of 31 °C, CO_2 is perfectly adapted for the food, aroma and essential oil industries.

The first CO_2 extraction process described was the decaffeination of coffee by Ludwig Roselius in 1905. Roselius's method used benzene, a carcinogenic and toxic hydrocarbon, to remove caffeine from premoistened green coffee beans. Modern decaffeination processes are much milder, and many make that point by claiming to be ''naturally decaffeinated''. Supercritical carbon dioxide decaffeination is very similar to direct solvent methods, except that in this case the solvent is carbon dioxide. High-pressure vessels (operating at roughly 250–300 times atmospheric pressure) are employed to circulate the carbon dioxide through a bed of premoistened green coffee beans. It typically can extract 96–98% of the caffeine originally present in the beans. The life cycle assessment (LCA approach) was used to evaluate the environmental performances of a coffee decaffeination process driven by carbon dioxide under supercritical conditions.[31]

The dyeing and allied industries have often been under observation for large-scale pollution of the environment, and in fact this situation has assumed the status of a global problem. Considerable research has been carried out in this field to develop technologies that are environmentally much cleaner,[32] and in 2012 NIKE announced that it had entered into a strategic partnership with DyeCoo Textile Systems. One of the technologies that evolved is the use of supercritical carbon dioxide as the dye solvent, instead of water. The dyeing system using supercritical carbon dioxide is composed of a three-component/three-phase system (Figure 8.6). The key components are gas, dyestuff and a substrate such as fibre polymer. While in the solid state, the dyestuff and polymer are present in three separate phases along with the supercritical mixture. The dyestuff is first dissolved in the supercritical fluid and is then transferred to the fibre, where it subsequently becomes absorbed and diffused into the fibre.

Like most other natural raw materials, cork can contain secondary metabolites produced by microorganisms. The most disadvantageous one is trichloroanisole (2,4,6-TCA), which is primarily responsible for the typical ''cork taint''. The cork industry made many efforts to solve this problem because it became very serious in recent years. Various processes with steam or alcohol were developed to reduce the TCA concentration in cork, but these processes usually did not achieve more than 85% efficiency on an industrial scale. In 1997, one of the leading cork producers, Sabaté, later Oeneo Bouchage, started the development of a cork cleaning process with

Figure 8.6 DyeCoo technology, which has been commercialized in Taiwan.[33]

supercritical gases in cooperation with the French Commissariat à l'Energie Atomique (CEA). The tests were very successful and consequently the process was patented. Supercritical CO_2 allows the TCA content to be reduced to below the detection limit. Nowadays, studies to define the best parameters for this extraction are continuing.[34]

The design of green and sustainable extraction methods for natural products is currently a hot research topic in the multidisciplinary area of applied chemistry and technology. Six principles of green extraction are defined, describing a multifaceted strategy to apply this concept at the research and industrial levels. The mainstays of this working protocol are new and innovative technologies, process intensification, agro-solvents and energy saving. Technologies could be hyphenated to improve the extraction (microwaves and SFE) of specific compounds such as antioxidants and saponins.[35]

Supercritical fluids combine the beneficial properties of liquids and gases. Likewise, supercritical fluid chromatography (SFC) combines the advantages of HPLC and gas chromatography (GC). SFC can be more advantageous than HPLC and GC when analysing compounds that are decomposed at high temperatures as in GC and do not have functional groups to be detected by HPLC. SFC enables some properties to be changed during the chromatographic process and this tuning ability brings an advantage to optimizing the analysis. Also, SFC utilizes the same range of detectors as HPLC. This technique can easily be coupled with mass spectrometry (MS). SFC surpasses GC when easily decomposable substances are to be analysed. These substances can be used in SFC because of its ability to work at lower temperatures than GC. Three major qualities are important for chromatography: selectivity, efficiency and sensitivity. GC is much better in terms of efficiency and sensitivity, and HPLC has better selectivity owing to changeable mobile phases and the use of various stationary phases. Although SFC has poorer

selectivity than HPLC, it shows good quality in terms of sensitivity and efficiency. With advanced features, SFC is both an alternative and a complementary technique in this field.

Supercritical fluid chromatography with mass spectrometric detection (SFC-MS) has been demonstrated over several years to be a powerful technique for the characterization of chiral mixtures. Owing to its orthogonality compared with commonly applied chromatographic techniques, SFC is becoming a really interesting method for enantiomeric separations.[36] Once the correct stationary phase had been selected, the role of the modifier was studied and in this case 2-propanol led to a faster analysis than with other solvents. Moreover, an additive was necessary, and indeed without using trifluoroacetic acid (TFA) there was no separation. The next step was scale-up for semipreparative chromatography and this was carried out successfully.

Bergamot essential oil is very important in the perfumes field, in particular for the production of eau de Cologne. However, during the extraction process some compounds such as furocoumarins were present, in this case bergapten. These furocoumarins (or furanocoumarins), also known as psoralens, are photosensitive toxic agents. Therefore, it was important to develop a method to analyse and monitor them during the extraction or fractionation process.[37] In this work, pressures and temperatures were selected to perform the process under operating conditions corresponding to six different densities of supercritical CO_2. Under the conditions of 80 bar and 70 °C, an extract without bergapten was obtained, although it still had a content of volatile oxygenated compounds similar to that of the essential oil starting material. Analysis could be performed using SFC, with ethanol as co-solvent, back pressure regulator at 100 bar and temperature fixed at 35 °C.[38]

By coupling the sample extraction on-line with chromatography and detection, sample preparation is minimized, diminishing sample loss and contamination and significantly decreasing the required extraction time. Liu *et al.* developed a rapid, simple and environmentally friendly off-line SFE-SFC-MS/MS method for the analysis of phenolic compounds in garlic.[39] The SFE parameters were optimized, indicating that extracting 15 phenolic compounds at 50 °C in 9 min with the addition of 30% methanol was the best set of off-line SFE conditions. Subsequently, phenolic compounds were separated by SFC on a Diol phase column using methanol and CO_2 as the mobile phase, with MS/MS detection. Use of the mobile phase additives 0.1 mM oxalic acid and 1 mM ammonium formate successfully resolved the appearance of the tailing peaks. Further, a matrix-matched standard was employed to remove matrix effects and only nine phenolic compounds showed acceptable selectivity, linearity, sensitivity, recovery and precision from the validation data.

8.5 Liquefied Gases

Liquefied gases are stored in the liquid phase at room temperature and under moderate pressure (between 1 and 100 bar). These compounds have boiling points between −90 and 10 °C. Inside a pressurized container, these

gases are liquefied under their own vapour pressure; the liquid is in equilibrium with the gas phase. Examples include propane, butane, dimethyl ether (DME) and HFO-1234ze [1,3,3,3-tetrafluoropropene, a hydrofluoroolefin (HFO)]. Under ''normal'' solid–liquid extraction conditions, most liquefied gases are chemically inert.

These gases were used from the beginning of the nineteenth century as refrigerants, fuels and propellant gases for aerosols. However, they can also be used as extraction solvents and numerous applications can be found in the literature.

To be used safely at the industrial scale, several criteria are important, and two thermodynamic parameters are especially essential:

- a boiling temperature at atmospheric pressure between −30 °C and room temperature, to allow the gas to condense at reasonably achievable temperatures;
- a vapour pressure of less than 10 bar at room temperature, to limit the cost of pressure equipment.

DME can be used in various industrial fields. It is non-toxic and environmentally benign. When it is pressurized above 5 bar, it condenses to the liquid phase. Gaseous DME is denser than air whereas liquid DME has a density two-thirds that of water. The vapour pressure is similar to that of liquefied petroleum gas (LPG) and it requires the same handling and storage precautions. It dissolves in water at up to 6% by mass.[40] As a first example, biofuel could be produced by direct extraction of the green crude from natural blue–green microalgae. This type of process showed a high extraction rate. Compared with conventional methods, this method omits drying, cell disruption and heating of the solvent.[41] As a second example, it could be used to produce free lutein from marigold flowers. Lutein is an important carotenoid, which is a yellow pigment produced by plants. It is a potent antioxidant and could offer a range of health benefits. Liquefied DME allowed the extraction of lutein esters and at the same time a de-esterification process was performed to obtain free lutein using KOH–EtOH solution.[42] As shown schematically in Figure 8.7, the apparatus used consists of a 120 mL stainless-steel batch extractor and a separation unit. The separation unit is composed of an 80 mL hyper-glass vessel in a polycarbonate housing. The extractor was heated by a heating jacket connected to a temperature control box. Agitation of the extraction system was provided using a magnetic stirrer.

Cannabis is the most widely used illicit substance globally and particularly in developed countries.[43] However, the use of medicinal cannabis, or medicinal marijuana, is a therapy that has garnered much attention in the USA in recent years.[44] The use of butane as the extraction solvent creates what is known as butane hash oil (BHO). The process starts with cannabis and liquid butane in a pressurized and heated system. By applying evaporation under a vacuum, it is then possible to remove the butane solvent. The

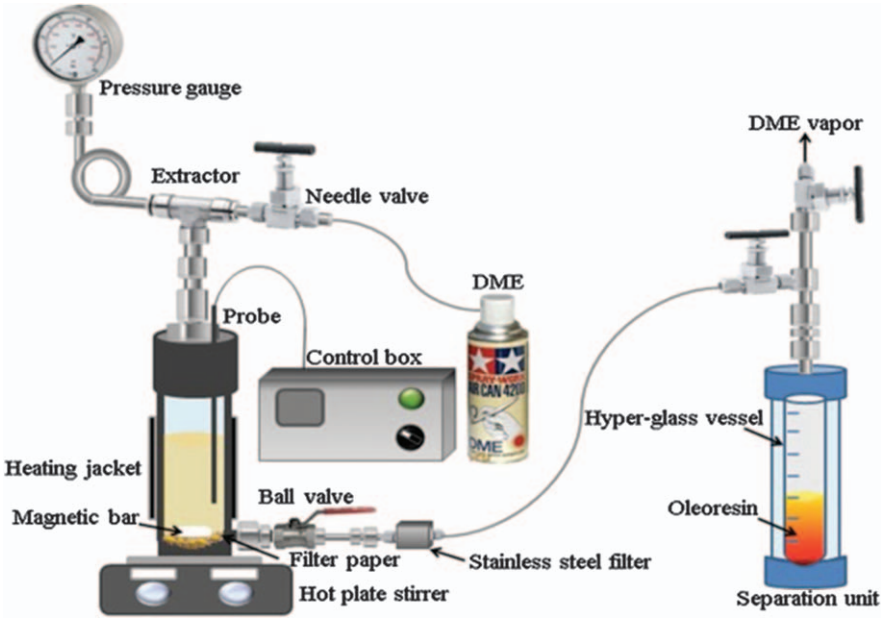


Figure 8.7 DME extraction apparatus.[39]

vacuum converts the butane from a liquid to a vapour, making it easier to remove. Instead of BHO, some manufacturers opt to produce propane hash oil.

Even without any added steps, this method includes some potential hazards. First, butane burns easily in its gas phase, hence the temperature applied must be managed carefully otherwise there is a serious risk of the gas exploding. In addition, the system should include circulators that remove and recycle the butane. The removal process should reduce any residual butane in an extract. In all cases, however, analytical testing should be carried out to ensure the removal of butane, because it is highly toxic to humans. This danger can make this method a less desirable choice, especially for medicinal products. As an alternative, it is currently possible to use supercritical CO_2 extraction, for example.

Extraction of rice bran lipids was performed using liquid propane.[45] Propane has several advantages over both hexane and CO_2. First, propane is relatively inexpensive and does not leave a toxic residue. Second, the pressures involved in oil extraction using propane are 10 times lower than those in supercritical CO_2 extraction. The maximum yield achieved with propane at 7.6 bar and ambient temperature was 0.224 ± 0.016 kg of oil per kilogram of rice bran. It is possible to achieve some selectivity for particular compounds by extraction with propane at different temperatures and pressures. To evaluate the selectivity effect, the lipid class profiles were determined for propane extracts. Only three lipid classes were detected: TG (triglycerides), FFA (free fatty acids) and cholesterol.

Another class of solvents with low boiling points are low hydrofluorocarbons (HFCs). R134a is 1,1,1,2-tetrafluoroethane, a haloalkane with formula CH_2FCF_3 and a boiling point of −26.3 °C at atmospheric pressure. It is a naturally polar solvent and therefore the solubility of polar compounds is sufficient without the addition of any modifier. Setapar *et al.* studied the extraction of α-tocopherol and β-carotenoids from crude palm oil.[46] R134a is an alternative low-pressure, non-reactive, non-flammable, non-toxic, non-ozone-depleting solvent and has comparable solvent properties to those of CO_2.

R1234yf is 2,3,3,3-tetrafluoropropene, an HFO with the formula $CH_2{=}CFCF_3$. It is the most likely replacement for 1,1,1,2-tetrafluoroethane (R134a), a widely used refrigerant, propellant and solvent, characterized by a very high global warming potential. Suberu *et al.* investigated experimentally and through computational modelling the solvation properties of R1234yf using artemisinin and its extraction from *Artemisia annua* as an example of a currently relevant industrial biopharmaceutical product.[47]

8.6 Subcritical Water: A Green Solvent for Analytical Chemistry

Water is the most abundant molecule on Earth, covering 70% of its surface. It is often recognized that water has tremendous benefits as a green solvent because it is not only inexpensive and environmentally benign but is also non-flammable and non-toxic, providing opportunities for clean processing and pollution prevention.

Subcritical water is defined as hot water that has a temperature in the range 100–374 °C, which is under high pressure to keep it in the liquid state. Subcritical water is also known as superheated water, hot water, pressurized hot water, pressurized low-polarity water, high-temperature water and hot liquid water. Under subcritical conditions, liquid water is less polar than at ambient temperature and it has an increased capacity for dissolving organic molecules. The polarity of subcritical water is measured by the value of the dielectric constant. When water is heated above 100 °C, its dielectric constant decreases and it becomes similar to those of organic solvents.[48] At 214 °C, the dielectric constant of water is the same as that of methanol at room temperature At 295 °C, water becomes similar to acetone. For this reason, it is possible to extract non-polar, moderately polar and polar chemical compounds. Low-temperature water extraction could obtain more water-soluble substances, whereas high-temperature water extraction could result in less soluble substances. Furthermore, liquid water at elevated temperature is a solvent of lower polarizability/polarity. Above 200 °C, water may be an acid or base catalyst because its H_3O^+ and HO^- ion concentrations are perhaps orders of magnitude higher than those in ambient water. Subcritical water is therefore a much better solvent than ambient water for hydrophobic organics. It can itself be a catalyst for reactions that normally require the addition of an acid or base. Subcritical water can be

used in several stages of analysis, for example, for the extraction and subsequent chromatographic separation of analytes.

Subcritical water extraction (SWE) is considered to be an environmentally friendly and efficient method for the extraction of less polar compounds without the use of organic solvents. Therefore, SWE has been successfully applied for the extraction of antioxidants (phenols and flavonoids), essential oils, fatty acids, oils, carotenoids, pectin, monosaccharides, *etc.* (Table 8.3, Figure 8.8).

Most studies on SWE have been investigations of the extraction of antioxidants, *e.g.* phenolic compounds, from a wide variety of sources (herbs, fruits, seeds, roots, by-products, waste, *etc.*). According to Plaza and Turner,[49] in SWE, phenolic compounds have usually been extracted at temperatures up to 150 °C with extraction times from 1 to 60 min, whereas

Table 8.3 Examples of extraction by SWE.

Source	Targeted compound	SWE operating conditions	Ref.
Chilli pepper	Capsaicinoids	$T = 200$ °C, $P = 20$ MPa, $t = 10$ min	51
Crocus sativus petals in saffron industry residues	Total phenols and total flavonoids	$T = 159$ °C, $P = 0.69$ MPa, $t = 54$ min	52
Ginger	Gingerol	$T = 130$–140 °C, $P = 2$ bar, $t = 40$ min	53
Tumeric rhizomes	Curcumin	$T = 160$ °C, $P = 10$ bar, $t = 22$ min	54
Thymbra spicata	Essential oil	$T = 150$ °C, $P = 20$ bar, $t = 20$ min	55
Pomelo peels	Pectin	$T = 120$ °C, $P = 30$ bar, $t = 100$ min	56

Figure 8.8 Subcritical water extraction system (www.buchi.com).

the extraction of terpenoids was realized at high temperatures (200 °C) and with long extraction times (27–120 min).

As another example, Lachos-Perez *et al.*[50] established the optimal conditions to extract the flavanones hesperidin and narirutin from orange peel after supercritical CO_2 extraction (to separate essential oil). The maximum yields of the flavanones were obtained at 150 °C. Compared with conventional methods, SWE showed significantly higher extraction yields for the recovery of bioactive compounds with high antioxidant activity.

In recent years, subcritical water has started to be used widely as an analytical extraction solvent and this application will be considered in this section. The changes in the polarity of water with increase in temperature have also been exploited in chromatographic methods using superheated water. Indeed, subcritical water can be used as an eluent in reversed-phase liquid chromatography (RP–LC) as an alternative to methanol–water or acetonitrile–water mixtures.[57–59] The use of subcritical water as an organic free eluent in RP–LC using a polystyrene–divinylbenzene (PS–DVB) column and octadecylsilyl (ODS)-bonded phase columns was demonstrated by Smith and Burgess.[60] The elution order of analytes corresponded to a reversed-phase separation and in some cases gave enhanced separations and shorter analysis times compared with conventional reversed-phase HPLC using water–organic solvent mobile phases. Furthermore, Li *et al.* applied subcritical water as an HPLC analytical solvent to extract and quantify caffeine, chlorophenols and anilines.[61]

In another study, Rodriguez-Meizoso *et al.*[62] demonstrated that the combined use of subcritical water and HPLC with diode-array detection (HPLC–DAD) was a good alternative for obtaining and characterizing nutraceuticals from natural sources, *e.g.* oregano. They also reported that changing the temperature of the subcritical water could be used as a means of fine tuning the extraction selectivity for the extraction of antioxidant compounds. Another advantage of subcritical water is its compatibility with many commercial detectors such as UV, FID, fluorescence, refractive index, electrochemical, light scattering and even MS detectors.[63]

8.7 NADESs as Green Solvents for Analytical Chemistry

8.7.1 Green Solvents from Ionic Liquids (ILs) and Deep Eutectic Solvents (DESs) to Natural Deep Eutectic Solvents (NADESs)

Over the past two decades, the trend towards alternative solvents has generated great interest in ionic liquids (ILs) as an alternative to traditional organic solvents. These ILs have been successfully applied in many analytical chemistry fields, such as chromatography, capillary electrophoresis, mass spectrometry and sample preparation.[64–67] Nevertheless, the

''greenness'' of ILs is often argued about, mainly owing to their poor biodegradability, biocompatibility and sustainability.[68,69] Deep eutectic solvents (DESs) are a subclass of ILs that are obtained by mixing solid compounds forming a eutectic mixture with a melting point much lower than those of the individual components. These solvents have been slowly emerging since 2004 as potential alternatives to ILs.[70] DESs and ILs have similar characteristics, such as low volatility, non-flammability, chemical and thermal stability, high solubility and tuneability.[71] However, DESs show advantages over ILs such as the their ease of storage and synthesis and also the low cost of their starting materials.[71] To date, the applications of these new media have shown great promise, especially in the medical, biological and biotechnological fields, unlike analytical chemistry where the application of DESs is still limited.[72] In addition to their high viscosity, the synthetic origin of the DES starting materials could be a limiting factor for their wider adoption. Therefore, in order to extend their applications and to meet the ''Twelve Principles of Green Chemistry'' proposed by Anastas and Warner,[73] particular attention has been given to natural sources of DESs for replacement of synthetic compounds.[74] In this case, we can consider natural deep eutectic solvents (NADESs), which are currently attracting considerable attention from researchers and there is a growing demand in many areas of technology. Both DESs and NADESs are mixtures of compounds that have a much lower melting point than those of any of their individual components, mainly owing to the generation of intermolecular hydrogen bonds between hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs).[72]

In addition to all of the advantages of DESs, NADESs are more environmentally friendly and considered to be ''readily biodegradable'' owing to their natural origin, which promoted their application in analytical procedures as a new type of green solvent.[70,72] These new green media were first introduced by Choi *et al.* as a third liquid phase that occurs in all living organisms and cells.[75] According to Choi *et al.*, these liquids dissolve a number of natural compounds that are poorly soluble in water and lipids such as taxol and rutin and also proteins. This explains many biological phenomena such as the biosynthesis of molecules that are insoluble in either water or lipids.

The compounds found to form NADESs are common primary metabolites such as sugars (glucose sucrose, fructose, *etc.*), organic acids (lactic, malic, citric acid, *etc.*), amino acids, alcohols and choline choride.[69,76] These natural compounds are reported to be enriched in various organisms under extreme biotic and abiotic conditions (drought, cold stress, external attack, *etc.*).[69,77,78]

Some examples of different molecules that can be combined to produce NADESs are presented in Figure 8.9.

According to the nature of their starting materials, NADESs can be classified into four groups: (1) derivatives from organic acids, (2) derivatives from choline chloride, (3) mixtures of sugars and (4) other combinations.[79]

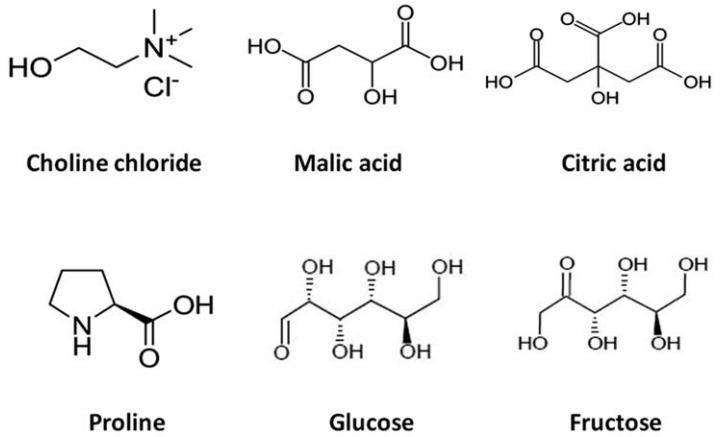


Figure 8.9 Structures of different compounds with the ability to form NADESs.

8.7.2 Preparation of NADESs

A major advantage of NADESs is their easy preparation methods and the large number of combinations that can be made.[69] Three methods are most commonly used for preparing NADESs: (1) heating and stirring as described by Dai *et al.*,[74] where a mixture of natural components is placed in a bottle with a stirring bar and cap and heated with agitation until a clear liquid is formed or following the conditions used by Abbott *et al.*,[80] *i.e.* heating and stirring at 80 °C; (2) an evaporation method described by Dai *et al.*,[74] where the NADES components are first dissolved in water before being evaporated at 50 °C with a rotary evaporator, and the liquid obtained is finally kept in a desiccator with silica gel until it reaches a constant weight; and (3) the freeze-drying method described by Gutiérrez *et al.*,[81] where aqueous solutions of the individual components are freeze-dried; this method is not used as frequently as the others.

8.7.3 Applications of NADESs

Nowadays, the field of applications of NADESs is very broad and includes, for example, the dissolution of DNA and acting as media for enzymatic or chemical reactions, electrochemical determinations, biotransformations, biomass processing, synthesis, biocatalysis, gas separations, *etc.*[69,82,83] The pharmaceutical industry is another field in which NADESs can be extremely useful; for instance, they can be used to improve the solubility of drugs that are poorly soluble in water and to enhance the bioactivities of dissolved species.[83–85]

NADESs can also be used for the preparation and pretreatment of analytical samples, which is a key step in almost every analytical procedure.[72] These solvents appear to be good candidates for this purpose owing to their high stabilization and solubilization abilities.[86]

In another area, NADESs have found applications in solid–liquid and liquid–liquid extractions of natural bioactive compounds. They have been successfully used as an alternative to organic solvents for the extraction of plants metabolites, allowing the extraction of compounds of different polarities, while being inexpensive, non-toxic and easy to prepare.[68,76] Flavonoids, phenolic acids, saponins, anthocyanins and essential oils are some examples of bioactive compounds that have been successfully extracted from natural matrices, typically from plant leaves using mixtures of NADESs. Here, it is worth mentioning that, as the definition of NADESs is novel, it is possible to find reported mixtures described as DESs that fit the definition of NADESs.

Some of these applications of NADESs are presented in Table 8.4. As can be seen, choline chloride-based NADESs are the most often used solvents.

Moreover, the compatibility of NADESs with many analytical methods (HPTLC, HPLC, UPLC, GC–FID and GC–MS) has been widely reported, and especially the recovery of the analytes from these solvents, which is the most challenging step.

It can be concluded that NADESs offer endless opportunities as promising new solvents that can be applied in different research fields, particularly as solvents for extraction. It is therefore expected that the field of active pharmaceutical ingredients, cosmetics and nutrition will greatly benefit from the advantages of NADESs. Moreover, NADESs could represent a new class of mobile phases increasing the possibilities of new separation selectivities while reducing the environmental impact of HPLC analyses. This was recently demonstrated by Sutton *et al.*,[109] who successfully applied NADESs as the major mobile phase in RP–HPLC.

8.8 Bio-based Solvents

The definition of ''biorefinery'' is the sustainable processing of biomass into a spectrum of marketable products and energy.[110] Bio-based solvents are typically categorized (Figure 8.10) into three types based on the agro-sector from which they are synthesized: (1) sugar/starch-rich biomass, (2) protein/oil-rich biomass and (3) lignocellulose-rich biomass.

The raw material undergoes a range of unit operations such as pretreatment, hydrolysis and biochemical processes to yield the solvent. Various components in this biomass and their intermediates aid in the biorefinery scheme to produce a diverse mixture of solvents. The solvents obtained are mostly then classified based on their functional groups such as alcohols, esters, ethers, *etc.* A classic example is bioethanol, which is predominantly produced from agricultural crops of which 60% is from sugarcane and the remaining 40% is from other sources.[111] Many novel innovations in the utilization of paper pulp and lignocellulosic biomass for the production of solvents, particularly ethanol, are patented each year.

Terpene hydrocarbons are mainly acyclic, bicyclic or monocyclic, exhibit relatively different physical properties and are principally recovered from

Table 8.4 Examples of applications of NADESs in chemical analysis.[a]

Nature of sample	Sample matrix	Analyte	NADES composition (molar ratio)	Method of detection	Ref.
Solid	*Ginkgo biloba* leaves	Phenolic acids Saponins	ChCl–MA (1 : 1)	HPTLC	87
	Panex ginseng leaves		ChCl–MA (1 : 1)		
	Prunella vulgaris leaves	Phenolic acids	ChCl–EG (1 : 4), 30–36% water	UPLC–UV	88
	Orange peel	Phenolic compounds	ChCl–EG (1 : 4), 10% water	HPLC	89
	Byrsonima intermedia leaves	Phenolic compounds	ChCl–Gly (1 : 1)	HPLC–DAD	90
	Cajanus cajan leaves	Phenolic compounds	ChCl–M (1 : 2)	UPLC	91
	Grape skin	Phenolic compounds	ChCl–OA (1 : 2), 25% H_2O	HPLC–UV	92
	Grape skin	Phenolic compounds	ChCl–MA (1 : 1)	HPLC–UV	93
	Camellia oleifera leaves	Flavonoids	ChCl–LA (1 : 2)	HPLC–UV	94
	Radix scutellariae	Flavonoids	L-Pro–Gly (1 : 4)	RP–HPLC	95
	Olive pomace	Total phenolic content	ChCl–LA (1 : 1), 20% water	HPLC	96
	Wine lees	Anthocyanins	ChCl–MA	HPLC–UV	97
	Chamaecyparis obtusa leaves	Bioactive terpenoids	ChCl–EG (1 : 4)	GC–FID	98
	Tobacco	Volatile compounds	ChCl–EG (1 : 3)	GC–MS	99
	Fish	Cu, Fe and Zn	ChCl–OA (1 : 2)	FAAS	100
	Sheep, bovine and chicken liver	Fe	ChCl–LA (1 : 1)	FAAS	101
Liquid	Virgin olive oil	Phenolic compounds	ChCl–Xy (2 : 1)	HPLC–UV	102
	Virgin olive oil	Total phenolic content	LA–Glu–H_2O (6 : 1 : 6)	UV–VIS	103
	Vegetable oils	Phenolic acids	ChCl–EG (1 : 2)	HPLC–UV	104
	Model oil	Phenolic compounds	ChCl–EG (1 : 3)	HPLC–UV	105
	Water	Polycyclic aromatic hydrocarbons	ChCl–ChPh (1 : 2)	HPLC–UV	106
	Tea samples	Phenolic compounds	ChCl–urea (1 : 2)	FAAS	107
	Fruit juices	Pesticides	ChCl–ChPh (1 : 2)	GC–FID	108

[a]ChCl, choline chloride; MA, malic acid; Pro, proline; Glu, glucose; EG, ethylene glycol; M, maltose; OA, oxalic acid; LA, lactic acid; CA, citric acid; Xy, xylitol; ChPh, *p*-chlorophenol; HPTLC, high-performance thin-layer chromatography; FAAS, flame atomic absorption spectrometry; HPLC, high-performance liquid chromatography; UPLC, ultra-performance liquid chromatography; GC–FID, gas chromatography with flame ionization detection; GC–MS, gas chromatography with mass spectrometric detection.

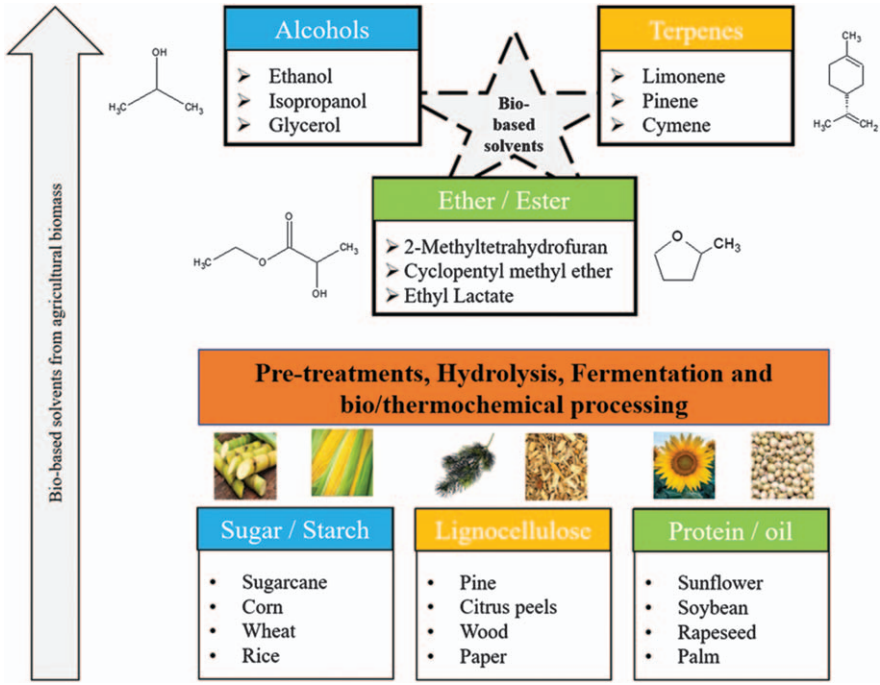


Figure 8.10 The purview of bio-based solvents.

conifers and fruit pomaces. Terpenes include hydrocarbons with C_5H_8 isoprene units generally derivable from essential oils, resins and vegetable aromatic products, which are considered as an economical renewable feedstock.[112] Limonene, a terpenoid solvent, is produced by steam distillation from peels and intermediates discarded by orange juice processors. Limonene is also the major component of citrus rind oil and was used as an alternative to *n*-hexane for the extraction of rice bran oil. The optimal solvent-to-solid ratio, extraction time and other parameters were compared with those of *n*-hexane.[113] Similarly, the feasibility of α-pinene for the extraction of fatty acids from food products was tested; it is of additional interest that the high recycling rate makes α-pinene an attractive bio-based solvent.[114]

Ethers also consitute useful solvents, such as 2-methyltetrahydrofuran (2-MeTHF) and cyclopentyl methyl ether (CPME), which can also be produced from lignocellulosic waste biomass comprised of 10–35 wt% lignin, 25–60 wt% cellulose and 25–40 wt% hemicellulose. In order to disrupt the rigid structure of the lignocellulosic biomass, pretreatment is often an essential prerequisite before fermentation or thermochemical processing. The Biofine process is a good example wherein the primary step is a rapid high-temperature-mediated dilute acid-catalysed hydrolysis of lignocellulosic biomass. The principal product obtained from this step is separated, purified and further processed to produce solvents. CPME, an aprotic solvent, is

a greener alternative to tetrahydrofuran and *tert*-butyl methyl ether (TBME). It is safer as it has a high boiling point of 106 °C and preferred characteristics, such as a low tendency to generate peroxides, relative stability under acidic and basic conditions, make it a suitable alternative to conventional ethers employed in extraction.[115]

A brief overview of bio-based solvents used for distinctive applications is presented in Table 8.5. Analytes belonging to a diverse class of matrices along with the use of bio-based solvents, replaced petrochemical solvents and extraction techniques employed to achieve the desired processes are compared. The advantageous schemes established by the utilization of agricultural, food processing and post-harvest wastes to extract bioactive compounds with bio-based solvents is a winning situation for industry and academia alike. Numerous research articles on this theme create a positive impact as they explore the possibility of the industrialization of such eco-friendly processes, thus giving industries an incentive to adopt such processes early, thereby alleviating pressure exerted by the statutory and regulatory bodies as they increasingly impose restrictive legislation when it comes to the disposal of toxic solvents and their environmental impact. The environmental rules are stricter than ever before and compliance by industries is heavily monitored and certain incentives are conferred to adopt green, clean technologies.

Natural carotenoids, the pigments present in vegetables and fruits, are one such class of compound that exhibit bioactive properties and they have been extracted using ethyl lactate. The recovery of lycopene from tomato wastes was three times higher in ethyl acetate extracts than in those of other polar solvents at ambient and elevated temperatures, thereby reducing the process energy costs significantly.[116] Additionally, the potential of ethyl lactate as a green solvent to extract curcumin, a biologically active compound with antimicrobial and anticancer activities, was elucidated.[117] The efficacy of the solubilization of α-mangostin in different green solvents was compared by predicting the theoretical solubility and validating the results with experimental data. Ethyl lactate proved to be the best solvent among the solvents compared, which included ethanol, D-limonene and the petrochemical solvent dichloromethane.[122]

Lipids, especially oil from oleaginous yeasts with almost 20% lipids in their dry cell mass, are a perfect candidate for the production of biofuel. Bio-based esters, ethers and terpenes were chosen for the removal of lipids from yeasts. Cymene had the highest recovery with a yield of almost 15.57%, closely followed by 2-propanol at 15.48%.[120] Rapeseed oil was recovered in a similar fashion; various green solvents were compared and 2-MeTHF was found to be the most suitable solvent. A pilot-scale study to elucidate the extraction efficiency and extrapolate the data for a scale-up operation was highlighted.[125]

The increasing concentration of ethanol in an ethanol–water solvent system was used to identify the highest polyphenol content and antioxidant activity in bilberry extracts[126] and rosemary plant.[127] In the biorefining

Table 8.5 Current scenario of bio-based solvents in the extraction domain.[a]

Analyte	Class	Matrix	Bio-based solvent	Petroleum solvent replaced	Remarks	Ref.
Lycopene	Carotenoids/ tetraterpenoids	Tomato waste	Ethyl lactate	Acetone, *n*-hexane	Maceration	116
Curcumin	Curcuminoids	Turmeric	Ethyl lactate	Methanol	Maceration	117
Nigrumin ferulate	Nitrile-containing metabolites	Blackcurrant waste epicarp	Ethyl acetate	Acetone	Maceration	118
Green oleoresins	Lipids	Lavender flowers	Liquefied DME	*n*-Hexane	Maceration	119
Oil	Lipids	Yeast	CPME	*n*-Hexane	Hot reflux	120
Rare earths	Metals/metal ores	Rare earth(III)	DMC	Esters	Maceration	121
α-Mangostin	Xanthones	*Garcinia mangostana* L.	D-Limonene	Dichloromethane	Hot reflux	122
Fatty acids	Lipids	Olive	α-Pinene	*n*-Hexane	Soxhlet	114
Biodiesel	Fuels	Soybean oil	DMC	Methanol	Hot maceration	123
Oil	Lipids	Rapeseed	*p*-Cymene	*n*-Hexane	Soxhlet	124
Tocopherol	Vitamin E	Rapeseed	2-MeTHF	*n*-Hexane	Soxhlet	125
Anthocyanin	Polyphenols	Bilberry	Ethanol	Methanol	Ultrasonication	126
Rosmarinic acid	Antioxidants	Rosemary	Ethanol	Methanol	Hot reflux	127

[a]DME, dimethyl ether; CPME, cyclopentyl methyl ether; DMC, dimethyl carbonate; 2-MeTHF, 2-methyltetrahydrofuran.

scheme for bilberry extracts, 100% ethanol was found to be the best solvent for the solubilization of phenolic acids, flavonols and anthocyanins. The antioxidant assay results were in good agreement with the theoretical predictions. In the case of rosmarinic and carnosic acid, the latter was found in abundance using 100% ethanol but the yield of rosmarinic acid using 30% ethanol was the highest. These examples prove that regardless of the solubilization capacity of solvents, the antioxidant histolocalization strongly influences their extraction rates.

Extraction using bio-based solvents is the primary topic of this discussion, but other major trends utilizing bio-based solvents for a wide range of applications are also gaining significant traction. For instance, the application of CPME in a biphasic system to enhance the yield of furfural, by selective conversion of lignocellulosic pentoses to furfural, was investigated.[128] The potential of the efficacy of the green solvent 2-MeTHF in the *N*-alkylation of phthalimide derivatives promoted by KF–alumina was investigated.[129] Solvent mixtures such as chloroform–methanol are an integral part of membrane protein extraction,[130] but owing to the toxicity and mutagenicity implications, a replacement for protein membrane purification using green solvents such as 2-MeTHF and CPME was found. This was the first time that such alternatives using a bio-based solvent was communicated. Similarly, the efficacy of ethyl lactate, a renewable solvent with low toxicity and minimal detrimental effects on air quality, was studied for the production of magnetic tapes.[131]

8.9 Future Perspectives

The post-harvest loss of agricultural produce and the inefficient streamlining of organic industrial by-products, residues and wastes are of grave concern. Up to 40% of food that is grown or processed ends up being wasted. Valorization and biorefining of these biomasses are a mammoth challenge, but for generating a circular economy or closing the loop, optimizing the revenue stream is of paramount importance. Agro- or bio-based solvent production is one such economically viable and sustainable process for stabilizing the production line and cutting off loose ends and waste.

References

1. S. Armenta, S. Garrigues and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2008, **27**, 497–511.
2. P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, New York, USA, 1998, p. 135.
3. F. Chemat, M. A. Vian and G. Cravotto, *Int. J. Mol. Sci.*, 2012, **13**, 8615–8627.
4. J. Hildebrand and R. L. Scott, *Regular Solutions*, Prentice-Hall, Englewood Cliffs, New Jersey, 1962.

5. C. M. Hansen, *Hansen Solubility Parameters- A User's Handbook*, CRC Press, Boca Raton, FL, 2000, pp. 1–25.
6. A. Klamt and G. Schueuermann, *J. Chem. Soc., Perkin Trans. 2*, 1993, **2**, 799–805.
7. A. Klamt, *J. Phys. Chem.*, 1995, **99**, 2224–2235.
8. A. Klamt, *Fluid Phase Equilib.*, 2003, **206**, 223–235.
9. A. Klamt and F. Ecker, *Fluid Phase Equilib.*, 2000, **172**, 43–72.
10. A. Klamt, *COSMO-RS: From Quantum Chemistry to Fluid Phase Thermodynamics and Drug Design*. Elsevier, Amsterdam, 2005.
11. A. Klamt, F. Eckert, M. Diedenhofen and M. E. Beck, *J. Phys. Chem. A*, 2003, **107**, 9380–9386.
12. E. Mullins, R. Oldland, Y. A. Liu, S. Wang, S. I. Sandler, C. C. Chen, M. Zwolak and K. C. Seavey, *Ind. Eng. Chem. Res.*, 2006, **45**, 4389–4415.
13. E. I. Alevizou and E. C. Voutsas, *Fluid Phase Equilib.*, 2014, **369**, 55–67.
14. L. Moity, M. Durand, A. Benazzouz, C. Pierlot, V. Molinier and J. M. Aubry, *Green Chem.*, 2012, **14**, 1132–1145.
15. A. Pozarska, C. C. Mathews, M. Wong and K. Pencheva, *Eur. J. Pharm. Sci.*, 2013, **49**, 505–511.
16. L. Y. Garcia-Chavez, A. J. Hermans, B. Schuur and A. B. de Haan, *Sep. Purif. Technol.*, 2012, **97**, 2–10.
17. A. Benazzouz, L. Moity, C. Pierlot, V. Molinier and J. M. Aubry, *Colloids Surf., A*, 2014, **458**, 101–109.
18. F. Chemat, J. Smadja and M. E. Lucchesie, Solvent Free Microwave extraction of volatile natural compound, *Eur. Pat.*, EP 1 439218 B1, 2004.
19. F. Chemat, M. E. Lucchesie and J. Smadja, Solvent Free Microwave Extraction of volatile natural substances, *U. S. Pat.*, US 0187340 A1, 2004.
20. F. Chemat, M. Abert Vian and F. Visinoni, Microwave hydro-diffusion for isolation of natural products, *Eur. Pat.*, EP 1 955 749 A1, 2008; U. S. *Pat.*, US 2010/0062121, 2010.
21. M. E. Lucchesie, F. Chemat and J. Smadja, *J. Chromatogr. A*, 2004, **1043**, 323–327.
22. M. E. Lucchesie, F. Chemat and J. Smadja, *Flavour Fragrance J.*, 2004, **19**, 134–138.
23. F. Benkaci-Ali, A. Baaliouamer, B. Y. Meklati and F. Chemat, *Flavour Fragrance J.*, 2007, **22**, 148–153.
24. B. Uysal, F. Sozmen and B. S. Buyuktas, *Nat. Prod. Commun.*, 2010, **5**, 111–114.
25. S. Riela, M. Bruno, C. Formisano, D. Rigano, S. Rosselli, M. L. Saladino and F. Senatore, *J. Sep. Sci.*, 2008, **31**, 1110–1117.
26. M. Abert Vian, X. Fernandez, F. Visioni and F. Chemat, *J. Chromatogr. A*, 2008, **1190**, 14–17.
27. N. Bousbia, M. Abert Vian, M. A. Ferhat, B. Y. Meklati and F. Chemat, *J. Food Eng.*, 2009, **90**, 409–413.
28. N. Bousbia, M. Abert Vian, M. A. Ferhat, E. Peticolas, B. Y. Meklati and F. Chemat, *Food Chem.*, 2009, **14**, 355–362.

29. S. Zill-e-Huma, M. Abert-Vian, A. S. Fabiano-Tixier, M. Elmaataoui, O. Dangles and F. Chemat, *Food Chem.*, 2011, **127**, 1472–1480.
30. S. Périno-Issartier, S. Zill-e-Huma, M. Abert-Vian and F. Chemat, *Food Bioprocess Technol.*, 2010, **4**, 1020–1028.
31. I. de Marco, S. Riemma and R. Iannone, *Chem. Eng. Trans.*, 2017, **57**, 1699–1704.
32. X. Luo, J. White, R. Thompson, C. Rayner, B. Kulik, A. Kazlauciunas, W. He and L. Lin, *J. Cleaner Prod.*, 2018, **20**, 1–10.
33. https://www.ecotextile.com/2019011823987/dyes-chemicals-news/in [consulté le 13 octobre 2009]dia-textile-sector-weighs-up-co2-dyeing.html [consulted on january 18th 2019].
34. M. L. Viguera, C. Prieto, J. Casas, E. Casas, A. Cabañas and L. Calvo, *J. Supercrit. Fluids*, 2018, **141**, 137–142.
35. L. Santos-Zea, J. A. Gutiérrez-Uribe and J. Benedito, *J. Supercrit. Fluids*, 2019, **144**, 98–107.
36. P. Balasubramanyam, M. Ashraf-Khorassani and J. S. Josan, *J. Chromatogr. B*, 2018, **1092**, 279–285.
37. V. Sicari, *J. AOAC Int.*, 2018, **101**(1), 293–297.
38. C. Desmortreux, M. Rothaupt, C. West and E. Lesellier, *J. Chromatogr. A*, 2009, **1216**, 7088–7095.
39. J. Liu, F. Ji, F. Chen, W. Guo, M. Yang, S. Huang, F. Zhang and Y. Liu, *J. Pharm. Biomed. Anal.*, 2018, **159**, 513–523.
40. C. Arcoumanis, C. Bae, R. Crookes and E. Kinoshita, *Fuel*, 2008, **87**, 1014–1030.
41. H. Kanda and P. Li, *Fuel*, 2011, **90**, 1264–1266.
42. P. Boonnoun, P. Tunyasitikun, W. Clowutimon and A. Shotipruk, *Food Bioprod. Process.*, 2017, **106**, 193–200.
43. G. C. K. Chan, W. Hall, T. P. Freeman, J. Ferris, A. B. Kelly and A. Winstock, *Drug Alcohol Depend.*, 2017, **178**, 32–38.
44. M. Barna Bridgeman and D. T. Abazia, *Pharm. Ther.*, 2017, **42**, 180–188.
45. D. Sparks, R. Hernandez, M. Zappi, D. Blackwell and T. Fleming, *J. Am. Oil Chem. Soc.*, 2006, **83**(10), 885.
46. S. H. M. Setapar, A. Khatoon, A. Ahmad, M. A. I. Che Yunus and M. A. A. Zaini, *Asian J. Chem.*, 2014, **26**, 5911–5916.
47. J. Suberu, P. Yamin, R. Cornell, A. Sam and A. Lapkin, *ACS Sustainable Chem. Eng.*, 2016, **4**, 2559–2568.
48. A. G. Carr, R. Mammucari and N. R. Foster, *Chem. Eng. J.*, 2011, **172**, 1–17.
49. M. Plaza and C. Turner, *TrAC, Trends Anal. Chem.*, 2015, **71**, 39–54.
50. D. Lachos-Perez, A. M. Baseggio, P. C. Mayanga-Torres, M. R. Maróstica Jr., M. A. Rostagno, J. Martínez and T. Forster-Carneiro, *J. Supercrit. Fluids*, 2018, **138**, 7–16.
51. T. Bajer, P. Bajerova, D. Kremr, A. Eisner and K. Ventura, *J. Food Compos. Anal.*, 2015, **40**, 32–38.
52. Z. Ahmadian-Kouchaksaraie, R. Niazmand and M. N. Najafi, *Innovative Food Sci. Emerging Technol.*, 2016, **36**, 234–244.

53. M. E. Yulianto, P. Kusumo, I. Hartati and A. Wahyuningsih, *J. Chem.*, 2017, **10**, 734–738.
54. M. V. Kiamahalleh, G. Najafpour-Darzi, M. Rahimnejad and A. A. Moghadamnia, *J. Chromatogr. B*, 2016, **1022**, 191–198.
55. M. Z. Ozel, F. Gogus and A. C. Lewis, *Food Chem.*, 2003, **82**, 381–386.
56. S. Q. Liew, W. H. Teoh, C. K. Tan, R. Yusoff and G. C. Ngoh, *Int. J. Biol. Macromol.*, 2018, **116**, 128–135.
57. R. M. Smith and R. J. Burgess, *J. Chromatogr. A*, 1997, **785**, 49–55.
58. D. J. Miller and S. B. Hawthorne, *Anal. Chem.*, 1997, **69**, 623–627.
59. T. M. Pawlowski and C. F. Poole, *Anal. Chem.*, 1999, **36**, 71–75.
60. R. M. Smith and R. J. Burgess, *J. Chromatogr. A*, 1997, **785**, 49–55.
61. B. Li, Y. Yang, Y. X. Gan, C. D. Eaton, P. He and A. D. Jones, *J. Chromatogr. A*, 2000, **873**, 175–184.
62. S. Rodriguez Meizoso, O. Chienthaworn, I. D. Wilson, B. Wright and S. D. Taylor, *Anal. Chem.*, 1999, **71**, 4493–4497.
63. R. M. Smith, O. Chienthavorn, I. D. Wilson, B. Wright and S. D. Taylor, *Anal. Chem.*, 1999, **71**, 4493–4497.
64. T. D. Ho, C. Zhang, L. W. Hantao and J. L. Anderson, *Anal. Chem.*, 2014, **86**, 262–285.
65. H. N. Abdelhamid, *TrAC, Trends Anal. Chem.*, 2016, **77**, 122–138.
66. A. A. C. Toledo Hijo, G. J. Maximo, M. C. Costa, E. A. C. Batista and A. J. A. Meirelles, *ACS Sustainable Chem. Eng.*, 2016, **4**, 5347–5369.
67. V. C. A. Orr and L. Rehmann, *Curr. Opin. Green Sustainable Chem.*, 2016, **2**, 22–27.
68. A. Paiva, R. Craveiro, I. Aroso, M. Martins, R. L. Reis and A. R. C. Duarte, *ACS Sustainable Chem. Eng.*, 2014, **2**, 1063–1071.
69. M. Espino, M. Á. Fernández, F. J. V. Gomez and M. F. Silva, *TrAC, Trends Anal. Chem.*, 2016, **76**, 126–136.
70. C. Florindo, F. Lima, B. D. Ribeiro and I. M. Marrucho, *Curr. Opin. Green Sustainable Chem.*, 2019, **18**, 31–36.
71. Y. P. Mbous, M. Hayyana, A. Hayyan, W. F. Wong, M. A. Hashima and C. Y. Looi, *Biotechnol. Adv.*, 2017, **35**, 105–134.
72. A. Shishov, A. Bulatov, M. Locatelli, S. Carradori and V. Andruch, *Microchem. J.*, 2017, **135**, 33–38.
73. P. T. Anastas and J. C. Warner, *Green chemistry: Theory and practice*, Oxford University Press, 1998.
74. Y. Dai, G. Witkamp, R. Verpoorte and Y. H. Choi, *Anal. Chem.*, 2013, **85**, 6272–6278.
75. Y. H. Choi, J. V. Spronsen, Y. Dai, M. Verberne, F. Hollmann, I. W. C. E. Arends, G. J. Witkamp and R. Verpoorte, *Plant Physiol.*, 2011, **156**, 1701–1705.
76. C. G. González, N. R. Mustafa, E. G. Wilson, R. Verpoorte and Y. H. Choi, *Flavour Fragrance J.*, 2017, 1–6.
77. F. Fahn, Structure and Function of Secretory Cells, in *Advances in Botanical Research Incorporating Advances in Plant pathology Plant Trichomes*, ed. D. L. Hallahan and J. C. Gray, Academic press, 2000, pp. 36–75.

78. R. Verpoorte, Secondary metabolism, in *Metabolic Engineering of Plant Secondary Metabolism*, ed. R. Verpoorte and A. W. Alfermann, Kluwer Academic, Dordrecht, 2000, pp. 1–29.
79. M. A. Fernandez, J. Boiteux, M. Espino, F. J. V. Gomez and M. F. Silva, *Anal. Chim. Acta*, 2018, **1038**, 1–10.
80. A. P. Abbott, G. Capper, D. L. Davies, R. K. Rasheed and V. Tambyrajah, *Chem. Commun.*, 2003, **9**, 70–71.
81. M. C. Gutiérrez, M. L. Ferrer, C. R. Mateo and F. del Monte, *Langmuir*, 2009, **25**, 5509–5515.
82. H. Vanda, Y. Dai, E. G. Wilson, R. Verpoorte and Y. H. Choi, *C. R. Chim.*, 2018, **21**, 628–638.
83. T. Jeliński and P. Cysewski, *J. Mol. Model.*, 2018, **180**, 1–17.
84. M. Faggian, S. Sut, B. Perissutti, V. Baldan, I. Grabnar and S. Dall'Acqua, *Molecules*, 2016, **21**, 1531–1542.
85. F. J. V. Gomez, M. Espino, M. de los Angeles Fernandez, J. Raba and M. F. Silva, *Anal. Chim. Acta*, 2016, **936**, 91–96.
86. S. C. Cunha and J. O. Fernandes, *TrAC, Trends Anal. Chem.*, 2018, **105**, 225–239.
87. X. Liu, S. Ahlgrena, H. A. A. J. Korthoutc, L. F. Salomé-Abarcaa, L. M. Bayonaa, R. Verpoortea and Y. H. Choi, *J. Chromatogr. A*, 2018, **1532**, 198–207.
88. B. Xia, D. Yan, Y. Bai, J. Xie, Y. Cao, D. Liao and L. Lin, *Anal. Methods*, 2015, **7**, 9354–9364.
89. B. Ozturka, C. Parkinsona and M. Gonzalez-Miquel, *Sep. Purif. Technol.*, 2018, **206**, 1–13.
90. K. Fraige, R. D. Arrua, A. T. Sutton, C. S. Funari, A. J. Cavalheiro, E. F. Hilder and V. S. Bolzani, *J. Sep. Sci.*, 2019, **42**, 591–597.
91. Z. Wei, X. Qi, T. Li, M. Luo, W. Wang, Y. Zu and Y. Fu, *Sep. Purif. Technol.*, 2015, **149**, 237–244.
92. M. Cvjetko Bubalo, N. Ćurko, M. Tomašević, K. Kovačević Ganić and I. Radojcic Redovnikovic, *Food Chem.*, 2016, **200**, 159–166.
93. K. Radošević, N. Ćurko, V. G. Srček, M. C. Bubalo, M. Tomašević, K. K. Ganic and I. R. Redovniković, *LWT-Food Sci. Technol.*, 2016, **73**, 45–51.
94. Y. Ma, M. Liu, T. Tan, A. Yan, L. Guo, K. Jiang, C. Tan and Y. Wan, *Phytochem. Anal.*, 2018, 1–10.
95. Z. Wei, X. Wang, X. Peng, W. Wang, C. Zhao, Y. Zu and Y. Fu, *Ind. Crops Prod.*, 2015, **63**, 175–181.
96. S. Chanioti and C. Tzia, *Innovative Food Sci. Emerging Technol.*, 2018, **48**, 228–239.
97. T. Bosiljkov, F. Dujmić, M. C. Bubalo, J. Hribarb, R. Vidrihb, M. Brncić, E. Zlaticb, I. R. Redovniković and S. Jokić, *Food Bioprod. Process.*, 2017, **102**, 195–203.
98. B. Tang, W. Bi, H. Zhang and K. H. Row, *Chromatographia*, 2014, **77**, 373–377.
99. J. Nie, G. Yu, Z. Song, X. Wang, Z. Li, Y. She and M. Lee, *Anal. Methods*, 2017, **9**, 856–863.

100. E. Habibi, K. Ghanemi, M. Fallah-Mehrjardi and A. Dadolahi-Sohrab, *Anal. Chim. Acta*, 2013, **762**, 61–67.
101. E. Yilmaz and M. Soylak, *Talanta*, 2015, **136**, 170–173.
102. A. Garcia, E. Rodriguez-Juan, G. Rodriguez-Gutierrez, J. J. Rios and J. Fernandez-Bolanos, *Food Chem.*, 2016, **197**, 554–561.
103. V. M. Paradiso, A. Clemente, C. Summo, A. Pasqualone and F. Caponio, *Food Chem.*, 2016, **212**, 43–47.
104. T. Khezeli, A. Daneshfar and R. Sahraei, *Talanta*, 2016, **150**, 577–585.
105. T. Gu, M. Zhang, T. Tan, J. Chen, Z. Li, Q. Zhang and H. Qiu, *Chem. Commun.*, 2014, **50**, 11749–11752.
106. M. A. Farajzadeh, M. Reza, A. Mogaddam and M. Aghanassab, *Anal. Methods*, 2016, **8**, 2576–2583.
107. M. B. Arain, E. Yilmaz and M. Soylak, *J. Mol. Liq.*, 2016, **224**, 538–543.
108. M. A. Farajzadeh, M. R. Afshar Mogaddam and B. Feriduni, *RSC Adv.*, 2016, **6**, 47990–47996.
109. A. T. Sutton, K. Fraige, G. Mazzi Leme, V. S. Bolzani, E. F. Hilder, A. J. Cavalheiro, R. D. Arrua and C. S. Funari, *Anal. Bioanal. Chem.*, 2018, **410**, 3705–3713.
110. E. D. Jong and G. Jungmeier, Biorefinery concepts in comparison to petrochemical refineries, in *Industrial Biorefineries & White Biotechnology*, ed. A. Pandey, R. Höfer, M. Taherzadeh, K. Madhavan Nampoothiri and C. Larroche, Elsevier B.V., pp. 3–33.
111. A. Gupta and J. Verma, *Renewable Sustainable Energy Rev.*, 2015, **41**, 550–567.
112. C. D. Tanzi, M. A. Vian, C. Ginies, M. Elmaataoui and F. Chemat, *Molecules*, 2012, **17**, 8196–8205.
113. P. K. Mamidipally and S. X. Liu, *Eur. J. Lipid Sci. Technol.*, 2004, **106**, 122–125.
114. S. Bertouche, V. Tamao, A. Hellal, C. Boutekedjiret and F. Chemat, *J. Essent. Oil Res.*, 2013, **25**, 439–443.
115. K. Watanabe, N. Yamagiwa and Y. Torisawa, *Org. Process Res. Dev.*, 2007, **11**, 251–258.
116. I. F. Strati and V. Oreopoulou, *Int. J. Food Sci. Technol.*, 2011, **46**, 23–29.
117. A. A. D'Archivio, M. A. Maggi and F. Ruggieri, *J. Pharm. Biomed. Anal.*, 2018, **149**, 89–95.
118. S. Farooque, P. M. Rose, M. Benohoud, R. S. Blackburn and C. M. Rayner, *J. Agric. Food Chem.*, 2018, **66**, 12265–12273.
119. V. Rapinel, C. Santerre, F. Hanaei, J. Belay, N. Vallet, N. Rakotomanomana, A. Vallageas and F. Chemat, *C. R. Chim.*, 2018, **21**, 590–605.
120. C. Breil, A. Meullemiestre, M. Vian and F. Chemat, *Molecules*, 2016, **21**, 196.
121. Y. Liu, L. Zhu, X. Sun and J. Chen, *AIChE J.*, 2010, **56**, 2338–2346.
122. K. Bundeesomchok, A. Filly, N. Rakotomanomana, P. Panichayupakaranant and F. Chemat, *LWT-Food Sci. Technol.*, 2016, **65**, 297–303.
123. X. Tian, X. Chen, L. Dai, W. Du and D. Liu, *Catal. Commun.*, 2017, **101**, 89–92.

124. Y. Li, F. Fine, A. S. Fabiano-Tixier, M. Abert-Vian, P. Carre, X. Pages and F. Chemat, *C. R. Chim.*, 2014, **17**, 242–251.
125. A. G. Sicaire, M. Vian, F. Fine, F. Joffre, P. Carre, S. Tostain and F. Chemat, *Int. J. Mol. Sci.*, 2015, **16**, 8430–8483.
126. H. K. Ravi, C. Breil, M. Vian, F. Chemat and P. R. Venskutonis, *ACS Sustainable Chem. Eng.*, 2018, **6**, 4185–4193.
127. M. Jacotet-Navarro, M. Laguerre, A. S. Fabiano-Tixier, M. Tenon, N. Feuillere, A. Billy and F. Chemat, *Electrophoresis*, 2018, **39**, 1946–1956.
128. M. J. C. Molina, R. Mariscal, M. Ojeda and M. Lopez Granados, *Bioresour. Technol.*, 2012, **126**, 321–327.
129. V. Pace, P. Hoyos, M. Fernandez, J. V. Sinisterra and A. R. Alcantara, *Green Chem.*, 2010, **12**, 1380–1382.
130. S. F. Tenne, J. Kinzel, M. Arlt, F. Sibilla, M. Bocola and U. Schwaneberg, *J. Chromatogr. B*, 2013, **937**, 13–17.
131. S. M. Nikles, M. Piao, A. M. Lane and D. E. Nikles, *Green Chem.*, 2001, **3**, 109–113.

CHAPTER 9

Green Chromatography: State-of-the-art, Opportunities and Future Perspectives

JUSTYNA PŁOTKA-WASYLKA,* MAGDALENA FABJANOWICZ, KAJA KALINOWSKA AND JACEK NAMIEŚNIK

Department of Analytical Chemistry, Gdańsk University of Technology, Gabriela Narutowicza Street 11/12, 80-233 Gdańsk, Poland
*Emails: juswasyl@pg.edu.pl; plotkajustyna@gmail.com

9.1 Introduction

The analysis of organic compounds in samples that are characterized by different matrix compositions is very important in many areas. Mainly gas and liquid chromatographic methods are applied to the determination of this type of compound. Chromatographic procedures are applied both in research and in routine environmental, food, industrial and medical analyses, which translates into a very large number of chromatographic determinations performed every day around the world.[1] It is well known that chromatographic methods can have a significant impact on the environment if laboratory practice is not followed in line with the principles of green analytical chemistry (GAC). Just as the responsibility of industrial chemists is to minimize pollution generated by their activities, that of analytical chemists is to obtain reliable analytical results within a short time, with little or preferably no negative environmental impact.[2] However,

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

chromatographic techniques have the potential to be greener in all steps of the analysis, from the collection and preparation of the sample to separation and final determination. Economy is usually on the side of GAC, as it promotes analytical methodologies that consume no or only small volumes of solvents and other reagents. This generates opportunities for savings for analytical laboratories, as they do not require the purchase of large amounts of these chemicals.

GAC is a branch of green chemistry related to different aspects of chemical analysis. A schematic representation of the way in which GAC can be used in sample preparation and the final determination step is presented in Figure 9.1.

Without a doubt, complete elimination of sample preparation would be an ideal scenario, but it is not always practical. In such cases, solventless extraction techniques offer a very good alternative. However, if solvents have to be used, the focus should be placed on the minimization of their consumption.

The approaches applied to make chromatographic separations greener differ depending on the type of chromatographic method used. For example, in gas chromatography (GC), it is desirable to move away from using helium as the carrier gas because it is a non-renewable resource. Application of low

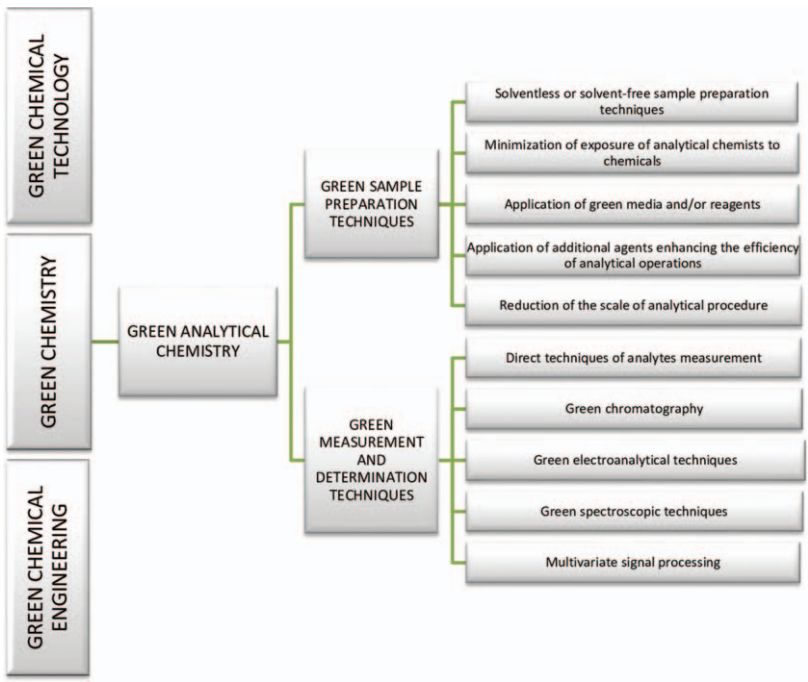


Figure 9.1 Green analytical chemistry principles applied to sample preparation and the final determination step.

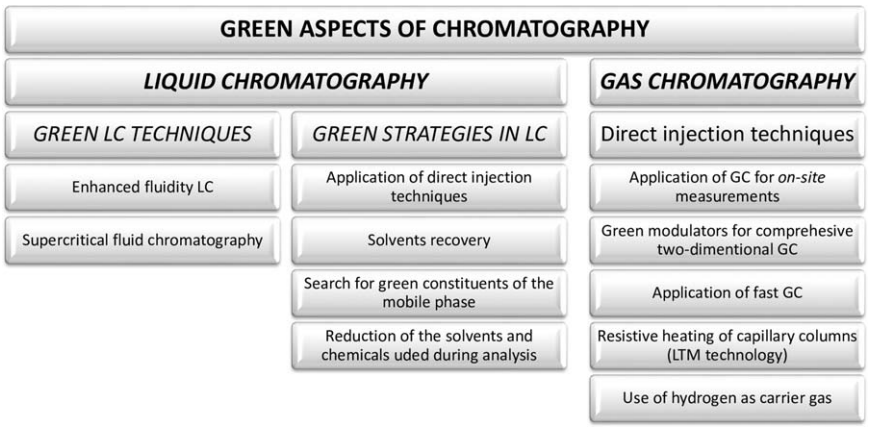


Figure 9.2 Schematic representation of different approaches to greening chromatographic methodologies.

thermal mass technology in GC separations can be more eco-friendly because of the energy savings that are offered by this technology. In the case of liquid chromatography (LC), the focus should be placed on the reduction of solvent consumption and the replacement of environmentally hazardous and toxic chemicals with more benign alternatives. It should be noted that multidimensional separation techniques also have the potential to make analyses greener in both GC and LC applications.

An important issue related to the environmental impact of the method is the location of the instrument with respect to the sample collection point. As all of the traditional approaches generate a negative environmental impact, the application of portable chromatographs or on-line process analysers is therefore recommended.

This chapter aims to present the ways in which chromatography can become greener (Figure 9.2). GC and LC are compared from the point of view of GAC. Moreover, the role of miniaturization in sample preparation and chromatographic separation is stressed. Portable chromatographs and on-line process analysers are also discussed.[5]

9.2 Direct Chromatographic Analysis

From the GAC point of view, one of the main features of chromatographic techniques is the presence or absence of a sample preparation step, as this is often the most polluting stage in the overall chromatographic analysis process.[3] It is well known that occupational exposure to toxic solvents and reagents during sample pretreatment may affect the health of the analyst. Hence, taking into account the perspective of GAC, it is highly advantageous to apply direct chromatographic methodologies, *i.e.* methodologies that do not require sample preparation. However, this mode of analysis has some

limitations, the main one of which is that such methodologies are applicable only to samples with relatively clean matrices, *e.g.* water, spirits and petroleum fractions.[4] Direct methodologies applied to samples characterized by complex matrix compositions impact on the chromatographic columns, which might deteriorate quickly owing to deposition of sample components that do not elute from the column.

GC methods are usually more readily adaptable than LC methods to the elimination of the sample preparation step.[2] However, the introduction of the sample (usually water but also water–ethanol mixtures) directly into the chromatographic capillary column used is not to be recommended for several reasons. As water is considered to cause increased column bleeding (mainly when polar stationary phases are used), dense, non-polar stationary phases and dense, polar, crosslinked phases are therefore applied. Recent developments in the quality of stationary phases and novel methods of crosslinking have improved resistance to deterioration caused by water. An additional limitation is that the on-column introduction of environmental water may cause problems with the column performance (mainly when the water has a high salt content) and has a negative impact on the detector sensitivity. However, deactivated columns can be installed in front of the analytical column to prevent inorganic salts and organic non-volatile compounds from entering the column.

Two decades ago, an alternative approach called programmed temperature vaporization (PTV) to inject a liquid sample into the column was introduced. This technique was developed in order to prevent the solvent from reaching the column by removing most or all of it in the injector.[4] However, it should be mentioned that it is debatable whether PTV sample introduction is a direct analytical method or on-line sample preparation to be applied before chromatographic analysis. In the PTV approach, the solvent evaporates before the sample reaches the column. The PTV device is similar to a split/splitless injector, but it is equipped with an efficient heating and cooling system. In the PTV mode, after introducing the sample into the injector, the solvent is slowly evaporated and vented at relatively low temperature, below its boiling point, in the split mode of the injector.[4] Such a solution allows the enrichment of analytes in the injector. Next, the analytes are introduced into the column by rapidly increasing the injector temperature in the splitless mode. PTV can be applied to the determination of analytes that are characterized by boiling points considerably higher than that of the solvent involved.

Application of a liner filled with sorbent is another approach to preventing the solvent from reaching the column.[6] In this approach, an important step is to select a suitable sorbent that retains solvent and thus enables the analytes to be introduced into the column. Inappropriate sorbent selection may impact on the degradation of the analytes or may strengthen their retention.

It is common practice to use greener solvents such as water or ethanol as the mobile phase in reversed-phase LC.[7] In such cases, the almost direct

injection of samples that often have polar matrices can be performed. Because in LC the injected sample needs to be absolutely clean, there are hardly any absolutely direct LC methods – some are almost direct because they require simple filtration or dilution of the sample, but without needing other operations.[4]

Summarizing, the application of direct chromatographic procedures meets the requirements of GAC in several ways. First, such methodologies are more economical than those performed with a sample preparation step, as fewer reagents and less energy are consumed. In addition, the consumption of materials that could have been applied during the sample pretreatment process, such as organic solvents, cartridges, sorbents, fibres, *etc.*, is avoided. The total analysis time is also shortened. It should also be noted that the lack of a sample preparation step allows the chromatograph to be positioned at-line or on-line, which may further reduce the analysis time, which is very important when near-real-time results are crucial.[4] Furthermore, the introduction of impurities is limited (this can occur when performing the sample preparation). Taking all of this into account, it can be stated that direct chromatographic procedures meet the 11th of the ''Twelve Principles of Green Chemistry'' that pushes analytical chemistry towards reducing the environmental impact of chemical processes through real-time monitoring.[2]

9.3 Portable Chromatographs and On-line and At-line Process Analysers

An important issue relating to the environmental impact of a method is the location of the instrument with respect to the sample collection point. Universal chromatographic analysis involves sample collection, transport to the laboratory, sample preparation and final analysis. This traditional approach generates a negative environmental impact, hence the application of portable chromatographs or on-line process analysers is recommended.

There are four possibilities for locating the analytical device with respect to the investigated medium,[8] as illustrated in Figure 9.3. Looking at these possibilities, it can be concluded that both on-line and at-line modes are the most desirable considering the principles of GAC. These approaches allow the consumption of chemicals to be minimized and the total analysis time to be shortened. Several applications of these modes are presented in Table 9.1.

Another opportunity to perform the analysis near the investigated medium is provided by the application of portable chromatographs. Such application satisfies the 11th principle of green chemistry calling for real-time analysis for pollution prevention. Several types of portable chromatograms are available, as summarized in Figure 9.4 with their salient features.

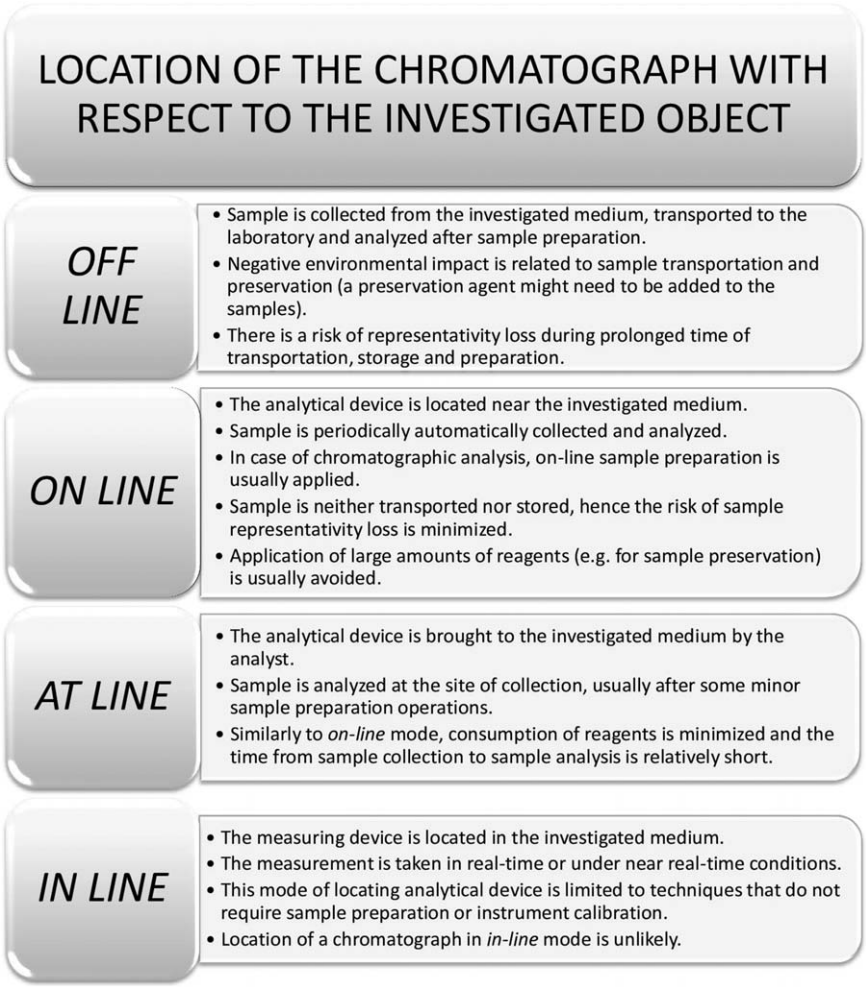


Figure 9.3 Location of the chromatograph with respect to the investigated object.

9.4 Green Aspects of Gas Chromatography

GC is often seen as relatively green since the separation is performed in the gas phase, hence the analysis itself does not require the use of solvents. However, it can be made even greener by the application of various approaches. First, the amount of solvent and the number of reagents used during preparation of the samples prior to the GC analysis can be decreased with the use of green sample preparation methods, or this step can even be omitted with the use of above-mentioned direct chromatographic methods. Moreover, several GAC principles can be implemented during the analysis itself, *e.g.* the use of an environmentally friendly carrier gas and direct resistive heating, and the application of either fast GC or two-dimensional GC (GC×GC) (Figure 9.5).

Table 9.1 Examples of at-line and on-line chromatographic methods.[a]

Investigated medium	Chromatographic mode	Analyte	Remarks	Ref.
At line mode				
Water	GC–μFID	BTEX	Analysis time 3 min, sample preparation by headspace SPME	9
		Amines	Derivatization step, SPME, analysis time 22 min	10
Groundwater	IC	Major cations	No sample preparation	11
	GC–MS	Explosives	Analysis time ~1 h	12
Air	GC–μFID	Acetaldehyde	SPME sample preparation, analysis time 30 min	13
	GC–MS	VOCs	Analysis time 3 min	14
	GC–TCD	Gaseous components	Analysis time 6 min	15
On-line mode				
Drinking water	GC–MS	Trihalomethanes	Analysis time 3 min	16
			Purge-and-trap sample preparation, analysis time 5 min	17
	GC–ECD		Capillary membrane sampler, analysis time 20 min	18
	IC-fluorescence detection	Haloacetic acids	Post-column derivatization with nicotinamide, analysis time 1 h	19
Ambient air	GC–FID	Hydrocarbons	Operating in trigger mode with NMVOC analyser	20

[a]ECD, electron capture detection; FID, flame ionization detection; GC, gas chromatography; IC, ion chromatography; MS, mass spectrometry; TCD, thermal conductivity detection; BTEX, benzene, toluene, ethylbenzene and xylene; SPME, solid-phase microextraction; VOC, volatile organic compound; NMVOC, non-methane volatile organic compound.

9.4.1 Instrumental Modifications

Selection of the appropriate environmentally friendly gas is one of the possible ways to improve the greenness of GC-based methodologies without affecting the efficiency or resolution to any significant extent. Nitrogen is one of the most commonly used carrier gases as it is an inert, non-toxic and non-flammable gas that is relatively easy to source. However, its optimal linear velocity is rather low, which leads to longer times needed to perform the analysis than in the case of other routinely used carrier gases, and consequently it is rarely recommended as the most suitable for GC-related applications. Another commonly used carrier gas, helium, has a higher

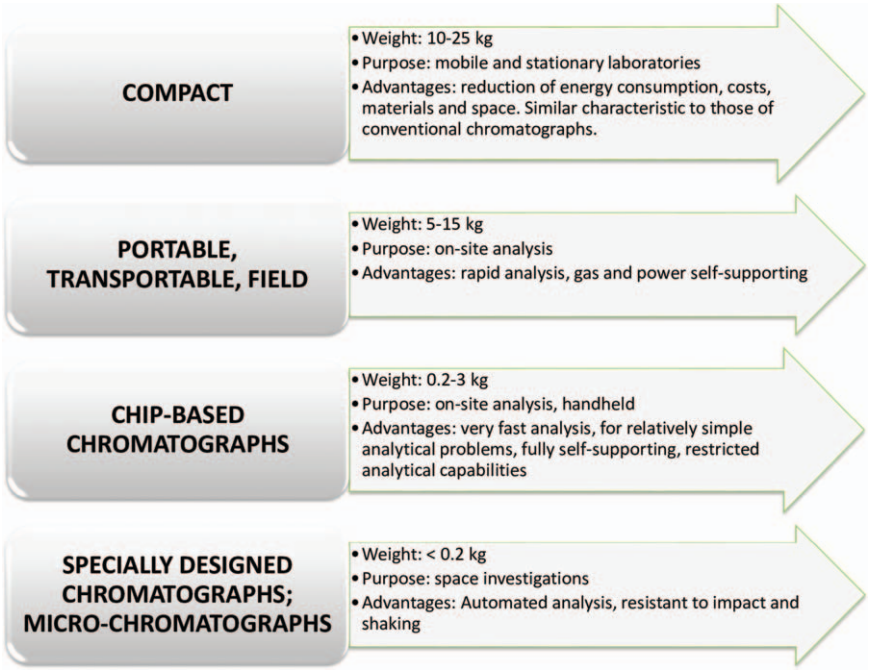


Figure 9.4 Classification of portable chromatographs together with a summary of their advantages.

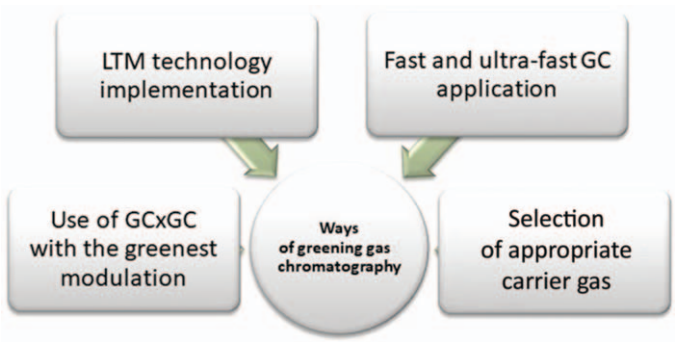


Figure 9.5 Different possibilities of implementation of GAC principles.

optimum linear velocity and therefore much better chromatographic properties, while also being non-toxic and non-flammable. However, since helium is a non-renewable resource, it is advisable to find a greener alternative.[2] With the application of hydrogen as a carrier gas, the analysis could be performed with satisfactory efficiency and resolution while being more environmentally friendly, as its use does not lead to the depletion of the resource.

Another area that could be improved in order to be more in accordance with GAC principles is temperature programming (TP). Since temperature is one of the most important parameters that can be controlled in GC, its programming is widely used in order not only to improve the detection limit or peak symmetry but also to shorten the time of analysis significantly.[21] Hence the application of TP is seen as a green approach. However, it can be made more environmentally friendly by the application of low thermal mass (LTM) technology. LTMGC was first introduced in 2001 and since then has become an important tool to increase considerably the greenness of GC. It typically consists of a capillary column, platinum resistor temperature sensor, heating wire made from nickel alloy, transfer line, electric fan and a metal tray supporting the whole module.[22] Owing to the small size of the system and its small heat capacity, raising of the temperature requires a smaller amount of heat and, thus, much less energy than in the case of conventional GC systems (the power consumption in LTMGC is estimated to be approximately 1% of that in standard GC).[22] Moreover, with the application of LTM technology, it is possible to achieve both ultra-fast TP (with the column heating rate reaching 1800 $^{\circ}C\,min^{-1}$) and significant shortening of the cool-down time, which results in a shortening of the time of the analysis.[23] Because of all of the above advantages, LTMGC has found application in various analyses for industrial purposes. With its use, it is possible to perform rapid diesel analysis without decreasing the separation power and to shorten the time of analysis of polycyclic aromatic hydrocarbons from 70 to less than 4 min.[24] However, there are some disadvantages of this method, such as the possibility of the negative impact that the elevated temperature may have on the tubing or the necessity for constant heating of the oven, hence there is still a possibility of increasing the greenness of LTMGC further.[2,22] Therefore, different modifications of LTMGC have been proposed. For example, Stearns *et al.*[24] suggested the use of a nickel-clad column instead of a nickel-wire column, where a fused-silica column was coated with nickel in order to provide insulation. Owing to higher heating and cooling rates, it can be implemented in, *e.g.*, portable GC instruments.[25,26]

Another solution worth mentioning is the application of fast GC and ultra-fast GC, which can significantly reduce the time of a single analysis while decreasing both carrier gas and energy consumption, without a significant decrease in the sensitivity of the system. Fast and ultra-fast GC instruments can be developed with the use of multicapillary columns, application of low pressure or by decreasing the length and diameter of the column. Multicapillary columns are a relatively new solution, the popularity of which is steadily increasing, as their use enables the separation to be performed in a short time with acceptable sample capacity. Moreover, many types of multicapillary columns are commercially available, which facilitates their use in different fields of analytical chemistry. The application of low-pressure (LP) GC makes it possible to reduce the time of the analysis even further. It has been reported that the time necessary to analyse organic compounds can be

reduced 3–5-fold compared with the application of commonly used GC instrumentation. In addition, increased sample throughput results in improved detection limits and the signal-to-noise ratio is reduced. Because of that, even though LP-GC has a rather low separation efficiency, it is often applied in environmental and food analysis.[27a]

9.4.2 Multidimensional Gas Chromatography

Two-dimensional GC was introduced for the first time in 1991 by Liu and Phillips.[27b] The main differences based on which it is possible to distinguish it from typical one-dimensional GC are the presence of two different columns, usually of complementary polarities and selectivity, instead of one, and the use of a modulator that continuously collects eluate from the first column and passes it to the second. There are two approaches for connecting the columns in multidimensional GC that differ in the way in which the eluate is transferred from one column to another: small portions of the eluate can be continuously injected, as in the case of comprehensive two-dimensional GC (GC×GC), or certain fractions of the eluate can be collected and introduced into the second column as in heart-cut two-dimensional GC (H/C GC–GC).[28] Owing to the presence of two different columns, in the case of both GC×GC and H/C GC–GC it is possible to obtain better resolution and lower detection limits in the same or a slightly longer time and with reagent consumption similar to that necessary for one-dimensional GC analysis.[29] However, GC×GC is usually used more often, as it facilitates obtaining the widest possible chemical profile. Its applications require amounts of reagents and sample volumes similar to those in one-dimensional GC but allow much better separation of sample components even in complex matrices. Owing to all of the above-mentioned advantages, GC×GC and its combination with time-of-flight mass spectrometry (TOF-MS) are implemented in various fields, particularly in food analysis.[30] Moreover, because of the possibility of obtaining satisfactory separations, determination of targeted analytes can be performed with little or no sample preparation, which significantly reduces both time and reagent consumption and thus increases the greenness of the analysis.

Even though comprehensive two-dimensional GC is relatively green, there is still the possibility of increasing its environmental friendliness. Cryogenic modulators, most commonly using a type of thermal modulator, use cryogens such as liquid nitrogen or carbon dioxide in order to trap analytes and therefore are rather expensive and difficult to work with. As a result, several different approaches have been proposed. One is to use a modification with a single-stage cryogenic modulator in which the delivery system of the liquid nitrogen is modified, which in effect lowers the coolant consumption.[31] Another possibility is to use a consumable-free modulator (CFM). This uses a capillary made of stainless steel in order to trap and then inject analytes into the second column. Its use does not require a cryogen and is ideal for performing analyses *in situ*, which makes it a much greener

solution that the application of the most common approaches. Another approach is to use flow modulators in place of thermal modulators. In these, eluate from the first column is collected in the sampling loop, which is periodically flushed with the carrier gas stream. The operation of flow modulators is simpler and cheaper than the operation of thermal modulators and does not require cryogens, which makes them a greener solution. However, their use usually leads to lower sensitivity as the modulation period is somewhat limited.[23]

9.5 Green Aspects of Liquid Chromatography

LC is an important technique, designed for the separation, identification and quantification of various compounds from a complex matrix composition. Although it is commonly considered less green than GC owing to the high volume of solvent used, it has a wide range of possibilities for making it more environmentally friendly. Nevertheless, during the greening process, one should remember to find the balance between ''greening'' and obtaining relevant separation results. In order to perform successful separations with the use of LC, a combination of several parameters related to the operation needs to be adjusted, such as[32,33]

- column [packing, length and internal diameter (i.d.)];
- mobile phase (type, flow rate, gradient elution);
- separation temperature;
- sample size;
- pump pressure.

The main problem associated with LC when green assessment is considered is the waste generation, since traditional LC systems were far behind the requirements for a green separation technique. With the simple assumption model when a conventional column of length 15–25 cm×4.6 mm i.d. packed with 5 μm particles is used, with a flow rate of 1 mL min^{-1}, one can realize that it creates around 1500 mL of waste per day, giving around 500 L of waste per year. Hence green strategies and techniques targeted at the improvement of LC are focused on the solvent issue, as presented in Figure 9.6.[2]

The use of solvents in LC is mostly affected by the time of analysis. All the efforts made to minimize the consumption of the mobile phase during LC analysis are closely related to shortening the time of analysis, keeping in mind that analytical characteristics such as separation efficiency, resolution, sensitivity, *etc.*, should be maintained at the highest possible level.[3]

9.5.1 Column-related Parameter Fitting

One of the practices commonly applied when it comes to dealing with the reduction of the organic waste generation by the LC system during a single

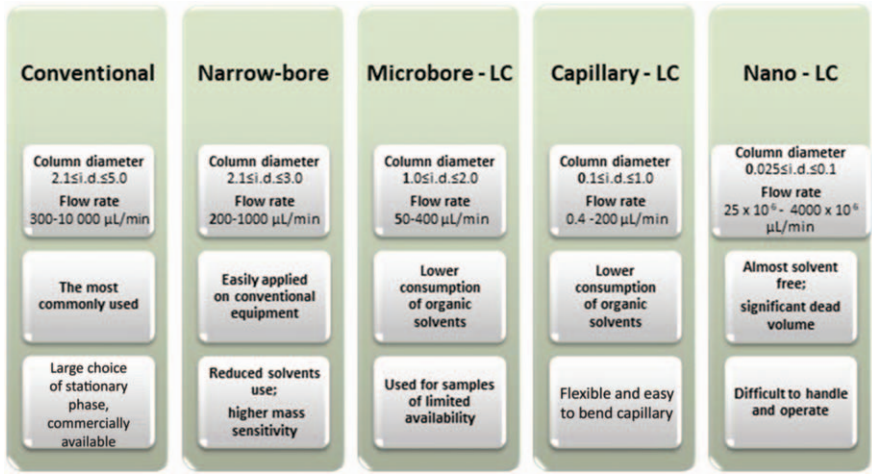


Figure 9.6 Classification and characterization of LC based on the column i.d.[2]

analysis is the adjustment of column-related parameters. It is clearly shown in Figure 9.6 that when the i.d. of the column is decreased, the amount of mobile phase flow is decreased. Once the i.d. of the column has been reduced, the flow has to be scaled down in order to maintain the efficiency of the separation, which can be calculated with the following equation:[34]

$$F_{\text{downscaled}} = F_{\text{conventional}} \left(\frac{\text{i.d.}_{\text{downscaled}}}{\text{i.d.}_{\text{conventional}}} \right) \tag{9.1}$$

Moreover, when the i.d. of the column is decreased, the sensitivity increases owing to reduced dilution of the analytes by the mobile phase. This therefore results in a higher signal intensity, since more concentrated solutes reach the detector, such as a UV, electrospray ionization mass spectrometric or fluorescence detector. However, apart from i.d. of the column, the particle size (from 5 to 2 μm) and the column length (from 25 to even 5 cm) might also be reduced in order to decrease organic solvent consumption. Reductions in these parameters positively influence the chromatographic productivity. They increase the throughput and at the same time make the analysis times shorter, which is highly desirable in many branches of analysis such as clinical, pharmaceutical, toxicological, forensic and environmental areas, when hundreds of samples need to be analysed.[34] However, according to Darcy's law, expressed by the equation

$$\Delta P = \frac{\mu L \eta \phi}{d_{\text{p}}^2} \tag{9.2}$$

where ΔP is the pressure drop, μ is the dynamic viscosity, L is the length of the column, d_{p} is the particle size, η is the viscosity of the solvent and ϕ is

the flow resistance, the pressure is inversely proportional to the particle size at the optimum linear velocity. This creates a certain limitation on using sub-2 μm particles with a standard high-performance liquid chromatographic (HPLC) system owing to the generation of a high column backpressure. Some instrumental modifications need to be made in order to allow the system to work at pressures above 400 bar. In 2004, the first ultra-high-performance liquid chromatographic (UHPLC) system, which can work even in the range 600–1200 bar, was developed and made commercially available by Waters Corporation.[34] Further, the separation was speeded up and the performance was maintained at the same level. Significant savings in the consumption of solvents are observed when small particles are packed into a column with small i.d. What is more, they reduce the frictional heating responsible for efficiency losses. However, it should be emphasized that UHPLC systems are also capable of working with conventional particles in the range 3–5 μm, but this is related to high solvent usage and in this case it is no longer a green technique. Nevertheless, systems with a narrow-bore column can fully satisfy the green chemistry approach and may be used to carry out analyses in a more environmentally safe manner.[2,34]

9.5.2 Temperature

Of the various the modifications to column parameters, temperature is the most frequently changed factor. As is commonly known, temperature is a powerful parameter in LC, affecting selectivity, efficiency and detectability. Temperature modifications represent the most preferred changes to be made during method development owing to their simplicity. Much less effort is required from the analyst to adjust the temperature of operation than to change the buffer pH or mobile phase composition. However, implementation of elevated temperatures requires the LC system to be equipped with a column fitted with a thermostat in order to maintain a fixed temperature for the whole time of analysis, a mobile phase preheater and a post-column effluent cooling system, because the temperature of the mobile phase must be increased before it enters the column and lowered after it leaves the column in order not to create disturbances to the detector signal. Adjusting the temperature brings several advantages for the separation performance and ''greening'' of the whole analysis since it has a strong influence on several factors:

- When the temperature is increased from 20 to 50 °C it reduces the viscosity by as much as 50%, which further results in a lower backpressure at the chromatographic column.
- Once the backpressure has been reduced, a higher flow rate of the mobile phase may be used, shortening the analysis time while at the same time achieving almost the same efficiency; taking advantage of the shortening of the time of analysis, a smaller particle size packing or

a longer column can be used in order to achieve a higher efficiency of separation.
- Higher temperatures reduce the polarity of green solvent alternatives, hence water (due to changes in its dielectric constant) and ethanol can be used as the mobile phase.
- Higher temperatures weaken secondary interactions with the silica support, resulting in better peak symmetry.
- Elevated temperatures are beneficial for the mass transfer from the mobile phase to the stationary phase thanks to the increase in the diffusion coefficient of the molecules; the analysis may be accelerated by a higher flow rate without loss of efficiency.
- A temperature-dependent stationary phase may be designed for selective separations.
- Trying various temperature changes can help to tune the retentions of many analytes since many properties are strongly related to temperature.

On the other hand, even though an LC system operating at higher temperatures is a promising approach, with many advantages, there are also some constraints, *e.g.* it is not applicable to the analysis of complex matrices containing thermally unstable molecules, a stable mobile phase should be used and caution must be exercised when operating with silica-based columns, as the temperature cannot exceed 60 °C, particularly when using acidic or basic eluents.[2,34]

9.5.3 Green Alternatives for Mobile Phases

Another approach in terms of greening LC is to use alternative mobile phase solvents. The conventional mobile phase used in LC consists of acetonitrile diluted in water or methanol diluted in water. Both acetonitrile and methanol are toxic and also the cost of acetonitrile disposal is high. Whenever possible, they should be replaced with green alternatives, such as superheated water, acetone or ethanol. Pure water at elevated temperature is favoured by many analysts. Pure water heated to 150 °C has a dielectric constant comparable to that of a 50 : 50 v/v mixture of methanol and water and it is safe for the environment. Nevertheless, it requires a thermally stable stationary phase to withstand such high temperatures.[35] Ethanol is another organic phase modifier considered as a green alternative to acetonitrile. The main limitation is its high viscosity, making it very difficult to implement in a conventional HPLC system. However, when UHPLC was introduced and the system could operate at pressures above 1000 bar, the viscosity was then no longer a problem.[2,34] Advantages and disadvantages of selected green alternatives of organic solvent are presented in Table 9.2.

Another solution is to use substances under their supercritical conditions. Some physical properties of given substances can be changed by making changes in pressure and temperature around their critical points. Together

Table 9.2 Characteristics of main green alternatives for organic solvents in LC.[2,34]

	Superheated water	Acetone	Ethanol	Supercritical CO_2
Pros	• Non-toxic • Non-flammable • Inexpensive • Lower dielectric constant resulting in higher solvent strength • Allows UV detection at a very short wavelength • Allows the use of a flame ionization detector, very desirable for UV-transparent compounds • Increases ionization efficiency and also the signal-to-noise ratio when an MS detector is used	• Good solubilizing power • Perfectly miscible with other solvents	• Similar boiling point, permittivity and density to acetonitrile and methanol • Lower volatility and lower toxicity in comparison with conventional solvents • Low disposal costs	• Low environmental impact • Non-toxic • Low disposal costs • Mostly non-toxic • Allows fast and efficient separation owing to low viscosity, high diffusivity and high solubilizing power
Cons	• Special instrumentation is necessary, silica column degrades at temperatures above 100 °C • Risk of on-column degradation of thermally unstable compounds • Problem with the solubility of hydrophobic compounds	• Not applicable with UV detector owing to strong UV absorption even up to 340 nm • Difficult to pump because of high volatility	• High viscosity • Difficulties in trading due to legal regulations	• Non-polar so some organic solvents need to be added, such as methanol, to increase its polarity. However, it is usually kept below 30%

with temperature and pressure fluctuations, the ranges of solubility and volatility may be broadened, giving high solubilizing power, mass transfer and selectivity. Moreover, supercritical fluids can be entirely recovered and have a low environmental impact and low disposal costs. They are mostly non-toxic apart from inorganic supercritical fluid liquids such as ammonia and nitrous oxide, which may cause neurotoxicity and are strong oxidants. Special attention should be devoted to their critical temperature, which may be high in some cases. Further, they may be flammable.[2] The most preferred supercritical fluid used in green LC is carbon dioxide, the advantages and disadvantages of which are presented in Table 9.2.

The strategy of using supercritical fluid chromatography (SFC) may be successfully applied to pharmaceuticals and for any other analyses where the preparative- or semipreparative-scale purification of chiral compounds is used. High diffusivity and low viscosity favour chiral separations. It has been found that in many cases HPLC may be replaced with SFC, which gives higher efficiency. SFC positively influences method development, column equilibration making it faster and, what is more, it gives a more flexible range of choice of solvent selection and also generates far less toxic and hazardous wastes.[2,34]

A comparative study was carried out by Toribio *et al.*[36] in which enantiomeric separations of antiulcer drugs such as omeprazole, lansoprazole, rabeprazole and pantoprazole were performed with the use of SFC and HPLC with a Chiralpak AD column. It was found that with HPLC only two compounds were separated (omeprazole and pantoprazole) whereas with SFC all four were well visible. Additionally, SFC provided higher selectivity and resolution and faster analysis (less than 10 min). HPLC had limitations regarding solvent selection since only ethanol and 2-propanol could be used whereas SFC had a much wider range of modifiers to choose from.[2,34]

Apart from approaches mentioned, there is one further possibility to make the mobile phase greener, which can be achieved using enhanced fluidity (EF) liquid mixtures. These can be successfully applied to separations of moderately polar to polar compounds. They are formed by adding to an alcohol high proportions of soluble gases such as carbon dioxide. EF liquid mixtures combine the properties of liquids and supercritical fluids. Similarly to supercritical fluids, together with changes in pressure the polarity of EF liquid mixtures may also be changed. Low pressures represent optimum conditions for EF liquids, making them stable in a single phase. Moreover, as a high proportion of CO_2 is added, several improvements in chromatographic performance may be observed, such as lower pressure drops, higher optimum linear velocity, higher efficiency and shorter analysis times. Thanks to the low viscosity of EF liquid mixtures, long capillary columns of 1 m or even more can be used, which results in very efficient separations. Nevertheless, to work efficiently with EF liquid mixtures, optimum operating conditions need to be set. They can be adjusted on the basis of the phase diagram, which for some of them can be found in the literature. However, for those for which no data are yet available, the phase diagram needs to be

obtained experimentally. A given type of fluid may be applied in reversed-phase and normal-phase chromatography and also in size-exclusion separations. EF liquid mixtures started to be used in the hydrophilic interaction liquid chromatography (HILIC) mode, in which a polar stationary phase is implemented together with aqueous–organic mobile phases in order to separate compounds basing on their hydrophilicity.[2] The separation process takes place by the partitioning between the enriched water layer on the surface of a polar stationary phase and a mobile phase with a high percentage of an organic solvent such as acetonitrile.[34] HILIC can be successfully applied in pharmaceutical analysis. As acetonitrile is a hazardous solvent for the environment, Pereira Ados *et al.* proposed to introduce an EF liquid (ethanol–water with carbon dioxide additive) in order to perform analyses in a greener way.[37] In addition to water–ethanol–CO_2, other environmentally friendly additives such as a combination of ammonium acetate with ammonia may be added in order to form an alkaline mobile phase or formic acid with acetic acid to form an acidic mobile phase. In such cases, HILIC may be considered as a green analytical technique.[2]

Further, ionic liquids can be used as a green solvent alternative. Their unique properties such as electrical conductivity, low volatility and thermal stability may be favourable for the performance of LC and may improve the peak shape by an ion-pairing mechanism. More and more analysts are showing interest in the use of ionic liquids as a stationary phase component in LC.[38]

9.5.4 Two-dimensional Liquid Chromatography

The two-dimensional LC technique is being applied increasingly often when it comes to dealing with highly complex matrices such as pharmaceuticals and environmental samples. In such a case, one-dimensional LC does not provide a complete resolution of the sample components, which necessitates multiple analyses, resulting in additional solvent generation, extended time and extra cost to obtain a full characterization of the sample. The development of multidimensional LC helped to achieve high resolution and increased separation space in a single run. It can be performed in two modes: heart cutting and comprehensive two-dimensional LC.[2]

Heart cutting is performed when only selected fraction(s) coming from the first-dimension LC system are directed to the second dimension, namely those which require additional separation. A six-port switching valve is used to change the flow from one column to another at a specific time.[2]

Comprehensive two-dimensional LC is applicable when many complex sample components need to be characterized. In this case, subsequent fractions of the entire effluent are directed from the first to the second dimension by an eight- or ten-port valve. It can be performed either off-line or on-line.[2]

The use of an LC×LC system provides several advantages, including prevention of sample contamination and/or sample losses, and it also creates

the opportunity for automation, which is highly desirable for routine analysis. On-line LC×LC can be considered green as the sample can be fully analysed in a single run. Additionally, less solvent is used and less time is needed for the analysis in comparison with off-line LC×LC. To make two-dimensional LC even greener, high temperatures and short columns can be utilized. Moreover, as in one-dimensional reversed-phase LC, acetonitrile and methanol are used owing to their low viscosity and low UV cutoff; they can easily be exchanged in the first LC system for more environmentally friendly solvents such as acetone or ethanol because there is no need for a low UV cut-off in the first-dimension separation. Nevertheless, to apply two-dimensional LC, complex instrumentation is necessary and data handling and optimization of the operating parameters of both LC systems are necessary to make them compatible with each other.[2]

9.6 Miniaturization in Chromatography

Miniaturization of analytical instruments is gaining popularity as a way to increase the greenness of both LC and GC. Application of miniaturized systems is usually related to reducing the number of consumables and energy consumption and also the volume of reagents needed to perform the analysis. Moreover, it leads to decreased waste production and costs, which means that it is not only more environmentally friendly but also cost-effective.[39] Furthermore, it is possible to improve the sensitivity of the method and shorten the time of a single analysis, which allows the use of miniaturized systems in both on-line and at-line analysis, which further improves the greenness of the method. Hence various miniaturized chromatographic systems (*e.g.* compact and portable chromatographs or micro-chromatographs, Figure 9.7) have found numerous applications.

One of the possibilities of miniaturization, as mentioned previously, is shortening the length and decreasing the diameter of the LC column. Since this makes it possible to reduce the flow rate of the mobile phase, the overall solvent consumption can be much lower than in the case of commonly used LC systems. In addition, this modification may lead to a lower sample volume requirement, which may be particularly useful in fields where obtaining larger samples may be difficult, *e.g.* in forensic and biomedical science. All of the advantages of decreasing the length and diameter of the LC column are the reason why this modification has also been applied in GC. The use of fast and ultra-fast GC systems permits the rapid determination of analytes even in complex matrixes, which makes them suitable for various industrial applications, *e.g.* spoilage assessment of food adulteration.[40,41]

Another approach that could be implemented is the ''lab-on-a-chip''. Even though the idea of GC-on-a-chip was first proposed in the 1970s, the first introduction of this method was made 20 years later. Since then, numerous efforts have been made to develop various chip-based systems.[42] Because of their small size, they are easily portable and cost-effective, as their use does not involve high solvent and energy consumption. For these reasons, it is not

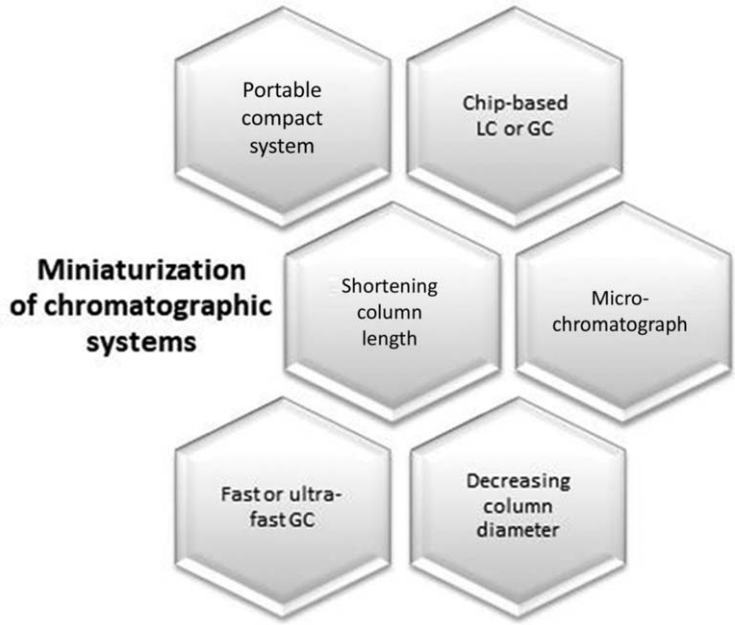


Figure 9.7 Possibilities of miniaturization of chromatographic systems.

only a notably environmentally friendly solution but also one that could and is being applied in various fields such as environmental analysis, biomedical and petrochemical science and even homeland security.[43] Moreover, there is a possibility that their mass production could be potentially cheap, which further increases their usability.[44]

9.7 Conclusions and Future Trends

Several ways to make chromatographic techniques more eco-friendly exist (Figure 9.8). These have the potential to make all the analysis steps greener, from sample collection through pretreatment to separation and final determination. The ideal chromatographic method would be performed in an in-line mode, without sample preparation; however, sample preparation prior to chromatographic analysis is needed in most cases. As a consequence, solvent-free or solventless miniaturized sample preparation techniques should be applied whenever possible to minimize solvent consumption. If this is not possible, it is recommended to use eco-friendly solvents, such as water or supercritical fluids.

Future progress in greening analytical chromatography is expected to be accomplished through miniaturization, shortening of the analysis time and improvement of the resolving power. It is believed that such progress will be made, as considering a literature survey on the state-of-the-art of green chromatographic techniques it can be deduced that there is general

Figure 9.8 Principles of green chromatography.

agreement between chromatographers and society on the need to avoid, or at least reduce, the deleterious side effects of the different steps of analytical procedures. Therefore, both researchers and manufacturers associated with analytical chemistry will strive to develop and adopt new avenues both for the education of new generations of chromatographers and for the revision of current practices in the chromatography laboratory. Efforts are being made to simplify, automate and miniaturize chromatographic systems.

It is obvious that additional developments must be introduced in order to move from the bench to the real world, and strong collaboration between equipment producers and method developers is needed in order to search for the incorporation of novel ideas in sample pretreatment and multiparametric analysis in addition to the real applicability of new and fast stationary phases and columns. In addition, efforts should be made to improve the information obtained from chromatograms by incorporating the recent advances in chemometrics. Such processing could help improve the information generated by chromatographic systems without increasing the consumption of chemicals and/or waste generation. Furthermore, additional attempts should be made to shift from large instruments to portable

systems, mainly regarding the portability of MS detectors. The introduction of a new generation of portable chromatographs would provide rapid information with a reduced impact on the environment. In addition, such instruments will impact on decreasing the side effects related to sample transport, preservation, storage and analytical sources of errors.

References

1. C. J. Welch, N. Wu, M. Biba, R. Hartman, T. Brkovic, X. Gong, R. Helmy, W. Schafer, J. Cuff, Z. Pirzada and L. Zhou, *TrAC, Trends Anal. Chem.*, 2010, **29**, 667.
2. J. Płotka, M. Tobiszewski, A. M. Sulej, M. Kupska, T. Górecki and J. Namieśnik, *J. Chromatogr. A*, 2013, **1307**, 1.
3. M. Tobiszewski, A. Mechlińska, B. Zygmunt and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2009, **28**, 943.
4. M. Tobiszewski and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2012, **35**, 67.
5. J. Teske and W. Engewald, *TrAC, Trends Anal. Chem.*, 2002, **21**, 584.
6. E. Hoh and K. Mastovska, *J. Chromatogr. A*, 2008, **1186**, 2.
7. C. J. Welch, N. Wu, M. Biba, R. Hartman, T. Brkovic, X. Gong, R. Helmy, W. Schafer, J. Cuff, Z. Pirzada and L. Zhou, *TrAC, Trends Anal. Chem.*, 2010, **29**, 667.
8. M. Tobiszewski, A. Mechlińska and J. Namieśnik, *Chem. Soc. Rev.*, 2010, **39**, 2869.
9. J. Ji, C. Deng, W. Shen and X. Zhang, *Talanta*, 2006, **69**, 894.
10. H. Lin, C. Deng and X. Zhang, *J. Sep. Sci.*, 2008, **31**, 3225.
11. I. K. Kiplagat, P. Kuban, P. Pelcova and V. Kuban, *J. Chromatogr. A*, 2010, **1217**, 5116.
12. A. J. Bednar, A. L. Russell, C. A. Hayes, W. T. Jones, P. Tackett, D. E. Splichal, T. Georgian, L. V. Parker, R. A. Kirgan and D. K. MacMillan, *Chemosphere*, 2012, **87**, 894.
13. H. Lin, Q. Ye, C. Deng and X. Zhang, *J. Chromatogr. A*, 2008, **1198**, 34.
14. J. D. Fair, W. F. Bailey, R. A. Felty, A. E. Gifford, B. Shultes and L. H. Volles, *J. Environ. Sci.*, 2009, **21**, 1005.
15. J. A. Dziuban, J. Mroz, M. Szczygielska, M. Małachowski, A. Gorecka-Drzazga, R. Walczak, W. Buła, D. Zalewski, J. Nieradko, J. Łysko, Koszur and P. Kowalski, *Sens. Actuators, A*, 2004, **115**, 318.
16. C. C. Chang and G. R. Her, *J. Chromatogr. A*, 2000, **893**, 169.
17. T. C. Chen and G. R. Her, *J. Chromatogr. A*, 2001, **927**, 229.
18. M. A. Brown and G. L. Emmert, *Anal. Chim. Acta*, 2006, **555**, 75.
19. P. S. Simone Jr., G. T. Anderson and G. L. Emmert, *Anal. Chim. Acta*, 2006, **570**, 259.
20. L. Zhou, Y. Zeng, P. D. Hazlett and V. Matherne, *Anal. Chim. Acta*, 2007, **596**, 156.
21. M. Van Deursen, *Novel Concepts for Fast Capillary Gas Chromatography*, Technische Universiteit Eindhoven, 2002.

22. J. Luong, R. Gras, R. Mustacich and H. Cortes, *J. Chromatogr. Sci.*, 2006, **44**, 253.
23. H. Shaaban, A. Mostafa and T. Górecki, Green Gas and Liquid Capillary Chromatography, in *The Application of Green Solvents in Separation Processes*, 2017, p. 453.
24. S. D. Stearns, H. Cai, J. A. Koehn, M. Brisbin, C. Cowles, C. Bishop, S. Puente and D. Ashworth, *J. Chromatogr. A*, 2010, **1217**, 4629.
25. M. R. Jacobs, E. F. Hilder and R. A. Shellie, *Anal. Chim. Acta*, 2013, **803**, 2.
26. B. Gilbert-López, J. A. Mendiola and E. Ibáñez, *TrAC, Trends Anal. Chem.*, 2017, **96**, 31.
27. (a) S. Armenta and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2016, **80**, 517; (b) Y. Liu and J. B. Philips, *J. Chromatogr. Sci.*, 1991, **29**, 227.
28. V. García-Cañas, C. Simó, M. Herrero, E. Ibáñez and A. Cifuentes, *Anal. Chem.*, 2012, **84**, 10150.
29. Y. Nolvachai, C. Kulsing and P. J. Marriott, *TrAC, Trends Anal. Chem.*, 2017, **96**, 124.
30. C. Cordero, J. Kiefl, P. Schieberle, S. E. Reichenbach and C. Bicchi, *Anal. Bioanal. Chem.*, 2015, **407**, 169.
31. A. Mostafa and T. Górecki, *Anal. Chem.*, 2016, **88**, 5414.
32. R. Malviya, V. Bansal, O. P. Pal and P. K. Sharma, *J. Global Pharma Technol*, 2010, **2**, 22.
33. L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley & Sons, Inc., 1979, p. 34.
34. H. Shaaban and T. Górecki, *Talanta*, 2015, **132**, 739.
35. R. M. Smith and R. J. Burgess, *J. Chromatogr. A*, 1997, **785**, 49.
36. M. Toribio, M. J. del Nozal, J. L. Bernal, J. J. Jimenez and M. L. Serna, *J. Chromatogr. A*, 1998, **823**, 163.
37. S. Pereira Ados, A. J. Giron, E. Admasu and P. Sandra, *J. Sep. Sci.*, 2010, **33**, 834.
38. M. Zhang, X. Liang, S. Jiang and H. Qiu, *TrAC, Trends Anal. Chem.*, 2014, **53**, 60.
39. A. Rios, A. Escarpa and B. Simonet, *Miniaturization of Analytical Systems: Principles, Designs and Applications*, 2009.
40. A. Różańska, T. Dymerski and J. Namieśnik, *Monatsh. f. Chem.*, 2018, **149**, 1615.
41. W. Wojnowski, T. Majchrzak, T. Dymerski, J. Gębicki and J. Namieśnik, *Monatsh. f. Chem.*, 2017, **148**, 1631.
42. K. D. Bartle and P. Myers, *History of Gas Chromatography*, 2002.
43. F. Haghighi, Z. Talebpour and A. Sanati-Nezhad, *Lab Chip*, 2015, **15**, 2559.
44. Y. I. Yashin and A. Y. Yashin, *J. Anal. Chem.*, 2001, **56**, 902.

CHAPTER 10

Chemometrics as a Green Analytical Tool

KANET WONGRAVEE,*[a] MIKA ISHIGAKI[b,c] AND YUKIHIRO OZAKI*[d]

[a] Sensor Research Unit (SRU), Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand; [b] Raman Project Center for Medical and Biological Applications, Shimane University, 1060 Nishikawatsu, Matsue, Shimane 690-8504, Japan; [c] Faculty of Life and Environmental Sciences, Shimane University, 1060 Nishikawatsu, Matsue, Shimane 690-8504, Japan; [d] School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda, Hyogo 669-1337, Japan
*Emails: kanet.w@chula.ac.th; ozaki@kwansei.ac.jp

10.1 Introduction

In past centuries, chemistry and chemists have made great contributions to and have documented many things that have been important parts of human history. The early alchemists conceived many developments based on studies of materials and their properties and reactions that changed civilization for a better quality of life in many cases. Even though most of the developments might seem to be simple by today's standards, at that time they were groundbreaking discoveries and inventions. Beginning with early chemical manufacturing, it incorporated simple chemical reactions to produce enormous amounts of products in a short period of time to serve customers, an example being daily products such as soaps, shampoos and detergents. Dye production contributed significantly to the creation of paints, fashion and art over the centuries. However, some chemical reactions

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

were regarded as ''black magic'' at that time because the fundamentals of chemistry were still unclear. Thanks to the development of theory in chemistry by many pre-eminent chemists, the usefulness and importance of chemistry became clear and more and more people changed their minds to pursue these endeavors. This dramatically increased the pace of new discoveries and applications. The production of different types of plastics altered people's lifestyles and also replaced traditional products in various areas (*e.g.* vehicle materials and electronic devices). Antibiotics were hot commodities that were widely used in therapeutics. The use of pesticides, including DDT, in agricultural manufacturing became popular as it can efficiently increase production and also improve product quality. Freons and other chlorofluorocarbons (CFCs) were typically introduced for use in refrigerants, air conditioners and aerosol propellants as they are stable, nonflammable, moderately toxic gases or liquids. Tetraethyllead (TEL) was added to gasoline in order to prevent ''knocking'' in combustion engines. These are only a few examples of the great discoveries in chemistry that changed human life.

In the past, the above-mentioned products were developed to serve and facilitate a better life for humans. However, the negative impacts on the ecosystem and the environment of these substances were not carefully considered and analyzed at that time. Plastics are very useful substances, but their degradation might take thousands of years. During the degradation process, plastics break down into smaller pieces that contaminate soil and water, entering the food chain and causing the deaths of over 100 000 marine animals each year. Residual pesticides can be dissolved in water and soil, which distorts the genetic diversity (fertility and neuron systems), and accumulate up the food chain in wildlife. Freons and CFCs have serious effects on the ozone layers that protect the Earth from harmful ultraviolet radiation. The coal industry spews pollutants into the atmosphere, contaminates rivers and produces millions of tons of toxic solid waste products that are among the primary causes of climate change and the global warming problem. All of these potential disasters initiated a different type of thinking among chemists and the general population. Now it is not just about chemistry; we must seriously consider the impacts on the environment and ecosystems in what is called *green chemistry.*[1–11]

The term green chemistry emerged from the prevention of pollution that was endorsed in 1990 in the United States. The US Environmental Protection Agency (EPA) defines green chemistry as the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances (https://www.epa.gov/greenchemistry). Green chemistry has recently been applied across the life cycle of chemical products, including chemical production, manufacture, design and ultimate disposal. In the early years of green chemistry, it was mainly oriented towards the planning of chemical synthesis methods and emphasized the product and the process used to produce it. This protocol should conform to the basic rules of sustainability. Nowadays, the applications of green chemistry concepts have

been gradually extended and are now widespread over all fields.[1] In 1998, Anastas and Warner[2] established the ''Twelve Principles of Green Chemistry'', with some being directly connected with analytical chemistry, such as using safer solvents and reagents, prevention of waste, energy and labor efficiency, renewability, reducing by-products, real-time analysis and prevention of accidents through the implementation of safer chemistry. These principles for analytical chemistry mainly relate to the creation of environmentally friendly methods and the development of powerful tools and instruments for minimization of the number of samples required, reduction of power and waste, using green reagents and reduction of energy, time and labor. These developments should have no effects on accuracy, sensitivity and reproducibility. In recent years, the subject has been discussed on the basis of the concept of *green analytical chemistry* (GAC), which has increasingly attracted attention in numerous papers,[3–5] books[6–8] and reviews.[9–11]

One of the keys to successfully meeting the principles of GAC is to develop the instruments, methods and protocols in such a way that they are faster and less labor intensive and reduce energy without increasing the cost of analysis and requiring fewer and smaller volumes of reagents and solvents.[10,12] In recent years, the power of computers and the performance of instruments have increased dramatically. These developments, together with other factors, provide new opportunities and challenges to chemists in research and development. Nowadays, the field of analytical chemistry is currently facing major changes that involve research on complex chemical systems. To study complex systems involving the presence of multiple coexisting factors, the application of statistical and mathematical techniques is often required and this is usually confined to studies in modern analytical chemistry.[13–15] In this development, new methodologies integrated with statistics, computation and mathematics are also employed to provide in-depth and broad-range analysis. This helps scientists to understand and interpret easily such complex systems, which might contain a huge amount of acquired data. In the past, the main problem facing analytical chemists was how to obtain good data containing a lot of information about the system. This involved labor-intensive measurements that were very tedious, expensive and time consuming. Furthermore, there were problems in preparing materials, a lack of high-performance techniques and inefficient instrumentation and technical support. Chemists in the early days aimed to extract as much information as possible about the composition, structure and other properties of the systems under routine investigation. After measurement, the collected data were often treated by different signal processing techniques to obtain high-quality data, eliminate irrelevant data and extract the maximum amount of meaningful information. However, it was not very easy to study or interpret the huge amounts of data generated directly. Therefore, powerful mathematical and statistical methods were needed to evaluate the results and extract and interpret information.

With the rapid development of advanced computer hardware and software, including the mobility of electronic instruments, several trends have

driven research towards new detection features such as miniaturization, automation, simplification and acceleration, which in turn allowed fast detection methods to be applied with the use of minimal resources. Implementation of these trends in analytical methods has usually not only provided enhanced analytical characteristics, but also significantly improved the greenness profiles. Data treatment and data interpretation in, for instance, spectroscopic and chromatographic studies are a part of the interdisciplinary science known as c*hemometrics*.[16–22]

10.2 Brief History of Chemometrics in Green Chemistry and Its Application to Green Analytical Chemistry

The origin of chemometrics can be traced back to the early 1960s, when computing became generally accessible. In the 1960s, Norris tried to use statistical methods to extract information about the quality of agricultural products from their near-infrared (NIR) spectra.[15] In the 1970s, the groups of Malinowski, Jurs and Massart published their work based on applications of statistical and mathematical techniques to complex chemical problems, which we now recognize as chemometrics.[17–20] However, the term *chemometrics* was invented by the Swedish scientist Svante Wold, who published the first paper using the word ''chemometrics'' in 1972.[21] He joined with Bruce Kowalski in 1974 to create the International Chemometrics Society[22] and defined the term chemometrics as follows: ''Chemometrics is the science of relating measurements made on a chemical system or process to the state of the system *via* application of mathematical or statistical methods''. In the 1980s, chemometrics as a discipline became organized with the inception of the journals *Chemometrics and Intelligent Laboratory Systems* (Elsevier) and *Journal of Chemometrics* (Wiley), meetings, conferences, books, societies, courses and program packages (*e.g.* UNSCRAMBLER, SIMCA, CAMO). The International Conference on Chemometrics in Analytical Chemistry (ICCAC Holland) was first held in Petten, The Netherlands (15–17 September, 1978), and was the first conference to have the word chemometrics in its title.[23,24] The conference still continues today with increasing numbers of participants. The breakthrough in chemometrics came at the beginning of the twenty-first century with the availability of ultrafast personal and clustering computers that not only process fast calculations but also provide an enormous storage space with easy accessibility.[25] The various software development companies have promoted equipment and tool intellectualization and they have also offered new methods for the construction of new and high-dimensional hyphenated equipment. These developments have driven the applications of chemometrics to other informatics fields such as genomics, metabolomics, bioinformatics and chemoinformatics.[16,25] Nowadays, the high-speed Internet is easy to access everywhere in order to search, download and remotely control even *via*

wireless technologies such as mobile phones and tablets. In the era of the Internet, big data and machine learning play important roles in our daily activities, and produce a massive amount of data in the form of documents, images, videos and media uploads. The large amounts of collected data can be securely stored in thousands of cloud servers. Therefore, sophisticated software tools with powerful mathematical and statistical algorithms must be developed to analyze and visualize these large amounts of data in real time. A brief history of the origination and applications of chemometrics is illustrated in Figure 10.1.

With the increase in the use of computing and communications technology in chemical data analysis, especially in information/data handling, process control and sensors, a large variety of chemometrics methods have been introduced in order to meet the principles of GAC such as to reduce the number of steps in chemical analysis, reduce the number and volume of reagents involved, consume less energy, be less labor intensive and decrease the resources used. These aims can be achieved by optimization, automation and robotization. Chemometrics has been increasingly mentioned in the literature relating to GAC and Figure 10.2 shows the evolution of publications devoted to GAC combined with chemometric techniques during 2000–2018 found using the search keywords ''green'' or ''greener'' or ''clean'' or ''cleanser'' or ''environmentally friendly'' and ''chemometrics''.

At present, there is dramatically increasing interest in the implementation of chemometric techniques in developing green analytical methods. This reflects the large increase in the number of publications based on the routine application of existing chemometric methods in major journals such as *Analytical Chemistry*, *Analytical Methods*, *Talanta*, *Food Chemistry* and *Journal of Agricultural and Food Chemistry*. From a literature search, there is no doubt that chemometrics is currently a predominantly important tool in achieving the aforementioned developments in GAC, especially in chemistry, food science, agricultural engineering and biochemistry, hence being an indirect way of saving time, labor, energy and reagents by implementing automated analysis. Significant savings can also be obtained by data analysis and visualization of large datasets to simplify and assist in reaching concise conclusions. Generally, the measurement results can be obtained by various techniques such as spectroscopic, chromatographic and electrophoretic methods, hyperspectral cameras and smartphones. These can be defined as a characteristic profile reflecting the complex chemical compositions of analyzed sample, patterns and fingerprints. Chemometrics helps us to extract significant information with a smaller number of experiments and prevent errors in an environmentally friendly way. The methodology based on chemometric approaches, including design of experiment (DOE),[26–28] signal preprocessing,[29] exploring data,[28–30] optimization,[28–30] calibration,[28–30] pattern recognition[28–30] and artificial neural networks,[31] is a powerful tool for analyzing and interpreting complex data in a simple way without using trial-and-error approaches.

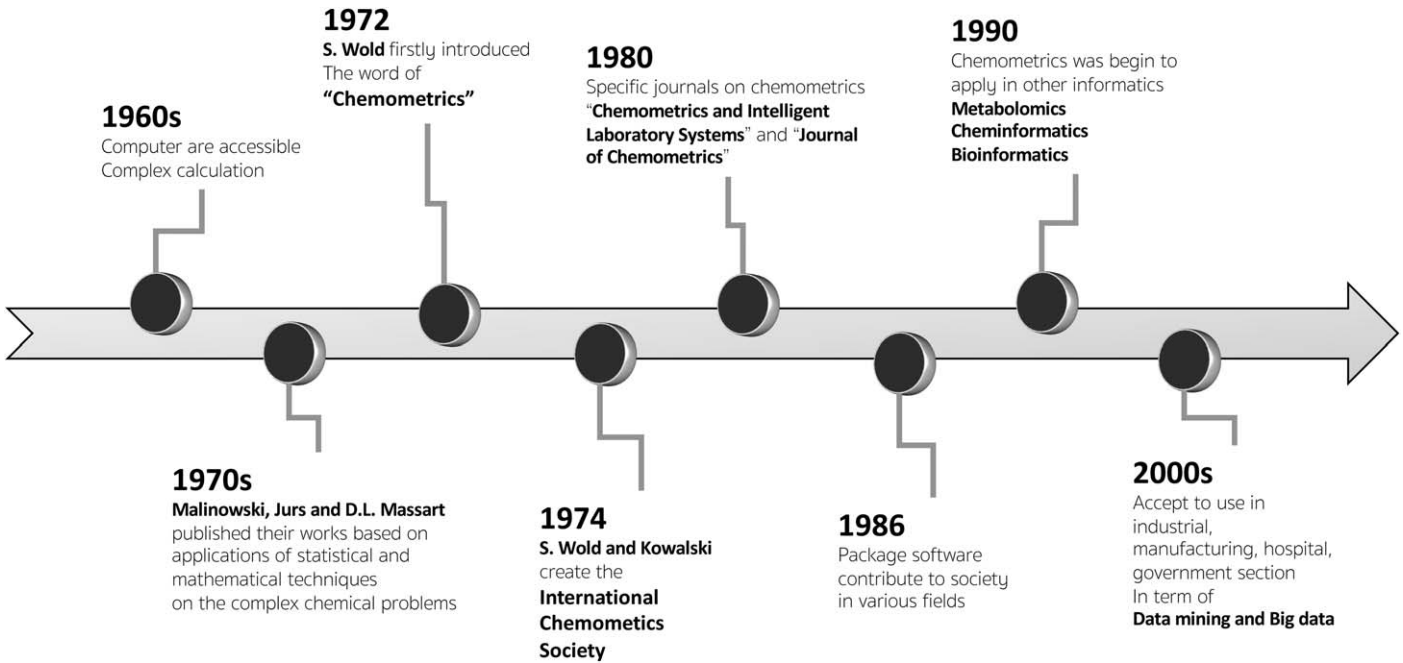


Figure 10.1 Progress and timeline of the development of chemometrics approaches in chemistry. More information on the history of chemometrics can be found in a review by Brereton.[16]

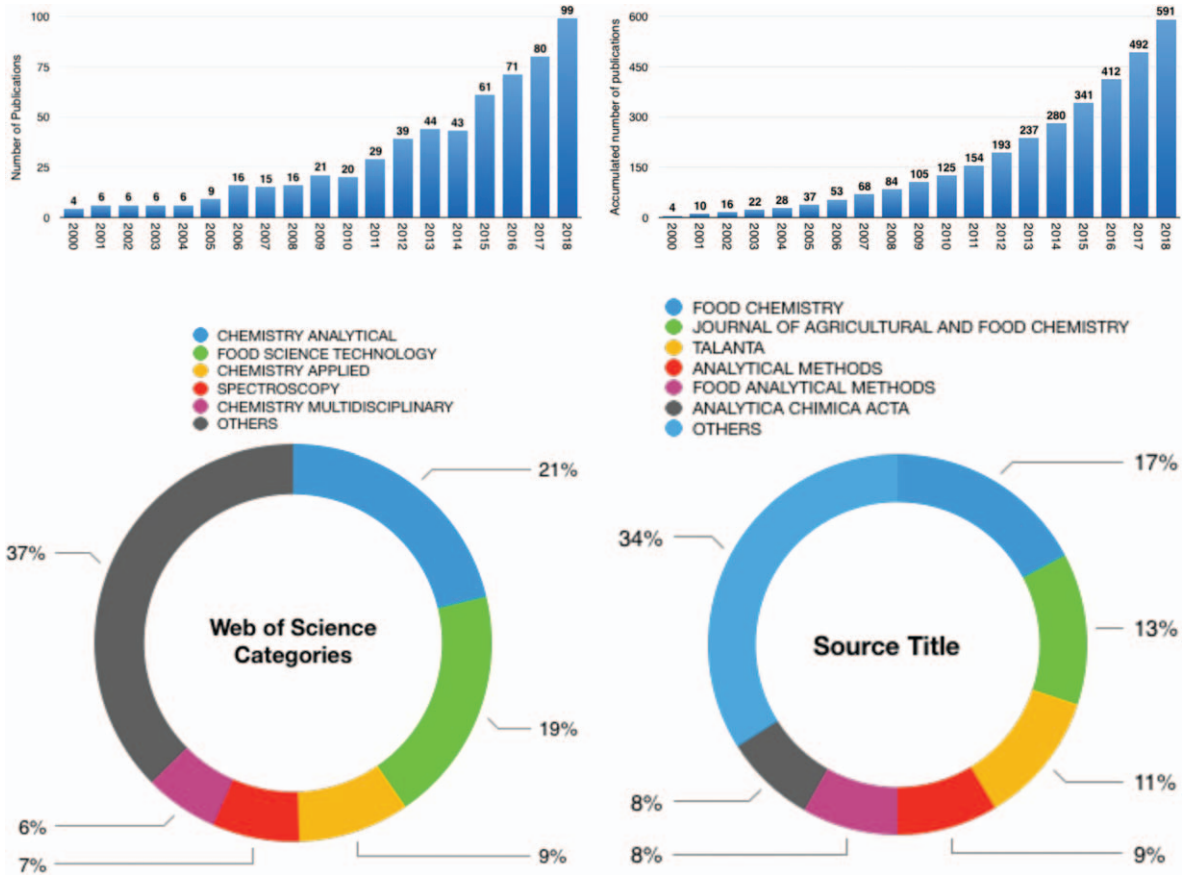


Figure 10.2 Bar graphs showing the evolution of publications devoted to the combination of chemometrics and green chemistry and circle graphs representing the literature sources from 2000 to 2018 using the keywords ''green'' or ''greener'' or ''clean'' or ''cleanser'' or ''environmentally friendly'' and ''chemometrics'', from ISI Web of Knowledge (Web of Science) – Thomson Reuters.

The combination of chemometric and analytical techniques to produce efficient and powerful methods according to the principles of green chemistry has become an important challenge in the last few decades. A large variety of chemometric methods have been applied in various fields, *e.g.* analytical chemistry, food chemistry, agricultural chemistry, spectroscopy and biological informatics. Figure 10.3 presents an overview of applying chemometrics approaches throughout the flow of information from the beginning to the end of the analysis process.

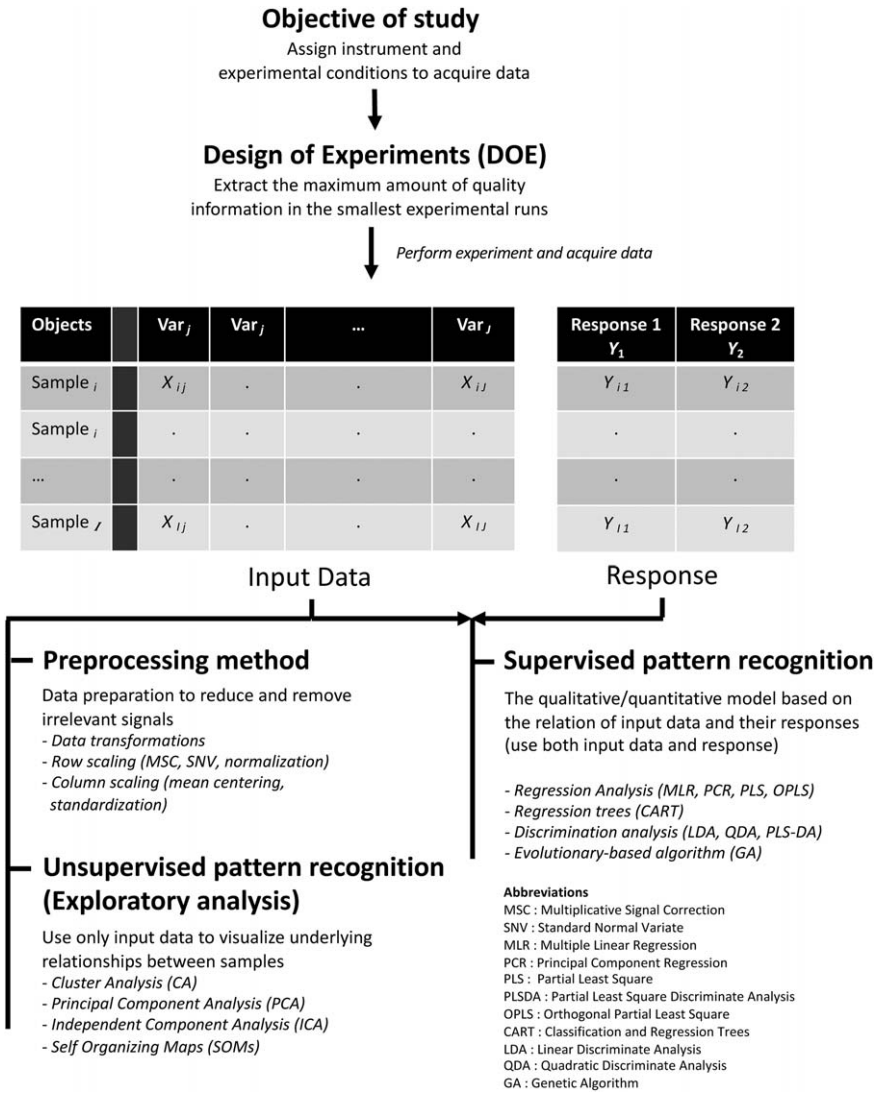


Figure 10.3 Generic chemometrics approaches as green chemical tools for data analysis.

Before performing the experiment, the strategy of design of experiment (DOE) should be applied in order to obtain the maximum amount of high-quality information with the smallest number of experimental runs.[32] This allows the evaluation of a large number of parameters in a relatively small number of experiments using statistical and mathematical approaches. Performing DOE in research minimizes the cost of operation without decreasing the information quality, quantifies the model and optimizes the experimental process. After the acquired data have been generated *via* the DOE strategy, the treatment of acquired analytical signals, often called ''pre-processing'', is first introduced. This pre-processing method should be considered as an important step in the overall strategy of data analysis. This step of data preparation can reduce and remove undesired perturbations in the signals by data cleaning, outlier detection, transformation, scaling and normalization. Second, the prepared data are projected to the calculation, which is often called ''unsupervised pattern recognition'' or ''exploratory analysis'', to reveal the main patterns or groups of samples in the data and also indicate outliers. The underlying relationship between samples is visualized with no further information such as response, class information or concentration required. In the experiment, the relationships between samples and the corresponding responses are important and crucial. The response can be recorded in terms of concentration, peak intensity or class member. Supervised pattern recognition is involved here to find the qualitative/quantitative models used in a variety of applications such as prediction, calibration, classification and regression.

Although chemometrics is a very powerful technique, it should be noted that it cannot operate on its own and cannot replace the knowledge of analytical chemists or scientists. It is useful to integrate this knowledge with these tools that allow a researcher to take full advantage of the modern analytical solutions available. In this perspective, chemometrics will continue to grow as long as it supports solutions to aid the community of chemists in their tasks, both in academia and in industry.

To assess green profiles, chemometrics could be used as an alternative tool to chemical data processing instead of using sample pretreatment and perform an additional set of experiments. This allows us to use much simpler measurement processes and obtain meaningful results in a very short analysis time. With the viability of software and also the availability of training courses for both commercial and academic users, not only chemometricians but also non-specialists can enjoy the advantages offered by the chemometrics approach in basic and applied research. The use of multivariate methods based on chemometrics has been endorsed and increased across broard ranges of scientific disciplines, as outlined in Table 10.1. The list of literature was extracted from the search results using the keywords mentioned earlier (for the period 2000–2018). Because it was not possible to include all references in the table, the representative articles cited are just examples that were selected based on the variety of the research and number of citations.

In general, chemometric methods are developed in order to prevent biased analysis by humans that might originate from their particular expertise, experiences and culture. To analyze complex data, a skilled person is required, in addition to hard work and extensive training, which might be specific for only a few analysis objectives. This means that it is not possible to prepare, train and provide such individuals across all research objectives. To avoid these problems, a multivariate statistical model can be used in a standard protocol in the form of programs and software to analyze such complex data. Since the model has been proven and satisfactory results have been obtained, it is easy to distribute it across the world. This might reduce the enormous costs originating from labor, analysis time and chemical tests. The valid software can run continuously for 24 h per day, 7 days per week to interpret enormous amounts of data without requiring assistance any from experts and specialists. Moreover, the programs are flexible and easy to modify for different objectives, which might not be possible for a human.

It can be seen that chemometrics has been used in different ways depending on the research objective and the acquired data. A relatively high proportion of research papers relate to the possibility of developing new applications using chemometrics in food science and technology.[33–39] In this case, chemometric methods are developed to extract useful information from complex data and provide qualitative/quantitative models to access and evaluate the food quality aspects, including authenticity, adulteration, food safety, contamination and freshness. These methods are potential analytical tools for further use in different industries and inspection agencies for the investigation and characterization of samples during the process of controlling and inspecting the quality of food products. In agricultural science and technology,[34,39–44] the perspective of chemometrics as an analytical tool to authenticate and classify agricultural products according to their geographic origins and other aspects, including planting season, sensory composition and quality, has been proposed. Owing to worldwide international trade, knowledge of the origin of agricultural products is nowadays essential for import and export trading to validate trackability for customers, traders and producers. Products from different origins have distinct qualities that may have consequences regarding different tastes, nutritional quality and prices. Moreover, the relationship between the planting environment, *e.g.* minerals, soils, fertilizers, humidity, and the characteristics of the products is of interest. Therefore, information about product origins is essential for verifying specifications and guaranteeing quality and efficacy.

In environmental research,[45–52] the process of collecting samples is complicated and difficult to control. Gaining insightful information might involve an enormous number of samples, and environmental data are mostly complex and contain several unpredictable factors with high variability. To interpret such complex data is extremely difficult. To extract and decipher the relationships within the data, multivariate analysis is required. It is possible to generate a model that can be used to analyze different environmental samples by remote detection with satisfactory results. Another

Table 10.1 Examples of applications of chemometrics in green analytical chemistry.[a]

Input data	Sample	Purpose	Chemometrics	Ref.
GCO	Cheese	Classify type of Ragusano cheese and build a sensory model	PCA, SIMCA, PLSDA	35
HPLC, GC, ICP-AES	Coffee	Discriminate geological growing areas of Arabica coffee	PCA, LDA	124
NIR	Olive oil	Classification and quantification of the adulteration of pure olive oils	MSC, PCA, SG, PLS	37
LC–UV	Coffee	Botanical and geographical characterization of green coffee	LDA, PLSDA	33
Raman	Coffee	Discriminate Arabica and Robusta green coffee	PCA	38
HPLC–DAD	Ginger	Discriminate geographical origins of ginger (*Zingiber officinale*)	HCA, PCA, LDA	125
MS, eNose, VIS-NIR	Wine	Measure sensory scores attributed in commercial Riesling wines from Australia	PLS	36
ICP-MS	Coffee	Control the authenticity of organic coffee	MLP, SVM, NB	126
VIS-NIR	Milk tea	Discrimination of variety of instant milk teas	BPNN, PLS, PCA, LS-SVM	41
UV–VIS	Sweeteners	Determination of the three different artificial sweeteners	PLS, PCR, CLS	42
GC–MS	Coffee	Discrimination and identify volatile compounds as markers for elephant dung coffee	CA, PCA, PLS	43
NIR	Honey	Determination of Chinese honey adulterated with high-fructose syrup	MSC, PLSD	44
Portable Raman	Vegetable juice	Determination of lycopene contents	PMIR	127
FT-IR microscopy	Potato chips	Determination and screening the acrylamide content in potato chips	PLSR	128
Raman	Tomato	Quantitative analysis of carotenoid contents	PLSR	108
LC–MS	Eggplant	Evaluation of eggplant genotype related to the total phenolic, flavonoid content and antioxidant capacity of 34 eggplant genotypes	CA, PCA	40
NIR	*Rosmarini folium*	Determination of the rosmarinic acid (RA) in dried and powdered *Rosmarini folium* (rosemary leaf)	PLSR	129

Table 10.1 (*Continued*)

Input data	Sample	Purpose	Chemometrics	Ref.
TD-NMR	Sealed package	Prediction of the total fat content in sealed packages	PCA, PLS	48
ATR-FTIR	Sediments	Quantitative determination of polyphosphate	PLS	51
GC–MS	Sediments	Qualitative and quantitative analysis of pollution [polycyclic aromatic hydrocarbons (PAHs)] in surface sediments collected from different areas	CA, PCA, MLR	50
LC	Pharmaceuticals	Determination of nine beta-blockers in river water	MCR–ALS	52
LC	Antibiotics	Analysis and quantification of six antibiotics in wastewater	MCR-ALS, U-PLS/RBL	47
% recovery	Waste Ag	Optimize the conditions to recover pure silver microcrystals from industry and laboratory wastes	CCD (DOE)	45
% recovery	As(III), As(IV)	Optimize the condition for coprecipitation conditions of As(III) and As(IV) from wastewater	CCD (DOE)	46
NIR	Soil	Prediction of heavy metal (Zn, Cu, Pb, Cr and Ni) contamination in soils	PLSR	49
Database	Organic solvents	Grouping of organic solvents according to their physicochemical, toxicological and hazard parameters	CA, PCA	68
Raman	Tissue	Discriminate and identify early-stage esophageal cancer	PCA, LDA, SOM, PLSR	54
NIR imaging	Fish eggs	Related molecular contents of egg growth	PCA, LDA	59, 60
Raman	Fish eggs	Discrimination of fish egg quality and viability	PCA, LDA	61
CE	Cabamazepine	Quantitation of carbamazepine in human serum	MCR-ALS	130
Raman	Tissue	Visualize the cancer region on the target tissue	PCA, PLS, ICA	53
GC–MS	Sweat, blood, urine	Discriminate groups of samples (age, stress and diet) using a large metabolite dataset	PCA, PLSDA	85

Table 10.1 (*Continued*)

Input data	Sample	Purpose	Chemometrics	Ref.
UV–VIS, SEM	Silver microplates	Obtain insight information on the shape evolution of silver nanoparticles induced by H_2O_2	OPA, SIMPLISMA, MCR-ALS	131

[a]GCO: Gas Chromatography Olfactometry, ICP-AES: Inductively Coupled Plasma Atomic Emission Spectroscopy, TD-NMR: Time Domain Nuclear Magnetic Resonance, MS: Mass Spectrometry, CE: Capillary Electrophoresis, MLP: Multilayer Perceptron, SVM: Support Vector Machine, NB: Naÿve Bayes, BPNN: Back Propagation Neural Network, LS-SVM: Least-Squares Support Vector Machines, CLS: Classical Least Squares Regression, PMIR: Product of Mean-Intensity Ratio, PLSR: Partial Least Squares Regression MCR-ALS: Multiple Curve Resolution Alternating Least Squares, U-PLS/BRL: Unfolded Partial Least Squares/Residual Bilinearization OPA: Orthogonal Projection Approach, SIMPLISMA: SIMPLe-to-use Interactive Self-modeling Mixture Analysis.

application is to optimize the factors for removing toxic substances from the environment (soil, water, air).[45,46] Furthermore, chemometrics combined with spectroscopic techniques [*e.g.* Raman, Fourier transform infrared (FT-IR), FT-NIR and hyperspectral cameras] is a powerful protocol that can be used to detect abnormal cells such as cancer cells.[53–55] As these are non-invasive analytical methods, they have been used extensively to assess molecular information about living organisms.[53–62] Ishigaki and co-workers[56–61] employed NIR imaging with chemometrics to monitor *in vivo* at the molecular level the development of the fertilized eggs of the medaka fish (Japanese rice fish, *Oryzias latipes*) over the period of embryonic body growth.[56–61] Other examples of applications of spectroscopy–chemometrics research are presented in Section 10.6.

Chemometrics is basically used to help researchers in terms of data analysis, automation and information extraction and can be further developed as software and installed in devices. In particular, data are obtained by measuring the spectra of samples with advanced instruments in a research laboratory and then the acquired data are exported to a computer with suitable software either to extract information or to develop a quantitative model. This protocol involves intensive built-in programming for very complex data; however, commercial software can also often be used. After validating the model, the detection procedure and developed software can be organized and installed in the instruments for real-time analysis, on-site analysis and automated analysis. Chemometrics calculations can be implemented with available programs, *e.g.* MATLAB (www.mathworks.com) and R-studio (www.rstudio.com). These might be a stand-alone program that can be installed and used for each personal computer separately. Therefore, to have several users, the developed program has to be distributed to the users who need to purchase the same program as the developer in order to use it. Moreover, the capacity of the instrument to generate and store the enormous volumes of data is limited. Most multivariate models are based on the defining model which is valid at the time, but it needs to be modified as time goes on. These are a kind of impact limitation of the

''progressing time window'' research to be applied in a practical way as the data may be ever-increasing, generated every day, and the developed model might not be valid when time passes quickly. Nowadays, there is an enormous amount of research and scientific data available on the Internet and cloud systems and sometimes they are free to access and use. This encourages the field of chemometrics to grow out of efforts to develop a toolbox of statistical and computer applications for processing, analyzing and visualizing the available big data, which is sometimes called *big data analysis*.[17,63–66] Some sort of statistical and mathematical technique, such as a machine learning algorithm, which can update and adapt itself (self-learning approximation) for better output without any external input, is needed in order to extract the essence in the data. This involves intensive programming and calculations combined with a search algorithm in a cloud system to reach the greenest profile of analytical chemistry because it does not involve any chemicals, experiments, waste or labor to accomplish the goal of research.[25] Figure 10.4 shows the data acquisition, process step and the role of chemometrics in recent research. It can be seen that the important change is how to obtain the data. In big data analysis, experiments and a wet laboratory are not always necessary. The significant information can be collected using an efficient search engine and powerful keywords.[25]

In a good example, Tobiszewski and co-workers[67,68] reported the application of chemometrics to big data analysis related to green chemistry. The solvent selection guide (SSG)[69–73] was developed to choose harmless solvents for green chemistry and GAC. SSG was prepared with 151 solvents (from different chemical classes, including aliphatics, aromatics, alcohols, aldehydes, ketones, ethers, esters, organic acids, chlorinated solvents, water, *etc.*) which were assessed. The significant parameters of the solvents, such as toxicological endpoints, physicochemical factors, environmental persistence, safety parameters and possibility to manage after use, were considered as the input data. All information was collected from the reported SSGs,[71] GSK (GlaxoSmithKline)[72,73] and American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable (ACS GCI-PR).[69,70] Some parameters that were not numbers were translated into numbers. After the data matrix had been prepared, the pre-processing step including auto-scaling was used to normalize and scale the real data values in a similar range. Cluster analysis (CA)[74] was performed by using the squared Euclidean distance and means of Ward's mode of analysis to calculate the distance between pairs of solvents and linkages, respectively. After CA, the solvents were organized into groups based on their similarity. This is represented as a dendrogram (Figure 10.5). It is clearly seen that three very defined groups, namely a cluster of non-polar and volatile solvents, a cluster of non-polar and sparingly volatile solvents and a cluster of polar solvents, were formed. Then, the ranking method based on TOPSIS (Technique for Order of Preference by Similarity to Ideal Solution)[75] was performed on each cluster separately to rank the solvents. The solvents with a high confidence ranking

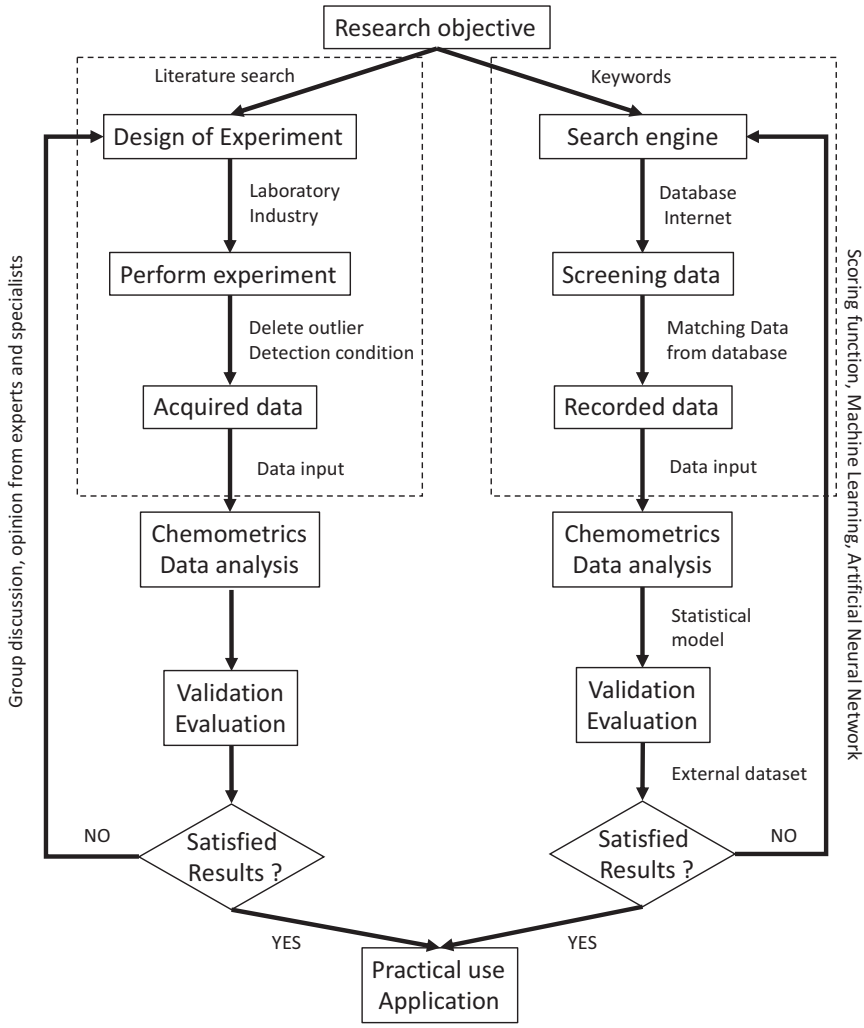
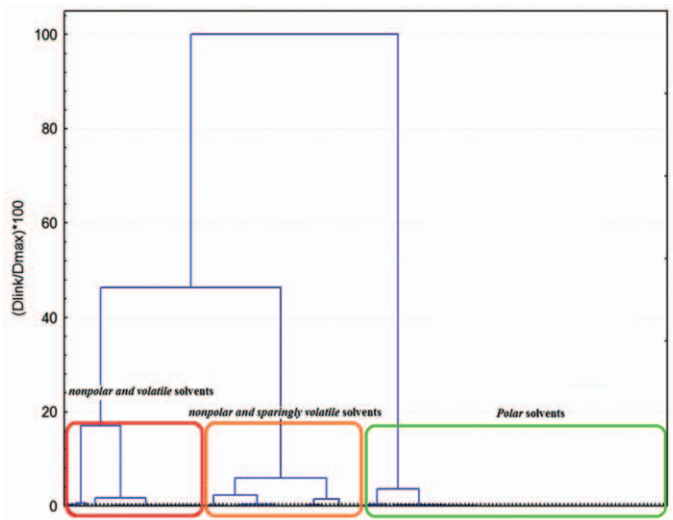


Figure 10.4 Scheme of process steps in applying a chemometric method developed for recent research.

were considered as preferred/usable solvents, whereas the solvents with a low confidence ranking were defined as banned/undesirable solvents.

In this chapter, the basic principles of data complexity and the most commonly used chemometric methods for data analysis, including DOE, signal pre-processing, unsupervised pattern recognition and supervised pattern recognition, with their brief principles and algorithms, are presented. The aspects have been simplified for readers who will likely come from different backgrounds in order to be able to follow and understand the concepts and algorithms of the methods. To obtain more details of the methods, comprehensive references have been added to allow readers to



Solvent	The highest confidence ranking	High confidence ranking	Medium confidence ranking	Low confidence ranking	Comparison with other SSGs			
					Pfizer[6]	GCI-PR[11]	GlaxoSmithKline[10]	AstraZeneca[7]
Diethyl ether	0.556	0.499	0.221	0.273	Undesirable	Undesirable	Undesirable	Undesirable
1,1,1-Trichloroethane	0.440	0.307	0.384	0.610	—	—	—	—
Pentane	0.432	0.374	0.271	0.317	Undesirable	—	Undesirable	—
1-Hexene	0.374	0.309	0.297	0.276	—	—	—	—
Cyclohexane	0.373	0.301	0.313	0.225	Usable	Undesirable	Usable	Undesirable
m-Xylene	0.367	0.276	0.302	0.413	Usable	Usable	Usable	Usable
Tetrachloroethene	0.361	0.230	0.274	0.452	—	—	—	—
p-Xylene	0.356	0.275	0.279	0.413	Usable	Usable	Usable	Usable
o-Xylene	0.348	0.264	0.294	0.415	Usable	Usable	Usable	Usable
Dichloromethane	0.346	0.244	0.217	0.318	Undesirable	—	Undesirable	—
Chloroform	0.342	0.233	0.215	0.346	Undesirable	Undesirable	Undesirable	—
Toluene	0.320	0.252	0.269	0.331	Usable	Undesirable	Usable	Undesirable
Trichloroethene	0.294	0.180	0.224	0.343	—	—	—	—
Benzene	0.292	0.202	0.203	0.263	Undesirable	Undesirable	Undesirable	—
1,1-Dichloroethene	0.285	0.191	0.176	0.208	—	—	—	—
Pentachloroethane	—	0.364	0.442	0.720	—	—	—	—
Furan	—	0.355	0.518	0.449	—	—	—	—
Chlorobenzene	—	0.258	0.260	0.380	—	Usable	Preferred	Undesirable
Styrene	—	0.250	0.304	0.426	—	—	—	—
1,3-Dichloropropene	—	0.239	0.268	0.401	—	—	—	—
trans-1,2-Dichloroethene	—	0.219	0.219	0.300	—	—	—	—
Carbon disulphide	—	0.202	0.200	0.246	—	—	Undesirable	—
1,2-Dichloroethane	—	0.185	0.236	0.374	—	Undesirable	Undesirable	—
Carbon tetrachloride	—	0.180	0.213	0.348	Undesirable	—	Undesirable	—
Cyclopentane	—	—	0.305	0.254	—	—	—	—
1 112-Tetrachloroethane	—	—	0.254	0.414	—	—	—	—
Dimethyl sulphide	—	—	0.253	0.355	—	—	—	—
Methoxycyclopentane	—	—	0.250	0.348	—	—	Usable	—
1-Chloropropane	—	—	0.206	0.247	—	—	—	—
1,1-Dichloroethane	—	—	0.197	0.255	Undesirable	—	—	—
1-Chlorobutane	—	—	0.184	0.209	—	—	—	—
Dioxolane	—	—	—	0.420	—	—	—	—
cis-1,2-Dichloroethene	—	—	—	0.287	—	—	—	—
1-Pentene	—	—	—	0.263	—	—	—	—

Figure 10.5 Left: clustering of solvents based on their physicochemical properties. Right: ranking of non-polar and volatile solvents (cluster 1, red square on the left diagram) within different confidence levels.
Reproduced from ref. 67 and 68 with permission from the Royal Society of Chemistry.

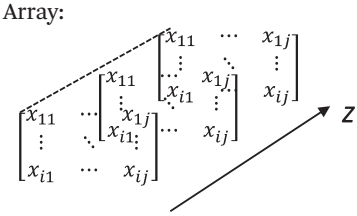
explore certain features further. At the end of chapter, the existing applications of chemometrics in chemical sensors and biomedical diagnosis are discussed.

10.3 Complexity of Datasets

Data analysis using chemometrics involves the use of mathematical models that relate multivariate instrumental signals to the response of samples, *e.g.* concentrations or sample properties. There are different ways to organize the data acquired from the instruments. The dimensions of the data are the first and the most important factor used to consider the appropriate analysis strategies. Zeroth-order data involve only the records of a single instrumental response per sample, whereas higher order data are acquired with multiple signals for each sample. A zeroth-order dataset is generated with detection by instruments that produce only a single response per sample. It can be in the form of the absorbance of UV–VIS or fluorescence emission at a single wavelength (usually as absorbance at λ_{max}). This type of data can be used for the basic calibration curve and a threshold for a test (*e.g.* police test in roadside alcohol breath testing). A first-order dataset can be acquired by the detection of a simple sample that can be rearranged into a vector obtained from either a spectrum or a chromatogram (*e.g.* UV–VIS spectrum, fluorescence emission spectrum). This type of dataset can be used in basic recognitions, for example, to match automatically an unknown spectrum with a library of reference spectra. A second-order dataset will come in a matrix for a single sample that can be obtained by multi-detections of the sample (*e.g.* investigation of chemical reaction profiles). On the other hand, it can also be generated by joining the first-order datasets together in either a row-wise or column-wise fashion. For example, the spectrum is recorded at each time interval in order to monitor chemical reactions. A third-order dataset can be developed by simply adding the second-order datasets together in the form of an array which is often called a *three-way data array*. The data type might be obtained from an image or a hyperspectral image where a layer of the array contains information on each pixel recorded at different parameters such as values of R, G and B presented as first-, second- and third-array layers, respectively. To perform the calculation on the data array, multi-way data analysis such as consensus PCA (CPCA),[76] multiblock partial least squares (MBPLS)[76] and parallel factor analysis (PARAFAC)[77] was implemented, which might involve a complicated calculation. To simplify the calculation, the data array can be rearranged into an augmented matrix by appending the columns (or rows) of matrices from each array with the same row (or column) of elementary operations. Therefore, multivariate analysis can commonly be performed.

Data types and data processing strategies are summarized in Table 10.2. At present, chemical information from experiments has been represented in the form of a high-order dataset that mostly involves matrix and data arrays. In this chapter, chemometric principles are outlined based only on the

Table 10.2 Dataset complexity including data order, type of data and multivariate analysis strategy.

Data order	Scheme of data	Type of data	Strategy
Zero	Scalar: $[x_{11}]$	Elements Chemical properties	Univariate
First	Vector: $[x_{11} \cdot x_{12} \cdot x_{13} \ldots x_{1j}]$ or Vector: $\begin{bmatrix} x_{11} \\ x_{21} \\ \vdots \\ x_{i1} \end{bmatrix}$	Spectrum Chromatogram	Multivariate
Second	Matrix: $\begin{bmatrix} x_{11} & \cdots & x_{1j} \\ \vdots & \ddots & \vdots \\ x_{i1} & \cdots & x_{ij} \end{bmatrix}$	Set of spectra Set of chromatograms	Multivariate
Third	Array:  *z* shows the number of matrix layers	Image Coupled data: GC–MS, LC–MS	Multi-way analysis Multivariate

data acquired in a matrix form where rows (i) represent the observations (samples) and columns (j) represent variables (factors, wavelengths, retention time, *etc.*).

10.4 Methodology of Chemometrics

In this section, chemometric principles in GAC are introduced based on the data acquired only in a matrix form. Several generic chemometric methods, which are frequently used in GAC, are considered in a comprehensive way.

10.4.1 Design of Experiments (DOE)

An initial step in any research is to formulate the objectives and goals of the study. A number of key questions have to be listed and taken into consideration in order to answer the chosen hypothesis. After defining the aim of the study, the selection of objects such as samples or individuals needs to be considered and should span the experimental setup in a balanced and systematic manner. Therefore, designing a study is required and plays as an important role when evaluating the outcome or results. An appropriate

experimental design is required to provide the results to answer the key questions. There is no point in continuing if the design of the study is not appropriate to answer the main questions. To reach the greenest profiles, the consideration of chemometric experimental designs is recommended at the beginning of the process in order to ensure quality, accuracy and richness of information with the smallest number of experiments.

There are numerous applications of chemometric experimental designs but they can be put into three main categories, namely screening,[26] optimization[27] and quantitative modeling.[78] In any experimental procedure, several experimental variables or factors may influence the results. A screening design is performed in order to determine which variables or factors and interactions have the greatest effect on the measured outcome, so that irrelevant variables are eliminated and the significant variables can be studied in more detail. For example, in an organic synthesis, there are various factors that can be investigated (*e.g.* solvent, reagents, temperature, reaction time) in one synthesis process. However, there may be only a small number of these factors, such as catalysts, pH and temperature, that have significant impacts on the product yield.[79] According to the design, the quantitative model can be coupled with statistical methods to discover the relation between response (product yield in this case) and significant factors. This model can be used to visualize and optimize the experimental conditions to give the maximum yield without performing any extra experiments. Generally, the aim of an analytical experiment is to determine a quantitative model to obtain an accurate outcome with the highest efficiency. The number of experiments is limited in practice as it is not possible to perform an infinite number of experimental runs to cover all possible outcomes. Therefore, it is sometimes necessary to determine how many experiments need to be performed and also the levels of each of the factors to determine the scope of the desired results. There are many different designs that can be used, such as full factorial design, fractional factorial design, central composite design and the Plackett–Burman design, all of which have been described elsewhere.[26,28,30] Each design has different rules to enable the maximum amount of information to be extracted from the experiment. DOE has been proven to be an important tool for various applications to eliminate the dissipation due to a trial-and-error method and save on costs in terms of time, money and labor.

10.4.2 Pre-processing Methods

Pre-processing methods should be considered as an important step to treat the data prior to performing data analysis. The data acquired from either spectroscopic or chromatographic measurements must be pretreated before applying further manipulations to obtain the true underlying information. Different results may be obtained by different pre-processing methods. In fact, it is difficult to collect samples in the same environment and analyze these samples using the same instrument under identical experimental

conditions. Variations due to collection date, stability of the instrument and background such as interference of chemical factors or errors in the experimental process are observed for different samples in terms of, *e.g.*, peak height, baseline, *etc.* This leads to a decrease in the signal-to-noise ratio and the mentioned factors may distort the important information extracted from the acquired dataset. Therefore, they must be scaled and reduced prior to data analysis by appropriate pre-processing methods so that they do not have an excessive influence on the final analysis. If the data in the form of a matrix are considered, there are three principal ways to scale the data matrix: (1) transforming the elements of the matrix,[80,81] (2) row scaling[29,80,81] and (3) column scaling.[29,80,81] These scaling methods can be used on a range of datasets as shown in Table 10.3. Moreover, there are many pre-processing techniques based on signal analysis (*e.g.* Fourier transformation[82]), but these will not be described here.

10.4.2.1 Transforming the Elements of the Matrix

Generally, the acquired data mostly contain very large signals compared with the others, which may exist in only a small number of samples. These signals will strongly influence and dominate the analysis if they are not suitably scaled. It is not necessarily the case that the informative signals are the most intense peaks. In some cases, small signals with large variations are the most interesting from a chemical point of view. Therefore, transforming the elements in the data is one of several approaches used to reduce the influence of large peaks. This pre-processing method is applied to each element of the data matrix independently of the other elements. They change the values of the data points without changing their ranks, which sometimes is called *monotonic transformations*.[80,81]

10.4.2.2 Row Scaling

This is an important step in preparing a data matrix before performing any data analysis. In the measurement or detection, the acquired data might be influenced by the non-linearities introduced in the experiments such as the acquired spectra from spectroscopic techniques. They can be strongly influenced by light scattering due to the particle size in the samples, which is not related to the target analyte. It is not possible to control this influence equally in each sample. Therefore, it will affect the signal, which leads to a background shift from undesired scattering effects. In chromatography, the amount of sample to be determined is difficult to control, especially with biological samples.[83–86] To compare the absolute amounts of each extracted compound in each sample will not reveal the real relationship between two samples. The goal of this pre-process step is to improve the subsequent data analysis, such as exploratory analysis, calibration model and classification model, without any use of response values.

Table 10.3 Pre-processing methods including element transformation, row scaling and column scaling.[29,80]

Methods	Explanation	Equation
Transformations		
Logarithmic	Modify the distribution of the data elements to be on a $\log_{10}$ scale	$\log_{10}(x_{ij})$
Box–Cox	Modify the distribution of the data elements to be more normally distributed	$x_{ij}^{\lambda} - 1/\lambda$ λ is the exponent value
Power	An alternative to log transformation but less drastic	x_{ij}^{p} $p = 0.5$ gives square root and *vice versa*
Row scaling		
Smoothing	Remove the random noise in the data Norris–Williams derivation and Savitzky–Golay (SG)	This involves an iteration process and polynomial fitting
MSC	Fit each row-data to a reference row-data to remove the baseline shifts This can be extended to extended MSC (EMSC), inverse MSC, extended inverse MSC	$\boldsymbol{x} = b_0 + b_{\text{ref}}\,\boldsymbol{x}_{\text{ref}} + \boldsymbol{e}$ $\boldsymbol{x}_{\text{corr}} = (\boldsymbol{x} - b_0)/b_{\text{ref}}$
SNV	Row-wise autoscaling to remove the baseline shifts by subtracting the mean value of row-data and dividing by its standard deviation	$(x_{ij} - \bar{x}_i)/\sigma_i$ σ is the standard deviation
Normalization	Operation performed within or across rows to scale the row profiles comparable in size. This can be done by dividing each row by an estimate of its intensity: maximum value, specific point, sum of values, *etc.*	$x_{ij}/\tilde{x}$ $\tilde{x}$ is the estimated value
Column scaling		
Mean centering	To subtract the variable mean from each value in the column After centering, each column is expressed as deviations from its mean	$(x_{ij} - \bar{x}_j)$
Autoscaling	To subtract the variable mean from each value in the column, then followed by dividing row entries of a column by the standard deviation within that column	$(x_{ij} - \bar{x}_j)/\sigma_j$
Pareto scaling	To subtract the variable mean from each value in the column, then followed by dividing row entries of a column by the square root of the standard deviation within that column	$(x_{ij} - \bar{x}_j)/\sqrt{\sigma_j}$

10.4.2.3 Column Scaling

Column scaling is necessary if variables in the data are on different scales and is needed to ensure that all variables have a similar influence on the

analysis. In the case of a property table, the data might be recorded in different scale ranges, *e.g.* temperature, boiling point, melting point, density, *etc.* In chromatography, often several hundred compounds are detected in each sample. A few compounds might have very high intensities compared with the others, and without column scaling the data analysis will be dominated by the large signals/peaks. In many cases, small peaks with high variations are much more interesting than large peaks.[81,85] For example, consider the chromatograms of urine collected from two groups of subjects (for instance, controls and diseased patients). The major chemical compounds in urine for both groups are in the form of the amide derivatives, which appear as huge peaks but with no large variations, hence they cannot be used to discriminate a particular group of samples. In this case, the discriminatory compounds may appear with small intensity with large variations between control and diseased subjects.[85,86]

10.4.3 Unsupervised Pattern Recognition

After the appropriate pre-processing methods have been applied, the data matrix is in a comparable scale and is ready for the data analysis. Nowadays, instruments, data storage methods and computers have undergone dramatic developments. They provide a lot of information even for a single sample, hence spectroscopic and chromatographic datasets are becoming more complex. It is impossible to interpret the underlying relationships between samples directly from these data by routine human analysis. Hence some techniques are needed to organize the information into easier forms for interpretation with no further knowledge such as class information, concentration, *etc.*, being required. Unsupervised methods can be split into two basic principles, involving dimension reduction and cluster analysis. For data dimension reduction, this is fundamentally based on a multivariate linear transformation in order to decompose the original data, which involves several variables and projects them into the new space involving only a couple of correlated variables (latent variables). In the other case, cluster analysis is an algorithm to discover the patterns in the data and then clusters of those samples with similar characteristics. In this section, hierarchical cluster analysis (HCA),[29] principal component analysis (PCA),[28,29] principal coordinate analysis (PCO)[87] and self-organizing maps (SOMs)[83,88] are outlined. In addition to these methods, there are also several other methods, such as independent component analysis (ICA),[89] developed based on the unsupervised principle, that are fully described elsewhere.[29]

10.4.3.1 Hierarchical Cluster Analysis (HCA)

Cluster analysis is a technique used to assign a set of samples into subsets, called *clusters*, so that samples in the same clusters are similar in some sense and character.[28,29,90] If meaningful groups are the goal of study, then the clusters should capture the natural structure of the data. It is commonly used in many areas, including data mining, pattern recognition and bioinformatics. There are two options for clustering the samples. First, all samples

are considered as independent clusters and then combined with those most similar into one cluster. Second, all samples are considered as one large cluster and then split into smaller clusters. The first option is chosen for this section; it is often called *agglomerative clustering*.[90] This technique provides a good similarity correlation of data beginning with one sample to the entire data for other samples. A pair of samples that have similar values numerically close to each other will be clustered. This process will continue until all samples in the entire data are clustered in the same group. In HCA, the similarity/dissimilarity is measured on the basis of the correlation/distance between all possible pair of samples. It should be noted that the calculation of similarity can easily be reformulated into a dissimilarity measure. There are several dissimilarity measures that can be calculated, sometimes also called distance measures, which result in a distance matrix ($\boldsymbol{D}$).[29] A distance matrix consists of pairwise dissimilarities between samples i and k, where d_{ik} is the dissimilarity value between these samples. Therefore, a dissimilarity matrix used in this context is always a symmetric square matrix with dimensions $I \times I$, where the diagonal values equal 0 (which means the dissimilarity between any sample and itself is 0). There are several methods to calculate dissimilarity/distance values;[29] however, only Euclidean distance (ED) and Mahalanobis distance (MD)[91,92] are discussed here and are calculated as follows:

$$\mathrm{ED}_{ik} = \sqrt{(x_i - x_k)(x_i - x_k)^{\mathrm{T}}}$$

$$\mathrm{MD}_{ik} = \sqrt{(x_i - x_k)\boldsymbol{S}^{-1}(x_i - x_k)^{\mathrm{T}}}$$

where x_i and x_k are the data acquired from samples i and k, respectively, T is a transpose operation and $\boldsymbol{S}$ is the covariance matrix.

The next step is to combine the samples (or clusters) with the highest similarity to form a new cluster and finally compute the distance between new clusters that have to be formed by merging. In this step, there are several approaches to calculate the distance between new clusters normally called *linkage*.[29,92] The linkage criterion used to determine the distance between sets of samples in cluster A (C_A) and cluster B (C_B) in this case can be generally divided into:

- *Single linkage* (*nearest linkage*): The new cluster that gives the smallest distance (highest similarity) is merged with the target cluster: $\min\{d(a, b)\colon a \in C_A, b \in C_B\}$
- *Complete linkage* (*furthest linkage*): The new cluster giving the largest distance (lowest similarity) is merged with the target cluster: $\max\{d(a, b)\colon a \in C_A, b \in C_B\}$. This is the opposite of single linkage.
- *Average linkage*: The average distance between the new cluster from the other clusters and the target cluster is calculated: $\frac{1}{|A||B|}\sum_{a \in C_A}\sum_{b \in C_B} d(a, b)$.

Once two clusters have been grouped together, the procedure is repeated until all samples have been agglomerated into a single cluster. The most

common method used to visualize the clustering of samples using HCA is a dendrogram. The group of samples is organized on the horizontal axis and the vertical axis represents the index of dissimilarity/distance. It should be emphasized that the analysis by HCA does not provide a stable outcome when different criteria are used and the number of existing clusters is decreased. A large number of other quantitative indices, linkage criteria and alternative clustering (*e.g.* *k*-mean clustering, fuzzy *c*-mean clustering) have been proposed in the literature,[29,30] but for brevity here only the most widespread and useful methods for pattern recognition are described.

10.4.3.2 Principal Component Analysis (PCA)

PCA is a useful statistical technique that has found applications in many fields *e.g.* pattern recognition[54,93–96] and image compression.[97,98] PCA is the most frequently used method for unsupervised pattern recognition. A data matrix can contain many variables that are not necessarily orthogonal to each other, *i.e.* they have some degree of correlation and therefore they can be difficult to interpret. Furthermore, some variables do not provide any significant information, for example, some variables may be present in only a few observations. The central aim of PCA is to find the patterns and reduce the dimensionality of the data to summarize the most important parts while simultaneously filtering out noise. Therefore, it will be easier to extract useful information from the reduced dataset. A key feature of PCA is to reduce the size of large data matrices to a few features by capturing the variance in terms of principal components (PCs). PCs are a set of variables that are uncorrelated (orthogonal) and ordered by the maximum amount of variance in the data.[99] Therefore, the first few PCs retain most of the variations in all the original variables. PCs are calculated until a sufficient amount of variance is described, resulting in A components. If a data matrix contains I samples and J variables, the maximum number of components should be equal to the smaller dimension of the data matrix, so it is equal to I when $J > I$ or equal to J when $I > J$.

In a PCA algorithm, a data matrix ($\boldsymbol{X}$) is decomposed into A PCs consisting of a scores matrix ($\boldsymbol{T}$), a loadings matrix ($\boldsymbol{P}$) and a residual matrix ($\boldsymbol{E}$). It can be written in the matrix form as follows:

$$\boldsymbol{X} = \boldsymbol{T}\boldsymbol{P}^{\mathrm{T}} + \boldsymbol{E}$$

PCA assumes that the variations in the data always deviate from the origin. Therefore, if this assumption is not held, it is possible that the first PC does not represent the direction of the largest variation of the data points. Because of this, column scaling (in particular mean or weight centering) on the data prior to performing PCA is very important and compulsory. There are several algorithms to perform PCA modeling. However, all methods should give the same results in the case when the variance in the data is unchanged. The two most common algorithms are based on singular value decomposition (SVD)[100] and non-linear iterative partial least squares (NIPALS).[28,101]

To use the SVD approach, the matrix $\boldsymbol{X}$ can be decomposed as

$$\boldsymbol{X} = \boldsymbol{U}\boldsymbol{D}\boldsymbol{V}^{\mathrm{T}} + \boldsymbol{E}$$

where $\boldsymbol{U}$ contains the same column vectors as the score matrix ($\boldsymbol{T}$), $\boldsymbol{V}^{\mathrm{T}}$ is identical with the loading matrix ($\boldsymbol{P}^{\mathrm{T}}$) but it is normalized to length one and $\boldsymbol{D}$ is a diagonal matrix containing the square roots of eigenvalues extracted from $\boldsymbol{X}^{\mathrm{T}}\boldsymbol{X}$. In the SVD algorithm, all possible PCs will be extracted in a single calculation. These PCs contain both useful and undesired information. Therefore, obtaining all PCs from especially the large data matrix will slow the calculation, be time consuming and waste memory and storage on the computer. To avoid these problems, an iterative protocol is proposed.

The NIPALS algorithm is an iterative method and calculates only a single component at a time until the desired number of PCs is reached. The method offers the opportunity to stop the calculation and get only the required PCs for the PCA model. The NIPALS algorithm is preferable when the datasets are very large. This can be very useful and reduce the time required for calculation. This method is explained using a data matrix $\boldsymbol{X}$ that is scaled for each dimension and involves this following steps:

1. A column of $\boldsymbol{X}$ with the greatest sum of squares (variance) is determined and used as the initial score vector ($\boldsymbol{t}_{\mathrm{initial}}$).
2. The loading vector $\boldsymbol{p}^{\mathrm{T}}_{\mathrm{unnorm}}$ is calculated: $\boldsymbol{p}^{\mathrm{T}}_{\mathrm{unnorm}} = \boldsymbol{t}^{\mathrm{T}}_{\mathrm{initial}}\boldsymbol{X}/(\boldsymbol{t}^{\mathrm{T}}_{\mathrm{initial}}\boldsymbol{t}_{\mathrm{initial}})$.
3. The loading vector is normalized to unit length: $\boldsymbol{p}^{\mathrm{T}}_{\mathrm{norm}} = \boldsymbol{p}^{\mathrm{T}}_{\mathrm{unnorm}}/(\boldsymbol{p}^{\mathrm{T}}_{\mathrm{unnorm}}\boldsymbol{p}_{\mathrm{norm}})$.
4. The new score vector is calculated: $\boldsymbol{t}_{\mathrm{new}} = \boldsymbol{X}\boldsymbol{p}^{\mathrm{T}}_{\mathrm{norm}}$.
5. Check for convergence by comparing the $\boldsymbol{t}_{\mathrm{initial}}$ and $\boldsymbol{t}_{\mathrm{new}}$. The sum of squared differences between all elements of the two consecutive score vectors is calculated. If the value meets the criterion (small enough), this indicates that the PC has been extracted; otherwise, replace $\boldsymbol{t}_{\mathrm{initial}}$ with $\boldsymbol{t}_{\mathrm{new}}$ and return to step 2, repeating until convergence is achieved.
6. Subtract the extracted PC from $\boldsymbol{X}$ to obtain a residual data matrix ($\boldsymbol{E}$): $\boldsymbol{E} = \boldsymbol{X} - \boldsymbol{t}\boldsymbol{p}^{\mathrm{T}}$.
7. If it is desired to compute further PCs, substitute the data matrix $\boldsymbol{X}$ with the residual matrix $\boldsymbol{E}$ and return to step 2.

Figure 10.6 shows the overall structure of the PCA model. Once the scores and loadings have been calculated, there are several properties for each component that can be determined (*e.g.* eigenvalues, percent variance).[28] These properties represent the importance of each component a. The eigenvalue (ξ) for each component is calculated by the sum of the squares of the scores vector of all I samples:

$$\xi_a = \sum_{i=1}^{I} t_{ia}^2$$

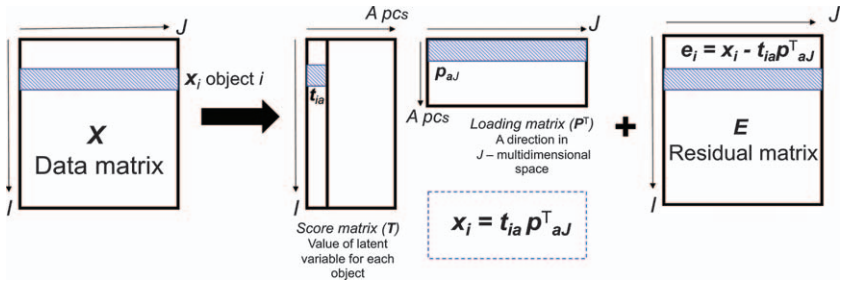


Figure 10.6 Structure of a PCA model demonstrating by projection the data of object i ($\boldsymbol{x}_i$) on to a loading axis ($\boldsymbol{p}_{aj}^{\mathrm{T}}$) to obtain a score of object i ($\boldsymbol{t}_{ia}$); a is the number of significant principal components.

where the sum of all eigenvalues is equal to the sum of squares of the data matrix:

$$\sum_{i=1}^{I} \xi_a = \sum_{j=1}^{J} \sum_{i=1}^{I} x_{ij}^2$$

The significance of each PC can be determined using the percentage of the total amount of variance calculated by

$$\% \, \xi_a = \frac{\xi_a}{\sum_{j=1}^{J} \sum_{i=1}^{I} x_{ij}^2} \times 100$$

From PCA, the first PC represents the greatest eigenvalue and percentage variance of the data, the second PC shows the second greatest eigenvalue and percentage variance and so on. The majority of useful information is contained in the first few PCs; the later PCs may represent only noise. However, there are some cases where the first few PCs correspond to the variance from instrumental and experimental conditions, especially in biological systems in which the first few PCs might correlate with the background of the biological samples.[84,102] Hence useful information may possibly be represented in the later PCs.

10.4.3.3 Principal Coordinate Analysis (PCO)

PCO is an alternative method closely related to PCA.[87] It is based on the same procedure as in PCA, namely to capture the maximum variance in the data matrix. The only difference between the two analyses is that PCO works on a pairwise distance matrix ($\boldsymbol{D}$) instead of the original data matrix ($\boldsymbol{X}$). To obtain the distance matrix, the distance between all possible pairs of samples in the data matrix $\boldsymbol{X}$ is calculated. The method to calculate the distance between samples has already been discussed in Section 10.4.3.1 on HCA. The major advantage of PCO is that many dissimilarity measures can be applied and therefore it is more flexible than PCA, which only uses the Euclidean distance. It should be noted that PCA and PCO using the Euclidean distance

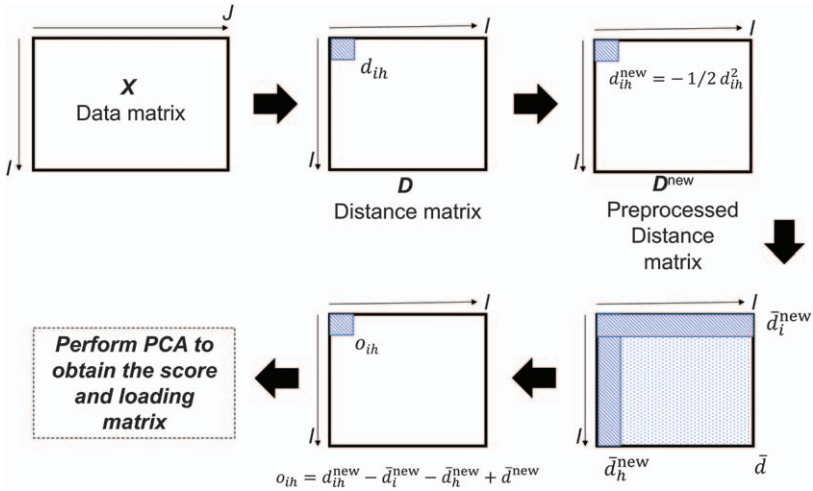


Figure 10.7 Calculation structure of a PCO model.

give comparable results. However, a disadvantage of PCO is that the method reveals the relationships only between the samples and not for the variables in the dataset.[87] To perform the PCO method as shown in Figure 10.7, the following steps are calculated:

1. The dissimilarity/distance matrix $\boldsymbol{D}$ with dimensions $I \times I$ is calculated and used as the input data matrix for further steps.
2. All elements in the matrix $\boldsymbol{D}$ are squared to obtain the matrix $\boldsymbol{D}^{(2)}$.
3. A new distance matrix $\boldsymbol{D}_{\text{new}}$ is computed whose elements are calculated by

$$d_{ih}^{\text{new}} = -\frac{1}{2d_{ih}^2}.$$

4. The elements in the matrix $\boldsymbol{O}$ are computed by

$$O_{ih} = d_{ih}^{\text{new}} - \bar{d}_i^{\text{new}} - \bar{d}_h^{\text{new}} + \bar{d}^{\text{new}}$$

   where $\bar{d}_i$ and $\bar{d}_h$ represent the mean of row (sample i) and column (sample h) of the matrix $\boldsymbol{D}_{\text{new}}$ and $\bar{d}$ is the overall mean.
5. The PCA method based on the NIPALS algorithm is performed on matrix $\boldsymbol{O}$ to obtain

$$\boldsymbol{O} = \boldsymbol{T}\boldsymbol{P}^{\text{T}} + \boldsymbol{E}.$$

   The scores $\boldsymbol{T}$ and loadings $\boldsymbol{P}$ are the same dimension apart from scaling issues if NIPALS is employed. To visualize the data, the rows of $\boldsymbol{P}$ can be plotted against one another.

PCO permits a wide range of approaches for visualizing data, especially for the case of binary variables.[87] The components obtained from PCO can also be used as input to the classifier just as for the scores from PCA.

10.4.3.4 Self-organizing Maps (SOMs)

The SOM is a neural network method that can be applied for both unsupervised and supervised learning.[54,83,86,88,103] SOM was first introduced by the Finnish computer scientist Teuvo Kohonen.[104,105] It is a powerful alternative to PCA and PCO for visualizing data. A SOM map provides information similar to scores plots, and reveals the relationship between samples and component planes that can be used to express characteristic variables. Units of the map can be generated in a hexagonal or square unit; however, only the hexagonal unit is discussed in this section. Each map unit (u) on a SOM map contains a weight (w) for each variable, resulting in a $1 \times J$ weight vector (note: J equals the number of variables in the dataset). If the number of map units is small it gives a rough picture, whereas if the number of map units is large a more detailed map of the samples is obtained. The calculation protocol of SOM for unsupervised learning[88] is described for brevity as follows:

1. An initial output map is determined in $M \times N = K$ units. A weight vector ($\boldsymbol{w}$) of each unit will be randomly selected between the maximum and minimum values of variable j in the input data. Note that the size of M and N should be carefully considered so as to cover most of samples to be matched in the next step.
2. Sample vectors ($\boldsymbol{x}_s$) in the dataset are then compared with the weight vector of each unit ($\boldsymbol{w}_k$) on the initial SOM map from step 1. The Euclidean distance between $\boldsymbol{x}_s$ and $\boldsymbol{w}_k$ for each map unit k is calculated:

$$d_{sk} = \sqrt{(\boldsymbol{x}_s - \boldsymbol{w}_k)(\boldsymbol{x}_s - \boldsymbol{w}_k)^{\mathrm{T}}}$$

   This process will be repeated until the distance of K units on the map is calculated.
3. The map unit that gives the smallest distance will be declared as the best matching unit (BMU) of the chosen sample ($\boldsymbol{x}_s$): $\mathrm{BMU} = \min_{\mathrm{k}}\{d_{sk}\}$
4. The BMU and the neighboring map units (N_{b}) within the length from the BMU are updated to become more similar to the sample vector $\boldsymbol{x}_s$. The learning rate which is used to determine the amount that a map unit can learn to represent a sample in each iteration is calculated:

$$\boldsymbol{w}_k = \begin{cases} \boldsymbol{w}_k + \omega\alpha(\boldsymbol{x}_s - \boldsymbol{w}_k) & k \in N_{\mathrm{b}} \\ \boldsymbol{w}_k & k \notin N_{\mathrm{b}} \end{cases}$$

   where α is the learning rate and ω is the neighborhood learning weight. The amount of learning decreases with each iteration of the algorithm, as does the neighborhood learning rate with distance from the BMU.

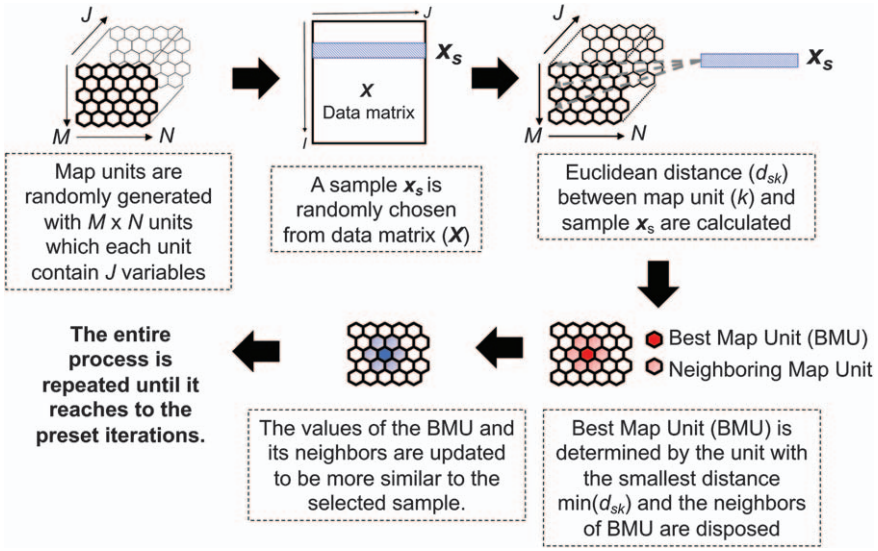


Figure 10.8 Calculation protocol of SOM for unsupervised learning.

The learning of the entire process is repeated until it reaches to the preset iterations. The calculation protocol of SOM is illustrated graphically in Figure 10.8.

It should be noted that an appropriate number of iterations used in a SOM calculation might be exceed 1000×the number of map units K in order to ensure that the map has a sufficient number of chances to learn about each sample. After the training process, the samples slowly become restricted to the region of the map to which they are similar. Therefore, samples with similar underlying information are mapped to SOM map units that are close together. However, it is very difficult to visualize directly the updated SOM map. For visualization, a color map has been created.[88] The shading of the color map units is updated in each iteration of the algorithm. The color map will help in interpretation and so it is possible to watch in real time as the training progresses. There are alternative ways of visualizing the relationship between samples, for example the U-matrix, hit histogram and supervised component plane, all of which, including the training and learning algorithms, are described elsewhere.[29,88,103]

Fisher's Iris database (http://archive.ics.uci.edu/ml/datasets/Iris) is chosen as an example to demonstrate the power of unsupervised pattern recognition methods performing on a multidimensional dataset. The dataset contains three groups of samples, namely *Iris setosa*, *Iris versicolor* and *Iris virginica*, with four variables, sepal length, sepal width, petal length and petal width. To simplify the visualization of the dataset, only the first 10 samples of each group are selected to simplify the calculation and visualization. The outputs of HCA, PCA, PCO and SOM are shown in Figure 10.9. It can be seen that the clusters of three groups from the multidimensional dataset (four variables)

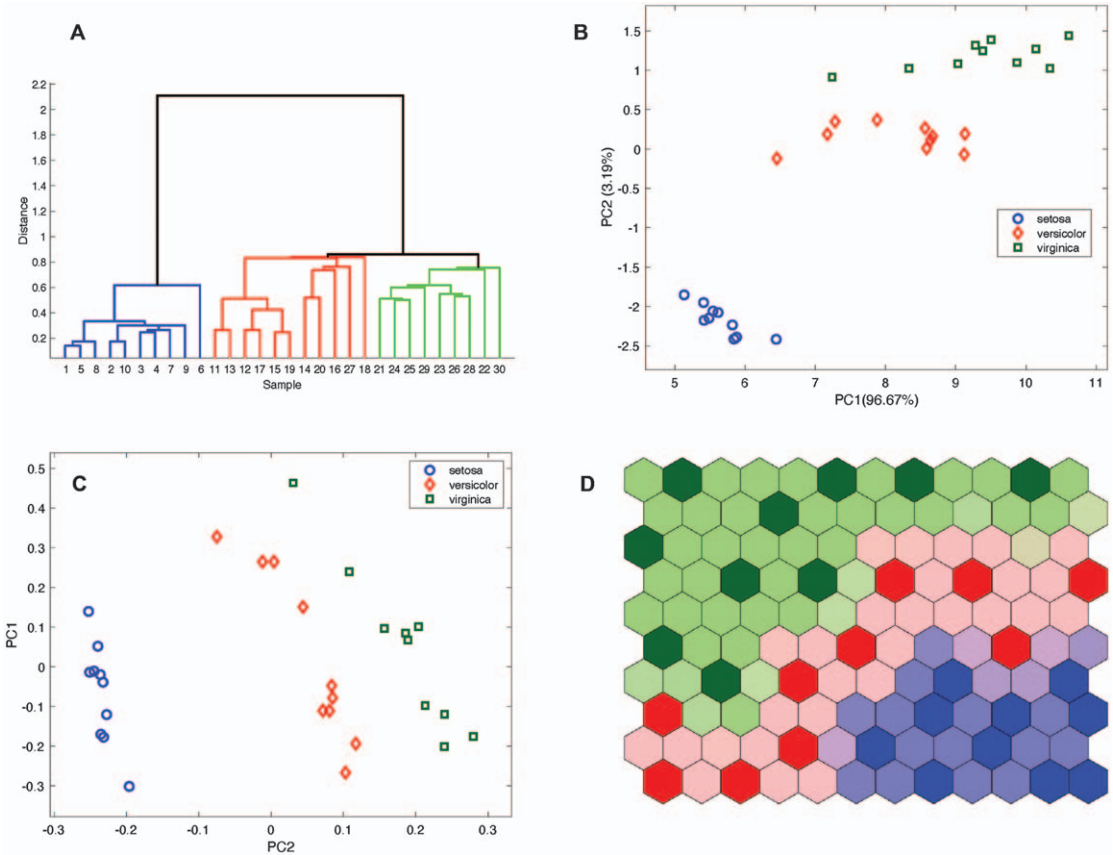


Figure 10.9 (A) Dendrogram of HCA with Euclidean distance and nearest linkage. (B) Graphical representation of samples with PC scores. (C) Graphical representation of the position of samples in PCO space using the Euclidean distance. (D) Graphical representation the BMU of each sample using the SOM algorithm with a 10×12 map.

are clearly revealed and visualized by using unsupervised pattern recognition.

10.4.4 Supervised Pattern Recognition

Supervised pattern recognition is mainly used for estimation, prediction and classification.[37,106–109] Prediction and classification problems are the most generic problems in chemometrics. The relationship between samples and responses (they can be class, group, concentration, intensity, *etc.*) are revealed by measuring a series of samples and then building the satisfied mathematical model. Once the model has been generated, it can be used either to predict the response or to classify a group of unknown samples. In fact, there are an enormous number of methods available in the literature; however, only commonly used supervised pattern recognition methods such as multiple linear regression (MLR),[28,30] principal component regression (PCR)[28,30,110] and partial least-squares regression (PLSR),[111,112] used to build the calibration model, and Euclidean distance (ED),[28,29] linear discriminant analysis (LDA)[28,29] and quadratic discriminant analysis (QDA),[28,29] used as classifiers, are described in this section.

10.4.4.1 Multiple Linear Regression (MLR)

MLR directly uses the features of the data matrix ($\boldsymbol{x}_j$) to determine the final latent variable with maximum correlation with the response vector ($\boldsymbol{y}$).[28,30] This method tries to minimize sum of squared residuals (e), where $e = y - \tilde{y}$ ($\tilde{y}$ is the estimated value of the response). If the data matrix contains I samples $\times$ J variables and the response vector is composed of I samples $\times$ 1, the coefficients of each variable ($\boldsymbol{b}$) can be calculated by using pseudo-inverse of the data matrix over the response as follows:

$$\boldsymbol{b} = (\boldsymbol{X}^{\mathrm{T}}\boldsymbol{X})^{-1}\boldsymbol{X}^{\mathrm{T}}\boldsymbol{y}$$

The response of unknown samples can be estimated by

$$\tilde{y} = \boldsymbol{X}_{\mathrm{unknown}}\boldsymbol{b}$$

It should be noted that $\boldsymbol{X}^{\mathrm{T}}\boldsymbol{X}$ is proportional to the covariance matrix of $\boldsymbol{X}$. This method cannot be performed if the covariance matrix of $\boldsymbol{X}$ is almost singular, which occurs when the features are highly correlated or the number of samples is much smaller than the number of variables. In the latter case, reduction methods such as PCA can be used to reduce the dimensions of variables prior to applying the MLR calculation.[110]

10.4.4.2 Principal Component Regression (PCR)

The linear regression model tries to model the response (y) as a linear combination of PCA scores. PCA scores are orthogonal and uncorrelated and

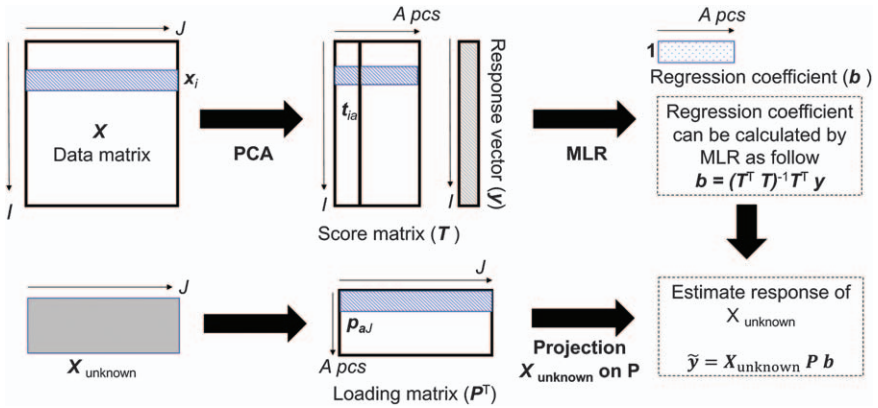


Figure 10.10 Structure of a PCR model to calculate the regression coefficients and to estimate of the response of unknown samples.

the dimensions of variables can be reduced into the latent variable as principal component (PC).[28,101] This causes no problems with the matrix inversion. To perform PCR, the first step is PCA of the data matrix $\boldsymbol{X}$ resulting in a score matrix ($\boldsymbol{T}$) and a loading matrix ($\boldsymbol{P}$). Only a subset of the PCA scores is used to build the regression model. The optimum number of PCA components is determined by cross-validation in order to achieve the goal of maximum prediction power of the model. The second step is to calculate the regression coefficients ($\boldsymbol{b}$) using the score matrix ($\boldsymbol{T}$) and the response vector ($\boldsymbol{y}$) as follows:

$$\boldsymbol{b} = (\boldsymbol{T}^{\mathrm{T}}\boldsymbol{T})^{-1}\boldsymbol{T}^{\mathrm{T}}\boldsymbol{y}$$

and the estimated values of the response ($\tilde{\boldsymbol{y}}$) by

$$\tilde{\boldsymbol{y}} = \boldsymbol{X}_{\mathrm{unknown}}\boldsymbol{b}$$

Figure 10.10 shows the schematic structure of a PCR model to calculate the regression coefficients and to estimate of the response of unknown samples. Although the PCR method is a powerful regression model and it is a standard method for regression problems in chemometrics, there is a major disadvantage of the method, namely that the regressor variables are computed without considering the relation of the response.[28,30,110] The optimized components might not be the real optima for building the model.

10.4.4.3 Partial Least-squares Regression (PLSR)

PLSR is one of the most common regression and supervised linear modeling techniques.[113] It is used to analyze strongly collinear and noisy data with

large numbers of variables (numerous *J*). Moreover, it can also simultaneously model for several response vectors. It has the advantage over MLR that it can be used to analyze the dataset with variables that are strongly correlated, whereas MLR is appropriate when the data are few and fairly uncorrelated.[113] The basic principle of PLSR is fairly straightforward. The method tries to relate the blocks of the dataset ($\boldsymbol{X}$) and the responses ($\boldsymbol{y}$) by maximizing the covariance between the two blocks.[28,30,113] The output of the PLSR model can be expressed by the following relations:

$$\boldsymbol{X} = \boldsymbol{T}\boldsymbol{P}^{\mathrm{T}} + \boldsymbol{E}$$
$$\boldsymbol{y} = \boldsymbol{T}\boldsymbol{q}^{\mathrm{T}} + \boldsymbol{f}$$

where $\boldsymbol{X}$ is an experimental data matrix, $\boldsymbol{y}$ is the response vector, $\boldsymbol{T}$ is the *X* scores, $\boldsymbol{P}$ is the *X* loading, $\boldsymbol{q}$ is the *y* loading and $\boldsymbol{E}$ and $\boldsymbol{f}$ are the residual matrix and vector for $\boldsymbol{X}$ and $\boldsymbol{y}$, respectively. It should be emphasized that $\boldsymbol{T}$ and $\boldsymbol{P}$ from PLSR are completely different from $\boldsymbol{T}$ and $\boldsymbol{P}$ obtained by PCA. In the brief algorithm, the *X* scores can be calculated by the estimation of the linear combination of variable x_j with coefficient of weight $\boldsymbol{W}^*$: $\boldsymbol{T} = \boldsymbol{X}\boldsymbol{W}^*$, where the coefficient of weight can be defined as $\boldsymbol{W}^* = \boldsymbol{W}(\boldsymbol{P}^{\mathrm{T}}\boldsymbol{W})^{-1}$. The $\boldsymbol{W}$ matrix can be obtained by $\boldsymbol{W} = \boldsymbol{X}^{\mathrm{T}}\boldsymbol{y}$.[114]

Generally, the regression model can be written as $\boldsymbol{y} = \boldsymbol{X}\boldsymbol{b}$, where $\boldsymbol{b}$ is the regression coefficient of each variable in the data matrix $\boldsymbol{X}$. In the case of PLSR, $\boldsymbol{b}$ can be calculated by

$$\boldsymbol{b} = \boldsymbol{W}^*\boldsymbol{q}^{\mathrm{T}}$$
$$\boldsymbol{b} = \boldsymbol{W}(\boldsymbol{P}^{\mathrm{T}}\boldsymbol{W})^{-1}\boldsymbol{q}^{\mathrm{T}}$$

At this stage, the response of an unknown sample can be estimated by using the calculated regression coefficient ($\boldsymbol{b}$): $\tilde{\boldsymbol{y}} = \boldsymbol{X}_{\mathrm{unknown}}\boldsymbol{b}$.[114] Figure 10.11 demonstrates the calculation structure of a PLSR model. It should be noted that the quality of the model is strongly dependent on the determined number of PLS components. There are many ways to determine the number of significant PLS components, such as the cross-validation approach.[29]

10.4.4.4 Euclidean Distance (ED)

A simple classifier is the Euclidean distance to centroids.[28,29] The basic principle of this method is to calculate the centroids of each class *g* ($\bar{\boldsymbol{x}}_g$) in a dataset. The centroids are calculated from the mean of all samples in a group for each of the variables. For this method, there is no other information relating to class distribution apart from the mean of each group and it is assumed that the distribution of samples around the centroid is symmetrical. The Euclidean distance of a sample *i* ($\boldsymbol{x}_i$) to the centroid of class *g* is calculated as follows:

$$d_{ig} = \sqrt{(\boldsymbol{x}_i - \bar{\boldsymbol{x}}_g)(\boldsymbol{x}_i - \bar{\boldsymbol{x}}_g)^{\mathrm{T}}}$$

where d_{ig} is the Euclidean distance between sample *i* and the centroid of class *g*.

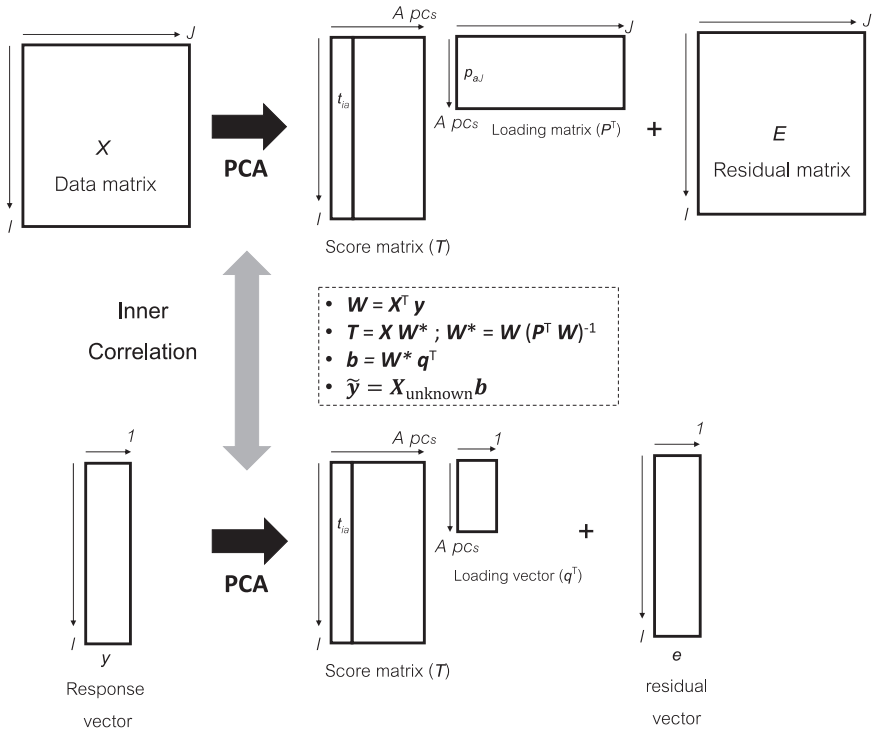


Figure 10.11 Calculation structure of a PLSR model.

10.4.4.5 Linear Discriminant Analysis (LDA)

LDA is an extension method of ED that includes the pooled variance–covariance matrix ($\boldsymbol{S}_p$) in the distance calculation.[28,29] The distance between samples to the class centroid is weighted according to the overall variance of each variable. Hence the correlation between any of the variables (if present) is now taken into account. The LDA distance to class centroid g is calculated using a measure called the *Mahalanobis distance* as follows:

$$d_{ig} = \sqrt{\left(\boldsymbol{x}_i - \bar{\boldsymbol{x}}_g\right)\boldsymbol{S}_p^{-1}\left(\boldsymbol{x}_i - \bar{\boldsymbol{x}}_g\right)^{\mathrm{T}}}$$

where $\boldsymbol{S}_p$ is the pooled covariance matrix, calculated for two classes as follows:

$$\boldsymbol{S}_p = \frac{\sum_{g=1}^{G}\left(I_g - 1\right)\boldsymbol{S}_g}{\sum_{g=1}^{G}\left(I_g - 1\right)}$$

where I_g is the number of samples in class g and $\boldsymbol{S}_g$ is the variance–covariance matrix for group g. However, the pooled covariance matrix is valid only if the classes have similar variance–covariance matrices, otherwise

this matrix will be inaccurate. It is important to realize that the LDA method uses the Mahalanobis distance based on a variance–covariance matrix for the entire dataset, rather than for each class independently.[29]

10.4.4.6 Quadratic Discriminant Analysis (QDA)

The correlation between variables is taken into account in the LDA method. QDA is similar to the LDA method but the former uses the variance–covariance matrix of each class rather than the overall pooled matrix. Hence QDA does not assume that the variances of different classes have a similar variance–covariance matrix. In some cases, it is possible to assume that there is a very different variance structure in each class itself in the dataset and therefore QDA will be more appropriate than LDA in this case. The distance can be calculated as follows:

$$d_{ig} = \sqrt{\left(\boldsymbol{x}_i - \bar{\boldsymbol{x}}_g\right)\boldsymbol{S}_g^{-1}\left(\boldsymbol{x}_i - \bar{\boldsymbol{x}}_g\right)^{\mathrm{T}}}$$

where $\boldsymbol{S}_g$ is the variance–covariance matrix of class g.

In the case of both LDA and QDA, it can be seen that it is necessary to calculate the inverse of the variance–covariance matrix ($\boldsymbol{S}$). Therefore, there is a limitation to these methods. If the number of variables is larger than the number of samples in a dataset, $\boldsymbol{S}$ will be a singular matrix that cannot be inverted. For EDC, LDA and QDA, the class of a sample is assigned to the class with the smallest distance. These methods can be used in multiclass classification (two or more classes).

10.5 Applications of Chemometrics in Optical Chemical Sensors

In recent decades, the field of optical chemical sensors has grown dramatically, according to the high selectivity and sensitivity to visualize and quantify the target being analyzed.[95,96,115,116] Optical chemical sensors employ optical transduction techniques to enhance the analyzed information. The most widely used techniques in optical chemical sensors are optical absorption, colorimetric and luminescence methods, and sensors based on other spectroscopic techniques, *e.g.* Raman, NIR and FT-IR spectroscopy, have also been developed. Optical chemical sensors can serve several possible purposes, such as to detect and discriminate the target analytes,[115] to monitor toxic volatile compounds,[117] to differentiate biomolecules,[95,96] to diagnose diseases using biomarkers[118] and to discriminate types of bacteria.[116] In spectroscopic techniques, a recent development in the field is to drive the availability of low-cost materials, miniature optoelectronic sources and high-efficiency detectors in order to acquire as much information from the sensors as possible. However, optical sensors using such spectroscopic

data have a major disadvantage as the instrument is expensive, difficult to move and sensitive. To operate the instruments, specialists or experienced users might be required. Recently, multiple array-based sensors combined with imaging technology have dramatically advanced the area of bio- and chemical sensing.[117,119,120] A number of studies have been conducted on the development of array sensors that provide signals for the detection and identification of the target analytes from changes in their electrochemical properties, fluorescence and color after interacting with the analytes. This analysis platform is considered to be the most convenient sensing technique as it minimizes the need for extensive signal transduction hardware as the samples are even possibly incorporated on pieces of paper and the signal can be simply observed with the naked eye. Such an advantage will lead to practical on-site analyses that can be delivered to non-technicians or end-users.

In the past, analysis with optical sensors was focused on the use of a single probe, which required high specificity with respect to the analyte in order to obtain accurate information. This involves a large number of sensors in order to detect and determine several target analytes because each sensor is used for only a single analyte. Recently, studies on sensors have moved from the search for selective sensors to the use of multi-sensor arrays to give more information about the sample composition – the more arrays, the greater is the complexity of the data obtained. In this respect, the application of chemometrics in multi-optical sensor analysis is rapidly expanding to the area of analytical chemistry. By using chemometrics, obtaining and interpreting complex datasets can be achieved without problems such as the contributions arising from analytes and interferents to be decoupled. The aim of applying chemometrics is to produce a method that will be a more reliable, intelligent process, capable of complex data interpretation, inexpensive and suitable for a wide range of applications.[115–120] The general platform of chemical sensors arrays combined with chemometrics for sensing target analytes is shown in Figure 10.12.

A set of sensing materials exhibit different binding affinities towards different analytes. Therefore, a response upon interaction of each analyte on the sensing material is measured in the form of either spectroscopic data, *e.g.* fluorescence or absorbance, or color. A more intense signal is produced when a stronger interaction occurs between the material and the analyte, whereas a weak interaction leads to a weaker response signal. The response signals from all the sensing materials in the array are collected for each analyte, resulting in a fingerprint-like response to the analyte. The group and class of the analyte can be determined and discriminated by the signal differences in the array, which can be simply visualized by applying chemometrics methods.

To acquire the data, a spectroscopic technique might be required in order to read out the information on the interaction between sensors and analytes. The fluorescence signal itself has attracted considerable attention recently as it is considered to be a suitable optical signal for incorporation into the design of a sensor array. Variations in fluorescence from the interaction between analytes and fluorophores have been used to screen, either

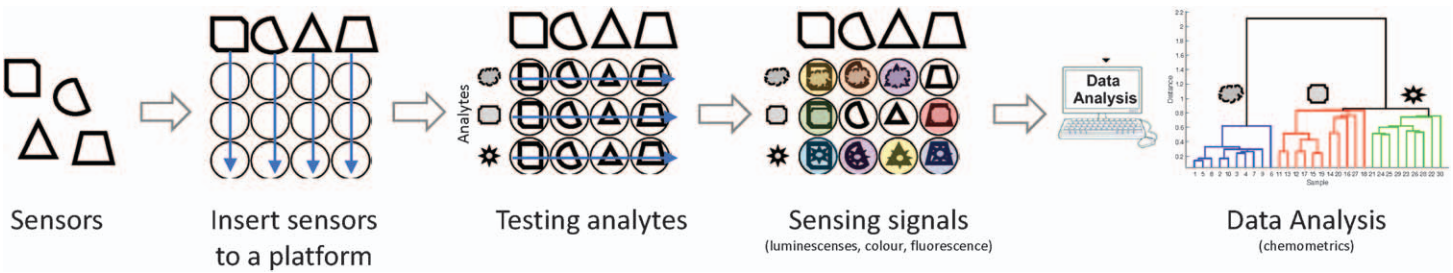


Figure 10.12 General platform of an optical sensor array for sensing target analytes. A set of sensors is provided and is then incorporated in the array platform (*e.g.* well plate, paper). The analytes are introduced into the array setup. The interaction between the sensors and analytes is measured as responses in terms of either spectroscopic signals, *e.g.* fluorescence or absorbance, or color. The collected responses create a unique fingerprint pattern for each analyte. The data that are generated are then analyzed by a suitable pattern recognition method where the different classes of analytes are determined and discriminated.

individually or simultaneously with other analytical targets. A chemosensor based on the Zn complex of 1-[bis(pyridin-2-ylmethyl)amino]methylpyrene was developed by Marbumrung *et al.*[96] for the detection of nucleotides in aqueous solution. Different recognition patterns of the fluorescence spectra from the Zn complex towards various phosphate anion-based nucleotides permitted the discrimination of the structurally similar nucleotide anions. PCA was applied to discriminate 10 nucleotides and the PC scores showed a good separation of those nucleotides. Furthermore, the PCA–LDA approach allowed the classification of the 10 analytes with nearly 100% accuracy. Discrimination of six proteins using a chemical nose approach based on fluorescence quenching of the fluorophore by gold nanoparticles stabilized by poly(*N*-isopropylacrylamide) has been reported.[95] Using PCA and LDA, a 100% accuracy of protein classification using the cross-validation approach was achieved. A sensitive fluorophore for trinitrotoluene (TNT) detection was developed using triphenylamine-based fluorophores containing pyrene or corannulene.[94] These fluorophores showed high quenching sensitivity and were highly selective towards explosive nitro compounds. The PCA scores showed good separations among the nitro compound groups and a classification accuracy of 100% was obtained by performing PCA followed by LDA based on the relative fluorescence intensity. A platform of a fluorescence sensor array containing polyelectrolyte fluorophores was developed to detect foodborne pathogens involving eight bacteria.[116] The interaction of the fluorophore and the cell membrane of the bacteria generates a response pattern that can be used to differentiate the type of pathogens. The response patterns of the eight bacteria were revealed by LDA with a classification accuracy of 100%. Concerning commercial and legal aspects of food authenticity, a fluorescence array platform was developed to identify processed milk by using the interaction patterns of fluorophores and protein content in the milk samples.[121] The fluorescence patterns involving $>50\,000$ responses were analyzed by multivariate analysis. The recognition of the milk samples according to their thermal processing was achieved by using LDA with 100% classification accuracy in a leave-one-out cross-validation approach.

For a different method of data acquisition, instead of recording spectral information, the readout based on the image of the sensor array before and after its exposure to the analytes has recently attracted considerable attention as it is simple, fast and involves an inexpensive instrument.[122,123] The images of sensor arrays can be obtained by a scanner, a digital camera or even a smartphone. The information on color (red, green and blue – RGB) was then digitally extracted pixel by pixel. The different interaction patterns between the sensor elements and the analytes will generate patterns of the color changes before and after introducing the analytes that can be used as the data input for further analysis. Eaidkong *et al.*[117] reported a novel platform with paper-based polydiacetylene (PDA) as a colorimetric sensor array to detect and identify 18 volatile organic compounds (VOCs) in the vapor phase. This is important for safety and quality assessment for environmental and industrial safety. The sensors showed color transitions when the

solvents were exposed to VOC vapors. The images of the changed color patterns were measured *via* RGB values and statistically analyzed by PCA and LDA. The score plots showed the capability of the sensor to distinguish all 18 VOCs in the vapor phase with a classification accuracy of 100%, which confirmed the high efficiency of the developed sensor. A similar platform was applied that used novel salicylidene fluorescence sensors to discriminate 15 VOC vapors.[119] The images of the paper-based sensors were captured by a mobile phone and the RGB values of the images were calculated. The patterns could be statistically categorized into 15 groups by using PCA with 100% classification accuracy evaluated by LDA.

A novel sensor array for geographic indications was developed by a unique and synergistic combination of chemical reaction arrays on paper.[120] This array was used to identify the geographic sources of turmerics, which are important as a food ingredient in several cultures. This study hypothesized that differences in chemical contents affect the reactivity of curcumins in reactions with various acidic reagents to control pH: 2,4-dinitrophenylhydrazine (2,4-DNP), vanillin, H_3BO_3, Cu^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+}. The photophysical changes of curcumins, which are major components reacting with various reagents, were used to differentiate and determine the sources of turmerics, as shown in Figure 10.13.

To allow for practical differentiations, all data were subjected to chemometric treatments. This could be done by first converting the reaction profiles from digital photographs into numerical data (RGB values) using ImageJ processing software. The data in the form of RGB numerical values in both visualization modes (white and UV light) of each reagent spot were then used to create a multidimensional dataset. In this case, the best geological discrimination of turmeric may not be obtained using all reactions. To achieve a greener detection process, a minimum reaction array might be required. In the study, the prediction of the all possible combination of reagent arrays ($=2^N - 1$, where N is the number of reagents) was performed to discover the best reagent array. Notably, the discovery of this best combination of reagents was made possible by the use of chemometrics, which permitted relatively rapid analysis of the prediction accuracies of all reagent combinations, as shown in Figure 10.14.

The number of possible combinations of reagent arrays is 2047 and predictions of all combinations were performed using LDA as shown Figure 10.14. This resulted in a set of eight reagents (H_2O + pH2 + 2,4-DNP + vanillin + H_3BO_3 + Fe^{2+} + Ni^{2+} + Pb^{2+}) that provided a satisfactory maximum prediction accuracy of 94%. To visualize the relation of samples, PCA was performed on the data using only the aforementioned eight selected reagents. A representative two-dimensional PC plot (Figure 10.15) provided clear differentiations of certain samples, with the most prominent ones being Cur9 (Myanmar) and Cur10 (China). The only problematic case was between Cur5 (Thailand) and Cur6 (Thailand), both of which were from the same country. No other obscure results were obtained for samples from different countries.

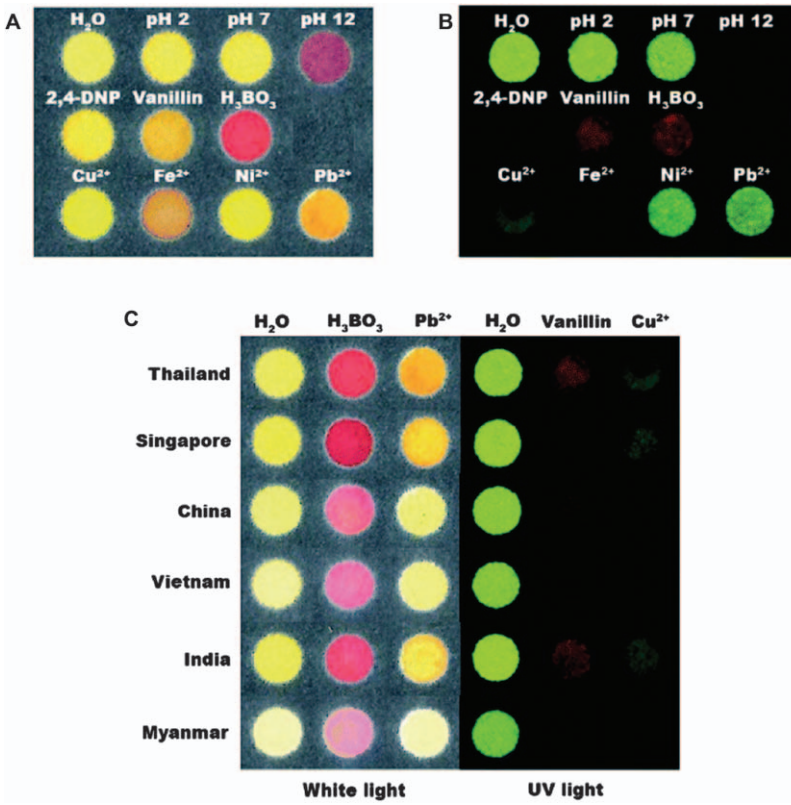


Figure 10.13 Paper arrays of chemical reactions probed under (A) white light and (B) 365 nm UV light. (C) A side-by-side comparison of reactions with H_3BO_3, Pb^{2+} (white light – left side), vanillin and Cu^{2+} (UV light – right side) from turmeric sources from various countries.
Reproduced from ref. 120 with permission from the Royal Society of Chemistry.

10.6 Some Examples of Applications of Spectroscopy–Chemometrics Research

This section presents three examples of spectroscopy–chemometrics research.

10.6.1 Moving Window Partial Least-squares Regression (MWPLSR) and Its Application to *In Vivo* Non-invasive Monitoring of Blood Glucose by Near-infrared Diffuse Reflectance Spectroscopy

MWPLSR was proposed for the purpose of searching for spectral regions that contain useful information for PLS model building.[106] MWPLSR develops a

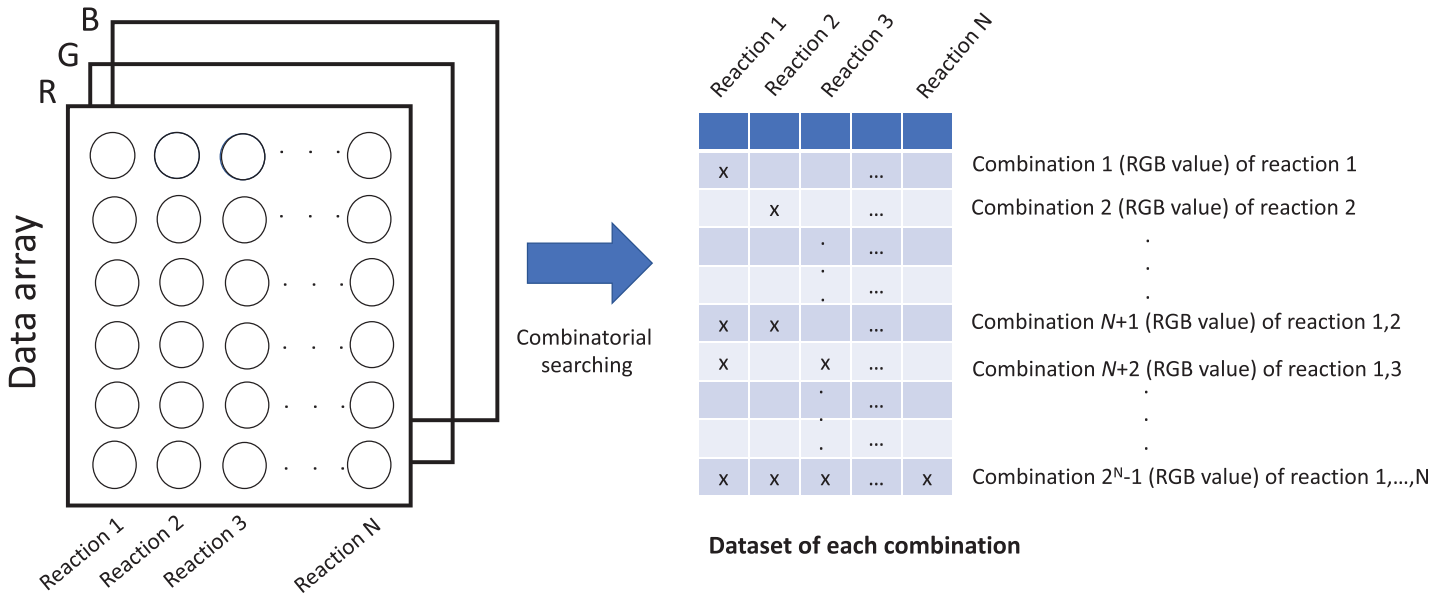


Figure 10.14 Setup of a dataset for all possible combinations of reagent arrays ($=2^N - 1$, where N is the number of reagents) in order to perform predictions for exploring the best reagent array.

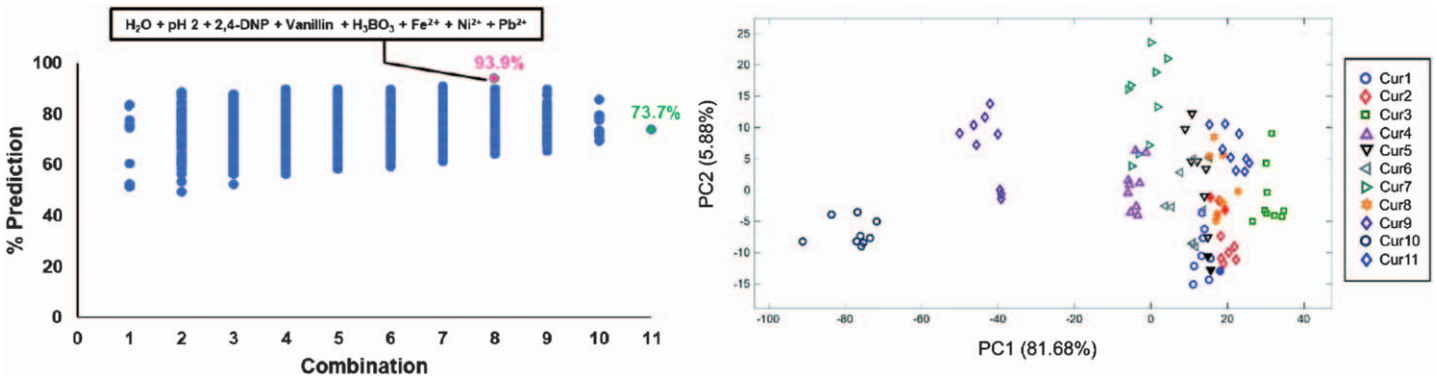


Figure 10.15 Left: plot of the LDA prediction accuracies (%) *versus* the number of reagents used in the differentiation arrays. Right: a two-dimensional PC score plot for the combination of eight reagents (H_2O; pH 2; 2,4-DNP; vanillin; H_3BO_3; Fe^{2+}; Ni^{2+}; Pb^{2+}) in discriminating 11 turmeric sources.
Reproduced from ref. 120 with permission from the Royal Society of Chemistry.

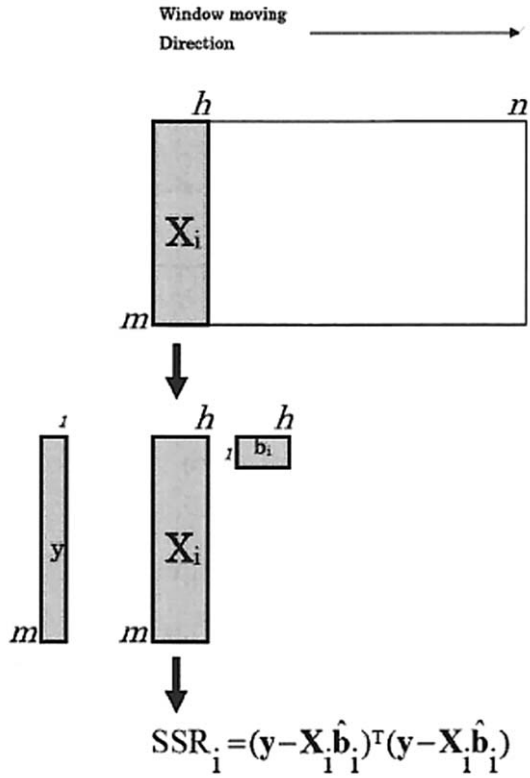


Figure 10.16 Scheme for explaining MWPLSR.
Reproduced from ref. 138 with permission from Elsevier, Copyright 2006.

series of PLS models, yielding PLS factor numbers (LVs) in a window that moves over the full spectra and then locates relevant spectral intervals in terms of the least complexity of PLS models reaching a desired error level. Moreover, one can reduce the size of a calibration sample set in calibration modeling by selecting spectral intervals in terms of the least model complexity.

Figure 10.16 illustrates the MWPLSR algorithm.[106] In MWPLSR, a spectral window is built starting at the ith spectral channel and ending at the $(I+h-1)$th spectral channel, where h is the window size. There are $(n-h+1)$ windows over the whole spectra, each window corresponding to a subset of the original spectra $\boldsymbol{X}$ ($m \times n$ matrix; m samples and n spectral channels; see Figure 10.16).

PLS models with different numbers of LVs can then be developed to relate the spectra in the window to the concentrations of the analyte, as follows:

$$\boldsymbol{y} = \boldsymbol{X}_i \boldsymbol{b}_{i,k} + \boldsymbol{e}_{i,k}$$

where $\boldsymbol{b}_{i,k}$ ($h\times1$ vector) is the regression coefficients vector, estimated by using PLS with k PLS components, and $\boldsymbol{e}_{i,k}$ is the residue vector obtained with k PLS components. The window is moved over the whole spectral region. At each position, PLS models with varying PLS component number are constructed for the calibration samples and with these PLS models the logarithms of the sums of squared residues [log(SSR)] are calculated and then plotted as a function of the position of the window.[106]

Each informative region yields low values of the SSR and often shows the shape of an upside-down peak, corresponding to a band in the same region. Thus, one can easily find the beginning and end points of the informative region. In practice, more than one informative region is often found by MWPLSR in vibrational spectra, because of the existence of many bands. In such cases, a search for the optimum combination of regions may be needed to extract more useful information from the spectra employed to develop the PLS models. To identify an optimized sub-region for each selected informative region and the optimized combination of informative regions, changeable size moving window partial least-squares (CSMWPLS) and searching combination moving window partial least-squares (SCMWPLS) methods were proposed.[107] Details of the basic principles of CSMWPLS and SCMWPLS can be found elsewhere.[107]

MWPLS, CSMWPLS and SCMWPLS were applied to the *in vivo* non-invasive monitoring of blood glucose by NIR diffuse-reflectance (DR) spectroscopy. NIR non-invasive blood glucose assays have long been investigated.[132–135] Robinson *et al.*[132] and Müller *et al.*[133] used a shorter wavelength NIR region, *i.e.* 800–1300 nm (1350 nm by Müller *et al.*[133]) for finger DR spectral measurements. Heise and co-workers[134,135] performed the *in vivo* determination of glucose on a single diabetic sample using a glucose tolerance test and that on a population of 133 different subjects. They measured the NIR DR spectra taken from the oral mucosa. They reported that the root mean square error of prediction (RMSEP) for the prediction of glucose was 36.4 mg dL^{-1}.

Non-invasive NIR blood glucose monitoring deals with very weak signals of glucose obtained directly from human skin. The physiological conditions of skin tissue, such as body temperature, always change with time.[132–135] One critical difficulty associated with *in vivo* blood glucose assays is the extremely low signal-to-noise ratio (S/N) of the glucose peak in an NIR spectrum of human skin tissue. This means that background noise easily hides the glucose signal when an NIR spectrum is measured *in vivo*. To overcome this difficulty, Maruo and co-workers[136,137] developed a novel NIR system that can detect very weak glucose signals in human tissue with reasonably good S/N in the 900–1300 nm region.

Figure 10.17 depicts the normalized NIR spectra of glucose powder (solid line) and human skin measured non-invasively (dashed line).[138] The human skin spectrum was calculated by averaging 48 skin spectra collected during an oral glucose tolerance test. It is noted that spectral features arising from blood glucose are significantly interfered with by those due to water and

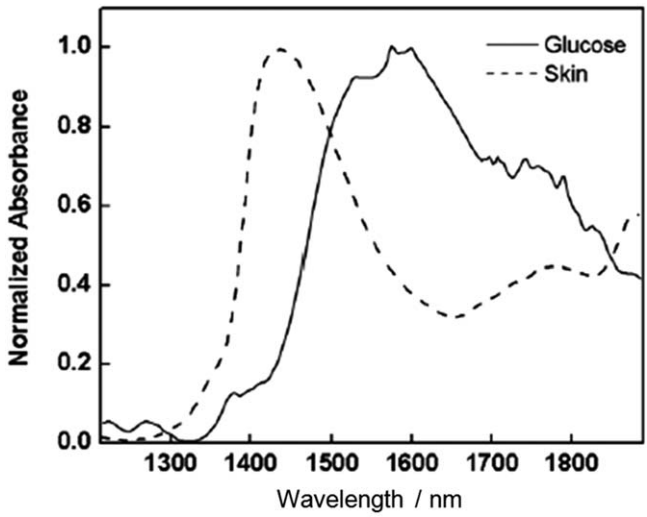


Figure 10.17 A normalized NIR spectrum in the 1212–1889 nm region of glucose powder (solid line) and a normalized average of 48 human skin spectra (dashed line) measured during an oral glucose tolerance test. Reproduced from ref. 138 with permission from Elsevier, Copyright 2006.

other components in the skin. To deal with this interference problem, MWPLSR and SCMWPLS were applied to the *in vivo* NIR spectra of skin for blood glucose assay.[138] The raw spectra were subjected to multiplicative scattering correction (MSC) before MWPLSR and SCMWPLS were applied. All 48 skin spectra were used to develop PLS calibration models. The model performance was validated by the four-segments cross-validation method (12 spectra for each segment) and the root mean square error of validation (RMSEV) was calculated.

Figure 10.18 exhibits 15 residue lines for blood glucose obtained by applying MWPLSR to the NIR spectra of skin.[138] Three informative regions (the 1228–1323, 1574–1736 and 1739–1800 nm regions) can be observed. Bands in the last two informative regions are assigned to the first overtones of the OH and CH stretching modes of glucose, respectively. The 1228–1323 nm region has only a weak absorption feature arising from glucose, but it has weak interference from water. For optimizing the combination of informative regions, SCMWPLS was applied to these three informative regions, and suggested the 1574–1736 nm region as the optimal combination.[138]

Table 10.4 summarizes statistical results of calibration models for blood glucose developed by the whole region, the individual informative regions, their direct combinations and the optimized informative region.[138] Table 10.4 reveals that the PLS calibration model based on SCMWPLS using the best optimized informative region of 1616–1733 nm gives the best validation results with the highest correlation coefficient of 0.9205 and the

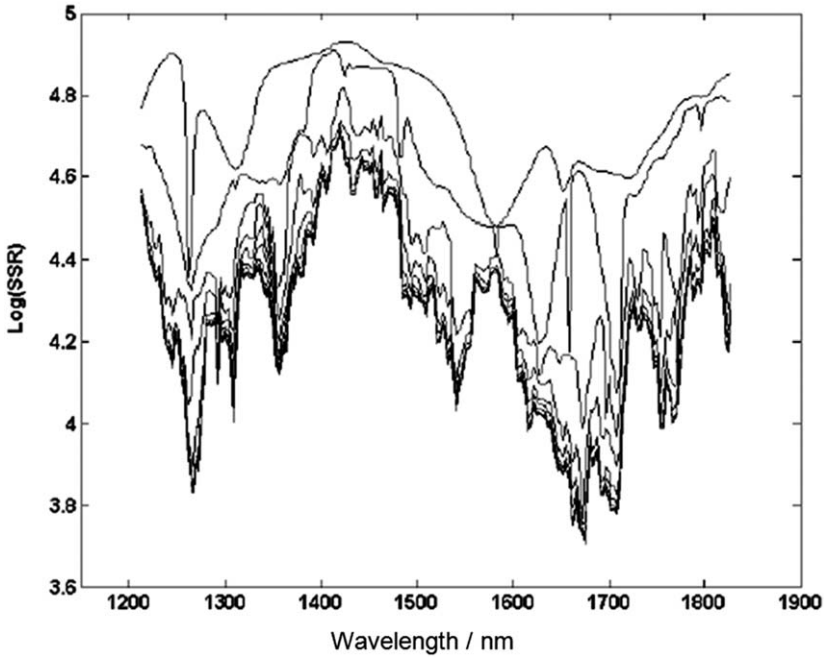


Figure 10.18 Residue lines obtained by MWPLSR for the NIR spectra of skin. Reproduced from ref. 138 with permission from Elsevier, Copyright 2006.

Table 10.4 Prediction results for PLS calibration models for blood glucose determination developed by use of the whole spectral region and the regions selected by MWPLSR and SCMWPLS. Reproduced from ref. 138 with permission from Elsevier, Copyright 2006.

Method	Spectral region/nm	PLS factor	Correlation coefficient	RMSE/mg dL^{-1}
Whole region	1212–1889	7	0.8936	20.1977
MWPLSR	1228–1323	6	0.8519	24.2398
MWPLSR	1574–1736	4	0.9091	18.3642
MWPLSR	1739–1800	4	0.8302	24.8947
MWPLSR	1228–1323, 1574–1736, 1739–1800	6	0.8840	20.9073
MWPLSR	1228–1323, 1574–1736	6	0.8984	19.4118
MWPLSR	1574–1736, 1739–1800	5	0.9060	18.7775
SCMWPLS	1616–1733	4	0.9205	17.1924

lowest RMSEV of 17.1924 mg dL^{-1} with a PLS factor of 4.[138] It is notable that this optimized region selected by SCMWPLS contains more information about blood glucose and less interference than the others.[138]

Exploratory graph analysis (EGA) plots for the three PLS models built by the whole region, the 1574–1736 nm region suggested by MWPLSR and the optimized 1616–1733 nm region yielded by SCMWPLS are shown in

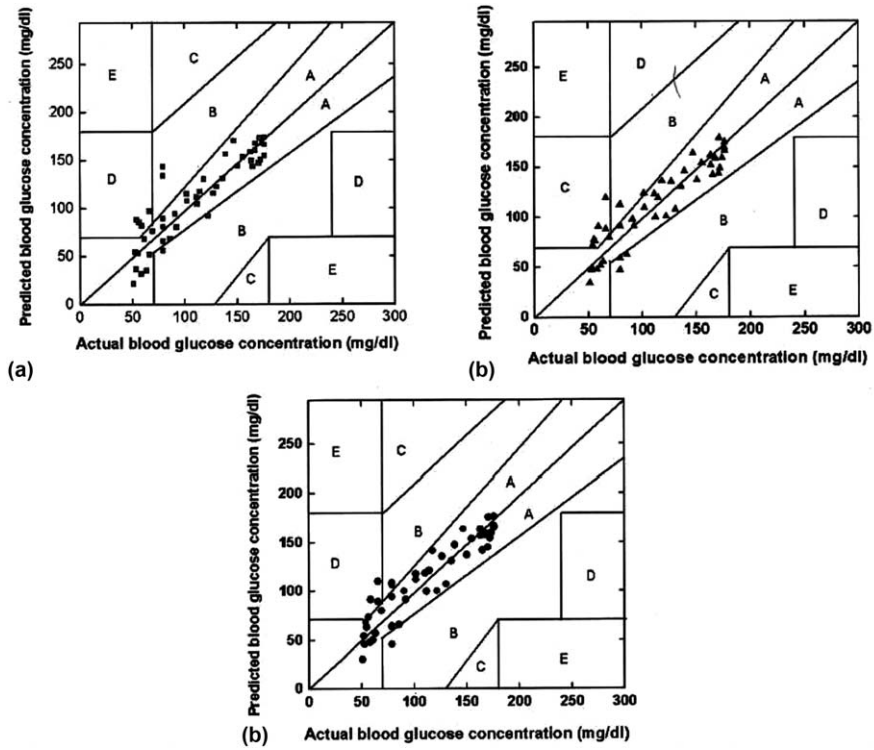


Figure 10.19 EGA plots between the actual and predicted blood glucose concentrations for (a) the PLS model based on the whole region, (b) PLS model obtained by using the informative region of 1574–1736 nm suggested by MWPLSR and (c) PLS model developed by use of the optimized informative region of 1616–1733 nm obtained by SCMWPLS.
Reproduced from ref. 138 with permission from Elsevier, Copyright 2006.

Figure 10.19.[138] All of the EGA plots illustrate that the mainstream of the prediction values is located within zone A, which is defined as clinically correct. These EGA plots confirm that the prediction results for the non-invasive blood glucose measurements by NIR spectroscopy are clinically acceptable. However, note that the EGA plots for the three models are significantly different from each other in terms of the numbers and positions of points in each zone. It was found that 85.5, 80.2 and 83.3% of the predicted blood glucose concentrations are located in zone A for the models obtained with SCMWPLS, MWPLSR and the whole region, respectively. Moreover, 7.2, 9.4 and 8.3% of the predicted values fall in zone B and 7.3, 10.4 and 8.4% are observed in zone D for these three models. Therefore, it was concluded that SCMWPLS not only demonstrated the best performance in statistical terms, but also yielded the best clinical accuracy among the three models.[138]

10.6.2 Near-infrared (NIR) Electronic Spectroscopy Study of a Calcination Reaction of Highly Reflective Green–Black (HRGB) Pigments

NIR spectroscopy consists of electronic spectroscopy and vibrational spectroscopy. Morisawa *et al.*[139] used NIR electronic spectroscopy for the non-destructive monitoring of a chemical reaction of an inorganic functional material. NIR spectra of highly reflective green–black (HRGB; $Co_{0.5}Mg_{0.5}Fe_{0.5}Al_{1.5}O_4$) pigments calcined at 1000, 1100 and 1200 °C and pigments that have the same components as HRGB but were calcined below 1000 °C (500–900 °C) (hereafter, these pigments are denoted Pigments A) were measured.[139] The HRGB pigment, which was developed at Toda Kogyo Co. (Hiroshima, Japan), is black in color, but absorbs little sunlight,[139] hence it is a sort of green material. It is made from Fe_2O_3, $Al(OH)_3$, $Mg(OH)_2$ and $CoCO_3$. Morisawa *et al.*[139] investigated the crystal structure of the components of the pigment and its transmittance characteristics based on the absorptions that are responsible for the decrease in the reflectivity.

NIR DR (diffuse reflectance) spectra in the 12 000–4000 cm^{-1} region and their second-derivative spectra in the 10 000–4500 cm^{-1} region of powders of HRGB, Co_3O_4 and α-Fe_2O_3 are shown in Figures 10.20 and 10.21, respectively.[139] It can be seen from the second-derivative spectra that the pigment yields bands at 6354, 7069, 7590 and 8024 cm^{-1} and that Co_3O_4, with a similar spinel structure to HRGB, has bands at 6094, 6713, 7569, 7951 and 8320 cm^{-1}. These bands of Co_3O_4 are due to *d–d* transitions, $^4A_2 \rightarrow {}^4T_1$, of Co(II) at a tetrahedral cite.[139] It is noted in Figure 10.20 that the NIR DR spectrum of α-Fe_2O_3 shows a long tail in the 12 000–10 000 cm^{-1} region arising from a charge-transfer (CT) transition, which has maxima at 17 000 and 14 000 cm^{-1}.[139] HRGB shows characteristic peaks of Co(II) in the spinel structure, but it does not show a tail due to Fe(III). Figure 10.22 presents NIR

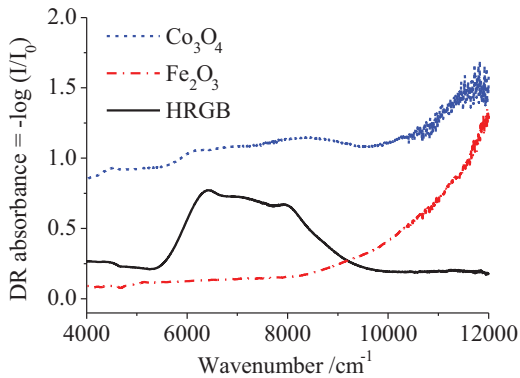


Figure 10.20 NIR DR spectra in the 12 000–4000 cm^{-1} region of powders of HRGB, Co_3O_4 and α-Fe_2O_3.
Reproduced from ref. 139 with permission from SAGE Publishing, Copyright 2012.

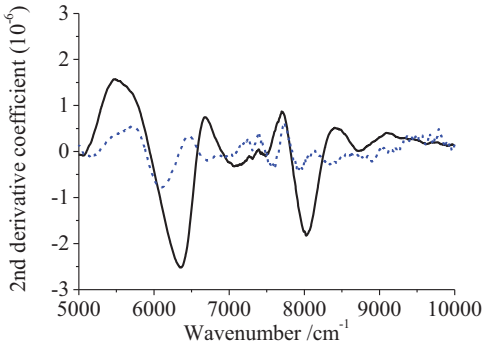


Figure 10.21 Second derivatives of the NIR DR spectra of powders of HRGB (–) and Co_3O_4 (-----) in the 10 000–5000 cm^{-1} region.
Reproduced from ref. 139 with permission from SAGE Publishing, Copyright 2012.

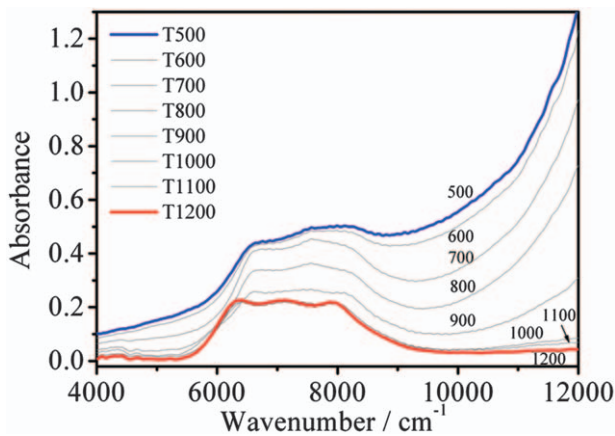


Figure 10.22 NIR DR spectra of HRGB calcined at 1000, 1100 and 1200 °C and Pigments A calcined below 1000 °C (500–900 °C).
Reproduced from ref. 139 with permission from SAGE Publishing, Copyright 2012.

DR spectra of HRGB calcined at 1000, 1100 and 1200 °C and Pigments A calcined below 1000 °C (500–900 °C).[139] The figure demonstrates that the three components of the *d–d* transition band of Co(II) in the 9000–6000 cm^{-1} region shift significantly to the lower wavenumber side with increase in calcination temperature from 900 to 1000 °C and that the CT band of Fe(III) decreases considerably.

NIR bands of Pigments A calcined at 500 and 900 °C and HRGB and Co_3O_4 are listed in Table 10.5. Referring to wide-angle X-ray diffraction (WAXD) data for HRGB and Pigments A, the bands at 6541, 7529 and 8208 cm^{-1} of Pigments A calcined in the temperature range 500–900 °C are assigned to

Table 10.5 Band positions in the NIR electronic spectra of Pigments A calcined at 500 and 900 °C, HRGB and Co_3O_4. Reproduced from ref. 139 with permission from SAGE Publishing, Copyright 2012.

	Pigment A	Pigment A	HRGB (TR)	HRGB (DR)	Co_3O_4 (DR)
Measured temperature	Room temp.	Room temp.	Room temp.	Room temp.	Room temp.
Calcination T/°C	500–900	700–900	1000–1200	1100	
Structure	Spinel	Inverse spinel	Spinel	Spinel	Spinel
Cobalt site	Th[a]	Th/Oh[b]	Th	Th	Th/Oh
Band positions/cm^{-1}	6541	5924	6264	6354	6109
				7069	6746
	7529	7220	7128	7590	7536
	8208	8208	7992	8024	8360

[a]Co(II) exists in a tetrahedral site.
[b]Co(II) exists in both a tetrahedral site and an octahedral site. TR: transmittance; DR: diffuse reflectance.

AB'_2O_4 [A = Co(II), Mg(II), B′ = Al(III)] with a spinel structure and those at 5924, 7220 and 8208 cm^{-1} of Pigments A calcined in the temperature range 700–900 °C are ascribed to $(A_{1-x}B_x)^{Th}(A_xB_{2-x})^{Oh}O_4$ (A = Co, Mg, B = Fe, Al) having an inverse spinel structure.[139] By comparing the calcination temperature-dependent NIR data with the corresponding WAXD data, it was found that the intensity of the CT band of Fe(III) (Figure 10.22) decreased with decrease in the α-Fe_2O_3 crystal in the pigments (Pigments A and HRGB). Therefore, it was concluded that when α-Fe_2O_3 is mixed with MgO and Al_2O_3, $(A_{1-x}B_x)^{Th}(A_xB_{2-x})^{Oh}O_4$ and AB_2O_4 [A = Co(II), Mg(II); B = Fe(III), Al(III)] are formed through AB'_2O_4 [A = Co(II), Mg(II); B′ = Al(III)].[139]

A PLS calibration model for the calcination reaction temperature was developed from the second-derivative NIR spectra in the 12 000–4000 cm^{-1} region of HRGB and Pigments A.[139] Figure 10.23a depicts loadings plots for LV1, LV2 and LV3 of the PLS calibration model constructed using the second-derivative NIR data for HRGB and Pigments A. Figure 10.23b and c represent score plots for LV1 and LV2 of the PLS calibration model, respectively.[139] It is noted that the negative peaks in LV1 correspond to the bands in the second-derivative spectra of HRGB calcined in the temperature range 1000–1200 °C whereas its positive peaks are attributed to the bands in the second-derivative spectra of Pigments A calcined in the temperature range 500–900 °C. Therefore, it is very likely that the scores of LV1 reflect the decrease in the initial products and the increase in the final products. The LV2 loadings plot shows a downward peak near 5900 cm^{-1}, attributable to a band in the second-derivative spectrum at 900 °C, assigned to the intermediate material [$(A_{1-x}B)^{Th}(A_xB_{2-x})^{Oh}O_4$; inverse spinel structure] (Figure 10.23a). Accordingly, Morisawa *et al.*[139] concluded that the PLS results reveal the existence of an intermediate. It is very likely that the score plots for LV1 and LV2 express the degree of progress of the calcination reaction (Figure 10.23b and c).

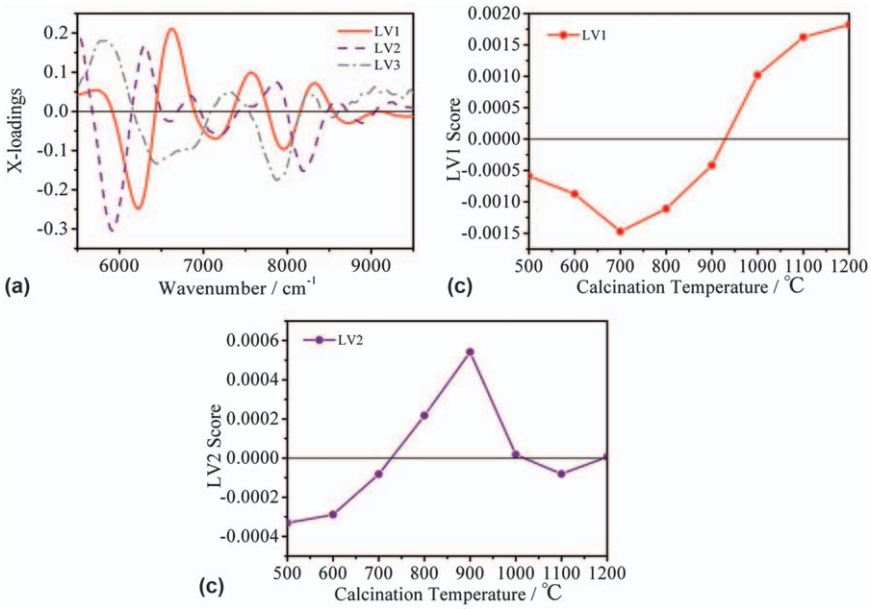


Figure 10.23 (a) Loadings plots for LV1, LV2 and LV3 of the PLS calibration model developed using the second-derivative NIR data of HRGB and Pigments A. (b), (c) Score plots for LV1 and LV2, respectively.
Reproduced from ref. 139 with permission from SAGE Publishing, Copyright 2012.

They succeeded in monitoring the behavior of the intermediate $[(A_{1-x}B_x)^{Th}(A_xB_{2-x})^{Oh}O_4]$ in the calcination process from the LV2 score plot; the intermediate appears in the 800–900 °C range (Figure 10.23c).

This study provided new insight into the development of chemometrics calibration models for the NIR electronic spectroscopy of complicated inorganic materials.[139]

10.6.3 Raman Imaging Study of the Lycopene Aggregation *In Vivo* in Tomato

Raman imaging is a powerful method that provides information about the distribution of components in a material and information on molecular structure at each spatial location.[140,141] Raman imaging was used for the *in vivo* investigation of lycopene with different aggregate conformations distributed inhomogeneously in tomato.[142] Raman images enabled the lycopene concentration in different parts of the tomato fruits to be visualized during the different ripening stages. A UV–VIS spectrometer with both microscopic and imaging functions is currently not available and as lycopene is a fluorescence-free molecule, fluorescence imaging cannot capture the lycopene distribution. Therefore, Raman imaging may be the only way to visualize the distribution of lycopene aggregates.

Figure 10.24 displays resonance Raman spectra of lycopene monomer and aggregates in acetone–water solutions and in dry microcrystals measured with (top) 532 nm and (bottom) 638 nm excitation.[142] Two intense Raman bands with ν_1 and ν_2 around 1514 and 1156 cm^{-1}, respectively, were assigned to C=C stretching and C–C stretching vibrational modes, respectively.[143–145] Both the ν_1 and ν_2 bands showed a significant peak shift with change in acetone concentration; the lycopene aggregates *in vitro* showed a shift of the ν_1 band to a lower frequency by 1 cm^{-1}, whereas the ν_2 band yielded a higher frequency shift by 3 cm^{-1} compared with the monomer. Corresponding significant shifts of the ν_1 and ν_2 bands were not observed for the 638 nm excitation.

For recording Raman imaging spectra, tomato fruits with a size of *ca.* 2.21×3.38 cm were placed on a motorized stage. A laser spot with 532 nm excitation was automatically refocused at each point prior to measurement to compensate for surface roughness. More details of the Raman imaging measurement can be found in the paper by Ishigaki *et al.*[142]

In order to analyze the conformations of lycopene and its spatial distributions, PCA was applied to the imaging data.[142] The PCA method enables one to extract characteristic variations of lycopene in tomato mathematically. Figure 10.25 shows Raman images of the section of a tomato fruit using PCA. Figure 10.25A, B and C were obtained by plotting the PCA scores of PC1, PC3 and PC4, respectively. The corresponding loadings plots are also depicted in Figure 10.25. The PC 1 loading shows the ν_1, ν_2 and ν_3 bands at 1516, 1157 and 1005 cm^{-1}, respectively. As can be seen in Figure 10.25, there are the three major components in tomato. By comparison with the Raman spectra of H-aggregate (spectra not shown here), the PC1 loading is likely to represent the H-aggregates. Therefore, it was concluded that tomato imaging drawn using the PC1 score depicts the distribution of H-aggregates within tomato. The concentration of H-aggregate changes with the location in the tomato; note that the mesocarp has more H-aggregates than the epicarp and endocarp. Moreover, the locular gel also contains a higher amount of H-aggregates.

It can be seen from Figure 10.25 that the PC3 loading represents the shifts of the ν_1 and ν_2 bands to lower and higher frequencies, respectively. As discussed above, these band shifts suggest the formation of J-aggregates. The tomato imaging from the PC3 scores demonstrates that the mesocarp and locular gel tend to contain a lower concentration of J-aggregates. Comparison of the images in Figure 10.25A and B allows us to view the complementary distribution patterns between two different structures of lycopene aggregates.

The PCA loading gives a peak at 1605 cm^{-1} as shown in Figure 10.25C. This peak is possibly due to the wax crystals in the cuticle matrix of tomato and assigned to the C=C stretching mode of aromatic rings.[142] Note that it can be observed clearly on the surface of seeds, hence the Raman image with PC4 scores highlights the epicarp and seeds.

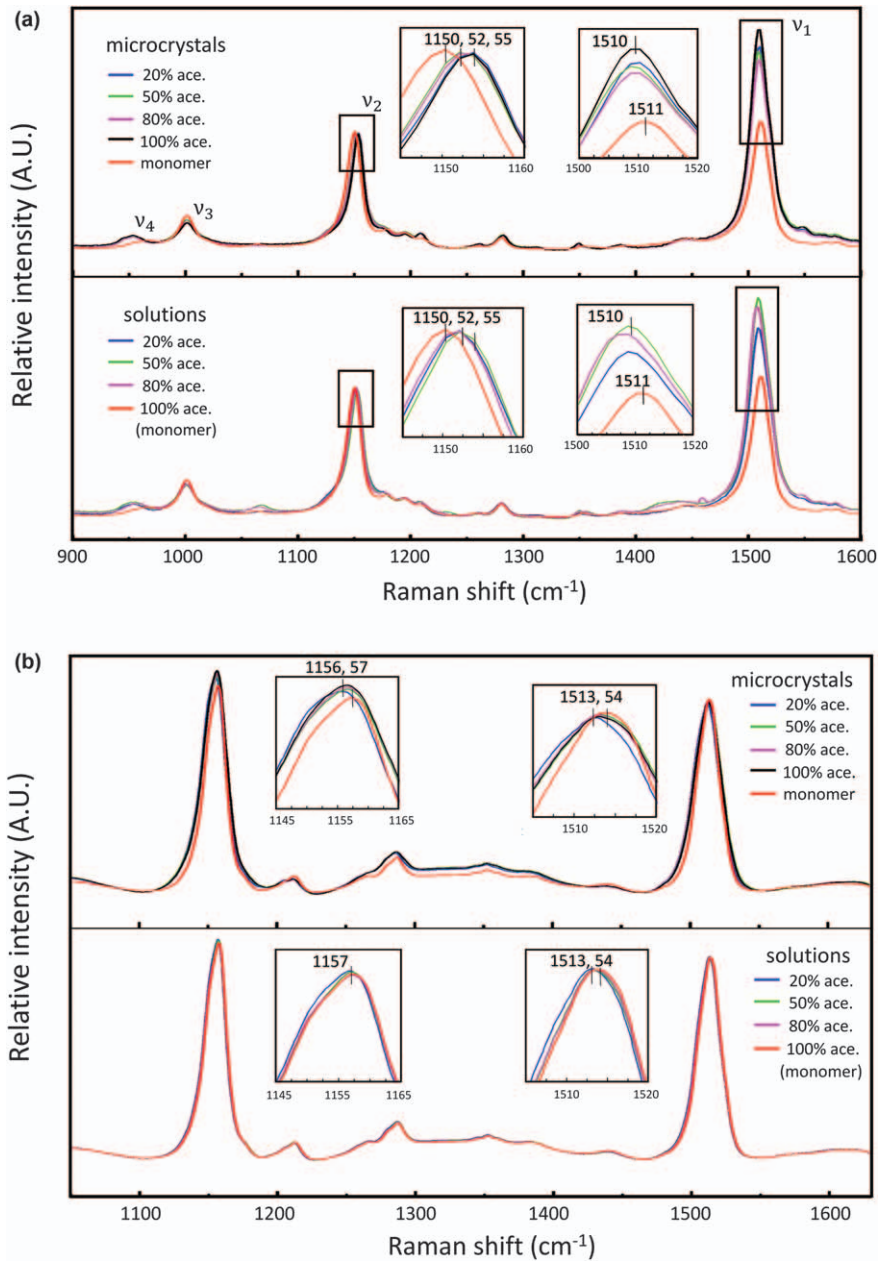


Figure 10.24 Resonance Raman spectra of lycopene monomer and aggregates in acetone–water solutions and in dry microcrystals measured with (top) 532 nm and (bottom) 638 nm excitation.
Reproduced from ref. 142 with permission from American Chemical Society, Copyright 2017.

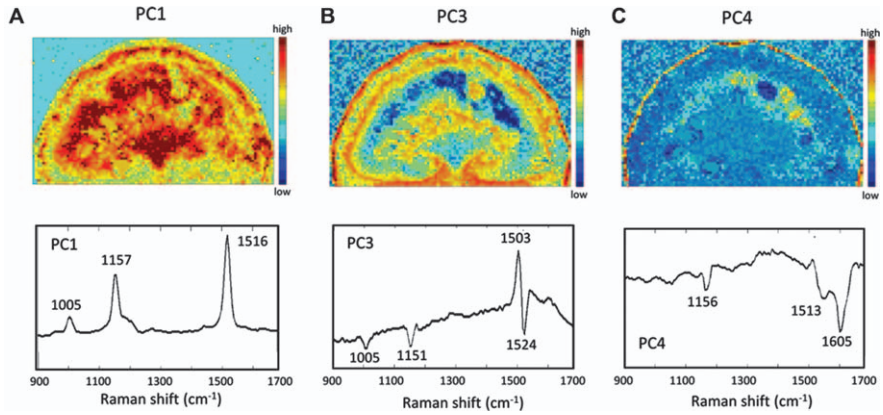


Figure 10.25 Raman images of the section of a tomato fruit using PCA. (A), (B) and (C) were drawn by plotting the PCA scores of PC1 (the H-aggregate of lycopene), PC3 (the J-aggregate of lycopene) and PC4 (wax component), respectively. The corresponding loadings plots are also depicted. Reproduced from ref. 142 with permission from American Chemical Society, Copyright 2017.

In this way, the distributions of lycopene with different aggregate structures were visualized by Raman imaging in conjunction with PCA results.[142] The PC loadings plots reveal that the mesocarp and locular gel had much higher H-aggregate contents, whereas the epicarp and endocarp had relatively high concentrations of J-aggregates.

References

1. P. Anastas, *Chem. Eng. News*, 2011, **89**, 62–65.
2. P. Anastas and J. Warner, *Green Chemistry: Theory and Practise*, Oxford University Press, New York, 1998.
3. M. Khanmohammadi and A. B. Garmarudi, *TrAC, Trends Anal. Chem.*, 2011, **30**, 864–874.
4. A. Gredilla, S. F. O. de Vallejuelo, N. Elejoste, A. de Diego and J. M. Madariaga, *TrAC, Trends Anal. Chem.*, 2016, **76**, 30–39.
5. J. A. L. Pallone, E. T. D. Carames and P. D. Alamar, *Curr. Opin. Food Sci.*, 2018, **22**, 115–121.
6. M. de la Guardia and S. Armenta, *Green Analytical Chemistry: Theory & Practice*, in Comprehensive Analytical Chemistry Series, Elsevier, Oxford, vol. 57, 2011.
7. M. Koel and M. Kaljurand, *Green Analytical Chemistry*, Royal Society of Chemistry, Cambridge, 2010.
8. M. de la Guardia and S. Garrigues, *Handbook of Green Analytical Chemistry*, John Wiley & Sons, Ltd., Chichester, 2012.
9. M. de la Guardia and J. Ruzicka, *Analyst*, 1995, **120**, N17.
10. P. T. Anastas, *Rev. Anal. Chem.*, 1999, **29**, 167–175.

11. J. Namieśnik, *Crit. Rev. Anal. Chem.*, 2000, **30**, 221–269.
12. S. Armenta, S. Garrigues and M. de la Guardia, *TrAC, Trends Anal. Chem.*, 2008, **27**, 497–511.
13. M. Watari and Y. Ozaki, *Bunseki Kagaku*, 2010, **59**, 379–397.
14. T. Hasegawa and Y. Ozaki, *Bunseki Kagaku*, 2005, **54**, 1–26.
15. D. R. Massie and K. H. Norris, *Trans. ASAE*, 1965, **8**, 598.
16. R. G. Brereton, *J. Chemom.*, 2014, **28**, 749–760.
17. P. C. Jurs, B. R. Kowalski, T. L. Isnehour and C. N. Reilley, *Anal. Chem.*, 1969, **41**, 1949–1953.
18. E. R. Malinowski, P. H. Weiner and A. R. Levinstone, *J. Phys. Chem.*, 1970, **74**, 4537–4542.
19. D. L. Massart, C. Janssens, L. Kaufman and R. Smits, *Anal. Chem.*, 1972, **44**, 2390–2393.
20. P. C. Jurs and T. L. Isenhour, *Chemical Applications of Pattern Recognition*, Wiley, New York, 1975.
21. S. Wold, *Kem. Tidskr.*, 1972, **3**, 34–37.
22. B. R. Kowalski, S. D. Brown and B. G. M. Vandeginste, *J. Chemom.*, 1987, **1**, 1–2.
23. T. A. H. M. Janse and G. Kateman, *Anal. Chim. Acta*, 1983, **150**, 219–231.
24. R. Kiralj and M. M. C. Ferreira, *J. Chemom.*, 2006, **20**, 247–272.
25. H. Martens, *J. Chemom.*, 2015, **29**, 563–581.
26. P. W. Araujo and R. G. Brereton, *TrAC, Trends Anal. Chem.*, 1996, **15**, 26–31.
27. P. W. Araujo and R. G. Brereton, *TrAC, Trends Anal. Chem.*, 1996, **15**, 63–70.
28. R. G. Brereton, *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*, Wiley, Chichester, 2003.
29. R. G. Brereton, *Chemometrics for Pattern Recognition*, John Wiley & Sons, Chichester, 2009.
30. R. G. Brereton, *Applied Chemometrics for Scientists*, John Wiley & Sons, 2007.
31. F. Marini, R. Bucci, A. L. Magrì and A. D. Magrì, *Microchem. J.*, 2008, **88**, 178–185.
32. S. A. Weissman and N. G. Anderson, *Org. Process Res. Dev.*, 2015, **19**, 1605–1633.
33. R. M. Alonso-Salces, F. Serra, F. Reniero and K. Heberger, *J. Agric. Food Chem.*, 2009, **57**, 4224–4235.
34. S. Yudthavorasit, K. Wongravee and N. Leepipatpiboon, *Food Chem.*, 2014, **158**, 101–111.
35. S. Carpino, T. E. Acree, D. M. Barbano, G. Licitra and K. J. Siebert, *J. Agric. Food Chem.*, 2002, **50**, 1143–1149.
36. D. Cozzolino, H. E. Smyth, K. A. Lattey, W. Cynkar, L. Janik, R. G. Dambergs, I. L. Francis and M. Gishen, *Anal. Chim. Acta*, 2006, **563**, 319–324.
37. A. A. Christy, S. Kasemsumran, Y. P. Du and Y. Ozaki, *Anal. Sci.*, 2004, **20**, 935–940.

38. R. M. El-Abassy, P. Donfack and A. Materny, *Food Chem.*, 2011, **126**, 1443–1448.
39. D. Villarreal, A. Laffargue, H. Posada, B. Bertrand, P. Lashermes and S. Dussert, *J. Agric. Food Chem.*, 2009, **57**, 11321–11327.
40. C. Kaur, S. Nagal, J. Nishad, R. Kumar and Sarika, *Food Res. Int.*, 2014, **60**, 205–211.
41. F. Liu, X. J. Ye, Y. He and L. Wang, *J. Food Eng.*, 2009, **93**, 127–133.
42. Y. N. Ni, W. Q. Xiao and S. Kokot, *Food Chem.*, 2009, **113**, 1339–1345.
43. P. Thammarat, C. Kulsing, K. Wongravee, N. Leepipatpiboon and T. Nhujak, *Molecules*, 2018, **23**, 1910.
44. L. Chen, X. Xue, Z. Ye, J. Zhou, F. Chen and J. Zhao, *Food Chem.*, 2011, **128**, 1110–1114.
45. H. Gatemala, S. Ekgasit and K. Wongravee, *Chemosphere*, 2017, **178**, 249–258.
46. I. Wittayarak, A. Imyim and K. Wongravee, *Desalin. Water Treat.*, 2016, **57**, 16571–16582.
47. M. Vosough, M. Rashvand, H. M. Esfahani, K. Kargosha and A. Salemi, *Talanta*, 2015, **135**, 7–17.
48. F. M. V. Pereira, A. P. Rebellato, J. A. L. Pallone and L. A. Colnago, *Food Control*, 2015, **48**, 62–66.
49. M. Todorova, A. M. Mouazen, H. Lange and S. J. W. Atanassova, *Water, Air, Soil Pollut.*, 2014, **225**, 2036.
50. A. Retnam, M. P. Zakaria, H. Juahir, A. Z. Aris, M. A. Zali and M. F. Kasim, *Mar. Pollut. Bull.*, 2013, **69**, 55–66.
51. A. Khoshmanesh, P. L. Cook and B. R. J. A. Wood, *Analyst*, 2012, **137**, 3704–3709.
52. M. M. Galera, M. D. G. Garcia, M. J. Culzoni and H. C. Goicoechea, *J. Chromatogr. A*, 2010, **1217**, 2042–2049.
53. P. Meksiarun, M. Ishigaki, V. A. C. Huck-Pezzei, C. W. Huck, K. Wongravee, H. Sato and Y. Ozaki, *Sci. Rep.*, 2017, 7.
54. M. Ishigaki, Y. Maeda, A. Taketani, B. B. Andriana, R. Ishihara, K. Wongravee, Y. Ozaki and H. Sato, *Analyst*, 2016, **141**, 1027–1033.
55. N. S. Eikje, K. Aizawa and Y. Ozaki, *Biotechnol. Annu. Rev.*, 2005, **11**, 191–225.
56. M. Ishigaki, P. Puangchit, Y. Yasui, A. Ishida, H. Hayashi, Y. Nakayama, H. Taniguchi, I. Ishimaru and Y. Ozaki, *Anal. Chem.*, 2018, **90**, 5217–5223.
57. M. Ishigaki, T. Nishii, P. Puangchit, Y. Yasui, C. W. Huck and Y. Ozaki, *J. Biophoton.*, 2018, 11.
58. P. Puangchit, M. Ishigaki, Y. Yasui, M. Kajita, P. Ritthiruangdej and Y. Ozaki, *Analyst*, 2017, **142**, 4765–4772.
59. M. Ishigaki, Y. Yasui, P. Puangchit, S. Kawasaki and Y. Ozaki, *Molecules*, 2016, **21**, 1003.
60. M. Ishigaki, S. Kawasaki, D. Ishikawa and Y. Ozaki, *Sci. Rep.*, 2016, 6.
61. M. Ishigaki, A. Taketani and H. Sato, *Anal. Methods*, 2014, **6**, 9206–9211.
62. M. Ishigaki, K. Hashimoto, H. Sato and Y. Ozaki, *Sci. Rep.*, 2017, 7.

63. B. C. Gibb, *Nat. Chem.*, 2013, **5**, 248–249.
64. J. Camacho, *Chemom. Intell. Lab. Syst.*, 2014, **135**, 110–125.
65. J. Camacho, A. Pérez-Villegas, R. A. Rodríguez-Gómez and E. Jiménez-Mañas, *Chemom. Intell. Lab. Syst.*, 2015, **143**, 49–57.
66. R. Tauler and H. Parastar, *Angew. Chem., Int. Ed.*, 2018, DOI: 10.1002/anie.201801134.
67. M. Tobiszewski, M. Nedyalkova, S. Madurga, F. Pena-Pereira, J. Namieśnik and V. Simeonov, *Ecotoxicol. Environ. Saf.*, 2018, **147**, 292–298.
68. M. Tobiszewski, S. Tsakovski, V. Simeonov, J. Namiesnik and F. Pena-Pereira, *Green Chem.*, 2015, **17**, 4773–4785.
69. ACS GCI Pharmaceutical Roundtable Solvent Selection Guide, 2011, http://www.acs.org/content/dam/acsorg/greenchemistry/industriainnovation/roundtable/acs-gci-pr-solvent-selection-guide.pdf (accessed 4 May, 2019).
70. C. R. Hargreaves and J. B. Manley, ACS GCI Pharmaceutical roundtable – Collaboration to deliver a solvent selection guide for the pharmaceutical industry, 2008, http://www.acs.org/content/dam/acsorg/greenchemistry/industriainnovation/roundtable/solvent-selection-guide.pdf (accessed 4 May, 2019).
71. A. D. Curzons, D. C. Constable and V. L. Cunningham, *Clean Prod. Processes*, 1999, **1**, 82–90.
72. C. Jimenez-Gonzalez, A. D. Curzons, D. J. C. Constable and V. L. Cunningham, *Clean Technol. Environ. Policy*, 2005, **7**, 42–50.
73. R. K. Henderson, C. Jimenez-Gonzalez, D. J. C. Constable, S. R. Alston, G. G. Inglis, G. Fisher, J. Sherwood, S. P. Binks and A. D. Curzons, *Green Chem.*, 2011, **13**, 854–862.
74. D. L. Massart and L. Kaufman, *The Interpretation of Analytical Chemical Data by the use of Cluster Analysis*, Wiley Interscience, New York, 1983.
75. C.-L. Hwang, Y.-J. Lai and T.-Y. Liu, *Comput. Oper. Res.*, 1993, **20**, 889–899.
76. J. A. Westerhuis, T. Kourti and J. F. Macgregor, *J. Chemom.*, 1998, **12**, 301–321.
77. R. Bro, *Chemom. Intell. Lab. Syst.*, 1997, **38**, 149–171.
78. P. W. Araujo and R. G. Brereton, *TrAC, Trends Anal. Chem.*, 1996, **15**, 156–163.
79. P. M. Murray, F. Bellany, L. Benhamou, D. K. Bucar, A. B. Tabor and T. D. Sheppard, *Org. Biomol. Chem.*, 2016, **14**, 2373–2384.
80. Å. Rinnan, F. v. d. Berg and S. B. Engelsen, *Trac, Trends Anal. Chem.*, 2009, **28**, 1201–1222.
81. L. Z. Yi, N. P. Dong, Y. H. Yun, B. C. Deng, D. B. Ren, S. Liu and Y. Z. Liang, *Anal. Chim. Acta*, 2016, **914**, 17–34.
82. A. A. Christy, Y. Ozaki and V. G. Gregoriou, *Modern Fourier Transform Infrared Spectroscopy*, Elsevier, 2001.
83. K. Wongravee, G. R. Lloyd, C. J. Silwood, M. Grootveld and R. G. Brereton, *Anal. Chem.*, 2010, **82**, 628–638.

84. M. L. Schaefer, K. Wongravee, M. E. Holmboe, N. M. Heinrich, S. J. Dixon, J. E. Zeskind, H. M. Kulaga, R. G. Brereton, R. R. Reed and J. M. Trevejo, *Chem. Senses*, 2010, **35**, 459–471.
85. K. Wongravee, N. Heinrich, M. Holmboe, M. L. Schaefer, R. R. Reed, J. Trevejo and R. G. Brereton, *Anal. Chem.*, 2009, **81**, 5204–5217.
86. G. R. Lloyd, K. Wongravee, C. J. L. Silwood, M. Grootveld and R. G. Brereton, *Chemom. Intell. Lab. Syst.*, 2009, **98**, 149–161.
87. Y. Xu, F. Gong, S. J. Dixon, R. G. Brereton, H. A. Soini, M. V. Novotny, E. Oberzaucher, K. Grammer and D. J. Penn, *Anal. Chem.*, 2007, **79**, 5633–5641.
88. G. R. Lloyd, R. G. Brereton and J. C. Duncan, *Analyst*, 2008, **133**, 1046–1059.
89. L. De Lathauwer, B. De Moor and J. Vandewalle, *J. Chemom.*, 2000, **14**, 123–149.
90. N. Bratchell, *Chemom. Intell. Lab. Syst.*, 1989, **6**, 105–125.
91. B. G. M. Vandeginste and D. L. Massart, *Handbook of Chemometrics and Qualimetrics: Part B*, Elsevier Science, Amsterdam, The Natherlands, 1998.
92. D. L. Massart, B. G. M. Vandeginste and L. Buydens, *Handbook of Chemometrics and Qualimetrics*, Elsevier Science, Amsterdam, The Natherland, 1997.
93. M. Shimoyama, S. Morimoto and Y. Ozaki, *Analyst*, 2004, **129**, 559–563.
94. N. Niamnont, N. Kimpitak, K. Wongravee, P. Rashatasakhon, K. K. Baldridge, J. S. Siegel and M. Sukwattanasinitt, *Chem. Commun.*, 2013, **49**, 780–782.
95. K. Kusolkamabot, P. Sae-ung, N. Niamnont, K. Wongravee, M. Sukwattanasinitt and V. P. Hoven, *Langmuir*, 2013, **29**, 12317–12327.
96. S. Marbumrung, K. Wongravee, V. Ruangpornvisuti, G. Tumcharern, T. Tuntulani and B. Tomapatanaget, *Sens. Actuators, B*, 2012, **171–172**, 969–975.
97. S. C. Ng, *Procedia Comput. Sci.*, 2017, **111**, 113–119.
98. C. Clausen and H. Wechsler, *Pattern Recogn.*, 2000, **33**, 1555–1560.
99. R. Bro and A. K. Smilde, *Anal. Methods*, 2014, **6**, 2812–2831.
100. J. Camacho, J. Pico and A. Ferrer, *Chemom. Intell. Lab. Syst.*, 2010, **100**, 48–56.
101. S. Wold and M. Sjostrom, *Chemom. Intell. Lab. Syst.*, 1998, **44**, 3–14.
102. R. Madsen, T. Lundstedt and J. Trygg, *Anal. Chim. Acta*, 2010, **659**, 23–33.
103. R. G. Brereton, *Chem. Cent. J.*, 2012, **6**, S1.
104. T. Kohonen, *Biol. Cybern.*, 1982, **43**, 59–69.
105. T. Kohonen, *Self-Organizing Maps*, Springer-Verlag, New York, 2001.
106. J. H. Jiang, R. J. Berry, H. W. Siesler and Y. Ozaki, *Anal. Chem.*, 2002, **74**, 3555–3565.
107. Y. P. Du, Y. Z. Liang, J. H. Jiang, R. J. Berry and Y. Ozaki, *Anal. Chim. Acta*, 2004, **501**, 183–191.
108. R. Hara, M. Ishigaki, Y. Kitahama, Y. Ozaki and T. Genkawa, *Food Chem.*, 2018, **258**, 308–313.

109. M. Watari and Y. Ozaki, *Appl. Spectrosc.*, 2005, **59**, 600–610.
110. I. T. Jolliffe, *J. R. Stat. Soc. Ser. C. Appl. Stat.*, 1982, **31**, 300–303.
111. A. Höskuldsson, *J. Chemom.*, 1988, **2**, 211–228.
112. P. Geladi, *Spectrochim. Acta, Part B*, 2003, **58**, 767–782.
113. S. Wold, M. Sjostrom and L. Eriksson, *Chemom. Intell. Lab. Syst.*, 2001, **58**, 109–130.
114. N. Kumar, A. Bansal, G. S. Sarma and R. K. Rawal, *Talanta*, 2014, **123**, 186–199.
115. A. Bigdeli, F. Ghasemi, H. Golmohammadi, S. Abbasi-Moayed, M. A. F. Nejad, N. Fahimi-Kashani, S. Jafarinejad, M. Shahrajabian and M. R. Hormozi-Nezhad, *Nanoscale*, 2017, **9**, 16546–16563.
116. R. Mungkarndee, G. Tumcharern, R. Thiramanus, I. Techakriengkrai and M. Sukwattanasinitt, *Anal. Methods*, 2015, **7**, 7431–7435.
117. T. Eaidkong, R. Mungkarndee, C. Phollookin, G. Tumcharern, M. Sukwattanasinitt and S. Wacharasindhu, *J. Mater. Chem.*, 2012, **22**, 5970–5977.
118. J. Sandlund, S. Lim, N. Queralto, R. Huang, J. Yun, B. Taba, R. Song, R. Odero, G. Ouma, R. Sitati, W. Murithi, K. P. Cain and N. Banaei, *Diagn. Microbiol. Infect. Dis.*, 2018, **92**, 299–304.
119. T. Jarangdet, K. Pratumyot, K. Srikittiwanna, W. Dungchai, W. Mingvanish, I. Techakriengkrai, M. Sukwattanasinitt and N. Niamnont, *Dyes Pigm.*, 2018, **159**, 378–383.
120. M. Rauytanapanit, T. Sukmanee, K. Wongravee and T. Praneenararat, *RSC Adv.*, 2018, **8**, 41950–41955.
121. R. Mungkarndee, I. Techakriengkrai, G. Tumcharern and M. Sukwattanasinitt, *Food Chem.*, 2016, **197**, 198–204.
122. C. Zhang and K. S. Suslick, *J. Agric. Food Chem.*, 2007, **55**, 237–242.
123. L. Feng, X. Li, H. Li, W. Yang, L. Chen and Y. Guan, *Anal. Chim. Acta*, 2013, **780**, 74–80.
124. B. Bertrand, D. Villarreal, A. Laffargue, H. Posada, P. Lashermes and S. Dussert, *J. Agric. Food Chem.*, 2008, **56**, 2273–2280.
125. S. Yudthavorasit, K. Wongravee and N. Leepipatpiboon, *Food Chem.*, 2014, **158**, 101–111.
126. R. M. Barbosa, B. L. Batista, R. M. Varrique, V. A. Coelho, A. D. Campiglia and F. Barbosa, *Food Res. Int.*, 2014, **61**, 246–251.
127. R. Hara, M. Ishigaki, Y. Kitahama, Y. Ozaki and T. Genkawa, *Food Chem.*, 2018, **241**, 353–357.
128. H. Ayvaz, M. Plans, K. M. Riedl, S. J. Schwartz and L. E. Rodriguez-Saona, *Anal. Methods*, 2013, **5**, 2020–2027.
129. C. G. Kirchler, C. K. Pezzei, K. B. Bec, S. Mayr, M. Ishigaki, Y. Ozaki and C. W. Huck, *Analyst*, 2017, **142**, 455–464.
130. L. Vera-Candioti, M. J. Culzoni, A. C. Olivieri and H. C. Goicoechea, *Electrophoresis*, 2008, **29**, 4527–4537.
131. K. Wongravee, T. Parnklang, P. Pienpinijtham, C. Lertvachirapaiboon, Y. Ozaki, C. Thammacharoen and S. Ekgasit, *Phys. Chem. Chem. Phys.*, 2013, **15**, 4183–4189.

132. M. R. Robinson, R. P. Eaton, D. M. Haaland, G. W. Koepp, E. V. Thomas, B. R. Stallard and P. L. Robinson, *Clin. Chem.*, 1992, **38**, 1618–1622.
133. U. A. Müller, B. Mertes, C. Fischbacher, K. U. Jageman and K. Danzer, *Int. J. Artific. Organs*, 1997, **20**, 285–290.
134. R. Marbach and H. M. Heise, *Appl. Opt.*, 1995, **34**, 610–621.
135. H. M. Heise, R. Marbach and A. Bittner, *J. Near Infrared Spectrosc.*, 1998, **6**, 361–374.
136. K. Maruo, M. Tsurugi, M. Tamura and Y. Ozaki, *Appl. Spectrosc.*, 2003, **57**, 1236–1244.
137. K. Maruo, T. Oota, M. Tsurugi, T. Nakagawa, H. Arimoto, M. Tamura, Y. Ozaki and Y. Yamada, *Appl. Spectrosc.*, 2006, **60**, 441–449.
138. S. Kasemsumran, Y. P. Du, K. Maruo and Y. Ozaki, *Chemom. Intell. Lab. Syst.*, 2006, **82**, 97–103.
139. Y. Morisawa, S. Nomura, K. Sanada and Y. Ozaki, *Appl. Spectrosc.*, 2012, **66**, 665–672.
140. R. Salzer and H. W. Siesler, *Infrared and Raman Spectroscopic Imaging*, Wiley-VCH, Weinheim, 2009.
141. S. Sasic and Y. Ozaki, *Raman, Infrared and Near-Infrared Chemical Imaging*, John Wiley & Sons, Inc, Hoboken, 2010.
142. M. Ishigaki, P. Meksiarun, Y. Kitahama, L. Zhang, H. Hashimoto, T. Genkawa and Y. Ozaki, *J. Phys. Chem. B*, 2017, **121**, 8046–8057.
143. Z. Movasaghi, S. Rehman and I. U. Rehman, *Appl. Spectrosc. Rev.*, 2007, **42**, 493–541.
144. G. J. Puppelsi, H. S. P. Garritsen, J. A. Kummer and J. Greve, *Cytometry, Part A*, 1993, **14**, 251–256.
145. M. M. Mendes-Pinto, E. Sansiaume, H. Hashimoto, A. A. Pascal, A. Gall and B. Robert, *J. Phys. Chem. B*, 2013, **117**, 11015–11021.

CHAPTER 11

Evaluation of the Greenness of Analytical Procedures

MARTA BYSTRZANOWSKA, JACEK NAMIEŚNIK AND MAREK TOBISZEWSKI*

Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, ul. Gabriela Narutowicza 11/12, 80-233 Gdańsk, Poland
*Emails: marektobiszewski@wp.pl; martobis@pg.edu.pl

11.1 Introduction

Analytical chemistry plays an important role in many areas of human activity, and owing to recent developments in industry, engineering, medicine, transport and agriculture its importance is continually increasing. New materials, products and drugs require many studies on their composition, effects on human and animal health, *etc.* Moreover, many daily human activities and also the development of economic sectors are sources of pollution in the environment. These are reasons for the increased interest in the quality and control of individual environmental components and industries such as pharmaceuticals and food, especially from the chemical point of view. This situation has led the development of analytical procedures to deal with new analytes, complex matrices and very low concentration levels. Such multiplicity and diversity have made choosing the right analytical procedure problematic.

Selection of a suitable analytical procedure depends on the nature of the analytes and matrix and also the aim of the analysis. These in turn are related to the quality of metrological factors, such as accuracy, precision and sensitivity. Selection of the appropriate techniques may be dictated by metrological and technical issues, and also economic and environmental

Green Chemistry Series No. 66
Challenges in Green Analytical Chemistry: 2nd Edition
Edited by Salvador Garrigues and Miguel de la Guardia
© The Royal Society of Chemistry 2020
Published by the Royal Society of Chemistry, www.rsc.org

factors, or sometimes by all of them at the same time. Therefore, choosing the best procedure is often not easy. Moreover, many available options and criteria or analyst preferences make the situation much more complicated. It is necessary to introduce a system of evaluation that will allow one to analyse the problem and help in making the right choice/decision.

In the case of green analytical chemistry (GAC), environmental assessment is necessary, and the question here is to know what parameters describe the greenness of the procedure. As shown in Figure 11.1,[1] an ideal analytical procedure should be based on a direct technique with low energy consumption and use of non-toxic reagents for the analysis of a small sample size. Moreover, the volume of wastes should be reduced and the safety of the analyst should be ensured.

Analytical chemists who develop new analytical procedures often state that their procedure is green. However, such statements are commonly not properly justified, as usually no metrics of greenness are applied. Analysts rely on their intuition (which often may lead to true conclusions) while assessing the greenness of a procedure. What is more, the conclusions drawn when developing a green analytical procedure should be put in context, so a newly developed method should be compared with previous methods.

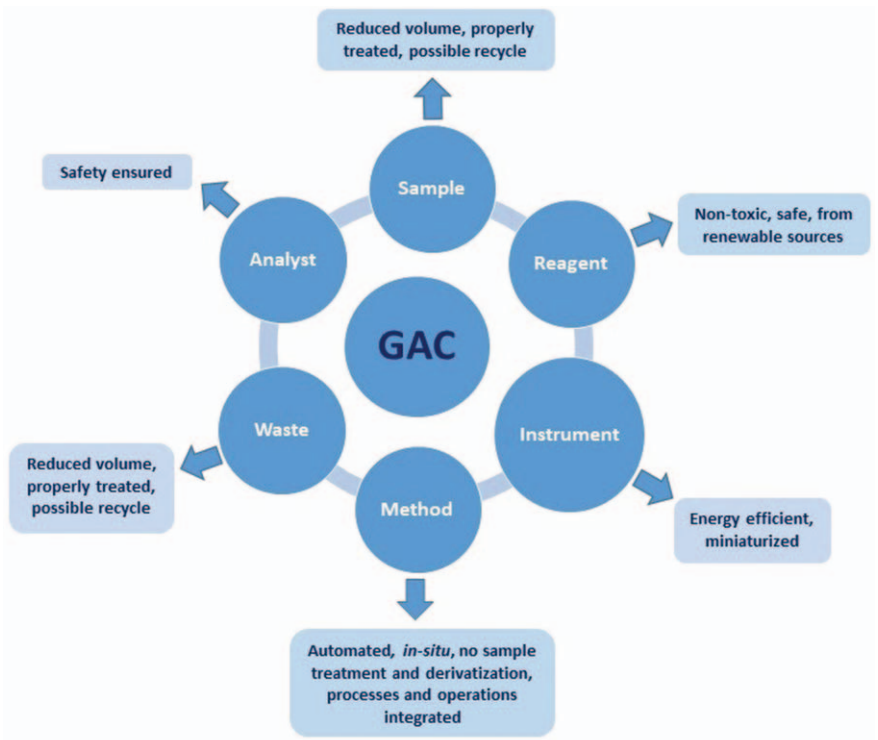


Figure 11.1 Definition of an eco-friendly analytical procedure according to the GAC concept.

GAC is the concept that brings the ideas behind the Twelve Principles of Green Chemistry to analytical laboratories. In fact, GAC introduces its adaptation of green chemistry principles in the form of the mnemonic SIGNIFICANCE, which defines a green analytical procedure as:

1. ***S***elect direct analytical technique.
2. ***I***ntegrate analytical processes and operations.
3. ***G***enerate as little waste as possible and treat it properly.
4. ***N***ever waste energy.
5. ***I***mplement automation and miniaturization of methods.
6. ***F***avor reagents obtained from renewable source.
7. ***I***ncrease safety for operator.
8. ***C***arry out *in situ* measurments.
9. ***A***void derivatization.
10. ***N***ote that the sample numer and size should be minimal.
11. ***C***hoose multi-analyte or multi-parameter method.
12. ***E***liminate or replace toxic reagents.

The most common way to assess the greenness of a chemical process is application of the *E*-factor,[2] which is calculated by dividing the mass of waste generated in the process by the mass of product from the process. It is used to express how problematic a chemical process is in terms of generated waste (without reference to the nature of the hazard of the generated waste). Typical *E*-factors are in the range from 0.1 (for the petrochemical industry) to 100 (in the pharmaceutical industry). Other metric systems have been developed to assess the greenness of chemical processes that consider also the nature of the generated waste and the amounts of solvents, catalysts and auxiliaries used.[3] As the metrics systems applied to assess chemical syntheses refer to the mass of the product, they are not applicable in analytical chemistry.[4]

The lack of applicability of metrics systems applied in chemical synthesis to analytical applications and the large number of assessment criteria are two main driving forces for the development of GAC metric systems. This task is difficult as metric systems should be simple to use and interpret and at the same time should consider the complexity of the analytical procedure. This chapter considers the achievements in the assessment of analytical procedures and solvents and reagents in the context of GAC.

11.2 Introduction to a Case Study

The case study of applications of GAC metrics presented here relates to analytical procedures that are applied in the determination of the insecticide DDT in honey samples. Readers familiar with the problem of DDT occurrence may wish to skip the next two paragraphs.

Dichlorodiphenyltrichloroethane or 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane, commonly known as DDT, is probably the most widely known insecticide in the world. It was first synthesized by Othmar Zeidler in 1874.[5] However, its insecticidal properties were not discovered until 1939 by Paul Müller, for which he was awarded the Nobel Prize in Physiology or Medicine in 1948.[6] DDT was used as an insecticide during World War II for the protection of soldiers from diseases such as typhus and malaria[6,7] Subsequently, its effectiveness and low price led to its commercialization and use for agricultural purposes. The mass production of DDT started in 1944 and the worldwide annual production of DDT in 1974 was around 60 000 000 kg.[8] In 1962, Rachel Carson noticed negative effects of DDT on the environment, which she published in her book *Silent Spring.*[9] It was proved that DDT and its metabolites had adverse effects on the reproduction of many animals.[5,9,10] As a result, despite the advantages DDT, it was banned in most developed countries during the 1970s.[8] Many years after its ban, DDT was recommended again by the World Health Organization (WHO) for malaria vector control in many African countries. Therefore, the production of DDT was continued in Mexico, India and several countries in Africa (*i.e.* Ethiopia, Mozambique, Zambia and South Africa).[11,12] However, so far, despite extensive research efforts, no other alternatives to DDT characterized by the same great efficiency have been found.[8] Hence there is also a risk that some illegal production may still exist, which is the reason why the amount of DDT in the environment or food should be monitored. Properties such as chemical stability, lipophilicity and bioaccumulation in the food chain reinforce the negative effects of DDT on humans and wildlife. Unfortunately, these properties are also characteristics of DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], which is a DDT metabolite.[5] It should be noted that technical DDT is a mixture consisting of 80% *p*,*p*′-DDT and 20% *o*,*p*′-DDT.[13] However, it may also contain DDE and DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] as contaminants.[14] Therefore, these related compounds are also determined during some chemical analyses. The structures of DDT and its metabolites and contaminants are presented in Figure 11.2.

DDT present in the environment may be released into the air, soil or water when it is sprayed on crops to control insects. Here the cycle of evaporation and deposition may play an important role and be repeated many times. DDE or DDD may be found as a result of contamination or breakdown of DDT. Moreover, DDD was also used as a pesticide in the past. Exposure to DDT and its metabolites is possible by eating food containing small amounts of these compounds. The amount of DDT in food has decreased greatly since DDT was banned. Nevertheless, in the period 1986–1991, the average adult in the United States consumed an average of 0.8 μg of DDT per day.[8] It is worth mentioning that according to levels established by the Food and Agriculture Organization (FAO)/WHO, the acceptable daily intake (ADI) of ΣDDT is 20 $\mu g\,kg^{-1}$ per day.[15] According to European Union Commission Regulations, the maximum residue level (MRL) for ΣDDT is 0.05 $mg\,kg^{-1}$.[16] However, because of the chemical characteristics of DDT, it may remain in

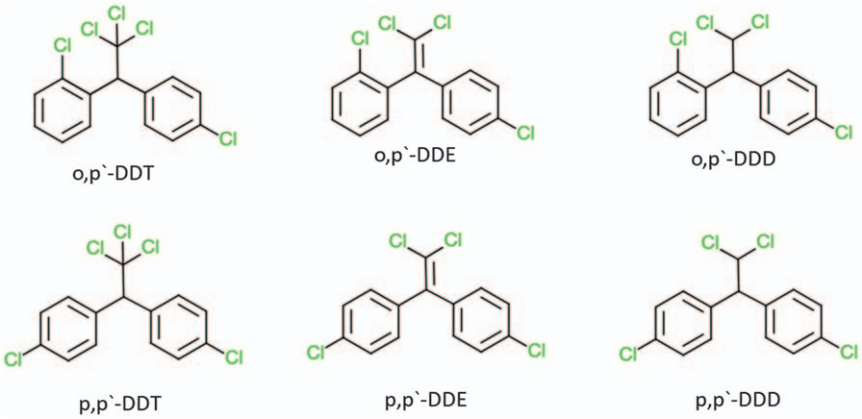


Figure 11.2 Structures of DDT and some related compounds.

the environment for many years. Hence DDT may be present in foods matrices at low concentration levels (including fruits, vegetables, honeybee products, meat, fish, *etc.*), and bees having direct contact with pesticide-treated plants collect pollen and produce honey contaminated with pesticides. In connection with the above, many studies have been performed on the determination of DDT and its metabolites in food samples.

Honey is a valuable commodity owing to its taste and medical, antimicrobial and antiseptic properties. Indirect contamination of honey may occur during the application of pesticides in agriculture, through soil, air and water. It is also possible through flowers, where bees visit and collect nectar to produce honey.[17] The chemicals are then carried to the hive on the bees' bodies or by forage. On the other hand, pesticides could be used in the treatment of plagues in the hive during honey harvesting, which may lead to some contamination.[18] Although regulatory agencies do not establish maximum residue limits for pesticides in honey samples, these concentrations must be below the MRLs established for pesticide residues in food samples of $<1\ \mu g\, g^{-1}$.[17]

In the case of the determination of DDT in food matrices such as honey, the final determination techniques involve gas chromatography coupled with mass spectrometric or electron capture detection. Owing to the complex matrix composition of honey, analytical procedures for DDT determination require prior sample preparation techniques such as solid-phase extraction, liquid–liquid extraction, solid-phase microextraction and QuEChERS (''quick, easy, cheap, effective, rugged and safe''). This is a key aspect of every analytical procedure for the determination of pesticides in bee products, hence a wide variety of pretreatments are available. Examples of frequently applied techniques for this particular pesticide determination in honey samples are summarized in Table 11.1. As they cover different sample preparation techniques they are used as examples in the presentation of different GAC metrics systems.

Table 11.1 Selected analytical procedures for the determination of DDT in honey samples for environmental evaluation.

No.	Analyte	Matrix	Analytical methodology	Abbreviation	Ref.
1	*p*,*p*′-DDT	Honey from markets (regions worldwide)	Accelerated solvent extraction–gas chromatography–ion trap mass spectrometry	ASE–GC–ITMS	19
2	*p*,*p*′-DDT	Honey from apiaries (Poland)	Dispersive liquid–liquid microextraction–gas chromatography–mass spectrometry	DLLME–GC–MS	20
3	*p*,*p*′-DDE	Multi-flowers, orange blossom and eucalyptus honey from local market	Headspace single-drop microextraction–gas chromatography–micro-electron capture detection	HS-SDME–GC–μECD	21
4	*p*,*p*′-DDT	Honey (Italy)	Solid-phase extraction–gas chromatography–tandem mass spectrometry	SPE–GC–MS/MS	22
5	*p*,*p*′-DDT	Honey (Mexico)	QuEChERS extraction (''quick, easy, cheap, effective, rugged and safe'')–dispersive solid-phase extraction–gas chromatography–electron capture detection	QuEChERS–d-SPE–GC–ECD	23
6	*p*,*p*′-DDT	Honey from apiaries (France)	QuEChERS extraction–gas chromatography–time-of-flight mass spectrometry	QuEChERS–GC–TOFMS	24
7	*p*,*p*′-DDT	Rosemary, heather and orange blossom honey form a local supermarket	Solid-phase microextraction–gas chromatography–atomic emission detection	SPME–GC–AED	25
8	*o*,*p*′-DDT	Floral honey (Iran)	Ultrasound-assisted emulsification microextraction–gas chromatography–mass spectrometry	USAEME–GC–MS	26
9	*p*,*p*′-DDT	Honey (Portugal, Spain)	Liquid–liquid extraction–gas chromatography–electron capture detection	LLE–GC–ECD	27

11.3 Assessment of Procedures by Scoring

Analytical procedures can be assessed in terms of their greenness by scoring. The aim of every assessment methodology is to combine a few or more parameters into a single score or very simple graphical output. The advantage of the scoring approach is the ease of interpretation of results. The main disadvantage in some cases is a tedious investigation of the nature of the threat related to each individual reagent.

11.3.1 NEMI Approach

One of the first approaches to express the greenness of analytical methodology is the National Environmental Methods Index (NEMI).[28] This uses a pictogram in the form of a circle divided into four equal parts, where each of them is coloured green if certain criteria are met:

1. Reagents used for the procedure are not hazardous, which means that none of the chemicals used in the method is present on the US Environmental Protection Agency (EPA) TRI75 list or the Resource and Recovery Conservation Act (RCRA)'s D, F, P or U hazardous waste lists.
2. Reagents are not persistent, bioaccumulative and toxic (PBT), so chemicals used in the method are not listed as a PBT on the EPA TRI75 list.
3. Corrosiveness criterion, so the pH during the entire procedure remains within the non-aggressive range 2–12.
4. No significant amounts of wastes are produced, in amounts greater than 50 g (or 50 mL).

An example of the assessment results is presented in Figure 11.3. The main advantages of the application of NEMI pictograms are simplicity of the expression and reading of results. Another advantage is that many standard

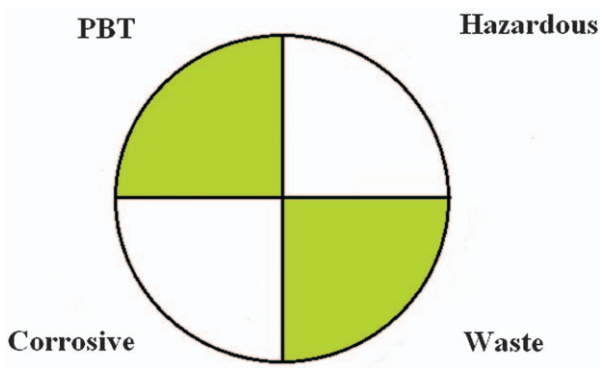


Figure 11.3 Example of an NEMI pictogram.

procedure results are available on-line in the NEMI database. The disadvantage is the need to search through lists of compounds to establish whether any compound applied in an analytical procedure is hazardous or PBT. Another disadvantage is that the result is semiquantitative at best. The result does not give information on what hazardous substance was applied or how much of it was used. The parameters that are included in the scoring seem not yet exhausted as energy demand is not included.

NEMI pictograms are an accepted greenness metrics system. Apart from their application in the NEMI database, the pictograms have been used in some published papers. In the case of scientific papers, NEMI is applied to assess whether a newly developed analytical procedure is greener than a previous, sometimes standard, procedure. The dispersive liquid–liquid microextraction technique for the determination of phenolic compounds in water and wastewater was assessed by the NEMI approach and it appeared that the newly developed procedure is a greener option than the standard American Public Health Association (APHA) method 5530.[29] An NEMI pictogram was also used for the assessment of a single-drop microextraction procedure for the determination of ethanol in cosmetics. Application of the microextraction technique and benign reagents resulted in a completely green NEMI profile being obtained.[30] A large number of microwave-assisted extraction-based procedures were assessed[31] and the results showed that mainly depending on the type of extraction solvent the procedures are completely green or with just one or two parts of the greenness profile missing.

11.3.2 Eco-scale

Another scoring method, proposed by Gałuszka *et al.*,[32] is the Analytical Eco-scale. The idea is based on an original study of the Eco-scale developed by Van Aken *et al.*[33] for the assessment of organic synthesis reactions. An assessment is related to a penalty points system, in which initially a procedure is assigned 100 points. If it differs from the ideal procedure, then it receives some penalty points, which are subtracted from the base value. The penalty points (PP) are given for:

- The amount of reagent:
  - <10 mL or g 1 PP
  - 10–100 mL or g 2 PP
  - >100 mL or g 3 PP

- The hazard related to the reagent:
  - Non-hazardous 0 PP
  - Less severe hazard 1 PP
  - Severe hazard 2 PP

The PP related to the amount of reagent is multiplied by the PP related to the hazard of the reagent. The type of hazard calculation is based on

pictograms present in a material safety datasheet and signal wording. Numerous hazards can be related to one chemical and a less severe hazard (multiplication by 1) is related to a ''warning'' word and a more severe hazard (multiplication by 2) to a ''danger'' word.

- Energy consumed:
  - $\leq$0.1 kWh per sample 0 PP
  - $\leq$1.5 kWh per sample 1 PP
  - $>$1.5 kWh per sample 2 PP
- Occupational hazard:
  - Hermetized process 0 PP
  - Emission of vapours 3 PP
- Waste generated:
  - $<$1 mL or g 1 PP
  - 1–10 mL or g 3 PP
  - $>$10 mL or g 5 PP
  - Recycling 0 PP
  - Degradation 1 PP
  - Passivation 2 PP
  - No treatment 3 PP

The score obtained with the Eco-scale is therefore calculated with the equation

$$\text{Eco-scale score} = 100 - \sum \text{PP} \tag{11.1}$$

The advantage of the application of the Eco-scale is a single numerical value that can be easily compared with the results for other procedures. It considers more assessment criteria and they are more quantitative than is the case with NEMI. The main disadvantage is the lack of information on the distribution of threats by the investigation of Eco-scale score alone, as it is not known where the penalty points are coming from.

The Analytical Eco-scale allowed the comparison of a newly developed high-performance liquid chromatographic (HPLC)-based procedure for the determination of antihypersensitive medications. A change of mobile phase from methanol-based to ethanol-based and removal of sodium perchlorate and acetonitrile allowed the greenness score to be improved from 46 or 60 (two procedures were used as references) to 86.[34] The Eco-scale was also applied to compare the greenness of a proposed analytical procedure with previously published procedures for the determination of drugs in wastewater samples.[35] An Eco-scale score of 80 was obtained, whereas eight other analytical procedures gave scores between 53 and 78. This assessment procedure was also applied to a procedure for the determination of mercury species in seafood by thermal mineralization, gold trapping and final determination by atomic absorption spectrometry.[36] Penalty points resulted from the application of HBr and toluene, and also waste generation and occupational exposure, with a final score of 73 points.

11.3.3 Other Scoring Approaches

The two scoring methodologies described above are well established in analytical practice, but there are other approaches for scoring that are not (yet) more widely applied. The first is the green analytical procedure index (GAPI), which is a hybrid of Eco-scale and NEMI with some new ideas.[37] This tool allows the evaluation of an entire analytical methodology, from sample collection to final determination. An assessment is based on five pentagrams used to evaluate and quantify the environmental impact, being coloured from green through yellow to red, reflecting a low, medium and high environmental impact, respectively. Figure 11.4 shows the elements used in evaluations with this method.

11.3.4 Scoring Case Studies

The greenness of the analytical procedures presented in Table 11.1 was assessed with Eco-scale and NEMI and the results are presented in Table 11.2.

11.4 Comparative Assessment of Procedures

An interesting way of dealing with multi-objective problems in analytical chemistry is the application of the multi-criteria decision analysis (MCDA) approach. This is a group of techniques that are able to evaluate many criteria (parameters of evaluation) and alternatives (possible options) constituting a large amount of data in decision-making problems.[38] Particular methods differ in their mechanisms owing to the use of various mathematical algorithms. For instance, the manner of assigning weighting values to criteria may be done in a specific and distinct way. The most widely known and used methods are TOPSIS (Technique for Order of Preference by Similarity to Ideal Solution), AHP (Analytic Hierarchy Process) and PROMETHEE (Preference Ranking Organization Method for Enrichment

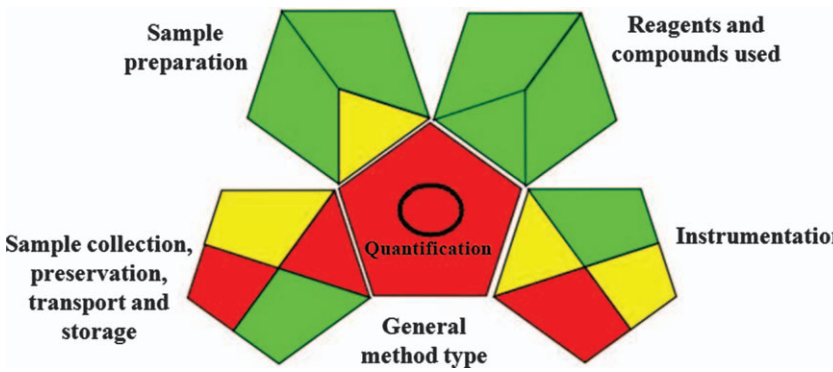
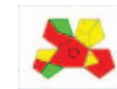
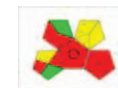


Figure 11.4 Green Analytical Procedure Index pictogram – an example.

Table 11.2 Assessment results with NEMI, GAPI and Eco-scale.

No.	Analytical methodology	Abbreviation	NEMI result	Eco-scale result	GAPI result
1	Accelerated solvent extraction–gas chromatography–ion trap mass spectrometry	ASE–GC–ITMS		69	
2	Dispersive liquid–liquid microextraction–gas chromatography–mass spectrometry	DLLME–GC–MS		84	
3	Headspace single-drop microextraction–gas chromatography–micro-electron capture detection	HS-SDME–GC–μECD		94	
4	Solid-phase extraction–gas chromatography–tandem mass spectrometry	SPE–GC–MS/MS		63	
5	QuEChERS extraction–dispersive solid-phase extraction–gas chromatography–electron capture detection	QuEChERS–d-SPE–GC–ECD		82	
6	QuEChERS extraction–gas chromatography–time-of-flight mass spectrometry	QuEChERS–GC–TOFMS		81	
7	Solid-phase microextraction–gas chromatography–atomic emission detection	SPME–GC–AED		95	
8	Ultrasound-assisted emulsification microextraction–gas chromatography–mass spectrometry	USAEME–GC–MS		83	
9	Liquid–liquid extraction–gas chromatography–electron capture detection	LLE–GC–ECD		82	

Evaluations). MCDA is often used to deal with complex problems in the areas of management, business, engineering, science and many others.[39] However since the burgeoning environmental problems and their seriousness (destruction of the ozone layer, greenhouse effect, *etc.*) have become to be appreciated, the interest in MCDA methods in the environmental field has increased drastically.[40] In turn, this has influenced the growth of interest in their application in the chemical sciences, including analytical chemistry.

There are many examples of the use of MCDA methods in cases of process/analytical procedure selection.[38] They can be used to assess GAC performance, when criteria describing the alternatives refer to their greenness character. MCDA algorithms were used in studies aimed at finding the most preferable process or analytical procedure. This has been described in detail by Fabjanowicz *et al.*[41] (determination of resveratrol in wine samples) and Serna *et al.*[42] (chemical process route to produce ethyl acetate) using AHP, and also by Jędrkiewicz *et al.*[43] (determination of chlorophenols in soy sauces) and Tobiszewski and Orłowski[44] (determination of aldrin in water samples) using PROMETHEE.

As has been mentioned, MCDA methods are used as an aid in complex decision-making problems. In general, they allow a problem to be described using numerical values and, with the application of a suitable algorithm, enable a ranking of available options in preference order to be obtained. Despite the mechanism of the algorithm, the general steps of MCDA application are similar. The scheme of operations is briefly presented in Figure 11.5.[45]

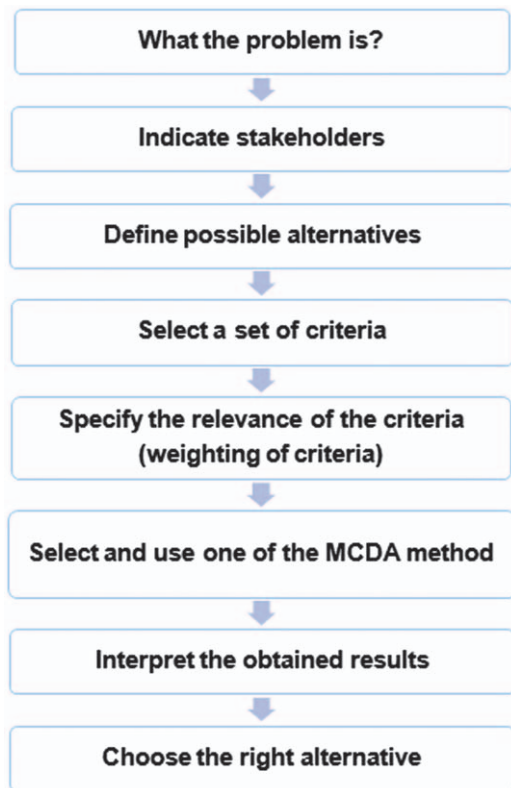


Figure 11.5 General steps in MCDA methods.

First, a problem should be defined, hence the purpose of an evaluation would be specified. Sometimes it is also worth choosing a group of stakeholders involved in the decision-making process. However, sometimes it could be a single decision-maker (DM). Then alternatives are identified, namely the possible options to be taken into consideration. Among them, the best solution will be selected with reference to the given assessment conditions. The next step is the definition of a set of criteria that allow available alternatives to be described and compared. In other words, they determine the requirements of stakeholders for the most suitable option. Their choice is important for obtaining reliable and satisfactory results of an analysis and also the most reliable characterization and comparison of the possible solutions. Weighting of criteria is a crucial step of the MCDA process. It reflects DMs' preferences by assessing the different importance of each criterion. This is also the stage at which the direction of the analysis can be indicated. This means that metrological, environmental or economic criteria could be taken into account. Then an appropriate MCDA method should be selected and applied. The last step is interpretation of the results, mostly presented as a ranking of available options, follow by choosing the most suitable alternative.

It is important to highlight several advantages of MCDA methods. The most important one is dealing with large amounts of data, including numbers of criteria and alternatives. This is possible as a decision-making problem is described by numerical values, where final results are also presented as numerical values. Therefore, it is easy to create a ranking of available options and select the best solution according to the analysis conditions. In this approach, subjective decision-making is avoided. It is worth mentioning that MCDA methods cope very well with conflicting criteria, in the case when a problem involves both benefit and cost criteria at the same time. The most characteristic for MCDA algorithms is the possibility of assigning weight values to criteria that reflect DM's preferences. All of this makes a comparison or assessment more systematic and comprehensive. Another advantage of MCDA tools is the use of commercial computer software such as Expert Choice, D-Sight, M-MACBETH, Super Decisions, Web-HIPRE, Visual PROMETHEE, *etc.* This is one of the reasons for their growing popularity in various fields of applications. Mathematical operations using software are not particularly labour intensive and time consuming because some parameters can even be calculated automatically. Moreover, the majority of the programs allow the results obtained to be presented in the form of clear charts and diagrams so that they can be easily understood by all recipients, not just by experts.

MCDA methods are a group of various evaluation approaches based on different mathematical algorithms. The most frequently used tools in the chemistry area are TOPSIS, AHP and PROMETHEE, but they are not the only ones available. Regardless, these methods are universal, hence they may be applied to solve various decision-making problems. It is worth mentioning that there are also some methods that were created particularly for only one

specific problem and cannot be easily adopted for other problems. An example is ORDREG,[46] an approach to assess the implementation of green chemistry principles by protocols for the synthesis of common nanomaterials. It was decided to apply TOPSIS and PROMETHEE algorithms for evaluation in this particular case study. Therefore, in the following only the basics of these two algorithms are considered.

11.4.1 TOPSIS

TOPSIS (Technique for Order Preference by Similarity to Ideal Solution) was developed by Hwang and Yoon in 1981.[47] Its mechanism is based on first allocating the positive ideal and negative ideal solutions and then placing the remainder of the alternatives between them. This leads to performing the ranking of available alternatives. According to its algorithm, the best option would be that which has the shortest distance from the positive ideal solution in a geometric sense. Only basic information on the TOPSIS algorithm is presented here; more details are available in articles describing its fundamentals.[48,49] In general, the input data are a matrix consisting of n alternatives, described by m criteria. The basic steps of the TOPSIS algorithm are as follows:

1. Construction of normalized decision matrix:

$$r_{ij} = \frac{x_{ij}}{\sum x_{ij}^2}, \quad i = 1,\ 2, \ldots, m \quad \text{and} \quad j = 1,\ 2, \ldots, n \tag{11.2}$$

   where x_{ij} and r_{ij} are original and normalized scores in the decision matrix, respectively.
2. Construction of the weighted normalized decision matrix:

$$v_{ij} = r_{ij} \times w_j,\ i = 1,\ 2, \ldots, m \quad \text{and} \quad j = 1,\ 2, \ldots, n \tag{11.3}$$

   where w_j is the weight of criterion j and $\sum_{j=1}^{n} w_j = 1$.
3. Determination of positive ideal (A^*) and negative ideal (A^-) solutions:

$$A^* = \left\{ \left(\max_i v_{ij} \,\middle|\, j \in C_b \right), \left(\min_i v_{ij} \,\middle|\, j \in C_c \right) \right\} = \left\{ v_i^* \middle| j = 1,\ 2, \ldots, m \right\} \tag{11.4}$$

$$A^- = \left\{ \left(\min_i v_{ij} \,\middle|\, j \in C_b \right), \left(\max_i v_{ij} \,\middle|\, j \in C_c \right) \right\} = \left\{ v_j^* \middle| j = 1,\ 2, \ldots, m \right\} \tag{11.5}$$

4. Calculation of the separation measures for each alternative:

$$S_i^* = \sqrt{\sum_{j=1}^{m} \left(v_{ij} - v_j^* \right)^2}, \quad j = 1,\ 2, \ldots, m \tag{11.6}$$

$$S_i^- = \sqrt{\sum_{j=1}^{m} \left(v_{ij} - v_j^-\right)^2}, \quad j = 1,\ 2, \ldots, m \tag{11.7}$$

5. Calculation the relative closeness to the ideal solution:

$$C_i^* = \frac{S_i^-}{S_i^* + S_i^-}, \quad i = 1,\ 2, \ldots, m \quad \text{and} \quad 0 < C_i^* < 1 \tag{11.8}$$

6. Arrangement of scenarios in order from closest to ideal to furthest from ideal – creation of a ranking.

The alternative with C_i^* closest to 1 is the most preferred among the possible alternatives.

11.4.2 PROMETHEE Algorithm

PROMETHEE (Preference Ranking Organization Method for Enrichment Evaluations) was developed by Brans and Vincke.[50] It is a group of non-parametric outranking methods for a finite set of alternatives. This family consists of PROMETHEE I–PROMETHEE VI methods. In general, it is based on positive and negative preference flows for each alternative in the valued outranking relation. In other words, its mechanism assumes the dominance of one alternative over another. As a result, the alternatives are ranked according to the DM's preferences basing on assessed appropriate weight values.[51] In this case study, PROMETHEE II was applied. This tool includes complete ranking as a result and is based on pair-wise comparison of alternatives along each criterion. Therefore, of the PROMETHEE family only the PROMETHEE II algorithm is briefly described below. More details may be found elsewhere.[50] The algorithm consists of several steps, as follows:[52]

1. Determination of deviations based on pair-wise comparisons:

$$d_j(a, b) = g_j(a) - g_j(b) \tag{11.9}$$

   where $d_j(a, b)$ is the difference between the evaluations of a and b on each criterion.
2. Application of the preference function

$$P_j(a, b) = F_j[d_j(a, b)] \quad j = 1, \ldots, k \tag{11.10}$$

   where $P_j(a, b)$ is the preference of alternative a with respect to alternative b on each criterion, as a function of $d_j(a, b)$.
3. Calculation of an overall or global preference index:

$$\forall a, b \in A, \quad \pi(a, b) = \sum_{j=1}^{k} P_j(a, b) w_j \tag{11.11}$$

where $\pi(a,b)$ of a over b (from 0 to 1) is the weighted sum $p(a,b)$ for each criterion and w_j is the weight associated with the jth criterion.

4. Calculation of outranking flow – the PROMETHEE I partial ranking:

$$\phi^+(a) = \frac{1}{n-1}\sum_{x\in A}\pi(a,x) \tag{11.12}$$

$$\phi^-(a) = \frac{1}{n-1}\sum_{x\in A}\pi(x,a) \tag{11.13}$$

where $\phi^+(a)$ and $\phi^-(a)$ are the positive and negative outranking flow, respectively, for each alternative.

5. Calculation of net outranking flow – the PROMTHEE II compete ranking:

$$\phi(a) = \phi^+(a) - \phi^-(a) \tag{11.14}$$

where $\phi(a)$ is the net outranking flow for each alternative.

In this case, an assessment is conducted with the aid of commercially available software – Visual PROMETHEE (version 1.4.0.0, 2011–2013, Academic Edition). The advantage of this software is the possibility of an evaluation using different methods of the PROMETHEE family.

According to this case study, the main aim of the assessment is to find the most suitable analytical procedure for the determination of DDT in honey samples. The analytes are various forms of DDT: *p*,*p*′-DDT, *o*,*p*′-DDT and *p*,*p*′-DDE. The most suitable alternative means here the best analytical procedure from the green analytical chemistry point of view. Therefore, mainly environmental or greenness parameters are taken into account; however, a metrological parameter is also included. Consequently, a solution that will be appropriate for the samples in terms of good analytical performance and greenness at the same time is desired.

11.4.3 Alternatives

Alternatives are subject to various considerations that represent possible options that may lead to the stated goal. In the present case, alternatives are analytical procedures for the determination of DDT in honey samples. The possible alternatives are summarized in Table 11.1.

The data are collected by searching databases of scientific articles. An attempt was made to choose relatively new analytical procedures. Selected procedures involve different extraction techniques as the sample preparation step prior to the main analysis. All of them are based on gas chromatography. However, various detection methods are applied, mostly mass spectrometry, electron capture detection and their modifications and improvements such as μECD. These analytical procedures have been used

successfully, as exemplified by numerous publications concerning the analysis of honeybee products.

11.4.4 Assessment Criteria

The technical evaluation of an analytical procedure is based on limits of detection (LODs). However, owing to the main aim of the analysis, almost all of the parameters are related to environmental assessment. One of the factors is the total time needed to perform an analysis. Moreover, this time is closely connected with another parameter – the number of procedural steps. This influences the time needed and also the consumption of energy and reagents. Additionally, the smaller the number of steps, the fewer potential errors and analytes losses occur during the operations. Another criterion is the amount of sample needed to perform the analysis. The last two parameters are related to the solvents and reagents used during the determination of analytes. According to the Twelve Principles of Green Chemistry formulated by Anastas and Warner,[53] the amounts and characteristics of the reagents and solvents should be included in the assessment, especially when the environmental point of view is taken into account. Scores for solvents in addition to scores for other reagents are applied. These criteria are assigned on the basis of a set of information, such as the amount, type and hazardous character of the chemicals used. In the application of MCDA algorithms, all the criteria must be expressed numerically. If they are not already in that format, it is necessary to change them into numerical values. Therefore, these two parameters had to be modified, and descriptive parameters were translated into numerical values. The evaluation of reagents was carried out according to an approach well known from the Analytical Eco-scale.[32] The hazardous character of reagents was determined by the number of pictograms multiplied by points for signal wording (danger, 2 points; warning, 1 point). On the other hand, the amounts of chemicals were calculated in accordance with the pattern. Penalty points were given depending on the volume of reagents from 1 to 3 points (<10 mL, 1 point; 10–100 mL, 2 points; >100 mL, 3 points). Another approach was used for solvents, where assessment was based on calculations proposed by Tobiszewski and Namieśnik.[54] The final result for solvents took into account both their volume and their chemical nature/properties. Therefore, the score for each solvent is the product of multiplication of the solvent volume (mL) and total analytical hazard value (taHV). The value of taHV is estimated according to an algorithm that depends on oral toxicity, inhalation toxicity, carcinogenicity, other hazardous effects, aquatic acute toxicity, aquatic chronic toxicity, biodegradability, hydrolysis half-life, bioconcentration factor and hazard value related to the volatility.

An important element of the analysis is also the definition of the preference functions for the criteria used in evaluation. The desirable dependences for given criteria should be determined, such as ''the higher the better'' or ''the lower the better'', *etc.* Applied dependences are summarized in Table 11.3. As can be seen, here for all criteria it was ''the lower the better''.

Table 11.3 Preference function for applied set of criteria.

Criterion	Units	Preference function
LOD	$\mu g\,kg^{-1}$	The lower the better
Amount of sample	g	
Time of analysis	Min	
Score for solvents	Points	
Score for other reagents	Points	
Number of procedural steps	—	

11.4.5 Weighting of Criteria

As described above, one of the most important and characteristic features of MCDA methods is the step of assigning appropriate weight values to each criterion. These weights are strictly correlated with the DM's preferences. Therefore, it is possible to judge which criterion has the greatest influence on the main goal and how strong this impact is. This contributes to creating rankings of available options in accordance with the stated requirements. In this particular case study, an environmental evaluation was performed, hence most of the criteria represent environmental parameters. However, because analytical procedures are the subject of consideration, a metrological parameter is also taken into account. Nevertheless, the weights assigned are equal, so their impacts on the main goal of the analysis are the same. The importance of greenness is stated by the number of criteria relevant to the main analysis goal.

11.4.6 Input Data

All the data values were taken directly or indirectly from scientific papers as indicated in Table 11.1. The set of data prepared for analysis with MCDA tools is presented in Table 11.4. The minimum and maximum values are designated with bold and italic type, respectively. It is clear that no obvious alternative can be found without the aid of MCDA.

11.4.7 Results of TOPSIS Analysis

According to the TOPSIS algorithm, it is necessary to find the best and the worst value within each criterion (maximum or minimum values). In other words, determination of a positive/negative ideal solution is conducted, whereas other alternatives are allocated between these two extreme values. Evaluation using the TOPSIS algorithm was performed with the Microsoft Excel program (2016). Results obtained with TOPSIS analysis for the ranking of available analytical procedures for the determination of DDT in honey samples are presented in Table 11.5.

The final score in the TOPSIS method is a ranking, where the order is based on values of similarity to the ideal solution. This value is calculated

Table 11.4 Dataset for the case study of the determination of DDT in honey samples.

No.	Abbreviation of analytical methodology	LOD/ $\mu g\,kg^{-1}$	Amount of sample/g	Time of analysis/ min	Score for solvents	Score for other reagents	No. of procedural steps
1	ASE–GC–ITMS	**0.01**	*20*	71	*1968*	**0**	6
2	DLLME–GC–MS	4	**0.5**	28	11.5	**0**	**3**
3	HS-SDME–GC–μECD	0.07	2	97	0.138	**0**	4
4	SPE–GC–MS/MS	0.94	10	*113*	1445	**0**	*7*
5	QuEChERS–d-SPE–GC–ECD	1.174	5	**25**	268	**0**	4
6	QuEChERS–GC–TOFMS	*21.9*	5	36	275	*4*	5
7	SPME–GC–AED	10	1.5	44	**0**	**0**	**3**
8	USAEME–GC–MS	0.06	*20*	63	1.79	**0**	**3**
9	LLE–GC–ECD	10	5	55	365	**0**	5

Table 11.5 Analysis with TOPSIS method: final ranking.

Alternative	Similarity to ideal solution
DLLME–GC–MS	0.916
QuEChERS–d-SPE–GC–ECD	0.890
HS-SDME–GC–μECD	0.814
SPME–GC–AED	0.797
LLE–GC–ECD	0.745
USAEME–GC–MS	0.706
SPE–GC–MS/MS	0.613
ASE–GC–ITMS	0.556
QuEChERS–GC–TOFMS	0.413

for each alternative taken into account during evaluation of the decision-making problem. According to the basics of its algorithm, the preferred alternative must be characterized by the shortest distance from the positive solution and the furthest distance from the ideal solution of the geometric point by using the Euclidean distance at the same time. The task of the algorithm is to determine the relative proximity of the optimal solution alternative.

11.4.8 PROMETHEE II Analysis

Similarly as before (during an assessment using the TOPSIS method), the first task of analysis is the definition of the main aim, set of criteria and alternatives. Then the assignment of appropriate weights for the criteria is done, which are estimated to be equal. An important step using Visual PROMETHEE software is the manual definition of minimum (MIN) and maximum (MAX) values within each criterion. Then, according to the DM's

preferences, the best value for each parameter is found. The final results of analysis as a complete ranking of alternatives are summarized in Figure 11.6.

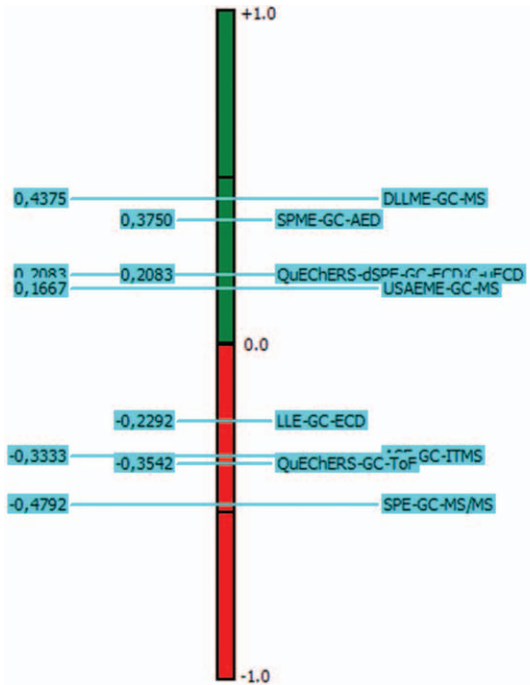
In the PROMETHEE tool, scores are expressed as Phi, Phi+ and Phi−, which can be explained as preference flows, *i.e.* the net flow, the positive (or leaving) flow and the negative (or entering) flow, respectively. They are designated to consolidate the results of the pair-wise comparisons of the actions. On the other hand, they allow all the actions to be ranked from the best to the worst one (according to the assumptions in the evaluation, including the DM's preferences). In general, the larger is Phi+ and the smaller is Phi− the better. The net preference flow is the balance between the positive and negative preference flows. Therefore, it takes them into account and aggregates both of them into a single score.

11.4.9 Comparison of Obtained Results

In the case of the selection of the greenest analytical procedure for determination of DDT in honey samples, two MCDA methods were applied: TOPSIS (Microsoft Excel program) and PROMETHEE II (Visual PROMETHEE software) algorithms were used to obtain rankings of the available options, as shown in Table 11.6, where previously calculated NEMI and Eco-scale results are also included.

Unquestionably, the best analytical procedure for the determination of DDT in honey samples in the case of MCDA greenness assessment is DLLME–GC–MS. As can be seen, HS-SDME–GC–μECD is a procedure that is ranked in the same positions using both MCDA methods. Unfortunately, other options have different positions in the ranking. However, it is worth noting that procedures such as ASE–GC–ITMS, QuEChERS–GC–TOFMS and SPE–GC–MS/MS are the less desirable techniques for DDT determination. In the case of ASE–GC–ITMS there is a high score for solvents due to the huge amount of dichloromethane and hexane used for extraction, which makes it not eco-friendly. The hexane consumption for SPE extraction and the long time needed for analysis placed the SPE–GC–MS/MS procedure at the lowest position. Moreover, this technique involves the highest number of procedural steps, which increases the possibility of analyte losses and the introduction of greater errors. The alternative involving GC with TOFMS is characterized by the worst LOD value among all available options. This makes the QuEChERS–GC–TOFMS procedure not very preferred from the point of view of the determination of DDT at trace levels in honey samples.

The application of two different MCDA methods produced slightly different results of the evaluation. The reason is the different algorithms in these applied MCDA tools, which involve different mechanisms. However, one can identify three well-distinguished groups within which there is a rotation of the positions of available solutions. These are procedures ranked at the following positions: I–IV, V–VI and VII–IX. It should also be noted that in all three groups the numerical scores are within narrow ranges. This may suggest that the considered analytical procedures ranked within these groups



Rank	Alternatives	Phi	Phi+	Phi-
1	DLLME-GC-MS	0.4375	0.6250	0.1875
2	SPME-GC-AED	0.3750	0.5833	0.2083
3	HS-SDME-GC-μECD	0.2083	0.5208	0.3125
4	QuEChERS-d-SPE-GC-ECD	0.2083	0.5000	0.2917
5	USAEME-GC-MS	0.1667	0.4792	0.3125
6	LLE-GC-ECD	-0.2292	0.2708	0.5000
7	ASE-GC-ITMS	-0.3333	0.2500	0.5833
8	QuEChERS-GC-ToF	-0.3542	0.2917	0.6458
9	SPE-GC-MS/MS	-0.4792	0.1875	0.6667

Figure 11.6 Obtained results of analysis based on the PROMETHEE II algorithm as an MCDA tool.

Table 11.6 Rankings of available alternatives obtained with the TOPSIS and PROMETHEE II algorithms and comparison with NEMI and Eco-scale results.

Alternative	TOPSIS rank	TOPSIS similarity to ideal solution	PROMETHEE rank	PROMETHEE phi	NEMI score (No. of green fields)	Eco-scale score
DLLME–GC–MS	I	0.916	I	0.4375	2	84
QuEChERS–d-SPE–GC–ECD	II	0.890	IV	0.2083	2	82
HS-SDME–GC–μECD	III	0.814	III	0.2083	4	94
SPME–GC–AED	IV	0.797	II	0.3750	4	95
LLE–GC–ECD	V	0.745	VI	−0.2292	4	82
USAEME–GC–MS	VI	0.706	V	0.1667	2	83
SPE–GC–MS/MS	VII	0.613	IX	−0.4792	2	63
ASE–GC–ITMS	VIII	0.556	VII	−0.3333	1	69
QuEChERS–GC–TOFMS	IX	0.413	VIII	−0.3542	2	81

are not very different. In general, the ranking positions depend strictly on the assessed weight values for the criteria taken into account in the evaluation. If we change the weights of importance, then the results may change. However, in this particular case study, the weight values were equal. In spite of that, both metrological and environmental criteria are involved and represented by green analytical chemistry and analytical performance factors also. Hence their impacts on the main goal are estimated to be the same.

The results obtained with NEMI correspond only slightly to those obtained as MCDA rankings. NEMI does not consider the quantities of solvents and reagents applied and their hazards are treated in a semiqualitative way. That is why microlitre volumes of a slightly hazardous solvent result in not fulfilling the PBT or hazard criterion (or both). The MCDA rankings proposed here also consider other criteria that produce different greenness assessment results. The results obtained with the Eco-scale are more correlated with the MCDA results, as the criteria of assessment are similar.

11.5 Assessment of Analytical Reagents and Solvents

Apart from the assessment of the greenness of an analytical procedure itself, it is very useful to apply greenness assessments of solvents or reagents that

are applied in analytical practice. This makes the procedure development and optimization more environmentally conscious. There are many systems and discussions allowing the selection of greener solvents but few studies on greener acids and bases or auxiliaries. Attention should be paid to the proper selection of such materials, because some of the green chemistry principles refer directly to these aspects.[55]

11.5.1 Solvents

As solvents are used in many areas of chemistry and are applied in huge amounts and in various processes, considerable attention has been given to their greenness assessment. In fact, the fifth of the Twelve Principles of Green Chemistry states that solvents should be avoided whenever possible, and whenever not then they should be as benign as possible.[56] Therefore, the search for solventless analytical extraction techniques is important,[57,58] and is usually pursued *via* application of solid-phase microextraction[59] or stir-bar sorptive extraction.[60] The approaches used to minimize the amount of an organic solvent used as an extracting agent are solvent microextraction techniques,[61] particularly single-drop microextraction,[62] hollow-fibre microextraction[63] and dispersive liquid–liquid microextraction.[64] The strategies used to avoid the application of organic solvents for extraction are the application of supercritical fluids,[65] hot water,[66] ionic liquids[67] and deep eutectic solvents.[68]

The first approaches to the greenness assessment of organic solvents were based on the Environment, Health and Safety (EHS) approach.[69] Within this approach, criteria such as release potential, fire/explosion and reaction/decomposition (as safety hazards), acute toxicity, irritation and chronic toxicity (as health hazards), persistence, air hazard and water hazard (as environmental hazards) are applied for assessment. Results of this assessment as an EHS indicator score, including some selected organic solvents, are presented in Figure 11.7.[69] Alcohols and esters are ranked as the greenest solvents with the EHS approach and formaldehyde and formic acid the worst.

Results of the assessment for different organic solvents are presented in Figure 11.7. On the basis of the EHS approach, a dedicated tool for the assessment of the greenness of LC separations was developed.[70] It is based on a simple scoring system, in which each solvent is given certain score values in environment, health and safety (E, H and S) categories that are multiplied by the mass of solvents consumed in the separation process and sample pretreatment.

Relatively similar tools dealing with solvent assessments are solvent selection guides (SSGs), developed by the pharmaceutical sector,[71,72] and others that are unpublished but are summarized in an article comparing different SSG results.[73] Similarly to the EHS approach, SSGs combine assessment criteria from E, H and S categories. The result is that every solvent is assigned a colour and the coding is based on traffic lights. The colour coding is presented in Table 11.7.

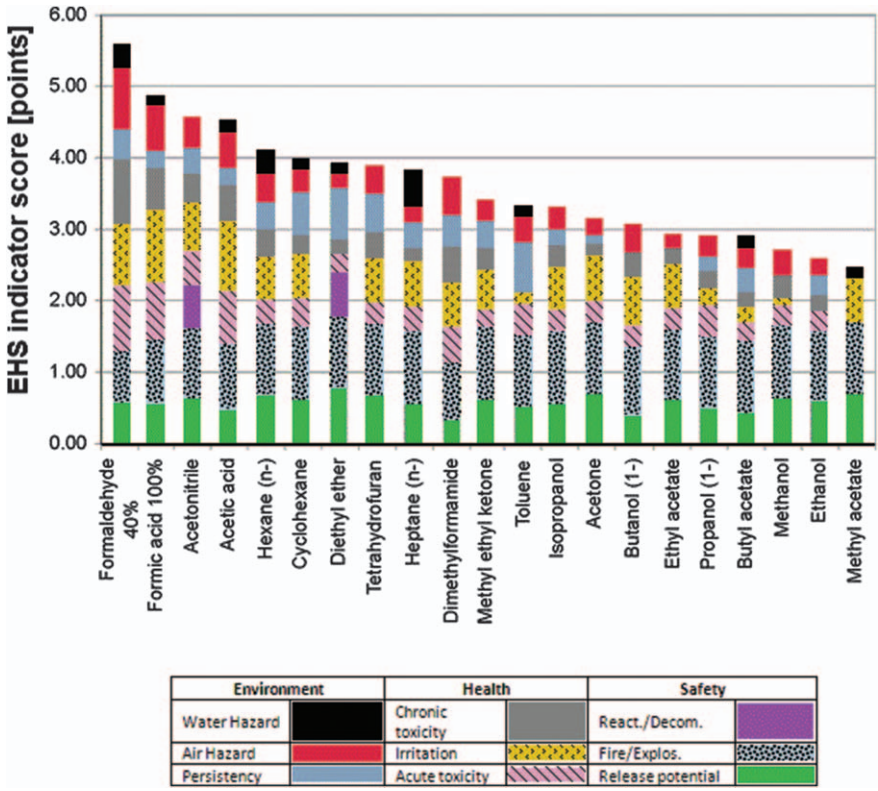


Figure 11.7 Results of the EHS method for organic solvents using the EHS tool according to Capello *et al.*[69]

Another SSG was based on MCDA methodology with the assignment of weights to criteria, and choosing criteria themselves the solvent selection can be more case specific.[74] The MCDA-based approach to solvent selection can easily be combined with other criteria relevant to a given application, such as extraction efficiency and repeatability of chromatographic response. Such a system has been successfully applied for the selection of a pair of dispersive and extraction solvents for the dispersive liquid–liquid microextraction-based determination of chlorophenols in water samples.[75] Different basic life-cycle assessment approaches were also included in the assessments of solvents, mainly in terms of their emissions and disposal.[76]

11.5.2 Acids and Bases

Acids and bases are used in analytical chemistry for the adjustment of pH and in the process of wet mineralization. In many applications, a few acids or bases can be applied with similar efficiency, so there is a possibility of

Table 11.7 Results of solvent assessments according to SSGs. Only solvents relevant to analytical chemistry are selected. Reproduced from ref. 73 with permission from the Royal Society of Chemistry.

Label, colour coding	Solvents
Recommended – dark green	Water, ethanol, 2-propanol, *n*-butanol, ethyl acetate
Recommended or problematic? – light green	Methanol, *tert*-butanol, acetone, 2-butanone, methyl isobutyl ketone, methyl acetate
Problematic – yellow	Methyltetrahydrofuran, heptane, methylcyclohexanone, toluene, xylenes, chlorobenzene, acetonitrile
Problematic or hazardous? – orange	Methyl *tert*-butyl ether, cyclohexane, dichloromethane
Hazardous – red	Pentane, hexane
Highly hazardous – dark red	Diethyl ether, benzene, chloroform, carbon tetrachloride

replacing more hazardous ones with greener substitutes. So far, not much attention has been paid to the greenness of these chemicals, but GSK has developed an acids and bases selection guide.[77] It was developed for the pharmaceutical industry but the assessment results can give some clues for the selection of greener acids and bases for analytical purposes.

The acids and bases were scored according to the E, H and S methodology. The other criteria included in the analysis were high molecular weight, generation of flammable or toxic by-products and generation of halogenated products during incineration.

The classification of various acids and bases is presented in Figure 11.8.[77] As can be seen, this method of evaluation consists of three main criteria scores, for EHS, clean chemistry and greenness. Each criterion involves some parameters, which are described in detail elsewhere.[78] First, the reagent is evaluated separately within each group of criteria, then information is summarized to rate the substance and colour it from green to red through yellow. Moreover, the authors introduced pK_a data values, which are often considered when selecting appropriate acids or bases, which makes the guide more useful.

Hydrochloric, hydrobromic, dilute phosphoric and sulfuric acid were assessed as green mineral acids. The main concerns are related to the generation of halogenated wastes during incineration of the first two and some issues with their disposal are present. Hydroiodic, sulfuric and phosphoric acid were assigned as ''yellow'' mineral acids so there are some major concerns related to their application. They cause severe burns and there are problems with their disposal. Nitric, hydrofluoric and perchloric acid are labelled as ''red'', undesirable alternatives. Nitric and perchloric acid are strong oxidants, hydrofluoric acid is very toxic and similarly to perchloric acid forms halogenated waste. They all cause severe burns and there are issues related to their disposal. Organic acids that have miscellaneous analytical applications are labelled as green acids – citric, ascorbic, benzoic, succinic and oxalic acid.

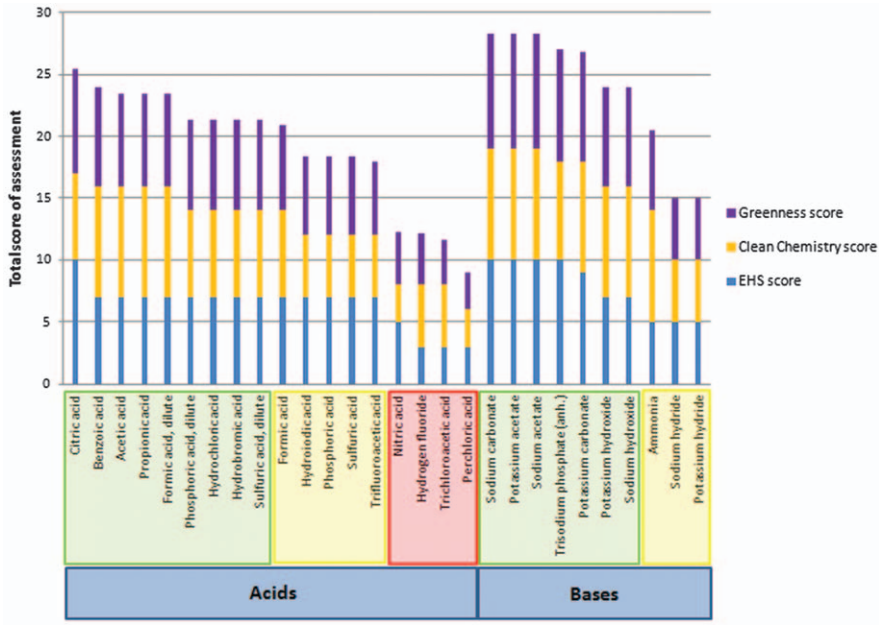


Figure 11.8 Classification of organic and inorganic acids and bases according to the approach proposed by Henderson *et al.*[77]

Potassium, sodium and calcium hydroxides are labelled as green bases, with the remark that first two can cause severe burns. Carbonates and bicarbonates of sodium and potassium are also green, similarly to sodium and potassium acetates.

A newly developed method for the assessment of HPLC-based procedures includes mainly the assessment of solvents.[79] The Analytical Method Greenness Score (*AMGS*) is calculated according to the equation

$$\mathrm{AMGS} = \frac{(mSn + mIn) \times \left[3\sqrt{Sn \times Hn \times En} + CEDn + (Ei \times R)\right]}{\text{No. of analytes}} \qquad (11.15)$$

where *mSn* is the mass *mS* of solvent *n* used for preparing a standard or sample, *mIn* is the mass *m* of solvent *n* used by the HPLC instrument, *Sn* is the safety index of solvent *n*, *Hn* is the health index of solvent *n*, *En* is the environmental index score of solvent *n* based on a given solvent's persistence, air and water hazard, *CED* is the cumulative energy demand for each particular solvent, based on production and solvent incineration, measured in units of kg solvent per MJ-equivalent, *Ei* is the instrument energy consumption or tabulated measured values determined for each instrument type selected in energy units of kW h, and *R* is the number of sample injections needed for a given analysis set, including standards and blanks.

Sn, *Hn*, *En* and *CED* values for various solvents are presented in Table 11.8.

Table 11.8 Scores for selected solvents in four categories.[79]

Solvent	*Sn*	*Hn*	*En*	*CED*
Acetone	2.29	1.6	3.23	40.7
Acetonitrile	2.72	1.2	3.44	58.8
N,*N*-Dimethylacetamide	0.7	0.63	3.6	65.2
N,*N*-Dimethylformamide	0.6	0.01	3.5	65.2
Dimethyl sulfoxide	0.9	1	3.21	44
Ethanol	0.8	0.28	3.99	18.4
Ethyl acetate	0.7	0.03	4.28	0.9
n-Heptane	0.6	0.43	4.87	7
n-Hexane	0.4	0.43	4.54	9.2
2-Propanol	0.6	0.28	3.92	29.1
Methanol	0.6	0.01	3.37	18.5
Tetrahydrofuran	0.5	0.53	3.1	233.3
Water	0.9	0.01	3.61	0.01

11.5.3 Derivatization Agents

Similarly to the case of acids and bases, not much attention has been devoted to the greenness of derivatization agents. The eighth of the Twelve Principles of Green Chemistry states that derivatization should be avoided, whenever possible. In chemical analysis this is not always possible[80] and derivatization agents are applied to reduce the polarity of analytes, improve volatility or improve the analytical signal during the final determination. One way to make the derivatization greener is to combine into one step the derivatization reaction and microextraction.[81] The combination of alternative ways of energy delivery to the system, such as the application of microwaves, ultrasound or UV irradiation, also contributes to greener derivatization.[82] As usually there are many alternatives to choose from, analysts should think about the selection of green agents. This is important as the safety of the operator is increased and environmental pollution is decreased. The latter could occur due to accidental spills or disposal of an analysed sample with unreacted derivatization agent. Suitable selection of less problematic derivatization agents is the second approach to making analytical derivatization greener.

A systematic approach to select greener derivatization agents was developed similarly to that presented before for solvent selection guides.[83] In the study, 267 compounds were assessed within three groups predefined by Sigma–Aldrich: GC, LC and chiral derivatization agents. As toxicological data for these chemicals were not available, the input assessment data were taken from material safety data sheets (MSDSs) and were predicted or modelled. In this way, derivatization agents were characterized with the following parameters:

- boiling point;
- flash point;
- vapour pressure;
- log K_{OW};
- log K_{OC};

- log BCF (bioconcentration factor);
- removal by wastewater treatment;
- persistence time;
- number and threat carried by hazards statements – from MSDS;
- number and threat carried by precautionary statements – from MSDS;
- signal wording – from MSDS;
- special hazards arising from the substance or mixture – from MSDS.

Table 11.9 Top rankings of LC, GC and chiral derivatization agents. Reproduced from ref. 83 with permission from the Royal Society of Chemistry.

Rank	Derivatization agent	CAS No.
LC derivatization agents		
1	(3*R*,4*R*)-2,5-Dioxotetrahydrofuran-3,4-diyl diacetate	6283-74-5
2	2-Hydroxy-1,2-diphenylethanone	119-53-9
3	2-Acetylbenzaldehyde	24257-93-0
4	9*H*-Fluorene-2-carbaldehyde	30084-90-3
5	4-Phenyl-3*H*-1,2,4-triazole-3,5(4*H*)-dione	4233-33-4
6	4-(Dimethylamino)benzaldehyde	100-10-7
7	(4-Formyl-5-hydroxy-6-methyl-3-pyridinyl)methyl dihydrogenphosphate	41468-25-1
8	1-[2-(4-Nitrophenyl)acetoxy]-2,5-pyrrolidinedione	68123-33-1
9	7-[(2-Aminoethyl)amino]-*N*-[2-(dimethylamino)ethyl]-2,1,3-benzoxadiazole-4-sulfonamide	913253-56-2
10	3-(Bromomethyl)-7-methoxy-2*H*-1,4-benzoxazin-2-one	124522-09-4
GC derivatization agents		
1	Butylboronic acid	4426-47-5
2	2,2,6,6-Tetramethyl-3,5-heptanedione	1118-71-4
3	*N*-(Trimethylsilyl)acetamide	13435-12-6
4	*N*,*O*-Bis(trimethylsilyl)carbamate	35342-88-2
5	1,4,7,10,13,16-Hexaoxacyclooctadecane	17455-13-9
6	(Diethoxymethoxy)ethane	122-51-0
7	*N*-Methyl-*N*-(trimethylsilyl)acetamide	7449-74-3
8	1,1-Diethoxy-*N*,*N*-dimethylmethanamine	1188-33-6
9	1-[Dimethyl(2-methyl-2-propanyl)silyl]-1*H*-imidazole	54925-64-3
10	*N*-[Dimethyl(phenyl)silyl]-1,1-dimethyl-1-phenylsilanamine	3449-26-1
Chiral derivatization agents		
1	(*S*)-6-Methoxy-2,5,7,8-tetramethyl-2-chromane-2-carboxylic acid	135806-59-6
2	(*R*)-6-Methoxy-2,5,7,8-tetramethyl-2-chromane-2-carboxylic acid	139658-04-1
3	(2*S*,3*S*)-2,3-Butanediol	19132-06-0
4	(1*S*,2*R*,4*S*,6*R*,7*S*,1′*S*,2′*R*,4′*S*,6′*R*,7′*S*)-4,4′-Oxybis(1,10,10-trimethyl-3-oxatricyclo[5.2.1.$0^{2,6}$]decane)	108031-79-4
5	*N*-Isobutyryl-L-cysteine	124529-02-8
5	*N*-Isobutyryl-D-cysteine	124529-07-3
7	(1-Isothiocyanatoethyl)benzene	24277-43-8
8	(2*R*)-2-Octanol	5978-70-1
9	(1*R*)-1-(1-Naphthyl)ethanamine	3886-70-2
10	3,3,3-Trifluoro-2-methoxy-2-phenylpropanoic acid	17257-71-5

As the data extracted from MSDS were of a descriptive nature, they had to be transformed into numerical values. More serious threats were given more points than less serious threats. Such datasets were treated with TOPSIS to obtain rankings within each of the three groups of derivatization agents. Part of the rankings results are presented in Table 11.9.

The-real life application of the derivatization agents selection guide was applied to select the most appropriate derivatization agent for the determination of chlorophenols.[84] Acetic anhydride, ethyl chloroformate, *N*-heptafluorobutyrylimidazole, hexamethyldisilazane, *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide, *N*,*O*-bis(trimethylsilyl)acetamide, chlorotrimethylsilane and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide–chlorotrimethylsilane mixture (99 : 1) were assessed as suitable alternatives according to their greenness and *N*-heptafluorobutyrylimidazole was selected as the greenest alternative. MCDA allowed assessment criteria other than those strictly referring to derivatization agents' greenness to be easily incorporated. In this way, the derivatization effectiveness of chlorinated phenols and the quality of the chromatograms obtained were introduced to other assessments. The most appropriate derivatization agent for the comprehensive approach with MCDA was *N*,*O*-bis(trimethylsilyl)trifluoroacetamide–chlorotrimethylsilane mixture (99 : 1).

11.6 Conclusion

So far, not much attention has been devoted to greenness assessments of analytical procedures. The assessment methodologies that have been developed include NEMI, Eco-scale and the application of MCDA, each characterized by its own advantages and disadvantages. The presented case study of the application of several methodologies for the assessment of the greenness of the determination of DDT in honey samples showed general agreement with GAC metrics results. The differences originated from consideration of different assessment criteria and their treatment with different degrees of quantitativeness.

Apart from the assessment of analytical methodologies, it is important to assess the solvents and reagents used in analytical laboratories. In this area, only a few systems have been dedicated to analytical chemistry but greenness assessment systems developed by the pharmaceutical industry for solvents, acids and bases provide some useful clues for analytical chemists.

All greenness assessments results rely strongly on the parameters that are the basis of the assessment. Therefore, some inconsistencies may appear during comparisons of results. There is still a need to develop metrics systems that treat analytical processes and materials in a comprehensive manner, giving simple and easy to interpret outputs.

References

1. A. Gałuszka, Z. Migaszewski and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2013, **50**, 78.
2. R. A. Sheldon, *Green Chem.*, 2007, **9**(12), 1273.
3. M. Tobiszewski, M. Marć, A. Gałuszka and J. Namieśnik, *Molecules*, 2015, **20**(6), 10928.
4. M. Tobiszewski, *Anal. Methods*, 2016, **8**(15), 2993.
5. J. Beard, *Sci. Total Environ*, 2006, **355**(1–3), 78.
6. K. R. Walker, M. D. Ricciardone and J. Jensen, *Int. J. Hyg. Environ. Health*, 2003, **206**(4–5), 423.
7. A. S. Purnomo, T. Mori, I. Kamei and R. Kondo, *Int. Biodeterior. Biodegrad.*, 2011, **65**(7), 921.
8. M. Alaee, *Dioxin and Related Compounds: Special Volume in Honor of Otto Hutzinger*, Springer, 2016, p. 49.
9. R. Carson, *Silent Spring*, Houghton Mifflin Harcourt, 2002.
10. E. Stokstad, *Science*, 2007, **316**(5832), 1689.
11. H. van den Berg, *Environ. Health Perspect.*, 2009, **117**(11), 1656.
12. J. J. Alava, S. Salazar, M. Cruz, G. Jiménez-Uzcátegui, S. Villegas-Amtmann, D. Paéz-Rosas and F. A. Gobas, *Ambio*, 2011, **40**(4), 425.
13. Z. M. Gong, S. Tao, F. L. Xu, R. Dawson, W. X. Liu, Y. H. Cui and B. P. Qing, *Chemosphere*, 2004, **54**(8), 1247.
14. K. L. Kezios, X. Liu, P. M. Cirillo, B. A. Cohn, O. I. Kalantzi, Y. Wang and P. Factor-Litvak, *Reprod. Toxicol.*, 2013, **35**, 156.
15. J. O. Okonkwo, L. Kampira and D. D. K. Chingakule, *Bull. Environ. Contam. Toxicol.*, 1999, **63**(2), 243.
16. European Union Commission Regulations, Part A of Annex I to Reg. 396/2005, available at https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.CurrentMRL&language=EN&pestResidueId=68, accessed 29.01.2020.
17. G. P. de Pinho, A. A. Neves, M. E. L. R. Queiroz and F. O. Silvério, *Food Control*, 2010, **21**(10), 1307–1311.
18. M. W. Kujawski and J. Namieśnik, *Food Control*, 2011, **22**(6), 914.
19. J. Wang, M. M. Kliks, S. Jun and Q. X. Li, *Food Res. Int.*, 2010, **43**(9), 2329.
20. M. W. Kujawski, E. Pinteaux and J. Namieśnik, *Eur. Food Res. Technol.*, 2012, **234**(2), 223.
21. E. G. Amvrazi, M. A. Martini and N. G. Tsiropoulos, *Int. J. Environ. Anal. Chem.*, 2012, **92**(4), 450.
22. S. Panseri, A. Catalano, A. Giorgi, F. Arioli, A. Procopio, D. Britti and L. M. Chiesa, *Food Control*, 2014, **38**, 150.
23. J. Ruiz-Toledo, R. Vandame, R. A. Castro-Chan, R. P. Penilla-Navarro, J. Gómez and D. Sánchez, *Insects*, 2018, **9**(2), 54.
24. L. Wiest, A. Buleté, B. Giroud, C. Fratta, S. Amic, O. Lambert and C. Arnaudguilhem, *J. Chromatogr. A*, 2011, **1218**(34), 5743.
25. N. Campillo, R. Penalver, N. Aguinaga and M. Hernández-Córdoba, *Anal. Chim. Acta*, 2006, **562**(1), 9.

26. M. M. Mousavi, S. Arefhosseini, A. A. AlizadehNabili, M. Mahmoudpour and M. Nemati, *J. Sep. Sci.*, 2016, **39**(14), 2815.
27. C. Blasco, C. M. Lino, Y. Picó, A. Pena, G. Font and M. I. N. Silveira, *J. Chromatogr. A*, 2004, **1049**(1–2), 155.
28. L. H. Keith, L. U. Gron and J. L. Young, *Chem. Rev.*, 2007, **107**(6), 2695.
29. I. Lavilla, S. Gil, M. Costas and C. Bendicho, *Talanta*, 2012, **98**, 197.
30. N. Cabaleiro, I. de la Calle, C. Bendicho and I. Lavilla, *Anal. Chim. Acta*, 2012, **733**, 28.
31. I. de la Calle and M. Costas-Rodrıguez, *The Application of Green Solvents for Separation Processes*, ed. F. Pena-Pereira and M. Tobiszewski, *Microwaves for Greener Extraction*, Elsevier, 2017, pp. 253–300.
32. A. Gałuszka, Z. M. Migaszewski, P. Konieczka and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2012, **37**, 61.
33. K. Van Aken, L. Strekowski and L. Patiny, *Beilstein J. Org. Chem.*, 2006, **2**, 3.
34. H. M. MoHaMed and N. T. LaMie, *J. AOAC Int.*, 2016, **99**(5), 1260.
35. M. Becerra-Herrera, L. Honda and P. Richter, *J. Chromatogr. A*, 2015, **1423**, 96.
36. M. Ruiz-de-Cenzano, A. Rochina-Marco, M. L. Cervera and M. de la Guardia, *Ecotoxicol. Environ. Saf.*, 2014, **107**, 90.
37. J. Płotka-Wasylka, *Talanta*, 2018, **181**, 204.
38. M. Bystrzanowska and M. Tobiszewski, *TrAC, Trends Anal. Chem.*, 2018, **105**, 98.
39. A. Mardani, A. Jusoh, K. M. D. Nor, Z. Khalifah, N. Zakwan and A. Valipour, *Econ. Res.-Ekon. Istraž.*, 2015, **28**(1), 516.
40. I. B. Huang, J. Keisler and I. Linkov, *Sci. Total Environ.*, 2011, **409**(19), 3578.
41. M. Fabjanowicz, M. Bystrzanowska, J. Namieśnik, M. Tobiszewski and J. Płotka-Wasylka, *Microchem. J.*, 2018, **142**, 126.
42. J. Serna, E. N. D. Martinez, P. C. N. Rincón, M. Camargo and D. Gálvez, *Chem. Eng. Res. Des.*, 2016, **113**, 28.
43. R. Jędrkiewicz, A. Orłowski, J. Namieśnik and M. Tobiszewski, *Talanta*, 2016, **147**, 282.
44. M. Tobiszewski and A. Orłowski, *J. Chromatogr. A*, 2015, **1387**, 116.
45. V. Hongoh, A. G. Hoen, C. Aenishaenslin, J. P. Waaub, D. Bélanger and P. Michel, *Int. J. Health Geograph.*, 2011, **10**(1), 70.
46. M. Kadziński, M. Cinelli, K. Ciomek, S. R. Coles, M. N. Nadagouda, R. S. Varma and K. Kirwan, *Eur. J. Oper. Res.*, 2018, **264**(2), 472.
47. C. L. Hwang and K. P. Yoon, *Multiple Attribute Decision Making: Methods and Applications*, Springer-Verlag, New York, 1981.
48. K. Yoon, *J. Oper. Res. Soc.*, 1987, **38**(3), 277.
49. C. L. Hwang, Y. J. Lai and T. Y. Liu, *Comput. Oper. Res.*, 1993, **20**(8), 889.
50. J. P. Brans and P. Vincke, *Manage. Sci.*, 1985, **31**(6), 647.
51. M. Herva and E. Roca, *J. Cleaner Prod.*, 2013, **39**, 355.
52. G. D. Betrie, R. Sadiq, K. A. Morin and S. Tesfamariam, *J. Environ. Manage.*, 2013, **119**, 36.

53. P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, Oxford, 1998.
54. M. Tobiszewski and J. Namieśnik, *Ecotoxicol. Environ. Saf.*, 2015, **120**, 169.
55. M. Poliakoff, J. M. Fitzpatrick, T. R. Farren and P. T. Anastas, *Science*, 2002, **297**(5582), 807.
56. P. T. Anastas and M. M. Kirchhoff, *Acc. Chem. Res.*, 2002, **35**(9), 686.
57. W. Wardencki, J. Curyło and J. Namieśnik, *J. Biochem. Biophys. Methods*, 2007, **70**(2), 275.
58. C. Nerín, J. Salafranca, M. Aznar and R. Batlle, *Anal. Bioanal. Chem.*, 2009, **393**(3), 809.
59. S. Risticevic, V. H. Niri, D. Vuckovic and J. Pawliszyn, *Anal. Bioanal. Chem.*, 2009, **393**(3), 781.
60. J. M. F. Nogueira, *Anal. Chim. Acta*, 2012, **757**, 1.
61. F. Pena-Pereira, I. Lavilla and C. Bendicho, *Spectrochim. Acta, Part B*, 2009, **64**(1), 1.
62. J. M. Kokosa, *TrAC, Trends Anal. Chem.*, 2015, **71**, 194.
63. M. A. Bello-López, M. Ramos-Payán, J. A. Ocaña-González, R. Fernández-Torres and M. Callejón-Mochón, *Anal. Lett.*, 2012, **45**(8), 804.
64. P. Viñas, N. Campillo, I. López-García and M. Hernández-Córdoba, *Anal. Bioanal. Chem.*, 2014, **406**(8), 2067.
65. M. Zougagh, M. Valcárcel and A. Rıos, *TrAC, Trends Anal. Chem.*, 2004, **23**(5), 399.
66. M. Plaza and C. Turner, *TrAC, Trends Anal. Chem.*, 2015, **71**, 39.
67. T. D. Ho, C. Zhang, L. W. Hantao and J. L. Anderson, *Anal. Chem.*, 2013, **86**(1), 262.
68. A. Shishov, A. Bulatov, M. Locatelli, S. Carradori and V. Andruch, *Microchem. J.*, 2017, **135**, 33.
69. C. Capello, U. Fischer and K. Hungerbühler, *Green Chem.*, 2007, **9**(9), 927.
70. Y. Gaber, U. Törnvall, M. A. Kumar, M. A. Amin and R. Hatti-Kaul, *Green Chem.*, 2011, **13**(8), 2021.
71. D. Prat, O. Pardigon, H. W. Flemming, S. Letestu, V. Ducandas, P. Isnard and P. Hosek, *Org. Process Res. Dev.*, 2013, **17**(12), 1517.
72. C. Jimenez-Gonzalez, A. D. Curzons, D. J. Constable and V. L. Cunningham, *Clean Technol. Environ. Policy*, 2004, **7**(1), 42.
73. D. Prat, J. Hayler and A. Wells, *Green Chem.*, 2014, **16**(10), 4546.
74. M. Tobiszewski, S. Tsakovski, V. Simeonov, J. Namieśnik and F. Pena-Pereira, *Green Chem.*, 2015, **17**(10), 4773.
75. P. Bigus, J. Namieśnik and M. Tobiszewski, *J. Chromatogr. A*, 2016, **1446**, 21.
76. M. J. Raymond, C. S. Slater and M. J. Savelski, *Green Chem.*, 2010, **12**, 1826.
77. R. K. Henderson, A. P. Hill, A. M. Redman and H. F. Sneddon, *Green Chem.*, 2015, **17**(2), 945.

78. J. P. Adams, C. M. Alder, I. Andrews, A. M. Bullion, M. Campbell-Crawford, M. G. Darcy and A. M. Redman, *Green Chem.*, 2013, **15**(6), 1542.
79. M. B. Hicks, W. Farrell, C. Aurigemma, L. Lehman, L. Weisel, K. Nadeau, H. Lee, C. Ginsburg-Moraff, M. Wong, Y. Huang and P. Ferguson, *Green Chem.*, 2019, DOI: 10.1039/C8GC03875A.
80. I. Lavilla, V. Romero, I. Costas and C. Bendicho, *TrAC, Trends Anal. Chem.*, 2014, **61**, 1.
81. M. Sajid, *TrAC, Trends Anal. Chem.*, 2018, **106**, 169.
82. M. Sajid and J. Płotka-Wasylka, *TrAC, Trends Anal. Chem.*, 2018, **102**, 16.
83. M. Tobiszewski, J. Namieśnik and F. Pena-Pereira, *Green Chem.*, 2017, **19**(24), 5911.
84. M. Bystrzanowska, R. Marcinkowska, F. Pena-Pereira and M. Tobiszewski, *Microchem. J.*, 2019, **145**, 664.

Subject Index