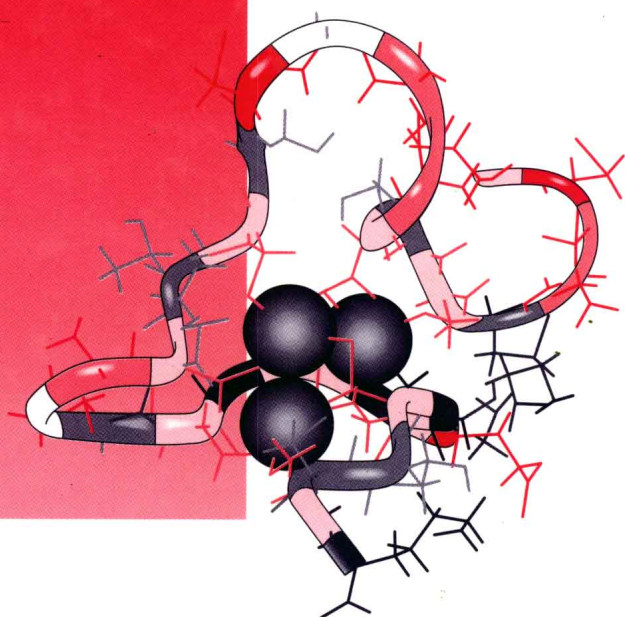


RSC CHROMATOGRAPHY  
MONOGRAPHS

RS•C

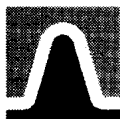


# *Hyphenated Techniques* in Speciation Analysis

---

JOANNA SZPUNAR  
and RYSZARD ŁOBIŃSKI

*series editor* ROGER M. SMITH



RSC  
CHROMATOGRAPHY  
MONOGRAPH

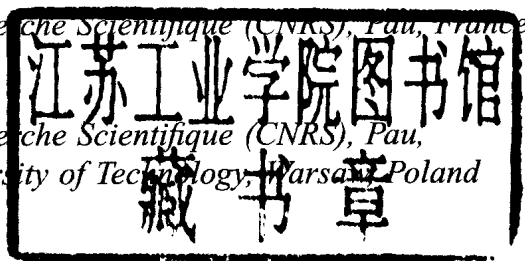
# *Hyphenated Techniques in Speciation Analysis*

**Joanna Szpunar,**

*Centre National de la Recherche Scientifique (CNRS), Pau, France*

**Ryszard Łobiński,**

*Centre National de la Recherche Scientifique (CNRS), Pau,  
France, and Warsaw University of Technology, Warsaw, Poland*



**RS•C**

advancing the chemical sciences

ISBN 0-85404-545-7

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

© The Royal Society of Chemistry 2003

*All rights reserved*

*Apart from any fair dealing for the purpose of research or private study, or criticism or review as permitted under the terms of the UK Copyright, Designs and Patents Act, 1988, 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 in the case of reprographic reproduction only in accordance with the terms of the 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.*

Published by The Royal Society of Chemistry,  
Thomas Graham House, Science Park, Milton Road,  
Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our web site at [www.rsc.org](http://www.rsc.org)

Typeset by Keytec Typesetting Ltd, Dorset, UK  
Printed by Athenaeum Press Ltd, Gateshead, Tyne and Wear, UK

## Hyphenated Techniques in Speciation Analysis

## RSC Chromatography Monographs

Series Editor: Roger M. Smith, *University of Technology, Loughborough, UK*

Advisory Panel: J.C. Berridge, *Sandwich, UK*; G.B. Cox, *Illkirch, France*; I.S. Lurie, *Virginia, USA*; P.J. Schoenmaker, *Amsterdam, The Netherlands*; C.F. Simpson, *London, UK*; G.G. Wallace, *Wollongong, Australia*.

This series is designed for the individual practising chromatographer, providing guidance and advice on a wide range of chromatographic techniques with the emphasis on important practical aspects of the subject.

Supercritical Fluid Chromatography

edited by Roger M. Smith, *University of Technology, Loughborough, UK*

Packed column SFC

by T.A. Berger, *Berger Instruments, Newark, Delaware, USA*

Chromatographic Integration Methods, Second Edition

by Norman Dyson, *Dyson Instruments Ltd, UK*

Separation of Fullerenes by Liquid Chromatography

edited by K. Jinno, *Toyohashi University of Technology, Japan*

HPLC: A Practical Guide

by Toshihiko Hanai, *Health Research Foundation, Kyoto, Japan*

Applications of Solid Phase Microextraction

Edited by Janusz Pawliszyn, *University of Waterloo, Ontario, Canada*

Capillary Electrochromatography

edited by Keith D. Bartle, *University of Leeds, UK*  
and Peter Myers, *X-tec Consulting Ltd, UK*

Cyclodextrins in Chromatography

By T. Cserhati and E. Forgacs, *Chemical Research Centre, Hungarian Academy of Sciences, Budapest, Hungary*

*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 upon publication. For further information please contact:

Sales and Customer Care, Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 0WF

Telephone: +44(0) 1223 432360 E-mail: [sales@rsc.org](mailto:sales@rsc.org)

***To our children: Izabella, Gabrysia and Robert***

# *Preface*

The last two decades have brought a surge of interest in speciation analysis, a field of trace element analytical chemistry that deals with the detection, identification and determination of individual chemical forms of metals and metalloids. Indeed, it is now generally accepted by environmental chemists, nutritionists and toxicologists that information on the total element concentration in the sample is not only insufficient to evaluate its toxicity, essentiality or bioavailability, but may even be misleading. The increasing awareness of the importance of elemental speciation is resulting in a growing demand from research and routine laboratories for analytical techniques capable of providing species-specific information for the environment, agriculture and nutrition, clinical chemistry and toxicology, medicine and pharmacology and industrial process chemistry.

Hyphenated techniques, based on the combination of high resolution separation techniques with element or molecule specific detectors, represent a unique analytical tool able to provide qualitative and quantitative information on element species at trace and ultratrace levels in complex matrices. A growing number of chromatographic and electrophoretic separations can be efficiently coupled with element specific detection, *e.g.* atomic emission or inductively coupled mass spectrometry as well as with molecule specific detection, *e.g.* electrospray mass spectrometry. Couplings such as GC-AAS, GC-MIP AES and GC-ICP MS, HPLC-ICP MS, CZE-ICP MS and HPLC or CZE-electrospray MS/MS, have become well established tools for elemental speciation analysis.

The field of speciation analysis itself has been undergoing a continuous evolution. The classical activities have involved species-specific determination of anthropogenic organometallic contaminants: organolead, organomercury or organotin compounds, and products of their environmental degradation. These are giving way to a search for endogenous metal and metalloid species, that are present in living organisms as a consequence of the biochemical evolution or have been bio-induced in response to a metal stress. In terms of analytical developments the demonstration of the analytical craft and skills of an analyst to determine a particular elemental species in a sample is being replaced by

exploratory investigations aimed at the detection of unknown elemental species in the tissues of a living organisms, their identification and/or structural characterisation.

The book is intended as not only an introductory text to newcomers to the field of elemental speciation analysis. It also offers a critical overview of the research carried out in the field that may serve an already practising analyst. The book is organised in two parts: the first is focused on the technical aspects of the different analytical techniques available and the second on their application to analytical problems in different disciplines. On the subject of analytical techniques individual chapters are devoted to gas chromatography, liquid chromatography and electrophoretic techniques with element specific detection, and electrospray mass spectrometry. The importance of quality control and assurance in speciation analysis is reflected by a dedicated chapter. On applications the selection and organisation of chapters reflect the different maturity of various research areas. The methodology for speciation analysis of methylated species, organolead, -tin and -mercury is well established so these chapters are focused on validated methods that are being implemented in routine laboratories. Regarding naturally occurring metallospecies preference has been given to approaches that have been allowing the exploration of the field in terms of the detection, characterisation and identification of new metallobiomolecules.

The representative coverage of the many facets of this broad and dynamically evolving field has been a difficult task. Indeed, during recent years speciation has become a fashionable area of inorganic trace element research that has resulted in the exponential proliferation of research and review publications. Over 2000 speciation-related papers have been published, many of which have unfortunately only contributed to the information noise. Therefore, the techniques, methods and applications discussed in this book had to be and are a critical selection from the massive literature available. The choice was made on the basis of our practical experience gathered over the last 12 years of research in the field where we have had the opportunity to follow the development of analytical methods in real time and to develop, test or adopt many of the applications in our laboratory.

Joanna Szpunar and Ryszard Łobiński  
Pau, December 2002



# *Terms and Abbreviations*

AAS	atomic absorption spectrometry
AE	anion exchange
AED	atomic emission detection
AES	atomic emission spectrometry
AFS	atomic fluorescence spectrometry
CE	cation exchange
CEC	capillary electrochromatography
CID	collision-induced dissociation
CRM	certified reference material
CT	cryogenic trap
CZE	capillary zone electrophoresis
DBT	dibutyltin
DDT	dithiothreitol
DDTC	diethyl dithiocarbonate
DFO	desferioxamine
DIN	direct injection nebuliser
DL	detection limit
DMAA	dimethylarsonic acid
DPhT	diphenyltin
ECD	electron capture detection
EI	electron impact
ES	electrospray
ET	electrothermal
ETV	electrothermal vaporisation
FAAS	flame atomic absorption spectrometry
FAB	fast atom bombardment
FID	flame ionisation detection
FPD	flame photometric detection
GC	gas chromatography
GE	gel electrophoresis
GF	graphite furnace

HG	hydride generation
HHPN	hydraulic high pressure nebulisation
HPLC	high performance liquid chromatography
HR	high resolution
ICP	inductively coupled plasma
ID	isotope dilution
IEF	isoelectric focusing
INAA	instrumental neutron activation analysis
LA	laser ablation
LC	liquid chromatography
LT	low temperature
MALDI	matrix assisted laser desorption ionization
MBT	monobutyltin
MMAA	monomethylarsinic acid
MPhT	monophenyltin
MC GC	multicapillary gas chromatography
MIP	microwave induced plasma
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MT	metallothionein
NMR	nuclear magnetic resonance
PAGE	polyacrylamide gel electrophoresis
PC	phytochelatin
PFPD	pulsed flame photometric detection
PIXE	proton induced X-ray emission
QF	quartz furnace
Q MS	quadrupole mass spectrometer
SBSE	stir bar sorptive extraction
SCID	source collision induced dissociation
SDS	sodium dodecylsulphonate
SE	size exclusion
SEC	size exclusion chromatography
SFC	supercritical fluid chromatography
SPME	solid phase micro extraction
TBT	tributyltin
TMAH	tetramethylammonium hydroxide
TPrT	tripropyltin
TPhT	triphenyltin
TOF	time of flight
TLC	thin layer chromatography
TXRF	total-reflection X-ray fluorescence
UV	ultraviolet
XRF	X-ray fluorescence

# *Contents*

## **Part I Principles and Fundamentals**

<b>Chapter 1</b>	<b>The Concept of Speciation Analysis and Hyphenated Techniques</b>	<b>2</b>
1	Introduction	2
2	Speciation Analysis: The Definition	3
3	Occurrence and Classification of Metal Species	4
4	The Concept of Hyphenated Techniques	7
5	The Choice of a Hyphenated Technique	9
	References	10
<b>Chapter 2</b>	<b>Element Specific Detection in Chromatography</b>	<b>14</b>
1	Introduction	14
2	Element Selective Detection in Gas Chromatography	15
3	Element Selective Detection in HPLC	20
4	ICP MS Detection in Chromatography and Electrophoresis	22
	References	28
<b>Chapter 3</b>	<b>Gas Chromatography with ICP MS Detection</b>	<b>30</b>
1	Introduction	31
2	Derivatisation Techniques for Gas Chromatography of Organometallic Species	32
3	Separation of Organometallic Species by GC	34
4	Interfacing GC to ICP MS	41
5	Choice of the Mass Spectrometer	45
6	GC-ICP MS Studies Using Stable Isotopes	46
	References	48

<b>Chapter 4</b>	<b>Liquid Chromatography with ICP MS Detection</b>	<b>53</b>
1	Introduction	53
2	Separation of Element Species by Liquid Chromatography	54
3	Interface Between HPLC and ICP MS	59
	References	62
<b>Chapter 5</b>	<b>Electrophoretic Techniques with Element Selective Detection</b>	<b>65</b>
1	Introduction	65
2	Flatbed Gel Electrophoresis	65
3	Capillary Zone Electrophoresis (CZE)	68
4	Areas of Applications	72
	References	72
<b>Chapter 6</b>	<b>Electrospray Mass Spectrometry in Elemental Speciation Analysis</b>	<b>76</b>
1	Introduction	76
2	Principles of Electrospray Mass Spectrometry	76
3	Speciation-Relevant Information from Electrospray MS	80
4	Areas of Application	84
	References	86
<b>Chapter 7</b>	<b>Quality Control and Assurance in Speciation Analysis</b>	<b>88</b>
1	Introduction	88
2	Definition of the Target Moiety	88
3	Stability of Species	89
4	Recovery	90
5	Contamination Risk	91
6	Standardisation	92
7	Isotope Dilution Analysis	93
8	Interlaboratory Studies and Certified Reference Materials	95
	References	96

## **Part II Applications**

<b>Chapter 8</b>	<b>Multielement Analysis of Organometallic Species in the Environment</b>	<b>100</b>
1	Introduction	100
2	Cryogenic Trapping Followed by Low Temperature GC-ICP MS	101
3	Identification, Calibration and Quantification	105

4 Overview of Applications	106
References	109
<b>Chapter 9 Speciation of Organotin Compounds</b>	<b>111</b>
1 Introduction	111
2 Analytical Techniques	112
3 Overview of Applications	114
4 Method Validation	119
References	119
<b>Chapter 10 Speciation of Organolead Compounds</b>	<b>122</b>
1 Introduction	122
2 Analytical Techniques	123
3 Overview of Applications	123
4 Method Validation	126
References	126
<b>Chapter 11 Speciation of Organomercury Compounds</b>	<b>129</b>
1 Introduction	129
2 Analytical Techniques	129
3 Overview of Applications	131
4 Sources of Error and Method Validation	133
References	134
<b>Chapter 12 Metal Speciation in Petroleum-Related Samples</b>	<b>135</b>
1 Introduction	135
2 Mercury in Natural Gas and Gas Condensates	135
3 Arsenic in Natural Gas and Gas Condensates	138
4 Metalloporphyrins in Coal and Shale Oil	139
5 Organolead and Organomanganese Species in Petrol	140
References	141
<b>Chapter 13 Speciation of Redox States</b>	<b>143</b>
1 Introduction	143
2 Analytical Methodology	143
3 Overview of Applications	145
References	147
<b>Chapter 14 Speciation of Organoarsenic Compounds in Biological Materials</b>	<b>149</b>
1 Introduction	149
2 Determination of Arsenic Species by HPLC-ICP MS	150

3 Identification of Arsenic Species by Electrospray MS/MS	153
4 Validation of Arsenic Speciation Analysis	158
References	159

## **Chapter 15 Speciation of Organoselenium Compounds in Biological**

<b>Materials</b>	<b>162</b>
1 Introduction	162
2 Volatile Selenium Species in Plants	164
3 SelenoAmino Acids and SelenoPeptides in Yeast and Plants	165
4 Selenoproteins	170
5 Selenium Metabolites in Urine	172
6 Optically Active Selenospecies	174
References	175

## **Chapter 16 Speciation of Metal Complexes in Microorganisms, Plants and Food of Plant Origin**

<b>1 Introduction</b>	<b>179</b>
2 Metal Complexes with Water-Soluble Proteins and Polypeptides	180
3 Metal Complexes with Polysaccharides	183
4 Metal Complexes with Phytometallophores	184
5 Other Metal Species in Plant Tissues	185
References	186

## **Chapter 17 Speciation of Metal Complexes with Metallothioneins**

<b>1 Introduction</b>	<b>189</b>
2 Recovery of Metal Complexes with Metallothioneins from Biological Tissues	190
3 Liquid Chromatography with ICP MS Detection	192
4 Capillary Electrophoresis-ICP MS	196
5 Identification of MT Isoforms by Electrospray MS	197
6 Analysis of Human and Animal Tissue Samples	198
References	198

## **Chapter 18 Speciation of Metal Complexes in Human Body Fluids and Tissues**

<b>1 Introduction</b>	<b>200</b>
2 Analytical Techniques	201
3 Overview of Applications	203
References	206

<i>Contents</i>	xiii
<b>Chapter 19 Metal Speciation in Pharmacology: Metallodrugs</b>	<b>209</b>
1 Introduction	209
2 Analytical Techniques	210
References	214
<b>Subject Index</b>	<b>216</b>

# **Part I Principles and Fundamentals**



## CHAPTER 1

# *The Concept of Speciation Analysis and Hyphenated Techniques*

## 1 Introduction

The analysis of metal(loid) organic compounds has become increasingly important in the last decade because the organic species of some elements have turned out to be much more toxic than their inorganic forms. The origin of metal species can be either anthropogenic input (*e.g.* as pesticides) or a result of biological transformations of inorganic forms of elements by living organisms. The harmful effects of trace metal species were fully recognised after the death of 50 residents of the Minamata fishing village in Japan who had experienced the biomethylation of mercury in their everyday food.<sup>1</sup> The spillage of tetraalkyl-lead in the Mediterranean due to the M/S Cavteat accident made the analytical community sensitive to organic forms of lead.<sup>2</sup> Extinction of the oyster population in the Arcachon Bay in Southern France stimulated interest in the possible release of butyltins from antifouling paints.<sup>3</sup> The above cases raised awareness of the importance of knowing the concentration of a particular species, defined the suspected analyte and analytical sample and, consequently, stimulated the development of analytical methodology.

It has also been widely recognised in biochemistry that the proper functioning of life is critically dependent on trace elements in a number of different ways. Some metals (*e.g.* Hg, Pb) and metalloids (As) are highly toxic whereas others (*e.g.* Mo, Mn, Fe, Co, Cu, Zn), considered essential, are needed for the accomplishment of life processes.<sup>4</sup> A number of other elements (*e.g.* V, Cr, Ni) are recognised as being beneficial to life. From a chemical point of view the intake, accumulation, transport and storage of essential or toxic metals and metalloids are realised by surrounding the element ion by electron pair donating biological ligands. Sometimes this process is accompanied by the synthesis of specific ligands such as metallothioneins, or by the formation of a metalloid-