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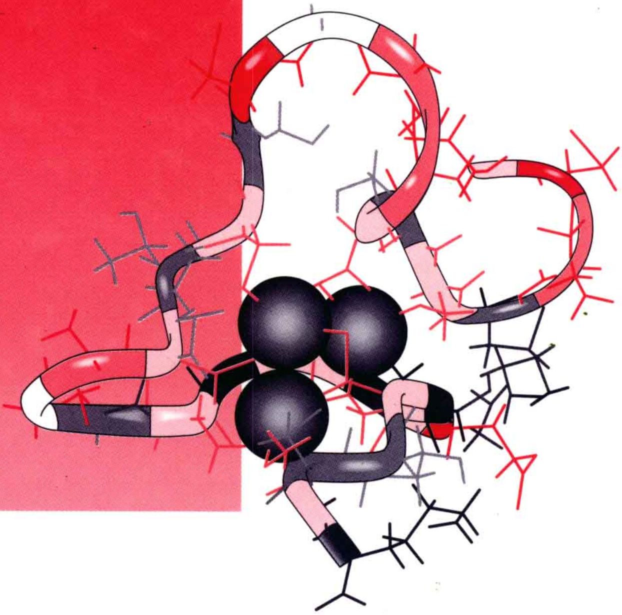

Hyphenated Techniques
in Speciation Analysis

JOANNA SZPUNAR
and RYSZARD ŁOBIŃSKI

series editor ROGER M. SMITH

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Hyphenated Techniques in Speciation Analysis

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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

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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.[1] The spillage of tetraalkyllead in the Mediteranean due to the M/S Cavteat accident made the analytical community sensitive to organic forms of lead.[2] Extinction of the oyster population in the Arcachon Bay in Southern France stimulated interest in the possible release of butyltins from antifouling paints.[3] 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.[4] 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-