Handbook of Elemental Speciation: Techniques and Methodology

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Preface

The recognition of the fact that, in environmental chemistry, occupational health, nutrition and medicine, the chemical, biological and toxicological properties of an element are critically dependent on the form in which the element occurs in the sample has spurred a rapid development of an area of analytical chemistry referred to as speciation analysis. In contrast to its biological meaning, the term speciation in chemistry refers to the distribution of an element among defined chemical species, i.e. among specific forms of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.

The areas of speciation analysis have been undergoing a continual evolution and development for the last 20 years. The area most frequently referred to is speciation of anthropogenic organometallic compounds and the products of their environmental degradation, such as methylmercury, alkyllead, butyl- and phenyltin compounds, and simple organoarsenic and organoselenium species. The presence of a metal(loid)–carbon covalent bond ensures a reasonable stability of the analyte(s) during sample preparation. The volatility of the species allows the use of gas chromatography with its inherent advantages, such as the high separation efficiency and the absence of the condensed mobile phase, that enable a sensitive (down to the femtogram levels) element-specific detection by atomic spectroscopy. Much effort has been devoted by the European Commission Measurement and Testing Program to raise the standards of accuracy of speciation measurements in terms of appropriate calibration and method validation using certified reference materials.

An insight into endogenous metal species in biological systems has remained for a long time a challenge to the analyst. Indeed, millions of years of evolution have resulted in a great variety of biological ligands with different functions and a significant coordinating potential for trace elements. They include small organic acids, macrocyclic chelating molecules, and macromolecules, such as proteins, DNA restriction fragments or polysaccharides. The complexity and the usually poor understanding of the system (the majority of trace element species with biological ligands have not yet been discovered!) have been the major obstacles on the way to the identification and characterization of the endogenous metal complexes with biomolecules. Their generally poor volatility in comparison with organometallic species calls for separation techniques with a condensed mobile phase that negatively affects the separation efficiency and the detection limits.

A fundamental tool for speciation analysis has been the combination of a chromatographic separation technique, which ensures that the analytical compound leaves the column unaccompanied by other species of the analyte element, with atomic spectrometry, permitting a sensitive and specific detection of the target element. Recent impressive progress toward lower detection limits in ICP MS, toward higher resolution in separation techniques, especially capillary electrophoresis and electrochromatography, and toward higher sensitivity in electrospray mass spectrometry for molecule-specific detection at trace levels in complex matrices allows new frontiers to be crossed. Analytical techniques allowing direct speciation in solid samples are appearing.

Speciation analysis is a rather complex task and a reference handbook on relevant techniques and methodology has been awaited by all those with an interest in the role and measurement of element species. These expectations are now fulfilled by the *Handbook of Elemental Speciation* of which the first (of the announced two) volume is now appearing. This first volume brings a collection of chapters covering comprehensively different aspects of procedures for speciation analysis at the different levels starting from sample collection and storage, through sample preparation approaches to render the species chromatographable, principles of separation techniques used in speciation analysis, to the element-specific detection. This already very broad coverage of analytical techniques is completed by electrochemical methods, biosensors for metal ions, radioisotope techniques and direct solid speciation techniques. Special concern is given to quality assurance and risk assessment, and speciation-relevant legislation.

Although each chapter is a stand-alone reference, covering a given facet of elemental speciation analysis written by an expert in a given field, the editorial process has ensured the volume is an excellent introductory text and reference handbook for analytical chemists in academia, government laboratories and industry, regulatory managers, biochemists, toxicologists, clinicians, environmental scientists, and students of these disciplines.

Ryszard Lobinski

Pau, France, October 2002

Technical Abbreviations and Acronyms

Abbreviations

AAS	atomic absorption spectrometry
AC	alternating current
AED	atomic emission detection
AES	atomic emission spectrometry
AF	atomic fluorescence
ANOVA	analysis of variance
CE	capillary electrophoresis
CGC	capillary gas chromatography
cpm	counts per minute
CRM	certified reference material
CVAAS	cold vapour atomic absorption spectrometry
CZE	capillary zone electrophoresis
DC	direct current
ES	electrospray
ESI	electrospray ionization
ETAAS	electrothermal atomic absorption spectrometry
ETV	electrothermal vaporization
EXAFS	extended X-ray absorption fine structure spectroscopy
FAAS	flame atomic absorption spectrometry
FID	flame ionization detector
FIR	far-infrared
FPD	flame photometric detector
FT	Fourier transform
FPLC	fast protein liquid chromatography
GC	gas chromatography
GD	glow discharge
GLC	gas–liquid chromatography
GSGD	gas-sampling glow discharge
HGAAS	hydride generation atomic absorption spectrometry
HPLC	high performance liquid chromatography
Hz	Hertz
ICP	inductively coupled plasma
i.d.	internal diameter
IEF	isoelectric focusing
IDMS	isotope dilution mass spectrometry
INAA	instrumental neutron activation analysis
IR	infrared
ISFET	ion-selective field effect transistor
ISE	ion-selective electrode
ITP	isotachophoresis
LA	laser ablation
LC	liquid chromatography
LED	light emitting diode
LOD	limit of detection
LOQ	limit of quantification
MAE	microwave-assisted extractions
MIP	microwave-induced plasma
MS	mass spectrometry
NIR	near-infrared
NMR	nuclear magnetic resonance
o.d.	outer diameter
OES	optical emission spectrometry
PBS	phosphate buffer saline
PIXE	particle/proton-induced X-ray emission
QA	quality assurance
QC	quality control
QF	quartz furnace
REE	rare earth element
RPC	reversed phase chromatography
RSD	relative standard deviation

SE	standard error
SEM	scanning electron microscope
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SFMS	sector-field mass spectrometer
SIMS	secondary ion mass spectrometry
SPME	solid-phase micro-extraction
TD	thermal desorption
TEM	transmission electron microscope
TIMS	thermal ionization mass spectrometry
TOF	time of flight
UV	ultraviolet
UV/VIS	ultraviolet–visible
XAFS	X-ray absorption fine structure
XRD	X-ray diffraction
XRF	X-ray fluorescence

Units

μg	micrograms
ng	nanograms
pg	picograms
fg	femtograms
mL	millilitres
L	litres
cL	centilitres

Symbols

M	molecular mass
M_r	relative molecular mass
r	correlation coefficient
s	standard deviation of sample
σ	population standard deviation

CHAPTER 1
Introduction

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'Speciation', a word borrowed from the biological sciences, has become a concept in analytical chemistry, expressing the idea that the specific chemical forms of an element should be considered individually. The underlying reason for this is that the characteristics of just one species of an element may have such a radical impact on living systems (even at extremely low levels) that the total element concentration becomes of little value in determining the impact of the trace element. Dramatic examples are the species of tin and mercury, to name just these two. The inorganic forms of these elements are much less toxic or even do not show toxic properties but the alkylated forms are highly toxic. No wonder analytical chemists had to study elemental speciation and devise analytical techniques that produce qualitative and quantitative information on chemical compounds that affect the quality of life.

Before embarking on the definitions of elemental speciation and species, it may be interesting to give a short historical setting of this emerging branch of analytical chemistry. Analytical chemistry began as a science in the early 19th century. A major milestone was the book by Wilhelm Ostwald 'Die Wissenschaftlichen Grundlagen der analytischen Chemie' (Scientific Fundamentals of Analytical Chemistry) in 1894 [1]. A personality who contributed substantially to the development of analytical chemistry and chemical

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analysis was Carl Remigus Fresenius. In 1841 he published a very interesting book on qualitative chemical analysis [2]. It was followed over the next 100 years by a series of standard works on qualitative and quantitative analysis by several generations of the Fresenius family and by the publications by Treadwell [3, 4], Feigl [5] and Kolthoff [6, 7], to name just these few. The interest remained largely focused on inorganic analytical chemistry. The term 'trace elements' dates back to the early 20th century, in recognition of the fact that many elements occurred at such low concentrations that their presence could only just be detected. During the following 60 years all efforts were focused on total trace element concentrations. Scientists developed methods with increasing sensitivity. It was only in the early 1960s that questions were raised concerning the chemical form of the trace elements and that the need for an analytical methodology developed subsequently. This development has been growing exponentially to the point that research on trace element analysis today appears almost exclusively focused on trace element species.

Extensive literature is available on the speciation and fractionation of elements. Newcomers have to absorb a wealth of highly specialised publications and they miss the broader overview to guide them. This handbook aims to provide all the necessary background and analytical information for the study of the speciation of elements.

The objective of this handbook is to present a concise, critical, comprehensive and systematic (but not exhaustive), treatment of all aspects of analytical elemental speciation analysis. The general level of the handbook makes it most useful to the newcomers in the field, while it may be profitably read by the analytical chemist already experienced in speciation analysis.

1 DEFINITION OF ELEMENTAL SPECIATION AND OF FRACTIONATION

The International Union for Pure and Applied Chemistry (IUPAC) has defined elemental speciation in chemistry as follows:

(i) *Chemical species.* Chemical element: specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.

(ii) *Speciation analysis.* Analytical chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.

(iii) *Speciation of an element; speciation.* Distribution of an element amongst defined chemical species in a system.

When elemental speciation is not feasible, the term fractionation is in use, being defined as follows:

(iv) *Fractionation.* Process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties.

As explained in the IUPAC paper [8], it is often not possible to determine the concentrations of the different chemical species that sum up to the total concentration of an element in a given matrix. Often, chemical species present in a given sample are not stable enough to be determined as such. During the procedure, the partitioning of the element among its species may be changed. For example, this can be caused by a change in pH necessitated by the analytical procedure, or by intrinsic properties of measurement methods that affect the equilibrium between species. Also in many cases the large number of individual species (e.g., in metal–humic acid complexes or metal complexes in biological fluids) will make it impossible to determine the exact speciation. The practice is then to identify various classes of the elemental species.

2 PROBLEMS TO BE SOLVED

While the incentive to embark on speciation and fractionation of elements is expanding, it becomes more and more evident that the matter has to be handled with great circumspection. Major questions include: What are the species

we want to measure? How should we sample the material and isolate the species without changing its composition? Can we detect very low amounts of the isolated species, which may represent only a minute fraction of the total, already ultra-trace element concentration? How do we calibrate the species, many of these not being available as commercial compounds? How do we validate methods of elemental analysis? All of these questions will be carefully dealt with in the first volume of the handbook. The second and third volumes will extensively address elemental species of specific elements and the analysis of various classes of species.

Advances in instrumentation have been crucial to the development of elemental speciation. There has been a very good trend towards lower and lower detection limits in optical atomic spectrometry and mass spectrometry. This has allowed the barrier between total element and element species to be crossed. While the limit of detection for many polluting species is sufficient for their measurement in a major share of environmental samples, this is not yet the case for human, animal and perhaps plant samples at 'background levels'. The background concentration of elemental species of anthropogenic origin was originally zero. Today they are present, because they have been and continue to be distributed in a manner that affects the life cycle. However, because we cannot measure them in living systems it does not mean that their presence is harmless. At the same time it is also highly plausible that a certain background level of these anthropogenic substances can be tolerated without any adverse effect. In order to assess the impact of low background levels of element species we will have to develop separation and detection techniques that surpass the performance of the existing speciation methodology.

In the mean time research teams are very resourceful in developing computer controlled automated systems for elemental speciation analysis. These are or will become tremendous assets for routine analyses. Preferably systems should be simple, robust, low cost and if at all possible portable, to allow for fieldwork. Although currently limited it can be postulated that once there are more regulatory or economic motives, this technology would develop rapidly. Moreover, sensors will play a major role in rapid detection of elemental species. Simple screening methods will be increasingly popular because they can provide speedy and reliable tests to detect elemental species and give an estimate of their concentration.

Good laboratory practice and method validation are a must to produce precise and accurate results. To this end, it is evident that provision has to be made for elemental species data in more certified reference materials (CRMs) reporting on elemental species. This need will become even more acute once legislation becomes specific and cites elemental species instead of the total concentration of the element and its compounds, as is presently the general rule. This type of legislation may be politically charged, because every species carries a different health risk or benefit. The toxicity may vary by several orders of magnitude among species of the same element. This may lead to some confusing and dangerous conclusions. A product may be legally acceptable on the basis of total concentration but when that total consists of some very toxic species it may constitute a real hazard. The opposite may also be true. This can be exemplified by the occurrence of arsenic in food. Whereas the total arsenic in some fish derivatives, such as gelatine, often exceeds the accepted limit, the product should not be rejected, because the arsenic is mainly present as arsenobetaine, a non-toxic arsenic species, as opposed to the toxic inorganic arsenic species.

3 SPECIATION STRATEGIES

'Strategy' signifies 'a careful plan or method' or 'the art of devising or employing plans or stratagems toward a goal' [9]. Ideally scientists hope to learn everything about the elemental species they study: to start with its composition, its mass, the bio- and environmental cycle, the stability of the species, its transformation, and the interactions with inert or living matter. This list is not exhaustive. The work involved to achieve this goal is, however, challenging, if not impossible to complete. Therefore a choice has to be made to identify

the most important issues as elemental speciation studies are pursued. A first group of compounds to be studied very closely are those of anthropogenic origin. Although they fulfil the requirements for which they were synthesised, unless they happen to be synthetic by-products or waste, their long-term effect on the environment, including living systems, has often been ignored or misjudged. One of the most striking examples is the group of organotin compounds. They have surely proven to be the most effective marine anti-fouling agents, fungicides, insecticides, bacteriostats, PVC stabilising agents, etc. However, the designers of these compounds never anticipated what the negative effect would be on the environment. Their disturbing impact on the life cycle of crustaceans constituted the first alarming event. In the mean time these components have become detectable, with concentrations now increasing in fish products and even in vegetables from certain areas. Little thought was given that organotin compounds would be serious endocrine disruptors, or that they would have such a long half-life. Hence they will continue to be a burden on the environment and ultimately on mankind itself into the distant future. The organotin compounds are only a minute part of the total amount of tin (mainly inert tin oxides) to be found in contaminated areas. Determination of the total tin concentration would surely not be appropriate.

In speciation studies, a lot of attention must be paid to the stability. Species stability depends on the matrix and on physical parameters, such as temperature, humidity, UV light, organic matter, etc. Next comes the isolation and purification of the species, the study of the possible transformation through the procedure, their characteristics and interactions. New analytical procedures have to be devised, including appropriate quantification and calibration methodologies.

Besides the suspect elemental species of anthropogenic origin, there is the barely fathomable domain of the species that developed along with life on earth. For many elements nothing is really known, or only a few uncertain facts can be stated. Whereas the total trace element concentration may be static, the species may be highly dynamic. They will change continuously with respect to changes in the surrounding environment, depending on chemical parameters such as pH value or concentration of potential ligands for complex formation, the physiological state of a cell, and state of health of a living entity. Therefore, thermodynamic but also kinetic stability of elemental species in the environment has to be taken into account. Unstable species in the atmosphere are predominant and this steady transformation requires special analytical procedures. Although species in living cells can be stable covalent compounds when the element forms the core of the molecule (such as Co in vitamin B_{12}), most elemental species exhibit very low stability constants with their ligands. These compounds are, however, very active in reaching the target organs. This to say that a reliable speciation strategy will include stability criteria for the species and awareness of possible transformations.

Understanding the fate of the trace elements in the life cycle is of paramount importance. When, through natural or anthropogenic activities, metal ions enter the environment and the living systems, only a small fraction will remain as the free ion. The major share will be complexed with either inorganic or organic ligands. Natural methylation of metal ions under specific conditions is prevalent. The new species can be much more toxic, as is the case with methylated mercury, or less toxic as in the case of arsenic. In the case of mercury, the concentration of ionic mercury in water may be very low (a few $ng\,L^{-1}$) and that of methylmercury only 1 % of total Hg. Unfortunately, this accumulates to $mg\,kg^{-1}$ levels in the top predators of the food chain, with methylmercury making up 90 to 100 % of the total Hg concentration. Metal ions will also be incorporated into large molecular structures such as humic substances. Elucidation of the many, often-labile species will take many more years. Trace element speciation has become important in all fields of life, and concerns industry, academia, government and legislative bodies [10].

It is obvious that it would have been impossible to accomplish the stated aims and objectives of this handbook without the wholehearted cooperation of the distinguished authors who contributed the

various chapters. To them we express our sincere appreciation and gratitude.

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CHAPTER 2

Sampling: Collection, Storage

2.1 Sampling: Collection, Processing and Storage of Environmental Samples

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1 INTRODUCTION

Public awareness and scientific understanding of the various compartments, processes and problems in the environment have been significantly improved particularly in the last three decades. To a large extent this can be attributed to the progress of environmental analysis. Analytical chemistry itself has gained from the needs of environmental sciences, technology and legislation. It is now widely accepted that human activities which influence the chemical composition of the environment have to be systematically controlled. Therefore, procedures for the analysis of an increasing number of elements and chemical compounds in air, water, sediment and soil have been developed and this is still going on. But modern environmental observation has to provide more effect-related information about the state of our environment and its changes with time. Therefore, it cannot be based only on investigations of abiotic environmental samples. Rather environmental studies and control need to take much more account of the situation in the biosphere. This includes the transfer of contaminants, mainly of anthropogenic origin, into plants, animals, and finally also into human beings. As a result, biomonitoring plays an increasing role in modern environmental observation programs. For that, selected biological organisms, called bioindicators, are used for the monitoring of pollutants either by observation of phenomenological effects (loss of needles, discoloring of leaves, etc.) or by measurement of chemical compounds taken up by the specimens. The latter approach is based on the chemical analysis of appropriate bioindicators and adds another dimension of complexity

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to the sample matrices encountered in environmental analysis.

In general environmental analysis should contribute answers to the following questions:

- Which pollutants appear where, when and at which concentration?
- How mobile and stable are pollutants in ecosystems?
- Which transformations occur with originally anthropogenic emissions in ecosystems?
- Where are pollutants accumulated or finally deposited and in which chemical form?
- Which short- and long-term effects do they have with respect to mankind and the environment?

All these questions are expanding both the chemical nature and the concentration ranges of analytes to be included when considering environmental studies and control. For evaluating the necessity, toxicity, availability, distribution, transformation and fate of chemicals, the identity and concentration of chemical species [1] rather than those of total elements have to be studied in very complex systems and samples. Speciation analysis adds new requirements to the usual boundary conditions in environmental analysis, in particular with respect to analyte stability and preservation of original chemical equilibria. Such aspects are also partially considered in the so-called 'organic analysis' of compounds outside the POP (persistent organic pollutants) group, but the thermodynamic and kinetic properties of different redox states or chemical complexes are adding new dimensions of lability and reactivity to analytical chemistry.

Sampling is always the first step of the total analytical process (Figure 2.1.1) and its design and implementation has a decisive influence on the final analytical result. For the purpose of this chapter the term 'sampling' will include the collection of specimens from the environment (accompanied in many cases by non-chemical operations for on-site sample preparation), followed by intermediate storage and often by a mechanical processing of the collected material up to samples which are appropriate for the subsequent steps of chemical analysis, namely analyte separation and determination. In the following text, general aspects and specific requirements for sampling, sample handling and sample storage, in the speciation analysis of environmental samples will be discussed.

2 GENERAL ASPECTS OF ENVIRONMENTAL SAMPLING

The second step after the definition of the analytical problem of interest consists of the careful selection and problem-specific design of the sampling procedure. For that one has to consider not only all relevant properties of the analyte of interest, the matrix and the chosen analytical techniques, but also a number of parameters that are necessary for the final evaluation and assessment of the analytical data. Unfortunately, a large number of environmental analyses are still wasted because of insufficient sampling and sample handling strategies.

Obtaining representative samples is of utmost importance. This includes accessing representative sampling sites for the purpose of the study, which

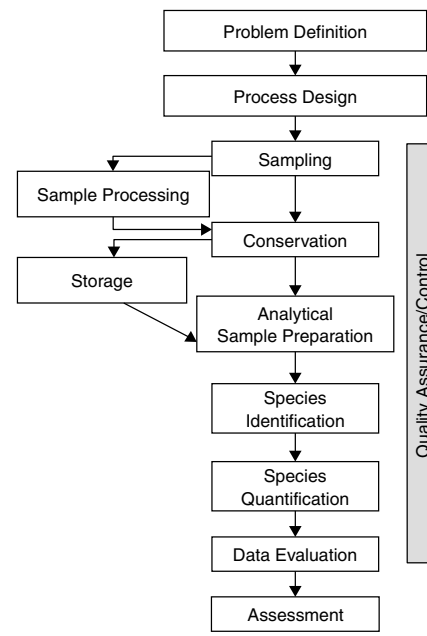

Figure 2.1.1. General steps in the total analytical process.

is often easier to achieve for the control of local emission sources than for the observation of larger 'normally' exposed areas or even background regions. One needs geographical, meteorological and biological data, and information about human activities for the site selection [2]. Sampling within a selected area is commonly planned by taking into account a combination of pre-existing environmental knowledge, statistical approaches (grid sampling) [3] and financial considerations. The heterogeneity of our environment on all scales, from ecosystems via populations and individual specimens down to the molecular level creates challenging demands on sampling concepts. Frequently, one or more screening studies have to precede the actual environmental sampling campaign in order to select the final sampling points.

Secondly, representativeness refers also to the kind of material to be sampled. The selection of representative environmental specimens from the various environmental compartments, ecosystems, etc. depends mainly on the question (groundwater quality, fate of industrial emissions, forest health, etc.), but also on the available knowledge about key indicators of the environmental situation. The frequently described 'flow circles' of elements between the atmosphere, hydrosphere, biosphere, pedosphere and lithosphere are certainly not sufficient for the proper design of sampling procedures for chemical speciation. The physicochemical properties of the target compounds have to be considered in more detail to estimate the transfer, transformation, deposition and accumulation of chemical species in environmental compartments and specimens. In this respect it may be useful to classify 'chemical species' from the point of view of the nature of their primary interactions with the surroundings, namely hydrophilic species with Coulomb forces or hydrogen bonding, and lipophilic species with hydrophobic interactions. In addition, characteristic pathways of species uptake, transport, accumulation and transformation within the biosphere, namely within food chains, should be considered. By taking these factors into account useful sample sets of environmental indicators can be composed which may significantly improve the information content of environmental analysis.

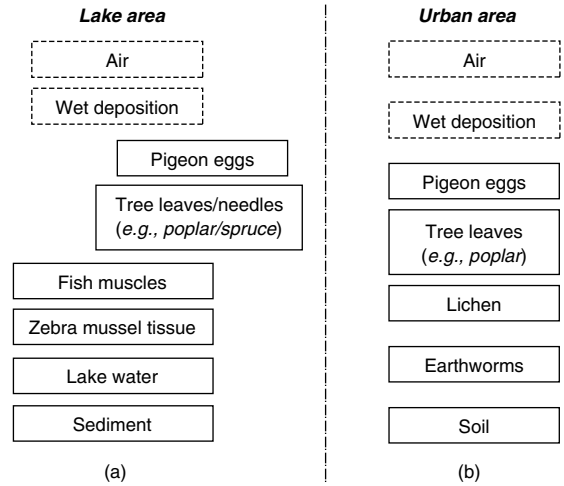

Figure 2.1.2. Examples of environmental indicator sets (modified from [31]): (a) limnic ecosystems: lake area; (b) terrestrial ecosystems: urban area.

Examples for two types of ecosystems are illustrated in Figure 2.1.2.

Another requirement for representativeness consists in the number of specimens which have to be sampled for subsequent analysis. Ideally this parameter should be calculated on the basis of the known variations in the chemical composition of the sampled population which arise from its geochemical/-physical or biochemical/-physical diversity and the variation in imissions in the studied area. An additional uncertainty for biological specimens originates from the natural heterogeneity within organisms and even organs (see also Section 4). All this information is rarely known in detail from screening investigations and therefore, this lack limits the precision (and often even the accuracy) of the environmental information decoded by the chemical analysis of the sampled material.

Representative (and reproducible) environmental sampling has also to consider the variations of climate/weather conditions, seasonal fluctuations of species concentrations in bioindicators and the different exposure time of the samples. An example for the variation of the methylmercury content in mussel tissue is shown in Figure 2.1.3a and can be compared with the corresponding data for total mercury (Figure 2.1.3b). Obviously,

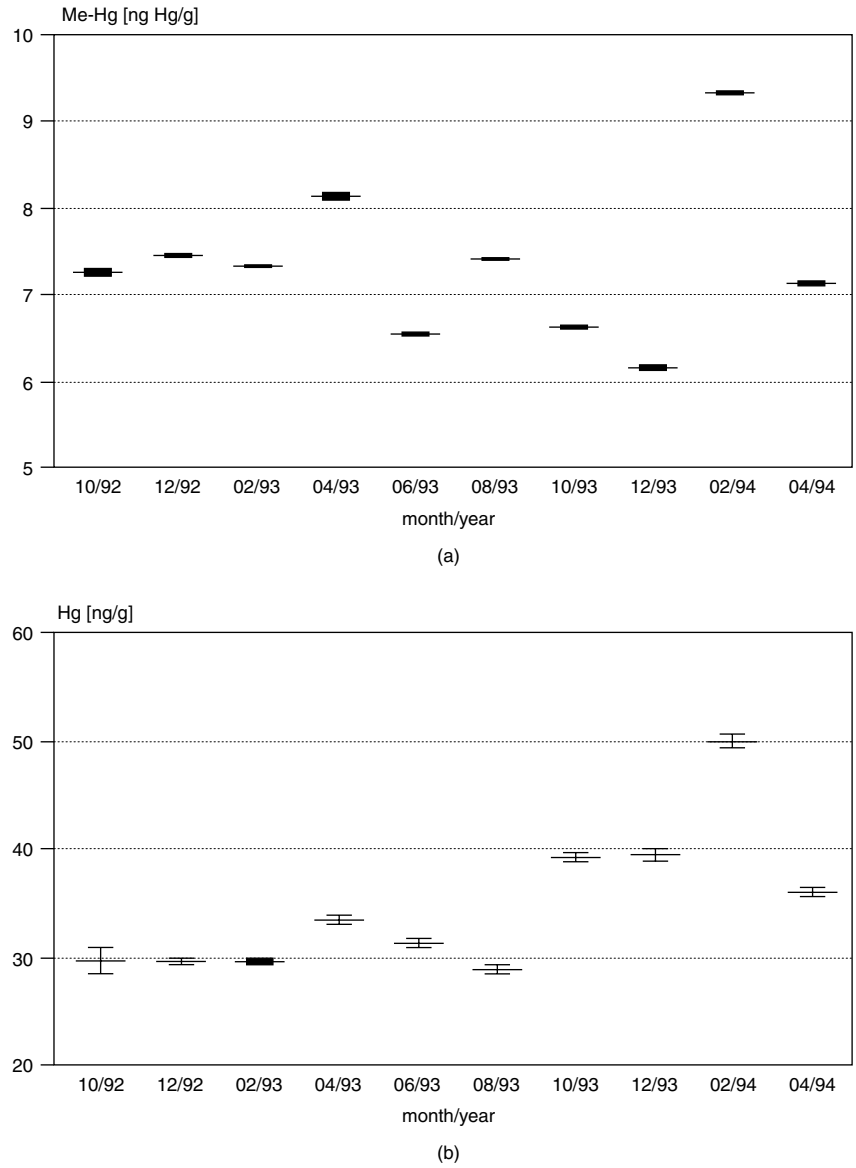

Figure 2.1.3. Seasonal variation of (a) methylmercury and (b) total mercury concentrations related to fresh mass in homogenates of common mussel tissue from the German Wadden Sea between October 1992 and April 1994 (means and standard deviations).

not only it is necessary to document extensively all available parameters (location, time, climate, emission sources, population data such as density, unusual damage etc.) in connection with environmental sampling campaigns, but also standardization of sample selection (and handling/manipulation) is recommended for obtaining useful environmental information from the sampled material. Corresponding standard operating procedures have been developed for various objectives of environmental studies and should provide also the basis for repetitive sampling (see below). But one should always keep in mind that the remaining variation in the representativeness

of sampled material makes often a greater contribution to the uncertainty in the final environmental information than any other subsequent analytical step.

In general the sampling procedure should be developed in advance with as much care and detail as possible. Experts with the necessary knowledge in environmental sciences have to be consulted, but the analytical chemist should be also involved from the beginning to avoid or at least reduce any sample manipulations which could influence the final analytical data.

3 SAMPLING FOR SPECIATION ANALYSIS

The key requirement of speciation analysis consists of the preservation of the species information during the whole analytical process (Figure 2.1.1). One could distinguish two principal strategies for achieving this goal: on the one hand, one may keep the chemical species of interest unchanged during all critical steps of their analysis, and on the other hand, the species may be quantitatively transformed at an early stage into suitable derivatives for further separation, accumulation and quantification methods. In practice there is usually a mixture of both extremes, but we will consider primarily the aspects of species-retaining sampling in the following. Therefore, chemical stability and volatility of the analytes of interest are of great importance for the sampling procedures.

In addition, one has to take into account that speciation analysis of environmental samples constitutes, in most cases, trace or even ultratrace analysis. Therefore, both contamination and loss of the analytes have to be avoided along the whole sample pathway from the sampling site to the analytical laboratory. All materials which are in contact with the sample must be checked in advance as a possible source of contamination or adsorption. Sampling devices with stainless steel surfaces which are often used for field sampling (knives, scalpels, drills, etc.) should be avoided in sampling for metal speciation. Careful cleaning of all sampling tools is mandatory. This can be done, for instance, by washing with concentrated or 10 % nitric acid, depending upon the material and the sample matrix, followed by several rinsing steps with distilled water. But the application of washing solutions which activate the adsorption sites at the surface of the tools (such as various concentrated acids for glassware) should be avoided to minimize species losses. The cleaning procedures for the sampling devices as well as the protective measures for the sampling team (wearing of gloves, obeying safety instructions etc.) have to be integrated into the planning and documentation of sampling.

3.1 Air

The collection of samples for ‘speciation analysis in the traditional meaning’ is mainly focused on the investigation of metal(loid) compounds in the gas phase. Hydrides, methylated and permethylated species of As, Hg, Pb, Se etc. have been sampled from terrestrial sources such as waste deposits or from aquatic ecosystems such as ocean water surfaces. The main techniques include the application of cryotrapping, solid adsorbent cartridges, polymer bags or stainless steel canisters (with coated inner surfaces). The gas phase can be transferred into a pre-evacuated sampling device [4] or sucked with the help of a pump. In most cases the air has to pass through a filter (often 0.45 μm) to remove particles and aerosols up to a predefined size from the sample. The advantage of trapping techniques lies in the integrated accumulation of the analyte and its partial separation from other sample constituents. On the other hand, any sample treatment has to be carefully validated with respect to possible changes of the original species pattern and contamination by used materials. Analyte loss can be caused by photolytic or surface-catalyzed decomposition, hydrolysis, oxidation, adsorption on container walls or absorption. Critical parameters during collection and sample preservation include, in particular, temperature, light intensity, humidity, oxygen content and aerosol concentration. For quantitative analysis one has to adjust for any temperature and pressure variations during the transfer of the original gas phase into the sampling device and during

any subsequent operations because of the much larger influence of these parameters on analyte concentrations in the gaseous state in comparison to condensed phases. Recently, the stability of various volatile As, Sb and Sn species was studied as a function of the temperature and a reasonable recovery was obtained for most of the species which were kept at 20 °C in the dark for 24 h [5].

At present the main problems occur in the quantitative sampling of the original air composition under environmental on-site conditions and its preservation. Therefore, the collection of the whole gas at the sampling location into containers, followed by its analysis within a relatively short time (about a day), seems to be the safest way at the moment. The sampling devices can be balloons, cylinders with inlet and outlet valves, canisters or bags. Special consideration should be given to the selected surface. Container walls coated with an inert polymer such as PTFE are recommended. Moreover, contamination by exhaust fumes from the pump has to be avoided. If the species of interest are stable enough, liquid absorbents, adsorbent cartridges or solid-phase microextraction can be applied. All adsorption techniques offer the advantage of sampling larger amounts of air and integrated analyte preconcentration. The same is true for the most widely used sampling approach for volatile species at present, cryotrapping. Volatile metal(loid) species have been sampled from urban air [6], landfill gas [7] or gases from domestic waste deposits [8] with the help of a U-shaped glass trap, filled with a chromatographic packing material (SP-2100 10 % on Supelcoport, 60/80 mesh), and cooled by liquid nitrogen or a mixture of acetone/liquid nitrogen. In most cases an empty cold trap has to be placed in front of the analyte trap for the removal of water vapor at −40 °C. Vacuum filling of stainless steel containers is the official sampling method of the US EPA for volatile compounds in monitoring urban air [9]. The sequential sampling of volatile Hg species using a noble metal trap in series with an activated carbon trap has been reported [10]. Gas from a sewage sludge digester has been collected in inert plastic Tedlar bags allowing sampling volumes of 10 L [5].

An almost unsolved problem is the validation of the sample integrity during all operations. Most of the compounds of interest for speciation studies in the air are not very stable. For instance, some of the volatile metal(loid) species can be transformed by reactions with co-trapped reactive air components such as ozone [11]. Therefore, the development of field-portable instrumentation for sensitive, reliable, fast and efficient on-site speciation analysis will be necessary.

Other targets for the speciation analysis of air are metal compounds in the liquid or solid state, i.e. aerosols and dust particles. The latter samples are collected on various filters (membranes of cellulose or quartz, glass fibers) and further treated as other solid material (see Section 3.4). Aerosols are sampled by using impactors, filters, denuders, electrostatic separators etc. [12]. Their preservation with respect to species integrity is very difficult because of their usually high reactivity and it seems to be more promising to apply *in situ* methods for the direct on-site analysis of aerosol components.

3.2 Water

The sampling technique varies with the properties of the species and the water type of interest (groundwater, freshwater: river or lake, seawater, tap water, wastewater, interstitial water of soil or sediment phases, atmospheric precipitation: rain, snow etc.). It is also influenced by the location (open sea, inner city or somewhere else) and the desired sampling depth below the water surface. Environmental waters are not as chemically homogeneous as water commonly available in the analytical laboratory and the aspects of representativeness (see Section 2) and homogeneity have to be obeyed for sampling.

Volatile analytes can be obtained by an on-line combination of purging the water with an inert gas such as helium followed by cryogenic trapping as described above. This approach has been used, for instance, for the investigation of methyl and ethyl species of Se, Sn, Hg and Pb

in estuarine water [13]. One has to be extremely careful to avoid contamination from the ambient air. Moreover, the formation of biofilms which can act as reaction sites for biomethylation on surfaces of the sampling devices has to be avoided.

Water sampling for speciation analysis should take into account all precautions for trace analysis of such matrices (see for instance [14]). They range from avoiding any contamination from the sampling vessel itself up to the selection of appropriate materials for the sampling and storage devices. Any metal contact has to be avoided, also within the pumping and tubing system. Polycarbonate or polyethylene bottles are recommended for most of the metal species. Mercury species have to be kept in glass bottles [15]. Wet precipitation (rain, snow) is preferably collected with the help of automatic wet-only samplers [16, 17], which can also be used for sampling such specimens for mercury speciation [18].

But several of the routine operations in water analysis cannot be applied to all cases of speciation studies. On the one hand, side filtration (usually with 0.45 μm membrane filters of cellulose or polycarbonate) or centrifugation is necessary for many purposes to remove bacteria and other reactive nondissolved constituents from the water sample. On the other hand, such separation techniques should be checked not only as sources of contamination but also with respect to their influence on original species distributions between the solution phase and the interfaces between particulate matter and water. The latter are prominent adsorption and reaction sites for many species of interest. The frequently recommended acidification of water samples not only stabilizes metal ions in solution by reducing their adsorption on container walls and bacterial activity, but also changes acid–base equilibria and coupled redox and complex formation equilibria, which would prevent the determination of such speciation patterns (see Chapter 5.9). Overall the validation of sampling techniques with respect to species-retaining operations has always to be performed with reference to the target species and general approaches do not exist.

Preservation of original water samples can be a major problem as discussed in Section 5. Therefore, a more promising approach for speciation analysis of dissolved species consists of the application of *in situ* measurements as described in other chapters of this handbook (see for instance Chapter 5.9).

3.3 Biological material

Most of the speciation analysis in biological environmental samples has been directed to the determination of organometallic constituents and redox states of trace elements. For the design of the sample collection one has to take into account that the biosphere varies much more widely in its physical and chemical properties relevant to species distribution and transformation than the abiotic environmental media air, water, sediment and soil. Therefore, only more general aspects will be discussed here. Detailed sampling protocols have to be developed separately for the specific studies depending on the particular requirements of the problem of interest.

Liquid samples from animals or plants (blood, urine, plant juices) have rarely been collected for speciation analysis until now. The main reason seems to be that environmental speciation studies were focused on such specimens which accumulate the compounds of interest and which are easily available in larger amounts for analyzing their ultratrace constituents. In principle, biological fluids from the biosphere could be handled in a comparable manner to the corresponding human samples (see other chapters of this handbook). This commonly includes filtration or centrifugation of the fresh sample material followed by short-term storage at −4 °C in the dark. Alternative techniques are shock-freezing and preservation as a frozen sample or lyophilizing and storage as a dried sample. Naturally, the latter method can only be applied to chemical species which are very stable. Any addition of chemical preservatives, including acidification, should be avoided.

'Solid' biological materials such as tissues have attracted much more attention for speciation analysis in recent years because they

are regarded as an important deposit of potentially hazardous compounds. The selection and identification of the biological specimens in the natural environment require appropriate biological and ecological knowledge. Moreover, a scientifically sound interpretation of the analytical data can only be performed if sufficient information about the environmental situation (exposure characteristics, population density and health, etc.) and ecological functions (trophic level, uptake and transport routes for chemical compounds) as well as relevant biological and biometric parameters (age, sex, variations of biological activity with daytime and season, surface area of leaves or needles, mass ratios of individual specimens and sampled organs, etc.) of the studied organism are available. In addition, the sampling strategy has to take into account not only the natural heterogeneity within a biological population, but also that within an individual organism. To fulfill the requirements of representativeness sufficient numbers of individual specimens should be sampled randomly within the selected area and for most studies they have to be mixed and homogenized (see Section 4). It has also been shown that different parts of plants (for instance, algae) [19] accumulate many trace elements to a different extent which has to be considered for the final sample composition. Therefore, detailed sampling procedures and protocols are mandatory. They can be developed for the specific purpose of the investigation on the basis of existing standards for long-term biomonitoring programs such as environmental monitoring and specimen banking [20–22].

During sample collection and further sample manipulations one has to consider that the so-called solid biota are from a chemical point of view very fragile materials with significant water content. Usually the amount of analyte in the chemically complete biological sample (leaf, plant stem, liver, kidney, muscle tissue, egg etc.) is of interest and one has to preserve during sampling the total chemical composition of the specimen as much as possible. Therefore, the removal of the specimens from their natural environment should be carefully planned. Species transformation and loss can already occur during collection at the sampling site. Degradation depends on the chemical nature of the species and may be influenced by biochemical processes such as enzyme activity. This is usually more critical in animal organs than plant samples. A key parameter for chemical reaction rates is the temperature. Consequently, one can diminish species transformations by decreasing the temperature as much and as early as possible. At present, shock-freezing of the desired samples in the gas phase above liquid nitrogen seems to be the safest technique and can be performed immediately at the sampling site. It offers the additional advantage of an inert gas atmosphere for the stored samples. If this approach is not feasible within the specific project a short-term preservation of the biological material at $-20\,^{\circ}\mathrm{C}$ is recommended.

The first preparation steps with the samples have to be performed mostly at the sampling site before freezing of the material. Plant samples from natural ecosystems are often modified by adhering material such as dust, soil or sediment particles. A general rule does not exist for separating such abiotic material and one has to decide which of the surface-attached constituents can be considered as an integral part of the sample. Even gentle washing of freshly cut plant organs can partly remove some of the relevant compounds and extensive washing procedures should be avoided in most cases. Marine samples such as algae or mussels can be carefully cleaned of sediment by shaking them in the surrounding water [20, 23].

Another operation of concern is the dissection of target organs which should preferably be performed immediately on the sampling site. Animal organs have to be extracted as fast as possible to minimize species transformations. All dissection tools must be selected with respect to possible contamination of the analytes of interest. Many speciation studies are aimed at metal(loid) compounds and therefore stainless steel knives etc. should be avoided. One can use titanium knives, tools which are coated with titanium nitride or ceramic scalpels. In general all precautions against contamination of the sample should be carefully carried out. Sample containers made from polymeric material such as PTFE, polyethylene or

polycarbonate are often adequate. The sampling crew has to wear protective gloves and the contamination is further minimized by working as early as possible on site in mobile laboratories equipped with clean-bench facilities. It has been shown that the dissection of mussels, i.e. the separation of mussel tissue from the shell, can be performed after deep-freezing and cryostorage of the whole mussels in the laboratory [24].

Overall the on-site preparation and preservation of fresh biological material, i.e. samples which still include all liquid components (such as water) belonging originally to the target specimen, is recommended for speciation analysis.

3.4 Sediment and soil

Most of the investigations which have been reported under the heading 'speciation' of sediments, soils or related samples were actually directed to operationally defined fractionation [1] mainly by sequential extraction procedures. This is not within the scope of this chapter on chemical speciation in the sense of analyzing redox states and binding partners for the trace elements of interest.

The most difficult and critical aspect of soil and sediment sampling is representativeness. Environmental specimens are very heterogeneous in their chemical composition and an extensive screening of each sampling site would be necessary for scientifically sound investigations. But usually the number and distances of the lateral and vertical (depth) sampling points are selected on the basis of available geological information by applying statistical models. Corresponding sampling grids and procedures are described in the literature [25–27].

One has to take into account for the adaption of sampling concepts for speciation analysis that larger amounts of such heterogeneous material must be collected in comparison to other environmental specimens for obtaining representative samples. Therefore, appropriately sized sampling tools (shovel, corer, spoon, knife etc.) and containers made from materials such as titanium, ceramics or plastics (e.g. polyethylene) are necessary. Depending on the problem of interest the soil can be collected by initial separation of the mineral layers or as intact depth profiles. The latter approach, which is also very common in the sampling of lake or marine sediments, is achieved by using different corer types (with plastic tubes inside) depending on the soil type, sampling depth and required sample mass. Recommendations for soil sampling can be found, for instance, in [28]. In general, large sample constituents (>2 mm) and larger parts of plants (roots, branches) are manually removed from the collected material.

Sediments can be collected with the help of grab or core samplers. Sediment traps are used in dynamic flow systems such as rivers for sampling at least part of the suspended matter. On-site operations include the decantation of water, the removal of particles larger than 2 mm and sometimes a wet sieving of the sediment. There are different approaches concerning the particle size separation, but 20 or 63 μm are the most commonly used filters. Particulate matter from aquatic ecosystems is collected either by filtration (often 0.45 μm) or by continuous flow centrifugation for processing larger sample volumes.

Special procedures for the species-retaining sampling of soil or sediment have not been described and validated until now. The preparation of harbor and coastal sediments as reference materials for matrix-matched speciation analysis of tributyl tin has been reported [29, 30]. But the application of air drying and the described sample processing (see Section 4) cannot be recommended for all other analytes of interest. Problems also arise from changes in the oxygen concentration during sampling, especially for originally anoxic sediments. Even the definition of the sample composition is difficult for speciation purposes, because the interfaces between solid particle and water, biofilms and pore water can be prominent reaction sites for species transformations and it is almost impossible to preserve their original state during sampling at present. Therefore, the development of both *in situ* methods for speciation analysis (in particular for sediments) and new approaches to species-retaining sampling procedures specifically designed for certain groups of chemical species, will be necessary.

4 SAMPLE PROCESSING

Most of the solid materials collected from the environment have to be prepared by physical operations (grinding, drying, etc.) before any analytical pretreatment in the narrow sense (extraction etc.) can be applied. The influence of such sample manipulations, called sample processing in the following, on the total speciation analysis is sometimes underestimated and should always be carefully controlled by the analyst with respect to contamination, loss or transformation of the analyte and relevant matrix modifications.

Usually the collected material has to be homogenized and divided into chemically authentic aliquots for repetitive analysis. If one is not interested in the 'fractionation' of the sample constituents [1] with respect to their surface attachment to different matrix components or the differentiation between inner- and extracellular species in biological specimens and comparable questions, grinding is performed for the purpose of homogenizing and creating large surface-to-volume ratios for subsequent species extractions.

For biological specimens sample processing with the requirement of minimizing possible changes in their chemical composition can be based on the developments and standards which have been acquired within the frame of environmental biomonitoring and specimen banking programs [22, 31]. The immediate shock-freezing of the sampled material in the gas phase above liquid nitrogen shortly after its removal from the natural environment on the sampling site and the further storage at temperatures below $-130\,^{\circ}C$ provides a raw material with very good mechanical properties for crushing. During grinding the samples should be continuously cooled (preferably with liquid nitrogen) to avoid species transformation or even loss [32] due to the local heating effects from friction. The cryogrinding of a broad range of biological specimens, including fatty or keratin-rich samples (such as liver, hair) and fibrous material (plant shoots etc.), is possible by using a vibrating rod mill (for instance, CryoPalla™) operated under continuous cooling with liquid nitrogen [33]. In many cases a pre-crushing of larger portions of frozen material has to be performed. The grinding at such low temperatures offers not only the advantage of a diminished probability for chemical transformations in the sample, but one can also achieve a fine powder with small particle sizes without sieving. This is shown for two different biological matrices in Figures 2.1.4 and 2.1.5. But

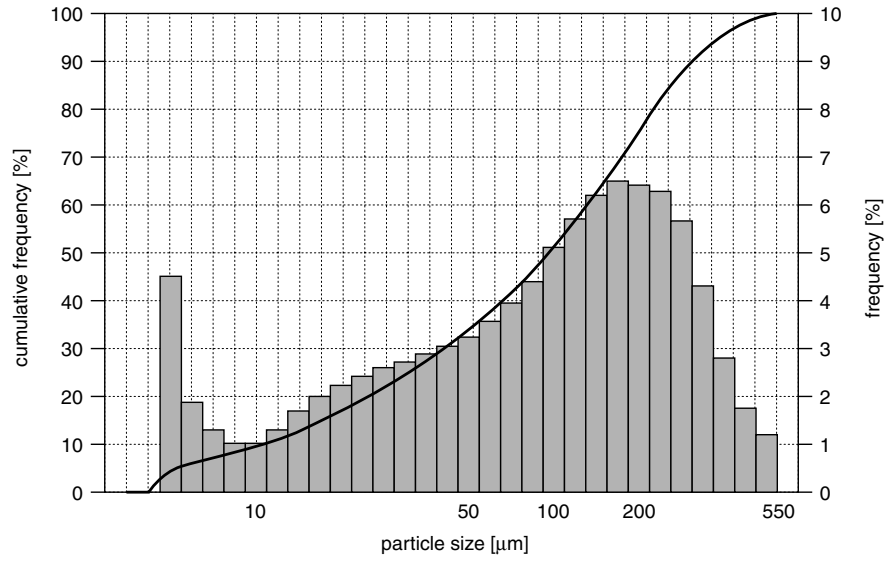

Figure 2.1.4. Particle size distribution after cryogrinding of pine shoots.