Title
Group Assessment: Elemental Speciation

Journal
Ecotoxicology and Environmental Safety, 56

Authors
Caruso, Joseph A.
Klaue, Bjorn
Michalke, Bernhard
et al.

Publication Date
2003

Peer reviewed

ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Ecotoxicology and Environmental Safety 56 (2003) 32–44

Ecotoxicology and Environmental Safety

http://www.elsevier.com/locate/ecoenv

Group assessment: elemental speciation

J.A. Caruso,[a,*] B. Klaue,[b] B. Michalke,[c] and D.M. Rocke[d]

[a] *University of Cincinnati, A&S Chemistry, P.O. Box 210172, Cincinnati, OH 45221-0172, USA*
[b] *University of Michigan, Department of Geological Sciences, Ann Arbor, MI 48109, USA*
[c] *GSF National Research Center for Environment and Health, 85764 Neuherberg, Germany*
[d] *Department of Applied Science, University of California, Davis, CA 98616, USA*

Accepted 19 March 2003

1. Definitions of terms related to speciation

Definitions for terms related to "elemental speciation" were discussed extensively in several Conferences on Trace Element Speciation and are given in a recent review. These definitions are derived from IUPAC[1] accepted terms (Templeton et al., 2000).

A *chemical species* is defined as a specific form of a chemical element, such as molecular or complex structure or oxidation state. Consequently, the *speciation of an element* is the distribution of defined chemical species of an element in a system. And finally, for elucidating speciation, a *speciation analysis*, defined as the analytical activity of identifying and measuring species is necessary. This term includes appropriate sampling, quantification, and quality-controlled analytical methods. Analytical procedures, which do not lead to an identification of chemical species but give characterizations of species groups are not considered chemical speciation analysis. They are distinguished as *operationally or functionally defined species characterization*. Operationally defined procedures characterize molecule groups, dependent on the selected analytical operation. The species identity is not considered. The functionally defined species characterization provides information about the function of species groups in biochemical paths.

2. Speciation analysis—overview

Trace metals in the environment may have associated risks or benefits. Although the need for trace and ultratrace analyses is clear, the required information about mobility, bioavailability, and finally the impact of elements on ecological systems or biological organisms is not necessarily given by total element concentrations alone. The knowledge of the elemental species provides a better understanding of chemical and biochemical processes and bioavailability and provides more complete information about toxicity or essentiality. Elemental species may involve their oxidation states, organometallic forms, isotopic composition, or complexation states. A meaningful risk assessment should then require speciation analysis. For example, speciation analyses can distinguish the more toxic inorganic arsenic species from relatively non-toxic arsenobetaine, one of the major As compounds in seafood. Depending on its oxidation state, chromium is essential (+3) or harmful (+6). Tin compounds are released into environment as toxic tributyl-tin compounds or as less dangerous mono-butyl forms.

Complete speciation schemes consist of sampling, sample preparation, species analysis, and evaluation. Without proper sampling and sample preparation procedures, there is little chance that any speciation analysis will provide reliable data upon which human health or environmental decisions can be based. Certain metal species can easily be lost, such as atomic mercury or the oxidation of Cr(III) to Cr(VI). Quality control approaches and statistical data handling are a must for

*Corresponding author. Fax: +513-556-9239.
E-mail address: joseph.caruso@uc.edu (J.A. Caruso).

[1] *Abbreviations*: AAS, atomic absorption spectrometry; AFS, atomic fluorescence spectrometry; BCR, now: standard measuring and testing program/EU; CE, capillary, electrophoresis; CUSUM, cumulative sum; CV, cold vapor (-AAS); DIN, direct injection nebulizer; ESI-MS, electrospray ionization mass spectrometry; EXAFS, extended X-ray absorption fine structure; GC, gas chromatography; HG, hydride generation (-AAS); HHPN, hydraulic high-pressure nebulizer; ICP-MS, inductively coupled plasma mass spectrometry; ICP-AES, inductively coupled plasma atomic emission spectrometry; IEC, ion-exchange chromatography; IPRPLC, ion-pairing reversed-phase liquid chromatography; IR, infrared spectrometry; IUPAC, International Union for Applied Chemistry; LC, liquid chromatography; MCN, microconcentric nebulizer; MMA, monomethyl arsonic acid; NIST, National Institute for Standards and Testing; NMR, nuclear magnetic resonance; RPLC, pairing reversed-phase liquid chromatography; SEC, size exclusion chromatography; USN, ultrasonic nebulizer; XANES, X-ray absorption near-edge spectroscopy; XRD, X-ray diffraction.

0147-6513/03/$ - see front matter
doi:10.1016/S0147-6513(03)00048-4

providing reliable results. Reviews on sample collection, pretreatment, and storage of a wide range of sample types have been published by Szpunar (2000). For quality control strategies, review articles are available from Michalke (1999), Quevauviller et al. (2000), and Caruso et al. (2000) and in this joint report. A full monograph on elemental speciation is now available from Elsevier (Caruso et al., 2000).

There are direct speciation methods. Most common are combinations of different separation and detection techniques. Following sample preparation, speciation analysis requires that the differing elemental species of the same or different elements must be separated as cleanly as possible prior to introduction to the detector. Liquid chromatographic methods are the most widely applied separation methods for elemental or metal speciation. These will be a focus of this overview, as will gas chromatography (GC) and capillary electrophoresis (CE) separations. Finally, atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) or electrospray ionization mass spectrometry (ESI-MS) as chromatographic detectors will be discussed.

2.1. Sampling

The sampling step is critical in speciation analysis, as sampling may alter species ratios randomly. Concerns arise about contamination, representativeness of the sample, species preservation or adequate tracking, and precipitation or wall effects (from sample containers). Alterations and errors produced at this step are typically irreversible (Dunemann and Begerow, 1995).

Sampling concepts are needed and must be used to preserve the original information about the native species and the species equilibria. Species alteration is a primary concern in the sampling and sample preparation steps. Minimal sample handling is desirable particularly for field sampling. Methods "freezing" the species composition by chelation, derivatization, or fractionation should be considered, especially when the samples cannot be preserved by other means such as freezing. Any chemical reaction utilized in these steps must be stoichiometric. Typically, mild extraction methods need to be employed to reduce the risk of species alterations. This requirement for species preservation and minimizing contamination also includes precleaned sampling containers of suitable material, high volume to surface ratios (low wall effects), or sampling under an inert gas to avoid oxidation. Depending on the sample matrix, e.g., biological matrices, contamination may come from syringes, metal scalpels, and other metal tools, which may convert the species. Such changes can secondarily alter species ratios of another element. Species alterations are also caused from bacterial activity (Dunemann and Begerow, 1995).

2.2. Sample preparation and derivatization

Storage should be for as short duration as possible and preferably at low temperature. Clean room conditions should be used if possible and as necessary (Schramel and Hasse, 1994). Sample preparation needs to be as simple as possible to reduce steps in the process that may lead to contamination or species conversion. An excellent review is that by Szpunar (2000) in a recent volume devoted to elemental speciation. A major section of the issue is devoted to sample handling prior to GC and liquid chromatography (LC) analyses. Sorption, extraction, distillation, leaching, stabilization, supercritical fluid extractions, and microwave-assisted extractions (MAEs) are discussed. MAE is currently one of the most favored techniques, because it allows fast, efficient, very controlled, and even heating of the solvents in open or closed vessels.

The review by Szpunar also discusses derivatization techniques, often used for GC separations. Although derivatization may increase detection sensitivity and provides matrix separation, it is sometimes less desirable, as derivatization is time consuming and may be tedious. The selectivity of such transformations can be problematic because of side reactions (Demuth and Heumann, 2001; Bloom et al., 1997). However, with standards also undergoing derivatization, good precision can be achieved. Different efficiencies of derivatization and different detector responses of the derivatized species are sometimes observed (Quevauviller et al., 1993).

3. Speciation approaches

3.1. Direct speciation methods

3.1.1. Isotopic methods

Determination of the isotopic composition of an element with multiple isotopes also qualifies as speciation analyses. The isotopic composition of an element can vary naturally because of radioactive decay or kinetic mass fractionation. The production of artificial radioactive or enriched stable isotopes allows their use as optimal internal standards for a given element or its species with near identical chemical properties. A detailed review of the use of isotopic tracers for the investigation of sources, pathways, and bioavailability of metals species is given by Klaue and Blum (2003).

The isotopic composition for natural *radiogenic* isotope systems is a function of the ratio of mother and daughter nuclides and the time period after mixing in geological systems based on the radioactive decay

constants (Faure, 1986). Depending on the isotope system and the geological time scales the isotopic variations can be very large (U–Pb) or very small (Nd–Sm) (Faure, 1986). The most prominent example regarding toxic heavy metals is the U–Pb system. Depending on the geologic origin of the Pb from mined ore deposits or natural background, lead compounds can have very distinct isotopic compositions, which can be used to track and quantify sources (Shirata and Patterson, 1980). Pb isotopic signatures have been used in a number of studies tracking lead contamination of atmospheric aerosols and deposition, soils, surface water, biota, and human subjects (Shirata and Patterson, 1980; Widmer et al., 2000; Labonne et al., 2001). The release of anthropogenic radioactive isotopes such as ^{137}Cs, ^{89}Sr, ^{239}Pu, and ^{240}Pu through nuclear power production or weapons testing can be used to track those sources.

A relatively new approach to isotopic signatures of heavier isotopes is the investigation of kinetic- or mass-dependent fractionation. This effect has been exploited for light stable isotopes (H, C, O, N, S) for the past 30 years (O'Neil, 1986; Arthur et al., 1983). New technologies in the form of multicollector ICP-MS allow the precise isotopic analyses of environmentally important metals such as Fe, Cu, Zn, Mo, Cd, Sn, and Hg (Williams et al., 2001; Zho et al., 2000; Belshaw et al., 2000; Zhu et al., 2000a, b; Marechal et al., 1999; Matthews et al., 2001; Klaue and Blum, 2000; Klaue et al., 2000). Because of the relatively high average mass and small mass differences of the isotopes in these systems, the degree of fractionation is small and typically only in the range of fractions of a percent. Nuclear spin or quantum effects are typically minor, although they cannot always be entirely excluded (Fujii et al., 2001). It is believed that certain processes such as partial evaporation or condensation, ion-exchange, redox reactions, forming or breaking of covalent metal–organic bonds, and, in particular, metabolic processing cause significant fractionation. Therefore, mass-dependent fractionation could become a tool for tracking sources and pathways of metals in the environment. At this point only few data are available (Williams et al., 2001). Natural processes for fractionation have not been carefully investigated. Additionally, unlike radiogenic systems, the isotopic signature from mass-dependent fractionation can be altered by subsequent reactions.

The use of stable or radioactive artificial isotopes represents by far the more common application of isotopic speciation. The major applications for using enriched and radioactive isotopes are isotope dilution quantification, method validation, mechanistic studies, and recovery and uptake experiments (IUPAC International Working Party, 1999), both with elemental- or species-specific spikes (Hook and Fisher, 2001; Encinar et al., 2002; Atkinson et al., 1993; Fox et al., 1994; Rao, 1994). For the application of isotopically labeled spikes, the definition of isotopic analyses as speciation analyses is probably surprising because their use for the quantification of an element by isotope dilution does by definition not qualify as speciation analyses. It is even more confusing in the case of species-specific labeling of compounds. For those applications it often also involves the chemical compound speciation before or combined with the isotopic speciation (Demuth and Heumann, 2001; Krupp et al., 2001). Isotopically labeled species are the best way to assess recovery and species transformation during speciation analyses. Limitations can arise from imperfect sample-spike equilibration (Demuth and Heumann, 2001; Bloom et al., 1997; IUPAC International Working Party, 1999).

The use of enriched stable isotopes offers a number of advantages over radioactive spikes. General laboratory safety issues can be minimized and no special permits will be necessary, as stable isotopes are accepted by both the public and the scientific community. Stable isotopes can also be more easily employed in human subject studies. However, in some cases such as monoisotopic elements like arsenic, there is no alternative to artificial radioactive isotopes. But for most other relevant heavy metal systems, there are sometimes multiple stable isotopes available, which allow experiments with multiple species-specific spikes.

The use of isotopic labeling is an invaluable tool for precise and accurate determinations of at least the marginal recovery rates (IUPAC International Working Party, 1999) and is therefore a very important tool for method validation. For bioavailability and mechanistic studies, the use of spikes eliminates background problems, which is particularly important for complex natural matrixes. Because of the high sensitivity for the detection of isotopic spikes, uptake experiments near or even below natural background levels are possible. Examples of method validation, recovery experiments, uptake experiments, mechanistic studies, and species-specific spiking for Fe, Hg, Zn, Pb, Cd, As, and Se are given by Klaue and Blum (2003).

The instrumentation for analyses of radioactive isotopes typically involves the use of beta, gamma, and scintillation counters (Shamaev, 1987). The analyses of stable metal isotopes are performed with mass spectrometric techniques. For most applications ICP-MS is by far the preferred technique over thermal ionization MS and other techniques.

3.1.2. Electrochemical methods

Voltametric or polarographic techniques can be used to speciate labile and stable metal complexes in aqueous solution. Electrochemical methods have been used to characterize a number of metal species and complexes in natural waters (Town and Filella, 2000). The observed

potential shifts for certain complexes are typically not truly species specific and further data about the matrix are needed. Any type of sample preparation, extraction, or addition of buffers can change the natural species in solution and make appropriate speciation by voltametric techniques impossible.

3.1.3. X-ray diffraction and absorption techniques

A number of techniques using either X-ray absorption or diffraction techniques (extended X-ray absorption fine structure (EXAFS), X-ray absorption near-edge spectroscopy (XANES), X-ray diffraction (XRD), microprobe analyses) allow the chemical characterization of certain metal compounds in solid samples (Kim et al., 2001; Bostick et al., 2001; Calmano et al., 2001). While some of the techniques allow high spatial resolution for solid samples, the direct quantification of certain species can be difficult and is typically not very sensitive. Sometimes the isolation of the compounds may be required, which necessitates large amounts of sample materials.

3.1.4. Other techniques

Basically any technique that allows the characterization of specific chemicals compounds can be used for speciation analyses. Most of these spectroscopic techniques require the isolation of the compounds because of sensitivity or specificity issues. Examples are NMR, UV–Vis, IR, Mossbauer, and ESR (Das et al., 1995). NMR is especially useful for defining the K_d of metal ligand complexes.

3.2. Combined speciation methods

3.2.1. Separation methods

3.2.1.1. Liquid chromatography. The relative polarity, solubility, and molecular weight of the species of interest determine the type of liquid chromatography used for a specific application. The various modes of liquid chromatography include reversed phase, reversed-phase ion pair, micellar, ion exchange, size exclusion, and chiral LC. These will be briefly defined here and further detail can be found in the detailed speciation papers that follow this joint report. Interfacing LC with element-specific detection is discussed in a following section of this report. The interface is simple, requiring only low dead volume fittings and inert small-bore tubing to connect the LC effluent to the nebulizer of the element-specific detector.

Normal-phase and reversed-phase partition chromatography: are terms that are used to describe the mobile- and stationary-phase polarities. Normal-phase chromatography uses polar alumina or silica stationary phases and non-polar phases with solvents such as hexane. However, by using a polar mobile phase and a relatively non-polar stationary phase, similar separations are obtained with a reverse of the elution order. Reversed-phase LC is now the most popular mode of partition chromatography with ICP-MS detection.

Siloxanes form a stationary-phase backbone when hydrophobic hydrocarbons of 2, 8, or 18 carbon atoms form the chain. The analytes partition between the stationary phase and mobile phase to a differing extent. Varying the type and the amount of the mobile-phase modifier may control selectivity. Acetonitrile and methanol are the most common mobile-phase modifiers.

Reversed-phase ion-pairing chromatography (ion-pairing chromatography): has the ability to separate ionic and non-ionic compounds by adding an ion-pairing reagent to the reversed-phase mobile phase. The ion-pairing reagent has a polar head group and a non-polar tail. Tetraalkylammonium salts, triethylalkylammonium salts, or anions such as alkylsulfonates are different types of ion-pairing reagents. They bind to the ionic analytes to form an ion pair, which is then retained by the reversed-phase column because of its overall electrical neutrality. Analyte selectivity may be adjusted by changing the mobile-phase composition.

Ion-exchange chromatography: involves the process of an analyte ion and ions of the mobile phase competing for oppositely charged functional group ions on the stationary phase. Both cation- and anion-exchange modes are used. At injection the mobile-phase ions are joined with functional group counter-ions, and charge neutrality is maintained. Upon injection of analyte(s), these ions compete with mobile-phase ions for the functional group sites. The separation of analyte ions (different species) takes place when analyte species displace mobile-phase ions. Since the different analyte ions will often have greater or lesser retention on the column than others, a separation takes place.

Chelating ion-exchange chromatography: adds the possibility of preconcentration. As an alternative to simple ion exchange, it is dependent on the attraction between a complexing ligand and the metal of interest. The greater selectivity of chelating ion exchange means that separations for divalent and trivalent ions may be markedly affected relative to those of monovalent ions.

Size exclusion chromatography: is used to separate compounds of high molecular weight, such as proteins and polymers. Certain molecular sizes may diffuse into a uniform network of pores. Molecules larger than the pore size are unretained by the column and elute first. Those of smaller size can diffuse into the pores and are thereby retained to a greater or lesser extent depending on size; these elute later. Coupling SEC with element-specific detection is the most popular technique for the first screening of an unknown sample, especially when elemental macromolecular species may be present. In many applications SEC-LC-ICP-MS has been used as a semiquantitative technique and in most applications is followed by a complementary chromatographic

technique. By definition size exclusion chromatography qualifies only as *fractionation analyses* (Michalke, 2003) and is often followed by other chromatographic separations.

Chiral liquid chromatography: is a newer type of separation used with element-selective detection. A compound with a carbon atom that has four different groups around it is chiral. Since the orientation of the groups around the central atom may vary, several enantiomers of the compound may exist. Separation of these optical isomers is accomplished by forming diasteriomers or transient diasteriomers depending on the specific chiral technique used. Further discussion follows in Caruso and Montes-Bayon (2003).

3.2.1.2. Gas chromatography. GC is a common form of partition chromatography in which the separation is effected by the ability of a gas-phase analyte to be retained on a liquid stationary phase. The combination of GC with element-specific detection has become a good method for the speciation of organometallic compounds in complex environmental samples. Many of the published papers devoted to the use of GC-ICP-MS address the construction and development of adequate interfaces. Capillary columns account for most of the speciation studies. Interfaces with element-specific detection are relatively simple, although more complex than the LC interfaces and are more fully described in the following reviews. Detection levels as low as sub-ppt are readily available. In the case of nonvolatile compounds, it is possible to derivatize these and make them amenable to GC. Forming esters with derivatizing reagents such as ethyl choloroformate is one example.

3.2.1.3. Capillary electrophoresis. CE is a high-resolution, fast separation technique that has the ability to separate a wide range of analytes from large biomolecules to small inorganic ions. It is able to separate positive, neutral, and negative ions in a single run with high separation efficiencies (Landers, 1997). Other advantages in comparison to LC are nanoliter sample volumes, small amounts of reagents, and low costs of capillary columns. On the other hand, nanoliter sample volumes compromise the low detection limits capable with the ICP-MS because it is a mass-sensitive detector. The mobility of CE depends on the size and charge of a particular analyte. A high voltage of 15–30 kV is applied in either the positive or the negative mode. The separation is accomplished by the combined effects of electroosmotic flow (EOF) and electrophoretic flow. The latter flow is due to movement of ions in the electric field and the EOF arises from a charged environment in the vicinity of the capillary wall that results in a second type of movement through the capillary. Both ions and neutrals are caught up in this movement, which ultimately results in the separation of neutrals as well as ions. Resolution is a function of electrolytic buffer, pH, and the use of modifiers. Modes for CE separation are highlighted in the Michalke review following this overview (Michalke, 2003).

Numerous examples of applications are given in the cited references and in the review articles following this joint report, such as the excellent recent review by Templeton et al. (2000). In summary, CE with element-specific detection can potentially yield ppb detection levels. The actual concentrations detectable may be in the ppm to sub-ppm levels because of the nanoliter sample volumes injected providing a lower analyte mass to the mass responsive element-selective detectors.

3.2.2. Detectors-element-selective and general detectors (Table 1)

3.2.2.1. Element-selective detectors. Element-selective detection for chromatography using primarily atomic spectrometric methods provides excellent selectivity and detectability. When coupled with chromatography, this detection provides sub-nanogram to femtogram detection levels, as well as allowing the additional selectivity provided through specific emission or absorption lines plus mass selectivity with mass spectrometric detection. For example, two co-eluting chromatographic peaks resulting from overlapping Se and As species can be separated by mass, although chromatographically not resolvable (Table 1).

3.2.2.2. Atomic absorption detection. The AAS system is a relatively inexpensive element-selective detector, which recently has advanced to a few multielement systems. There are flame (FAAS), cold vapor (CVAAS), hydride generation (HGAAS), and graphite furnace (GF-AAS) AAS systems. FAAS shows detection levels generally too high for environmental or physiological speciation studies. CVAAS and HGAAS use selective derivatization for matrix separation and detection of the respective species. The detector response is strongly species dependent and is often compromised by interferences. GF-AAS uses sample sizes of a few microliters and provides low detection limits between 0.1 and 5 μg/L. Matrix interferences are widely eliminated via Zeeman background correction and matrix modifiers. Even with these attributes, GFAAS is usually unsuitable for online LC coupling as the chromatographic data points are generated as far as minutes apart, not sufficient to construct a reasonable chromatogram. Due to these limitations, AAS systems are less useful for speciation investigations, although they are viable where higher concentrations are available. It is possible to achieve trace level concentrations for Hg by the cold vapor technique and for As, Se, Bi, Sb, and Te by hydride generation. Commonly used detectors are ICP-AES and ICP-MS. More restricted than these two

Table 1
Instrumentation type vs attributes or performance

	AAS	AFS	ICP-OES sequential	ICP-OES simultaneous	ICP-MS quad	ICP-MS sector	ICP-MS TOF	ICP-MS MC
Investment cost	1		2	3	3	4	3	5
Operating cost	1		3	3	3	3	4	4
Required operator expertise	2	2	3	3	4	4	5	5
Portability	1–3	1–2	4–5	4–5	4–5	5	5	5
Sensitivity	2–4	2	2–3	2–3	1–2	1–2	2–3	1–3
Linear range	5	3–4	2–3	2–3			1	1–2
Robustness	4	3		1	2	2	2	2
Interferences	3	2–3	1–2	1–2	2–3	1–2	1–2	1–2
Multielement/transient signal	NA	NA	3–4	1	2	3	1	NA
Isotopic composition	NA	NA	NA	NA	3	2	3	1

Note: 1, excellent; 2, very good; 3, good; 4, fair; 5, poor; NA, not applicable.

techniques because of the limited number of elements accessible are atomic fluorescence detection methods.

3.2.2.3. Atomic fluorescence spectrometry. AFS is a popular method for Hg speciation and speciation of hydride forming elements (As, Se, Sn, Bi, Te, Ge, Pb, Sb). It requires coupling sample preparation/separation prior to the AFS detection. AFS is based on detection of fluorescence arising from excitation of the elemental resonance lines of the above elements, although the commercial instrumentation allows interrogation of only one line at a time. For these elements fluorescence emission as an energy decay mechanism for the excited element serves for highly specific elemental detection. Low detection levels are achieved since the detection step relies on mercury vapor (atomic mercury) or hydrides of the other elements, which readily decompose to their atomic states making them available for excitation and subsequent fluorescence decay at specific but different wavelengths. The presentation of Hg or the elemental hydrides (easily taken to the elemental state) of various species eliminates the need for desolvation and matrix compensation, resulting in low detection levels in the picogram range.

3.2.2.4. Atomic emission detection. The big advantage of ICP-AES is its multielement capability and high sensitivity. Online coupling with LC or GC is readily done. The excitation source is typically inductively coupled plasma to provide minimal chemical interference, such as molecular emissions, which are minimized through the high plasma temperatures. Sample introduction is performed via a nebulizer and spray chamber. Respective nebulizer types, related problems, and some solutions are given elsewhere in this document. There are sequential and simultaneous systems available. Sequential systems are flexible in allowing tuning to different elements or groups of elements. However, since the instrument scans individual emission lines for each element, multielement analyses of fast transient signals are limited. Modern simultaneous systems do not suffer from this limitation and analyze all programed elemental emission lines at the same time.

3.2.2.5. ICP-MS detection. ICP-MS is now the technique of choice for total element analysis with a wide range of samples and metal concentrations in the ppm to ppt range. It has become a highly versatile technique with low detection levels and high sensitivity. Coupled with metal specificity, it is a technique of choice for chromatographic detection including GC, LC, SFC, and CE. ICP-MS also offers a wide linear dynamic range, low limits of detection, high throughput, multielement capability, relatively simple spectra, and the ability to perform isotopic analysis. Numerous metals have received wide attention. Generally, the separation and the interface need to be optimized prior to ICP-MS detection. Several types of mass analyzers are used with ICP-MS (Caruso et al., 2000). The least expensive and most commonly used types are quadrupole mass spectrometers. Modern quadrupole instruments offer low dark noise, high sensitivity, and high scan speeds. Cold plasma technique, collision cells, chemical separation, or solvent removal can in some cases overcome spectral interference. Magnetic sector or high-resolution ICP-MS instruments can resolve many spectral interferences and offer superior sensitivity particularly when used in low resolution. Multicollector magnetic sector ICP-MS allows very high-precision isotope ratio determinations for many isotope systems. Time-of-flight mass spectrometers offer the simultaneous detection of the entire mass range, allowing the tracking of multielement and isotopic compositions of fast transient signals but suffer from relatively low sensitivities at the time of this writing.

ICPs, as high-temperature sources, atomize and ionize various metal species. The plasma is formed within a quartz torch, made up of three concentric quartz tubes, with argon at different flow rates flowing through each. An RF field provides the energy to sustain the argon plasma and the plasma transfers energy to the analyte(s) for excitation and ionization. For excellent discussions

of plasma theory, structures, and applications, see Taylor et al. (2000). The aerosol generated moves from spray chamber to the plasma, where it is desolvated, vaporized, atomized, excited, and ionized. Further discussions of nebulizer behavior are given in Montaser (1998), Montaser and Golightly (1992), and Caruso et al. (2000).

The ICP very efficiently produces singly charged ions for mass spectrometry. These are transported to the mass spectrometer via a multiple-stage differentially pumped system. Complete discussions are given following this joint report. For ultimately introducing sample into the ICP, LC columns of 0.2–1.0 mL flow rates must be connected to a nebulizer. These then match normal flow to microflow columns.

3.2.3. Non-element-specific detection

3.2.3.1. Electrospray mass spectrometry. Electrospray ionization is a process for preserving the molecular species intact under optimal circumstances. ESI is suitable for very low flow rates. It is based on "ion evaporation", where charged droplets of the analytes are transferred into gas phase. A volatile buffer consisting of considerable amounts of methanol supports this ion evaporation. The success of this detection method is based on the capability to produce multiple charged ions from high-molecular-weight elemental species such as metal-containing protein, thus allowing the analysis of compounds up to MW = 150,000–200,000. ESI-MS allows coupling of LC and CE separations to make a very important analytical technique. The soft ionization of elemental species yields a molecular ion as well as fragment ions, allowing characterization of the species to a degree unavailable with ICP-MS, although at higher levels of detection. ESI-MS can provide detailed information about molecular weight and even structure of analyzed compounds. On the other hand, as with all analytical techniques, ESI has challenges as well. Ion/solvent clusters may split signals from one species into multiple signals, degrading detection limits and increasing spectral complexity. Electrolytic processes occur at the metallic ESI needle tip and can transform species leading to erroneous conclusions.

3.2.3.2. UV–visible spectrometry. A major research effort in the application of CE to metal speciation is the development of more sensitive detectors through element selectivity. Conventional non-specific detection can lead to "false" detection of element species or be insufficiently sensitive at ppm or lower concentrations. Because of insufficient UV response, many elemental species at ppm levels or lower will not be seen. On the other hand, indirect UV absorbance detection is currently the most versatile method for universal detection of metal species by CE. Unfortunately, even with indirect UV detection, sensitivity is rarely suitable enough for monitoring species at natural concentrations. The chromophores added for indirect detection may react with the species of concern.

4. Interfacing separation methods with element-specific detectors

4.1. Interfacing to ICP-MS—LC, GC, CE

4.1.1. LC

The direct connections between separation systems and plasma detectors, predominantly ICP-MS, have led to a ready availability for elemental speciation. Data may be quickly obtained with minimal additional contamination. The actual interface from LC to ICP-MS is a nebulizer to convert the column effluent to an aerosol. Typical systems in use are the pneumatic nebulizer, ultrasonic nebulizer (USN), the direct injection nebulizer (DIN), and the hydraulic high-pressure nebulizer (HHPN). They differ widely in efficiency and operation. For example, the DIN was developed specifically for LC-ICP-MS interfacing. It provides high nebulization efficiency (up to100%) at flow rates of 30–150 μL/min. However, problems with stability need to be addressed. Therefore, Meinhard or cross-flow nebulizers are used more commonly. Another interface is the microconcentric nebulizer (MCN), used for low flow rates between ca. 30 and 150 μL/min with high nebulization efficiency. It is most useful when combined with microbore LC.

The problems of interfacing LC to ICP-MS are due to the salt load of eluents leading to torch or nebulizer clogging changes in ionization, or plasma stability by the introduction of organic modifiers, altering plasma stability or ionization characteristics. Ion-exchange chromatography utilizes buffers and up to 0.1% salt concentrations. Such column effluents must be diluted postcolumn or new developments in column technology need be developed. Cooling the spray chamber minimizes organic vapor to the plasma, especially when the nebulizer gas contains small amounts of oxygen. This approach reduces the carbon load, leading to fewer ArC^+ interferences, and increases plasma stability. Membrane desolvation systems are suggested, too, allowing high methanol concentrations, but also removing volatile elemental species. For reduction of salts and organic modifiers, combinations of micro-LC with low-flow-rate interfaces, such as the MCN, are increasingly used for LC-ICP-MS.

4.1.2. GC

The coupling of GC to ICP-MS is appropriate for volatile species such as organotins. It gives the advantage of the plasma energy going to the analyte ionization instead of drying and desolvating an aerosol.

The sample input into ICP-MS thus can reach 100%. Unfortunately, interfacing is more difficult than with LC. The transfer line, including the interface near the induction coil, must be at elevated temperature, either by a preheated sheath gas (Montaser and Golightly, 1992) or by wrapping with heating tape. Here the proximity of the metal substances to the induction coil causes problems such as arcing.

4.1.3. CE

For CE, too, the interface actually must be a nebulizer, typically a pneumatic nebulizer, providing an aerosol of the capillary's effluent. In addition, stable electrical connections for closing the electrical circuit from the CE must be implemented. Furthermore, the low flow rate must be adapted to the nebulizer uptake rate. A major challenge in CE-ICP-MS is irreproducible performance caused by suction or backpressure produced by the nebulizer gas when it exits the nebulizer, since it flows from the capillary into the nebulizer.

To make the electrical connection, the sheath flow around the capillary is varied. The grounded outlet electrode is in contact with this electrolyte flow. Optimizing the nebulization efficiency may be done by varying the flow rate when MCN- or DIN-based systems are used or via exact positioning of the CE capillary within the interface (Orellana-Velado et al., 2001). Fortunately, problems with high salt load, organic modifier introduction, and changes in ionization characteristics play little role in CE coupling to ICP-MS, as the total mass intake into the plasma is very low.

Complete discussion of CE-ICP-MS covering theory, application, and instrumentation is given by Olesik et al. (1995) and Caruso et al. (2000).

4.2. Interfacing to ESI-MS

LC or CE may be interfaced to ESI-MS for gaining molecular or even structural species information. Generally, the electrospray source operates with very low flow rates, which makes CE a good system for such coupling. Microbore LC is commonly coupled with ESI. ESI interfaces for CE are commercially available. This requirement introduces some limitation concerning the choice of suitable separation buffers. With ESI, ion production efficiency still is insufficient to yield the sensitivity levels of ICP-MS. Closing the electrical circuit from CE during ion evaporation is provided by a volatile electrolyte sheath flow. Effective ion production is controlled by spray voltage via instrument software.

5. Quality control in speciation

Quality control, or more broadly quality assurance, is an essential part of the conduct of an analytical laboratory. A full treatment of the many management, planning, record keeping, and audit procedures is beyond the scope of this article (see Garfield, 1991for these concerns); we will concentrate on a few critical issues in quality control as they impact analytical laboratories. Some issues of sample handling, calibration, certified reference materials, and other aspects of quality control specifically indicated for speciation are described more fully in Michalke (2003) and in Quevauviller et al. (1996).

5.1. Sampling, sample handling, and matrix effects

Sampling strategies need to be specifically designed to meet study objectives. For environmental sampling, a sampling plan should be developed to track or control for spatial and temporal variations. Selection of human subjects needs to be conducted by a formal sampling plan. In both cases, formal random sampling should be employed rather than haphazard selection or an attempt at "representative" units, since the latter often results in artificially suppressing natural variation that is part of the universe to be sampled. Great care is required in taking samples, sample handling, and accounting for matrix effects to avoid contamination, sample degradation, or other artifacts. Use of certified reference materials, if available, is also required (Michalke, 2003).

5.2. Calibration and added standards

In most cases quantitation is not possible without either calibration samples or added standards. For calibration, spiked samples must be carefully prepared at two or more concentrations that span the expected range to be encountered (Michalke, 2003). Ideally, each concentration would be prepared in duplicate, and different concentrations would be separately prepared, rather than using, for example, serial dilution. The full set of spiked samples should be run in a formally randomized order; in particular, duplicates should not be run in sequence. The calibration curve can then be estimated using ordinary least squares, weighted least squares (preferable), or in an integrated fashion with the precision determination (Rocke and Lorenzato, 1995). Added standards methods are described in Rocke (2003). Some methods, such as isotope dilution and neutron activation analysis, are handled differently (Currie, 1968).

5.3. Estimating precision

An important issue in an analytical laboratory involves the attachment of standard errors to measured values. Although every effort should be made to avoid bias, it may be difficult to estimate the remaining bias, so standard errors are usually based on the precision of

measurements, that is, the standard deviation of repeat measurements made under identical conditions. This estimation is necessarily only a lower bound on the true error standard deviation. It will hardly ever be the case that the error standard deviation is constant, independent of the level measured. For large concentrations of the analyte, the standard deviation will often be approximately a multiple of the concentration (more generally, this may be a power function). If small concentrations (near the limit of detection) are not of interest, then a linear equation describing the relationship between the precision and the concentration can be estimated from a series of repeat measurements at several concentrations, by regressing the standard deviation of the repeats on the mean of the repeats. This can often be done during the process of producing a calibration curve for the instrument.

If values near the limit of detection are of interest, then a more complex method is called for. Rocke and Lorenzato (1995) and Rocke (2003) present a model in which an additive error and a multiplicative error are both present, which allows realistic behavior at both high and low concentrations. This model can also be estimated during the calibration process.

5.4. Limits of detection and related concepts

Following the most recent IUPAC terminology, we can define the critical level L_C as the measured amount of the analyte, which would occur only infrequently if there were, in fact, none of the analyte present. This is sometimes called the detection limit, but critical level is now the preferred terminology to distinguish it from the second IUPAC level, called the limit of detection or minimum detectable value L_D, which is the amount of an analyte in the sample so that the measured value is almost always above L_C. These are defined and discussed in Currie (1968, 1995, 1997) and in Rocke (2003).

There is sometimes also a third level defined called the quantitation level, which might be the point at which the relative standard deviation falls to a given, prespecified level. Rocke (2003) shows how to estimate this quantity, but also argues that it is a concept of limited utility. So long as a measurement and its uncertainty can both be produced, a quantitative measurement exists.

In no case should measured values be unreported or replaced by a code such as ND for non-detect. This makes subsequent statistical analysis of the measured data artifactually difficult. Measured values should be censored in this fashion only when it is believed that the instrument has malfunctioned or that the measurement is unreliable for some reason other than merely that the analyte is reported at a low level. An example may make this clear. Suppose that a metal speciation method has a standard deviation of measured blanks of 1 ppt, and that the high level coefficient of variation is 5%. Then the critical level L_C is between 2 and 3 ppt depending on the chosen significance level, and the minimum detectable value is between 4 and 6 ppt roughly. If a measured value is 10ppt, then we can say with 95% confidence that the actual amount in the sample is between about 8 and 12 ppt. If the measured value is 4 ppt, then with 95% confidence, the actual amount is between 2 and 6 ppt. If the measured value is 1.5 ppt (below L_C), then we can still say that the actual amount is between 0 and 3.5 ppt. If the measured value is 0.5 ppt, then the actual amount should be between 0 and 2.5 ppt. Note that the two last measured values are both below L_C, but imply different estimated concentration ranges. If both are replaced with ND, then we know less about the possible range of concentration than if both are reported as measured.

5.5. Control charts

There are many problems, systematic and sporadic, that can interfere with the accurate determination of values in the analytical laboratory. Reagents may lose potency, temperature, or humidity may affect the results, and operators may differ in their technique. There are many methods of statistical quality control that can be used to detect these problems; detailed descriptions may be found in Wadsworth et al. (1986) and Montgomery (1996). Two of the most important are Shewhart charts, also known as $\bar{X}$ and R charts, which are used to detect general departure from a state of statistical control, and cumulative sum (CUSUM) charts, which are used to detect a shift in the mean. This "state of statistical control" is, in the context of laboratory errors, one in which measurement errors have zero mean and a standard deviation that does not change with time (although it may differ with the concentration of the analyte), and in which successive measurement errors are not correlated.

The general principle for the use of control charts is that, when the analytical system is in a state of statistical control, the chart should stay within certain defined bounds almost all of the time. When the system fails to be in control in one way or another, the chart will more often pass the bounds. Once a chart "signals" by passing the bounds, there should be an investigation to discover and correct the source of the problem. Over time, attention to control charts, and subsequent investigation of problems, should place the measurement process in a state of statistical control. Continued attention is required to detect later departures from this desirable condition.

The distinction between the two types of charts is that Shewhart charts are designed to detect many kinds of departure from a state of statistical control, so that almost all problems will eventually cause a signal.

CUSUM charts are aimed specifically at one frequent concern—a shift in the process mean, which would involve in the laboratory context the development of a bias in the measurements. CUSUM charts are specifically designed to detect this type of departure and are more sensitive to this problem than are Shewhart charts. More details on this methodology may be found in Wadsworth et al. (1986) or in Hawkins and Olwell (2000).

5.6. Proficiency testing

Proficiency testing is used internally by analytical laboratories to evaluate their own performance and externally to develop new analytical methods or to validate the performance of laboratories. This will usually involve the submission of spiked samples to the analytical process that is blind (the amount present is not known to the analyst) or double-blind (the analyst does not know that the sample is a check sample and not a routine sample). Detailed discussion may be found in Garfield (1991) and Youden and Steiner (1975).

5.7. Intralaboratory studies

These studies are used by laboratories to check themselves or to improve operations. Blind or double-blind samples can be routinely run and used as the input for control charts or for examination of specific results. Another useful method is Youden's ruggedness testing (Youden and Steiner, 1975), in which a designed experiment is used deliberately to vary the conditions of the analysis in order to find out what factors influence the variability of the results. An example of this type of study for immunoassays may be found in Jones et al. (1995).

5.8. Interlaboratory studies

Interlaboratory studies, or round robins, are an essential component in the development of analytical methods. Different laboratories receive a series of samples to analyze by a proposed method. The variability of a measured result then can be partitioned into within-laboratory variance (repeatability or precision) and between-laboratory variance. The total variability, which is the sum of the within- and between-laboratory variance, is called the reproducibility and is a measure of accuracy. Ideally, the between-laboratory variance would be small, but in practice it is often considerably larger than the within-laboratory variance. This may be due to inadequately described methods or to the influence of identifiable factors that can be determined with ruggedness testing and controlled in a revised procedure. Poorly performing laboratories may also be identified in an interlaboratory study; Youden and Steiner (1975) give a rank test for this purpose.

5.9. Outliers

Outliers can cause a significant disruption in quality control procedures, as well as inaccurate measurement values. Especially, outliers in the initial samples used to determine the control limits for Shewhart charts or to estimate the process standard deviation for CUSUM charts can reduce the effectiveness of these tools. Outliers can also seriously distort the analysis of an interlaboratory study. Robust procedures are available for standard Shewhart charts (Rocke, 1989, 1992), CUSUM charts (Hawkins, 1993), and interlaboratory studies (Rocke, 1983, 1991). If outliers are frequent in the checked samples used to produce the control charts, it would be essential to discover and eliminate the source of the outliers, since detection of outliers in routine samples would be difficult.

6. Examples of elemental speciation studies

Given below are brief summaries for a few elements. Excellent discussions of elemental toxicities, physiological responses, etc., are given by Seiler et al. (1994) and Lippard and Berg (1994) and elsewhere in this issue.

6.1. Arsenic speciation

Arsenic exposure mainly occurs via the food chain. The body eliminates As predominantly by urine excretion. The chemical form of As strongly influences its bioavailability and toxicity of this element. Inorganic species are highly toxic; methylated forms are generally far less toxic. Speciation is used to assess the risk for exposed persons, e.g., in occupational health. In this issue Caruso and Montes (2003) present a wide overview of As speciation methodology and investigated As compounds, whereas Michalke (2003) reviews As speciation, e.g., in soil, grass, rice, or methylation capability by humans.

6.2. Selenium speciation

Selenium is both a toxic and an essential element. The incorporation in active Se proteins or erroneously into Se-carrying proteins (instead of sulphur) depends on the Se species available in nutrition. Michalke (2003) and Caruso and Montes (2003) give overviews on Se speciation methodology or Se speciation in different matrices.

6.3. Tin speciation

It is important to determine the various forms of tin, both inorganic and organic, from water to seafood samples, since Sn species have different levels of toxicity.

A primary source of tin contamination is associated with the widespread use of organotin antifouling bottom paints. Since many of the organotins are reasonably volatile, GC combined with ICP-MS is good for determining sub-picogram levels. LC-ICP-MS studies also have been done and in some cases, less volatile organotins are derivatized to more volatile forms. Examples are provided in the reviews that follow.

6.4. Mercury speciation

Mercury species long have been of concern. Elemental speciation studies extend to a wide number of sample types, including seawater, air, foods, etc. Fortunately, relatively inexpensive instrumentation can be dedicated to mercury analyses via AFS techniques giving sub-ppb detection limits. LC and GC with ICP-MS may also be utilized.

6.5. Cr speciation

Chromium is of concern because Cr(III) is essential to life while Cr(VI) is carcinogenic. Speciation analyses are complicated by the fact the chromium(III)/chromate/dichromate equilibria are highly pH dependent and additionally the two forms are cation and anion. These make the separation task challenging since it is usually necessary to convert to the anionic form by adding a complexing agent such as EDTA. This approach then introduces additional competitive equilibria. However, anion-exchange columns are commonly used and with careful experimental control.

7. Concluding remarks

Speciation analysis is necessary to better inform the important issues of bioavailability and risk assessment of heavy metals. Total element concentrations give insufficient information to adequately inform these issues. We need to know the chemical forms of elements—the chemical species—to better understand the true elemental impact on human or the environment. Methods used for speciation analysis are primarily coupling techniques with LC-ICP-MS predominant for liquid samples. Capillary electrophoresis is receiving increasing interest due to its superior resolution capabilities. The selective detection of ICP-MS when coupled to CE provides detection levels in the low ppb range.

A variety of problems, including adequate analytical quality control strategies, still need further study and application. Issues of sampling and sample preparation are critical to ensuring species integrity for the analysis. Further, the current method of identification by matching retention time standards is inadequate and much study is necessary using mass spectral techniques other than ICP-MS for identification. Also, further research will need to focus on sample preparation from solids and the necessary species preservation.

Elements of major interest at this time include As, Se, Sn, Cr, and Hg. Others of importance are given in references below and in the specific reviews that follow this overview.

8. Recommendations

- Sampling, sample storage, species extraction, preconcentration, and species separation maintaining the "true" concentration and compositions or structure of a given species are the most important steps for speciation analyses. The importance of a particular elemental detector such as ICP-MS often is overemphasized and most often only defines detection limits. Those necessary at this time can be achieved.
- Method validation and quality assurance and control are essential parts of any speciation analysis. The inherent additional complications of speciation analyses over a total concentration range still pose one of the major challenges. Reference materials such as NIST or BCR are sparse. Commercial availability of certain species stock standards for method development is also limited.
- Round robin analysis is of great importance to validate and standardize speciation methods for specific matrixes.
- The development of simple field methods for sample species preservation or even simplified speciation analyses is desirable. Nevertheless, it is probably more important to develop appropriate sample storage and preservation techniques because some of the sophisticated speciation techniques will not be portable in the foreseeable future.
- In general, it is desirable to develop simple and robust speciation methods. Nevertheless, in some cases the distinction between true speciation analyses and operationally defined speciation must be considered.
- Total metal analyses will remain a key quality assurance tool regarding the mass balance of a metal in a specific matrix. Typically, the total metal analysis is the first step of any speciation analysis.
- It is also important to look beyond element specific techniques for species identification through comparisons with retention time standards. For example, ESI-MS and atmospheric pressure chemical ionization MS are powerful techniques for detection of online separation and can provide nanogram to subnanogram detection levels with excellent potential species identification capability.

References

Arthur, M.A., Anderson, T.F., Kaplan, I.R., Veizer, J., Land, L.S., 1983. Stable isotopes in sedimentary geology. SEPM Short Course No. 10. SEPM, P.O. Box 4756, Tulsa, OK.

Atkinson, S.A., Shah, J.K., Webber, C.E., Gibson, R.S., 1993. A multielement isotopic tracer assessment of true fractional absorption of minerals from formula with additives from calcium, phosphorus, zinc, copper and iron in young piglets. J. Nutr. 123, 1586–1593.

Belshaw, N., Zhu, Z., Guo, Y., O'Nions, R.K., 2000. High precision measurement of iron isotopes by plasma source mass spectrometry. Int. J. Mass Spectrom. 197, 191–195.

Bloom, N.S., Colman, J.A., Barber, L., 1997. Artifact formation during aqueous distillation and alternative techniques for the extraction of methyl mercury from environmental samples. Fresenius J. Anal. Chem 358, 371–377.

Bostick, B.C., Hansel, C.M., La Force, M.J., Fendorf, S., 2001. Seasonal fluctuations in zinc speciation within a contaminated wetland. Environ. Sci. Technol. 35, 3823–3829.

Calmano, W., Mangold, S., Welter, E., 2001. An XAFS investigation of the artifacts caused by sequential extraction analyses of Pb-contaminated soils. Fresenius J. Anal. Chem. 371, 823–830.

Caruso, J.A., Montes-Bayon, M., 2003. Elemental Speciation Studies–New Directions for Trace Metal Analysis. Ecotoxicol. Environ. Saf. 56, 148–163.

Caruso, J.A., Sutton, K.L., Ackley, K.L. (Eds.), 2000. In: Barcelo, D. (Ed.), Elemental Speciation: New Approaches for Trace Element Analysis, 1st edition. Comprehensive Analytical Chemistry, Vol. 33. Elsevier.

Currie, L., 1968. Limits for qualitative detection and quantitative determination—application to radiochemistry. Anal. Chem. 40 (3), 586.

Currie, L., 1995. Nomenclature in evaluation of analytical methods including detection and quantification capabilities. Pure Appl. Chem. 67 (10), 1699–1723.

Currie, L.A., 1997. Detection: international update, and some emerging dilemmas involving calibration, the blank, and multiple detection decisions. Chemomet. Intell. Lab. Syst. 37 (1), 151–181.

Das, A.K., Chakraborty, Cervera, M.L., de la Guardia, M., 1995. Metal speciation in solid matrixes. Talanta 42, 1007–1030.

Demuth, N., Heumann, K.G., 2001. Validation of methylmercury determinations in aquatic systems by alkyl derivatization methods for GC analyses using ICP-MS. Anal. Chem. 73 (16), 4020–4027.

Dunemann, L., Begerow, J., 1995. Kopplungstechniken zur Element-speziesanalytik. VCH, Weinheim, New York, Basel, Cambridge, Tokyo.

Encinar, J.R., Gonzales, P.R., Alonso, J.I.G., Sanz-Medel, A., 2002. Evaluation of extraction techniques for the determination of butyltin compounds in sediments using isotope dilution GC-ICP-MS with ^{118}Sn and ^{119}Sn-enriched species. Anal. Chem. 74, 270–281.

Faure, G., 1986. Principles of Isotope Geology. Wiley, New York.

Fox, T.E., Fairweathertait, S.J., Eagles, J., Wharf, S.G., 1994. Assessment of zinc bioavailability—studies in rats on zinc—absorption from wheat using radio-isotopes and stable isotopes. Br. J. Nutr. 71, 95–101.

Fujii, T., Hirata, T., Shibahara, Y., Nishizawa, K., 2001. Mass-dependent and mass independent isotope effects of zinc in chemical exchange reactions using liquid chromatography with a cryptand stationary phase. Phys. Chem. Chem. Phys. 3, 3125–3129.

Garfield, F.M., 1991. Quality Assurance Principles for Analytical Laboratories, 2nd edition. Association of Official Analytical Chemists, Washington, DC.

Hawkins, D.M., 1993. Robustification of cumulative sum charts by Winsorization. J. Qual. Technol. 25, 248–261.

Hawkins, D.M., Olwell, D.H., 2000. Cumulative Sum Charts and Charting. Springer, New York.

Hook, S.E., Fisher, N.S., 2001. Reproductive toxicity of metals in calanoid copepods. Mar. Biol. 138, 1131–1140.

IUPAC International Working Party on Harmonisation, 1999. Harmonized guidelines for the use of recovery information in analytical measurement (technical report). Pure Appl. Chem. 71, 337–348.

Jones, G., Wortberg, M., Kreissig, S.B., Hammock, B.D., Rocke, D.M., 1995. Sources of experimental variation in calibration curves for enzyme-linked immunosorbent assay. Anal. Chim. Acta 313, 197–207.

Kim, C.S., Brown, G.E., Rytuba, J.J., 2001. Characterization and speciation of mercury-bearing mine wastes using x-ray absorption spectroscopy. Sci. Total Environ. 261 (1–3), 157–168.

Klaue, B., Blum, J.D., 2000. Mercury isotopic analyses by single- and multi-collector magnetic sector inductively coupled plasma mass spectrometry. J. Conf. Abstr.: Goldschmidt 2000 (5), 591.

Klaue, B., Blum, J.D., 2003. Isotopic speciation: the use of artificial and natural isotopic tracers to study the sources and bioavailability of metal species. Ecotoxicol. Environ. Saf., in press.

Klaue, B., Kesler, S.E., Blum, J.D., 2000. Investigation of natural fractionation of stable mercury isotopes by inductively coupled plasma mass spectrometry. Proceedings of the International Conference on Heavy Metals in the Environment, Ann Arbor, MI, August 6–10; contribution 1101.

Krupp, E.M., Pecheyran, C., Meffan-Main, S., Donard, O.F.X., 2001. Precise isotope-ratio measurements of lead species by capillary gas chromatography hyphenated to hexapole multicollector ICP-MS. Fresenius J. Anal. Chem. 370 (5), 573–580.

Labonne, M., Othman, D.B., Luck, J.-M., 2001. Pb isotopes in mussels as tracers of metal sources and water movements in a lagoon (Thau Basin, S France). Chem. Geol. 181, 181–191.

Landers, J.P., 1997. In: Landers, J.P. (Ed.), Handbook of Capillary Electrophoresis, 2nd edition. CRC Press, Boca Raton, FL.

Lippard, S.J., Berg, J.M., 1994. Principles of Bioinorganic Chemistry. University Science Books, Mill Valley, CA.

Marechal, C.N., Telouk, P., Albarède, F., 1999. Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry. Chem. Geol. 156, 251–273.

Matthews, A., Zhu, X.K., O'Nions, K., 2001. Kinetic iron stable isotope fractionation between iron (-II) and (-III) complexes in solution. Earth Planet. Sci. Lett. 192, 81–92.

Michalke, B., 1999. Quality control and reference materials in speciation. Fresenius J. Anal. Chem. 363, 439–445.

Michalke, B., 2003. Element specification: definitions, analytical methodology and some examples. Ecotoxicol. Environ. Saf. 56, 122–139.

Montaser, A., 1998. Inductively Coupled Plasma Mass Spectometry, 1st Edition. Wiley-VCH, New York, p. 964.

Montaser, A., Golightly, D.W., 1992. Inductively Coupled Plasmas in Analytical Atomic Spectrometry, 2nd edition. VCH Publishers Inc., New York.

Olesik, J.W., Kinzer, J.A., Olesik, S.V., 1995. Capillary electrophoresis inductively coupled plasma spectrometry for rapid elemental speciation. Anal. Chem. 67, 1–12.

O'Neil, J.R., 1986. Theoretical and experimental aspects of isotopic fractionation. In: Valley, J.W., Taylor Jr., H.P., O'Neil, J.R. (Eds.), Stable Isotopes in High Temperature Geological Processes, MSA Reviews in Mineralogy, Vol. 16. Mineralogical Society of America, pp. 1–40.

Orellana-Velado, N.G., Pereiro, R., Sanz-Medel, A., 2001. Solid phase micro-extraction gas chromatography-glow discharge-optical emission detection for tin and lead speciation. J. Anal. Atom. Spectrom. 16, 376–381.

Quevauviller, Ph., Maier, E.A., Griepnk, B., 1996. Quality Control of Results of Speciation Analysis. In: Caroli, S. (Ed.), Element Speciation in Bioinorganic Chemistry. Wiley, New York, Chichester, Brisbane, pp. 195–222.

Quevauviller, Ph., Martin, F., Belin, C., Donard, O.F.X., 1993. Appl. Organomet. Chem. 7, 149–154.

Rao, B.S.N., 1994. Methods for the determination of bioavailability of trace-metals–a critical evalution. J. Food Sci. Technol. Mysore 31 (5), 353–361.

Rocke, D.M., Lorenzato, S., 1995. A two-component model for measurement error in analytical chemistry. Technometrics 37, 176–184.

Rocke, D.M., 1983. Robust statistical analysis of interlaboratory studies. Biometrika 70, 421–431.

Rocke, D.M., 1989. Robust control charts. Technometrics 31, 173–184.

Rocke, D.M., 1991. Robustness and balance in the mixed model. Biometrics 47, 303–309.

Rocke, D.M., 1992. $\bar{X}_Q$ and R_Q charts: robust control charts. Statistician 41, 97–104.

Rocke, D.M., 2003. Modeling uncertainty in the measurement of low-level analytes in environmental analysis, Ecotoxicol. Environ. Saf. 56, 78–92.

Schramel, P., Hasse, S., 1994. Iodine determination in biological materials by ICP-MS. Mikrochim. Acta 116, 205–209.

Seiler, H.G., Sigel, A., Sigel, H. (Eds.), 1994. Handbook on Metals in Clincal and Analytical Chemistry. Dekker, Inc., New York.

Shamaev, V.I., 1987. New possibilities of radioanalytical chemistry. J. Radioanal. Nucl. Chem. 111 (1), 233–248.

Shirata, H., Patterson, C., 1980. Chronological variations in concentrations and isotope compositions of anthropogenic atmospheric lead in sediments of a remote alpine pond. Geochim. Cocmochim. Acta 44, 149–162.

Szpunar, J., 2000. Bio-inorganic speciation analysis by hphenated techniques. J. Anal. Atom. Spectrom. 55 (7), 963–988.

Taylor, A., Branch, S., Fisher, A., Halls, D., White, M., 2000. Atomic spectrometry update: clinical and biological materials, foods and beverages. JAAS 16 (4), 421–446.

Templeton, D.M., Ariese, F., Cornelis, R., Danielsson, G., Muntau, H., van Leeuwen, H.P., Lobinski, R., 2000. Guidelines for terms related to chemical speciation and fractionation of elements: definitions, structural aspects and methodical approaches. Pure Appl. Chem. 72, 1453–1470.

Town, R.M., Filella, M.A., 2000. A comprehensive systematic compilation of complexation parameters reported for trace metals in natural waters. Aquat. Sci. 62 (3), 252–295.

Wadsworth, H.M., Stephens, K.S., Godfrey, A.B., 1986. Modern Methods of Quality Control and Improvement. Wiley, New York.

Widmer, C.R., Krähenbühl, U., Kramers, J., Tobler, L., 2000. Lead isotope measurements on aerosol samples with ICP-MS. Fresenius J. Anal. Chem. 366, 171–173.

Williams, R.J.P., O'Nions, K., Zhu, X., Burgess, B.K., Canters, G.W., de Waal, E., Matthews, A., Salvato, B., Weser, U., 2001. The use of heavy metal fractionation in the study of the uptake of elements into cell. J. Inorg. Biochem. 86 (1), 18–18.

Youden, W.J., Steiner, E.H., 1975. Statistical Manual of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Washington, DC.

Zho, X.-K., O'Nions, R.K., Guo, Y., Belshaw, N.S., Rickard, D., 2000. Determination of natural copper variations by plasma-source mass: implications for use as geochemical tracers. Chem. Geol. 163, 139–149.

Zhu, Z., O'Nions, R.K., Guo, Y., Reynolds, B.C., 2000. Secular variation of Fe-isotopes in North Atlantic deep water. Science 287, 2000–2002.

Zhu, X.K., O'Nions, R.K., Guo, Y., Belshaw, N.S., Rickard, D., 2000. Determination of natural Cu-isotope variation by plasma-source mass spectrometry: implications for use as geochemical tracers. Chem. Geol. 163, 139–149.