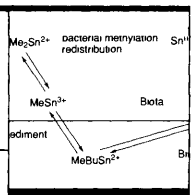
Speciation of trace metals in the environment



CHEMICAL SOCIETY REVIEWS

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With the recognition of the importance of metal speciation in many areas of environmental study, there has been a tremendous increase in research to aid our understanding of the role of specific metal species in terms of toxicity, bioavailability, bioaccumulation, mobility and persistence in the environment. In turn this has led to the development of analytical techniques that are both sensitive and specific to meet this challenge. This review provides an overview of trace metal speciation in the environment, an insight into some of the analytical techniques that are suitable for metal speciation studies on environmental samples, and a more detailed look at some selected elements for which the environmental chemistry has now been better characterised.

1 Introduction

The determination of distinct chemical species, often referred to as speciation analysis, is now widely acknowledged to be of vital importance in environmental chemistry. The term chemical speciation may be used to encompass both functionally defined speciation, that is, the determination of species that are, for example, available to plants or present as 'exchangeable' forms, and operationally defined speciation which refers to the determination of 'extractable forms' of an element. Whilst it is often possible to define a particular compound or oxidation state when dealing with solutions, for example, natural waters, it is far more difficult to characterise the actual chemical form of an element in solids such as soils and sediments. Thus, speciation tends to be defined somewhat differently by workers to reflect their field of study. However, one of the most comprehensive formal definitions of speciation is the one recommended by the International Union of Pure and Applied Chemistry (IUPAC) which states that speciation is 'the process yielding evidence of the atomic or molecular form of an analyte'. The determination of such specific chemical entities is of course not new to analytical chemists. For example, the determination of NO_3^-, NO_2^-, NH_4^+ and NH_3 where the nitrogen is characterised into its most environmentally important forms is long established. However, the characterisation of metals does not have the same history, although with the increased awareness of the importance of speciation in terms of toxicity, bioavailability, bioaccumulation, mobility and persistence in the environment, a range of sensitive yet specific analytical techniques have now been developed to address a wide range of complex problems.

It should be stressed that the identification and quantification of specific metal species in environmental samples is no longer an academic curiosity or indeed confined to a few specialist laboratories. As more information is collected on the importance of specific species, the determination of total concentrations of the metal in a sample is increasingly inadequate for many purposes since this information alone will not reflect, for example, the toxicity of the sample. Thus, the importance of metal speciation is now incorporated in new legislative requirements which often list specific species. The EC Council Decision 75/437/EEC (Marine Pollution from Land Based Sources) specifically includes As, Cd, Cr, Cu, Pb, Ni and Zn and their compounds in the list of substances requiring strict control. Other Council Directives mention Cd and Hg and their compounds (80/68/EFC—Groundwater) and As, Be, Cd, Cr, Hg, Sb and Tl and their compounds (78/319/EEC—Toxic and dangerous wastes).[1] In some cases individual species are listed, for example, in the UK tributyltin is listed specifically in terms of water quality. Further details on some important elements are given below, although clearly the introduction of such legislation has provided further impetus to develop reliable techniques to detect and quantify these species and presented a considerable challenge for the analytical chemist.

2 The practice of metal speciation

There are a number of important environmental factors which may affect the speciation of metals in the environment and these should be borne in mind during speciation studies. One of the most important of these is the prevailing redox conditions which not only determine the oxidation state of some metals, but may also influence the bioavailability and toxicity of the element. For example, Fe^{II} and Mn^{II} are soluble in natural waters deficient in oxygen but will precipitate out at higher oxidation states. In other cases photoreduction may be important, and changes in pH may shift the acid–base equilibrium and redox conditions.

In addition there are also a number of practical difficulties associated with metal speciation studies. The first, and perhaps most obvious problem, is to obtain a representative sample in which the integrity of the species of interest remains intact. Thus the collection of samples can lead to errors if suitable precautions are not taken. It is obviously necessary to ensure that the container used is not made of the same material as the analyte and some plastics are more suitable than others depending on the metals to be determined. Mercury, for example, is known to escape from some types of plastic container. Although the preservation of samples is frequently achieved by the addition of acid, as mentioned above, this may not be appropriate for speciation studies since it may lead to

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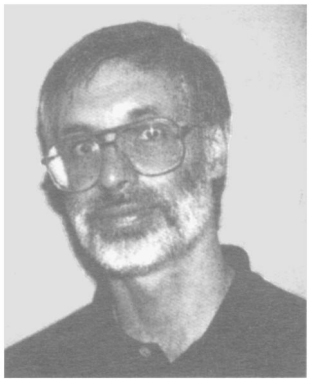

changes in oxidation state and alterations in the speciation. Filtration may also alter the species present and lead to a redistribution between the mobile and stable metal forms, as for example in the case of aluminium. Storage of some samples may add to the problems and it is normally recommended that analysis should take place as soon after sampling as possible. Some species, *e.g.* the alkyltin species and arsenobetaine are photosensitive and so if exposed to light following collection a radical induced change in the speciation may occur. In other cases specific precautions may aid the collection of samples for speciation work. Chloride, for example, should be added to solutions where selenium speciation is to be performed since this will prevent oxidation of Se^{IV} to Se^{VI}. In most cases samples should be kept at low temperature since this will reduce the risk of thermal rearrangement or thermal degradation. Often placing the sample in a refrigerator at 4 °C is sufficient, but occasionally freezing may be required.

The next stage to consider is the preparation of the sample for analysis. Quantitative extraction of the species without alteration to the speciation can prove to be very difficult. For liquid samples, the problem is not so bad. The sample may be centrifuged or filtered and if a pre-concentration procedure is not required, it may be analysed directly. For solid samples, the extraction may be far more problematic. Clearly an acid digestion is inappropriate and so the species must be taken into solution by another method. There are a number of different approaches in common use. For biological samples, an extraction using an organic solvent (often methanol or toluene) is fairly common. The use of an ultrasonic bath increases the efficiency and speed of the extraction. Soxhlet extraction is a more lengthy method and care must be taken not to lose labile species or to change the speciation at elevated temperatures. An alternative method that has been employed by some laboratories is the use of enzymes and a Potter homogeniser to disrupt the samples. Trypsin has been used successfully to extract arsenic species from proteinaceous materials. The use of ammonium carbonate to obtain the optimum pH for trypsin ensures the best possible extraction efficiency. For cereals or plant materials, an alternative enzyme is required. The use of cellulase has been found to be efficient at digesting this type of material.

To prepare soil and sediment samples for speciation studies, 5–20 g (to obtain a representative subsample) of an unground < 2 mm particle size air dried sample should be used. The use of fresh, field moist soils is not considered a practical possibility for general use.[2] However, the use of dried material may affect the soil speciation and this should be considered when interpreting the results. In addition, storage of dried samples may be a problem, particularly if anoxic sediments are being used. Separation by sieving may also discriminate against some elements that are associated with the larger particle size fractions and so the use of a 'conserved' element such as aluminium may be required to act as an internal standard.[2] Once a representative sample has been obtained different extractants can be used not only to assess the bioavailability of particular metals but also to isolate and determine elements bound or associated with defined soil fractions or phases. An improvement in the specificity of the extraction can be achieved by using a sequential extraction scheme in which the residue from one extract is extracted by the next extractant in the sequence.[3] Typical extracting reagents include calcium chloride, ammonium acetate, sodium nitrate and ammonium nitrate solutions. To extract organometallics organic solvents are often the method of choice. Occasionally tropolone may be used since it will aid very efficient extractions although this in itself may be problematic since it may also extract unwanted concomitants that could affect the analytical methodology, if for example, chromatography is used. Supercritical fluid extraction is also gaining in popularity for organometallic extractions in speciation studies since it yields high extraction efficiencies under relatively mild conditions. Thus, although relatively new, this approach would seem to offer much potential for the future. Solid phase extraction has also achieved great popularity with some laboratories.

One of the major challenges in speciation studies is that often the analytical scientist is being asked to quantify species present at very low concentrations, well below the detection limits of current techniques. Under such circumstances, it is often necessary to perform some kind of preconcentration procedure. A variety of methods are available including the use of liquid–liquid extractions, freeze drying, gentle evaporation (assuming none of the species are labile) and preconcentration using an ion exchange resin. Of these different methods, the liquid–liquid extractions probably yield the best preconcentration factors, with factors of over 100 sometimes being possible. This is especially useful for liquid samples such as seawater. In addition, the process removes the vast majority of the matrix which could otherwise interfere with the determination. As with all sample preparation procedures, care must be taken that analyte loss does not occur. More accurate results may be obtained if standard solutions of the analytes of interest are treated in an identical manner, since this should overcome many of the problems associated with different species of an analyte having a different affinity for the extractant.

A final practical consideration associated with speciation studies is that of quality control and quality assurance.[3,4] Most competent laboratories will use either certified reference materials (CRMs) or spiking/recovery experiments to ensure the accuracy of their determinations. However, for speciation studies this is not straightforward. At the moment very few CRMs with species specific data are available and so finding one that closely matches the matrix of interest is difficult. This is an area where the agencies producing CRMs are paying increasing attention and a number of new programmes to produce a range of reference materials specifically for speciation work are now under way. These include roadside dust and rainwater for lead species, fish tissues for mercury species, various fish and mussel tissues and algae for arsenic species and fish tissue and sediments for tin species. A summary of available reference materials for speciation studies is presented in Table 1. Although this list is not comprehensive, it does reflect the increasing demand for CRMs to validate speciation data. However, the preparation of such materials is not trivial. For instance, many CRMs produced for use in other areas are sterilised by gamma irradiation. However, this is not always appropriate in the case of CRMs certified for metal speciation since in some cases, for example, tin, dealkylation may take place, thereby making valid speciation impossible. The importance of matrix matching should also be emphasised. For example, if a sediment is to be speciated for butyltins (mono-, di- and tri-butyltin), the CRM PACS-1 (Harbour Marine Sediment, National Research Council, Canada) is available. Unfortunately, not all sediments have the same chemical composition. A procedure using glacial acetic acid is very

Table 1 Certified reference materials available for metal speciation analysis

DBT and TBT in marine sediment (PACS-1)—NRCC[a]
TBT in fish tissue (NRES 11)—NIES[b]
Hg and MeHg lobster hepatopancreas (TORT 1)—NRCC
MeHg in dogfish muscle (DORM 1)—NRCC
DBT and TBT in coastal sediment (CRM 462)—BCR[c]
MeHg in tuna fish (CRM 463/464)—BCR
Other materials in preparation:
Cr^{III} and Cr^{VI} in freeze dried solution and welding dust (BCR)
Se^{IV} and Se^{VI} in solution (BCR)
As in fish (NIES and BCR)
MeHg in sediments (BCR)
Organoleads in rainwater and urban dust (BCR)

[a] NRCC—National Research Council, Canada. [b] NIES—National Institute for Environmental Studies, Japan. [c] BCR—Bureau of Community Reference (Standards, Measurements and Testing Programme).

efficient at extracting the butyltin species from PACS-1, which has a very high organic composition, but it is considerably less efficient at extracting them from sediments that contain significant proportions of silica or alumina. In such cases, it is impossible to assume that because a procedure works for one reference material, that it will work for all such samples and serious underestimates of the true content can be made. The efficiency of an extraction procedure can be measured by first determining total analyte concentration by conventional means, *e.g.* acid digestion of a sub-sample. An appropriate extraction procedure for the species of interest may then be used on a second sub-sample. After extraction, the species specific analysis is performed. The concentration of each of the species is then summed and if the sum of the concentrations of the species is the same as the total concentration, the extraction efficiency may be assumed to be 100%. If the extraction efficiency is reproducible *e.g.* 80 ± 5%, the method is under control and may still be valid. If, however, the extraction procedure is not reproducible, *i.e.* an extraction efficiency of 80 ± 20% is obtained, the method is not under control and will not provide accurate results. As mentioned previously, the alternative method of quality control is the use of spiking/recovery experiments. Again, however, care must be taken in speciation studies since different species may have different extraction efficiencies. If a species is contained within fatty tissue, it may be extracted less efficiently than species that are simply bound to more hydrophilic materials. This means that even if a small volume of an aqueous standard is allowed to soak into the sample for 24 h (the recommended time for spiking experiments), the spike may not penetrate the lipid layers. The spike will therefore be more readily extracted and have a better extraction efficiency than the analyte in the sample (assuming methanol or some other polar solvent is used for the extraction). A further complication in many cases is that the standards to perform such spiking experiments are not always commercially available. For example, for arsenic speciation most of the species of interest are now available, although standards for some arseno-sugars are hard to obtain.

3 Analytical approaches to speciation studies

In terms of analysis, it is possible to identify species in a specific fraction separated during the sample preparation stage, or as is often desirable, by direct measurement from the sample matrix. Speciation studies on environmental samples are often performed using a chromatographic method to separate the species of interest directly interfaced with an element specific detector.[5–8] Electroanalytical methods, for example, anodic stripping voltammetry (ASV) in the differential pulse stripping mode may also be used but such studies are generally confined to 'labile' *versus* 'strongly bound' metals in aqueous samples.[9] Such methods have, however, proved particularly popular for use on board ships for selected elements such as Cd, Cu, Pb and Ni.[4] A comparison between electroanalytical techniques and those utilising atomic spectroscopy has been made elsewhere.[10] Similarly, ion selective electrodes may be used to detect a specific chemical form although electrodes are not available for organometallic cations. A range of other approaches have also been used including techniques such as neutron activation analysis, radioanalytical methods[11] and more exotic methods such as photoacoustic spectroscopy and thermal lens spectrometry but their use is not widespread. Tables 2 and 3 summarise the more common approaches to speciation studies and provide a number of examples of their use.

4 Chromatography

Although gas chromatography (GC) is an extremely common analytical technique for volatile analytes, its use for speciation of inorganic analytes has been relatively limited. This possibly reflects the fact that most GC detectors are not element specific, and that there may be problems associated with transferring the analyte from the end of the GC column to the atom/ion source when atomic spectroscopy is used for detection. The GC eluent is obviously in the gas phase and is usually at an elevated temperature. This temperature must be maintained all the way along the transfer line to the detector since failure to do this leads to cool spots and condensation of the analyte. A heated transfer line is therefore obligatory. For many analytes that are volatile, *e.g.* organolead species, the transfer line may be kept relatively simple as the temperatures required need not exceed 200–250 °C. For species that have been separated using high temperature GC techniques, *e.g.* metalloporphyrins, the transfer line may need to be heated to temperatures of approximately 400 °C.[12] Ensuring that no cool spots exist in such a transfer line is much more complex. In addition, coupling GC with plasma instruments may lead to the metallic transfer line acting like an aerial and thus coupling to the radio frequency (RF). This is potentially dangerous and may cause instability of the plasma, and instrument failure. The majority of work performed

Table 2 Analytical approaches to metal speciation

Technique	Comments	Analytical examples
Gas chromatography	GC has been used with a number of different detectors to effect speciation. Examples include the use of electron capture or flame photometric detectors. These tend to be non-specific, and so peaks from interfering matrix constituents may cause confusion.	The determination of organometallic compounds of tin, arsenic, lead and mercury.[23]
High performance liquid chromatography	UV–VIS detectors have been used occasionally, but suffer from a lack of sensitivity. Electrochemical and fluorescence detectors have also been used.	Speciation of aluminium in waters. Speciation of vanadium or chromium using HPLC with spectrophotometric detection.[24]
Polarography/anodic/cathodic stripping voltammetry	These have been used for differentiating between different oxidation states of species. They have also been used to determine speciation in the presence of humic and fulvic acids.	Electrochemical behaviour of metallothioneins.[25] Speciation of butyltin compounds by CSV.[26] Such methods have proved popular for work on board ships.
Ion selective electrodes	Although they have found use in differentiating between different oxidation states, they tend to suffer from a lack of sensitivity and so are used only for polluted waters.	Speciation of cadmium or copper in the presence of humic or fulvic acids.[27] Determination of fluorides in the presence of aluminium.
Auger electron spectroscopy and electron paramagnetic resonance	These are rarely used techniques.	Iron speciation at physiological pH in the presence of ascorbate and oxygen.[28]
Nuclear magnetic resonance	This has found several different uses, but the sensitivity is limited.	Aluminium complexation with nucleosides. Determination of Al^{3+}, hydroxy and other Al species of geological interest. Actinide speciation using complexation.[29]

coupling GC with atomic spectrometry has so far been achieved using flame spectrometry as a detector.[5] This approach has been used most commonly for analytes that are present in relatively high concentration *e.g.* determining organolead species in fuel or roadside dust. A potential limitation of using GC is that often the sample must be derivatised to make it voltaile enough for analysis. This can greatly increase sample preparation time, may cause loss of analyte and uncertainty about the identity of the original species in the sample.

High performance liquid chromatography (HPLC) is one of the most common methods used for the separation of non-volatile analytes and has been extensively coupled to atomic spectroscopy for detection. There are a large number of resin supports that may be used as the stationary phase with anion exchange, cation exchange, size exclusion, chelating and reversed phase functionality. The reversed phase resins *e.g.* octadecylsilane (ODS) are often used in conjunction with an ion pairing reagent, *e.g.* tetrabutylammonium phosphate, diethyl dithiocarbamate or 8-hydroxyquinoline. In this way, analytes with different oxidation states, *e.g.* Cr^{III} and Cr^{VI} may be separated. Arsenic has been speciated into As^{III}, As^{V}, monomethyl arsonic acid, dimethyl arsinic acid and arsenobetaine using an anion exchange resin with an ammoniacal potassium sulfate mobile phase. Other approaches have used a reverse phase column with a mobile phase of sodium dodecyl sulfate in 5% methanol and 2.5% acetic acid or cation exchange columns. More recently, the HPLC separation of arsenic species has been coupled with on-line microwave digestion and hydride generation to facilitate the direct determination of both reducible and non-reducible forms of arsenic.[13] In order to allow the optimum conditions for the separation of species to be maintained it is important that the detector facilitates as wide a range of solvents as possible. Since the majority of analyses using HPLC are undertaken at room temperature there are no real problems associated with the transfer line coupling the chromatograph to the detector. Thus, couplings with HPLC can often be made cheaply and quickly without major modification to either instrument.[6,7]

The use of capillary electrophoresis is rapidly growing in popularity. At present the flow rate used (10 μl min^{-1}) is not readily compatible with atomic spectrometric detection since there are fundamental problems associated with sample transport. Conventional nebulisers for example would be starved of liquid and thus not function properly. It is, however, probable that a union will eventually be developed which will facilitate a successful coupling between the two techniques.

Hydride generation, although not a chromatographic technique, can be used in conjunction with a cryogenic trap to effect speciation.[14] As^{III}, As^{V}, monomethylarsonic acid and dimethylarsinic acid all form hydrides. If the hydrides formed are flushed from the gas–liquid separator into a liquid nitrogen trap, the hydrides freeze. Removal of the transfer line from the liquid nitrogen trap will allow the hydrides to boil off at their own respective boiling points, and subsequently be swept to the atomic spectrometer for detection. This technique has found a niche for certain applications since it offers a means of improving sensitivity. It is not used very frequently, but does demonstrate that chromatography is not always necessary to obtain separation.

5 Element specific detection

Flame atomic absorption spectrometry (FAAS) is one of the most common techniques employed for trace inorganic determinations, although it is often not sensitive enough for environmental work. Flame AAS is extremely tolerant of organic solvents, indeed the nebuliser efficiency may be increased when compared with water and this in turn may lead to increased sensitivity and better detection limits. Clearly, this is an advantage for some HPLC applications, although care must be taken when using organic solvents to prevent a build up of carbon on the burner. This may be readily removed but failure to do so will adversely affect the flame and result in a decrease in sensitivity.

For HPLC, in which mobile phases with high dissolved solids may be employed, the nebuliser and burner system of flame

Table 3 Overview of coupled techniques used for metal speciation

Technique	Ease of coupling	Potential problems	Analytical examples
Gas chromatography–atomic spectrometry	A heated transfer line is usually required. Holes must be machined in ICP torch boxes to accommodate the transfer line.	Analytes must be volatile or derivatised into a more volatile form. Analytes may condense on any cool spots, hence losing sensitivity. Transfer lines may pick up RF.	Determination of organolead species in petrol.[5] Determination of organotin compounds in waters and molluscs. Determination of metalloporphyrins in crude oils.[12]
High performance liquid chromatography–atomic spectrometry	Easy. The end of the column may simply be attached to the capillary of the nebuliser, although an air bleed may be necessary to overcome differences in flow rate. For ICPs a desolvation device may be necessary if organic solvents are employed.	Some eluents contain high dissolved solids that may block the nebuliser. Eluents containing organic solvents may thermally decompose and block the burner head with carbon when using flame AAS. Organic solvents may extinguish ICPs and also clog cones and the ion lens stack in ICP–MS.	Determination of organotin compounds in water, molluscs and sediments.[30] Determiantion of arsenic species.[13] Speciation of antimony, selenium,[31] mercury and lead.[32]
Flow injection–atomic spectrometry	Very simple. FI has been coupled with numerous detection systems.	Simialr to those described for HPLC.	Determination of inorganic selenium and chromium species in waters.[33] Determination of Cr species using XRF (a batch method).
Capillary zone electrophoresis–atomic spectrometry	Difficult. The low flow rates of CZE are not readily compatible with atomic spectrometric methods.	Difficulty in coupling the two together.	A few research papers have been published.[34]
Hydride generation–atomic spectrometry	Usually quite easy.	Cryogenically trapped hydrides of species are slowly ramped to room temperature, volatilisng individual hydrides at their respective boiling point.	Speciation of reducible arsenic species.[35]

atomic absorption spectrometers may occasionally block, especially after extended use. It may therefore be necessary to have a wash out period at the end of each chromatographic run. A small air bleed into the nebuliser is frequently used to compensate for the different flow rates of the HPLC (1–2 ml min^{-1}) and the uptake rate of nebulisers used in flame AAS (5–8 ml min^{-1}). This air bleed often improves peak shape and decreases noise. The coupling of GC with atomic spectrometric detectors also needs care; the importance of maintaining the interface temperature has been discussed above. Most reports in the literature where GC has been used as the method of separation in speciation studies have used flame AAS for detection. The sensitivity of the analysis may be increased if the interface leads directly to the atom source rather than going through the conventional nebuliser/spray chamber arrangement. The use of a quartz or ceramic tube placed in the flame to increase the residence time of the atoms in the flame is a convenient way of accomplishing this.[15]

Electrothermal atomic absorption spectrometry (ET–AAS) may also be used although the batch nature of this technique precludes its use for routine on-line speciation analysis despite its increased sensitivity when compared with flame AAS. On-line couplings can be made but these require the use of either an electrospray or thermospray device, and even then, running the spectrometer isothermally at the atomization temperature will lead to rapid tube wear. Chromatographic fractions may also be collected off-line and then analysed, but this is laborious and prone to error.

Inductively coupled plasma atomic emission spectrometry (ICP–AES) has also been used as the detection method for speciation analysis. Such instruments have better sensitivity and longer dynamic ranges than flame spectrometers. In addition, they also allow alternative spectroscopic lines to be monitored either simultaneously or at least far more rapidly than AAS instrumentation. The use of an appropriate type of nebuliser/spray chamber configuration rather than simply using the type supplied with the instrument will lead to greatly improved results in terms of peak shape and signal to noise properties. However, the effects of organic solvents on the plasma can be substantial. Some instruments operate readily with close to 100% methanol without having an excessively high reflected power. Other instruments have very high reflected powers under the same conditions, thereby risking damage to the (RF) generator. In extreme cases, the plasma may be extinguished by even relatively small proportions of organic solvent (*e.g.* 30% acetonitrile). It is worthwhile remembering that if a mobile phase containing a high percentage of organic solvent is to be used, it should be introduced in stages to give the matching network time to compensate, as for example when using gradient elution chromatography. This is less likely to lead to plasma extinction. Typical flow rates of HPLC pumps and the uptake rate of an ICP nebuliser are compatible (1–2 ml min^{-1}) and since both usually operate at room temperature, the interface coupling can theoretically be very simple. There are occasions, however, when an on-line solvent removal system incorporating membrane drier tubes, Peltier coolers or a similar device is necessary between the spray chamber and the plasma to prevent problems due to excess solvent.[16] GC is less simply interfaced with ICP–AES instruments requiring custom built interfaces to be built.

Many of the same considerations are required with inductively coupled plasma–mass spectrometry (ICP–MS). These instruments offer detection limits as low as 1 ng dm^{-3} and have a linear range of five to six orders of magnitude. Therefore, such instruments are particularly attractive for speciation studies although they do have additional drawbacks. For example, the interface between the plasma and the mass spectrometer can be seriously affected by organic solvents. The sample and skimmer cones on the interface region between the atmospheric pressure plasma and the mass spectrometer, can become clogged with carbon which in turn leads to excessive signal drift until the cones become completely clogged, whereupon no signal is obtained. The problem can be overcome in most cases by the addition of oxygen (*ca.* 3% v/v) into the nebuliser gas flow although excess oxygen will lead to rapid ablation and to substantially reduced cone lifetime. The cones themselves are usually made of nickel, although platinum ones are available (but more expensive) and are not attacked by HPLC/ion chromatography mobile phases containing sulfate or phosphate. The opportunity to utilise isotope dilution in conjunction with speciation studies is also possible using ICP–MS and again offers potential for the future.[3] Several other element specific detectors have also been used to good effect and a number of commercial instruments have been produced for specific applications. Mercury, for example, has been speciated using GC coupled with an atomic fluorescence detector producing limits of detection below the ng ml^{-1} level. Microwave induced plasmas (MIP–AES) have been linked to GC. The helium MIP has an extremely high ionisation energy and may therefore be used to determine analytes such as the halogens, sulfur, nitrogen, oxygen and phosphorus. The use of MIP has been limited to analysing gaseous samples since even a few microlitres of solvent tend to extinguish the plasma, although research continues into the introduction of liquid samples using direct injection nebulisation, 'thermospray' and 'particle-beam' approaches.[17] Finally, new approaches are also being developed such as the use of liquid chromatography–mass spectrometry and low power ICP–MS which when coupled to GC has the potential to determine both atomic and molecular species.[18]

6 Selected elements of special interest

Although the above text has stressed a number of precautions that must be taken when performing speciation analysis, if appropriate care is taken in terms of sampling, sample preparation and choice of analytical technique, very good results may be obtained on a routine basis. Many elements exist as different species; however, this final section will consider only a few examples where there has been extensive environmental interest: arsenic, mercury, tin and lead. For many elements the organometallic forms tend to be substantially more toxic than the inorganic forms. This is true for tin, where inorganic tin is virtually non-toxic, but where the toxicity of the tin species increases as the percentage of organic moiety in the molecule increases. Thus, in the marine environment where organotin compounds have been widely used as the active ingredient in antifouling paints on boats, compounds such as tributyltin (TBT) and triphenyltin (TPhT) are known to have toxic effects on a number of organisms. Eventually these compounds are known to degrade to their progressively less toxic di-, mono- and inorganic derivatives.

There are, however, cases where the inorganic forms of the analyte are more toxic than the organometallic forms. Examples include arsenic and selenium, where the inorganic forms (arsenite and arsenate or selenite and selenate) are extremely toxic whereas many of the organic forms, *e.g.* arsenobetaine or selenomethionine are regarded as being non-toxic. Inorganic arsenic has been cited as a poison in numerous murder cases; however, the organic forms are found at relatively high concentrations in some fish and may be so stable that they do not degrade to the inorganic form even when treated with concentrated acid.

Some analytes, *e.g.* lead and mercury, are poisonous regardless of the chemical form in which they are present; but the degree of toxicity will again depend on the species. Methylmercury for example is substantially more toxic than inorganic mercury. The increased toxicity of the organic form in this case is due to the increased efficiency with which it crosses the blood–gut barrier. The following sections give a brief overview of some of the better known species of each of the selected metals and the methods that have been used to separate and determine them.

7 Tin

Organotins (mono- and di-organotins) are widely used as stabilizers for rigid PVC. However, it is their use as biocides (triorganotins) that has given rise to environmental concern. Triorganotin biocides (mainly triphenyltin) are used in pesticides and tributyltin (TBT) and triphenyltins in antifouling paints. It is this latter usage that has received most attention with regard to the effects of organotin compounds in the environment. In particular TBT has been the subject of many studies. For example, the concentration of TBT has been found to reach 1.5–2 μg l^{-1} in seawater, approximately 7 μg l^{-1} in fresh water, 25–30 mg kg^{-1} in marine sediments and 3.5–4.0 mg kg^{-1} in fresh water sediments. In addition, TBT is also known to enter the flora and fauna of these environments and in many cases lead to severe deformity and in some cases death. Concentrations of TBT of 6, 2 and 11 μ kg^{-1} have been reported for bivalves, gastropods and fish, respectively. As TBT is relatively insoluble in water and is lipophilic, it readily adsorbs onto particulates and bio-accumulates in the fat of organisms. Accumulation factors of several thousand have been observed. It is also present in seawater as either the oxide, the hydroxide or the carbonate. It degrades through various mechanisms (*e.g.* photolysis, hydrolysis and by the action of micro-organisms), where it is slowly de-butylated in a stepwise manner by breaking of the tin–carbon bond. It is known that dibutyltin (DBT) is more readily degradable than TBT, and the DBT then forms monobutyltin (MBT), which is slowly transformed to inorganic tin. Under natural conditions, hydrolysis and photolysis are limited and the bio-degradation by the micro-organisms predominates. This bio-degradation is important environmentally since the toxicity of the species decreases in the order: TBT

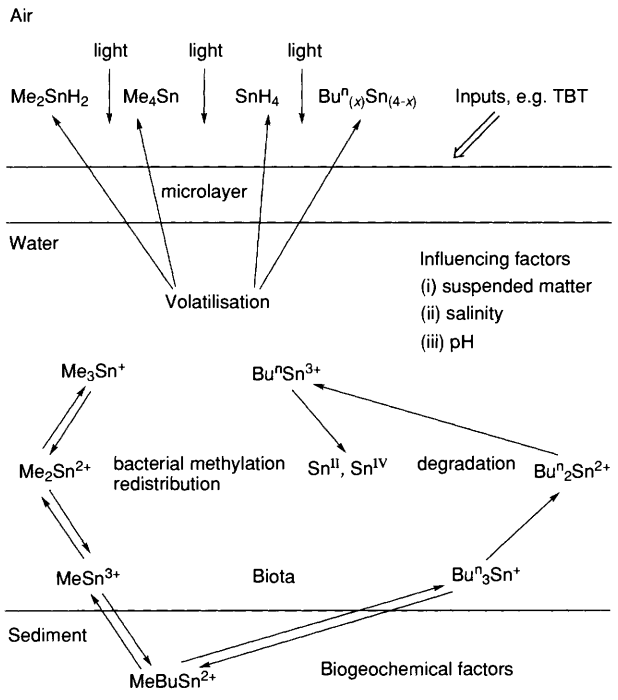

Fig. 1 Biochemical pathway (simplified) of tin compounds in the environment

> DBT > MBT > inorganic tin. Fig. 1 shows some of the biochemical pathways that have been suggested for tin compounds in the environment. A large number of research papers have been published that determine different tin species in a variety of matrices. The environmental aspects of non-biocidal organotin compounds have also been reviewed.[19] In terms of analysis, the tin compounds are often extracted from the matrix, derivatised into a more volatile form (usually either by a Grignard type reaction or by the formation of a hydride) and then separated using GC. Detection by flame ionisation detection (FID), flame photometric detection (FPD), AAS, GC–AED and ICP–AES is possible. The detection limits reported have been very impressive, with levels for different tin species at the ng dm^{-3} level being obtained. Other methods have coupled HPLC with ICP–MS with success. Here, the extraction methods employed will differ depending on the matrix. For example with sediments, glacial acetic acid or a method involving the use of tropolone may be used whilst for mussels enzymatic digestion followed by extraction with organic solvents is preferable.

8 Arsenic

As described above, there are a large number of arsenic species present in the environment. These range from the extremely toxic inorganic forms arsenate and arsenite through the mildly toxic species such as dimethyl arsinic acid and monomethyl arsonic acid to the harmless arsenobetaine and arsenocholine. A number of relatively harmless arseno-sugars are also known. Fig. 2 shows the structures of some of the better known arsenic species. Arsenic has found a variety of uses including being an active ingredient of herbicides, fungicides, insecticides, wood preservatives and growth promoting agents for poultry (although this use has now ceased). Once in the environment, arsenic and its compounds may undergo physical, chemical and biochemical transformations which may or may not involve a change of oxidation state, and/or mineralization, adsorption and precipitation processes. It has been demonstrated conclusively that arsenic can be methylated in the environment. Mould, for example, has been shown to produce trimethylarsine from arsenite. Bacteria and algae have also been known to methylate arsenic from sediments and waters. It is also known that some fish and higher animals can readily synthesize methylated forms of arsenic. It is assumed that this process occurs in the liver and is a result of de-toxifying the inorganic forms. Arsenobetaine is the end product of arseno-riboside metabolism and is the principal form of arsenic found in many aquatic organisms and sediments.

It is worth stating that the total levels of arsenic can be very high for some types of sample although the arsenic content differs markedly between samples. For sediments and soils the level can be as high as 1 g kg^{-1}, although a concentration of 7–20 mg kg^{-1} is more normal. In extreme cases, *e.g.* from a spoil tip close to an arsenic mine, arsenic can be at the percentage level. Natural levels in waters are usually very low (0.1–0.5 μg l^{-1}), but this again can depend upon a number of other factors. Some vegetation will take in arsenic from soil (and possibly from insecticides). It has been shown that spinach contains approximately 10 mg kg^{-1} arsenic and that radishes can contain levels far in excess of this (up to 100 mg kg^{-1}). However, such total levels reveal little about the potential toxicity of the sample. The main source of arsenic in the human diet, for example, is seafood. Benthos feeders and shellfish can contain extremely high levels of arsenic (mussels and prawns 50–200 mg kg^{-1}) although fortunately this is present mainly as arsenobetaine and is thus non-toxic and readily excreted from the body if consumed by man. Plankton are also known to contain arsenic at concentrations up to 2.4 g kg^{-1}. Fishmeal fed to farm animals has increased the arsenic levels in these species but to a far lesser extent than for the fish themselves.

A large amount of literature has been devoted to arsenic speciation.[3] Much of this has concentrated on the analysis of seafood samples, although some workers have analysed poultry or urine in diet trials. The vast majority of methods utilise HPLC coupled with a sensitive detector such as ICP–MS or ICP–AES. Other laboratories do partial speciation. For example, hydride generation is useful for determining the reducible forms of arsenic, *e.g.* the inorganic and methylated forms, but will not give a response for arsenobetaine or arsenocholine. In this way

a distinction between the reducible 'toxic' forms and the non-toxic non-reducible forms can be made. Extraction methods used have again concentrated very much on the use of organic solvents such as methanol, although enzymes are increasingly being used for biological samples. As always, the use of acids for sample destruction is precluded because although species such as arsenobetaine are stable, the methylated forms will become de-methylated.

9 Lead

Lead is not only ubiquitous in the environment but is also a well-known poison. Although used in numerous industrial applications the concentration of lead in the environment has increased substantially over the last fifty years largely because of its use in antiknock additives in petroleum. The alkylated lead compounds used for this purpose are extremely toxic and therefore legislation has been introduced in many countries to reduce the amount of leaded petrol. The toxicity of alkyllead species increases in the order $Pb^{2+} < R_2Pb^{2+} < R_3Pb^{+} < R_4Pb$ where R is a methyl- or ethyl-group. In the environment the ionic forms of lead have been found to be the most persistent. In algae and higher plants alkyllead compounds have been shown to inhibit growth and cause disturbances of mitosis and ultrastructural alterations. Fish have also been shown to be affected by organoleads and fatalities in man through chronic exposure are also known. Between 0.1–2.0% of the organolead (principally tetramethyllead and tetraethyllead) in petrol passing through the engine is not combusted and thus ends up in the environment.

Natural bio-methylation of lead also occurs, but only very slowly and it must be noted that, like tin, lead in the environment can also become de-alkylated. This is again due to factors such as photolysis. In the absence of light and at reduced temperature the decomposition of tetraethyllead is very slow (2% over 77 days) but under atmospheric conditions it is likely to be decomposed rapidly (half-life of 2–8 h). However, due to the non-polar nature, high vapour pressure and lipophilic character of tetralkylleads they are likely to volatilize from water, be absorbed into organisms (*e.g.* fish tissue) or directly absorbed onto sediments.

Many different procedures have been used for the analysis of organoleads in a variety of different samples. Gas chromatographic procedures have often been used because of the volatility of many lead species and this technique has been linked to numerous types of detector including atomic absorption and atomic emission spectrometers. More detailed reviews of lead speciation including the occurrence, chemical transformations, sampling, storage and pretreatment for water, air and solids together with an overview of the analytical methods that have been used are available.[20,21]

10 Mercury

Mercury is an unusual metal with a number of unique properties and a wide range of industrial applications. There are three main species of mercury: inorganic (Hg^{II}), methylmercury (MeHg) and dimethylmercury (Me_2Hg). All forms of mercury are considered poisonous, although methylmercury is of particular concern since it readily undergoes biomagnification in food chains. Indeed, one of the best known cases of poisoning involving metal speciation was that in Minamata, Japan where methylmercury was accidentally released into the sea and was consequently taken up by fish. The fish were later ingested by local people including some women who were pregnant, and resulted in severe abnormalities in the newly born children. In a second case, this time in Iraq, seed grain was sprayed with methylmercury-containing fungicides and again resulted in large-scale poisoning since the seed was eaten and not planted as intended. In many cases of mercury poisoning micro-organisms are responsible for the natural formation of the methylated species, although there are also micro-organisms that de-methylate. The methylmercury balance is therefore a result of two competing reactions. The monomethyl species is soluble in water whereas the dimethyl species is not. However, the pH of the sample can also have an effect on the formation of

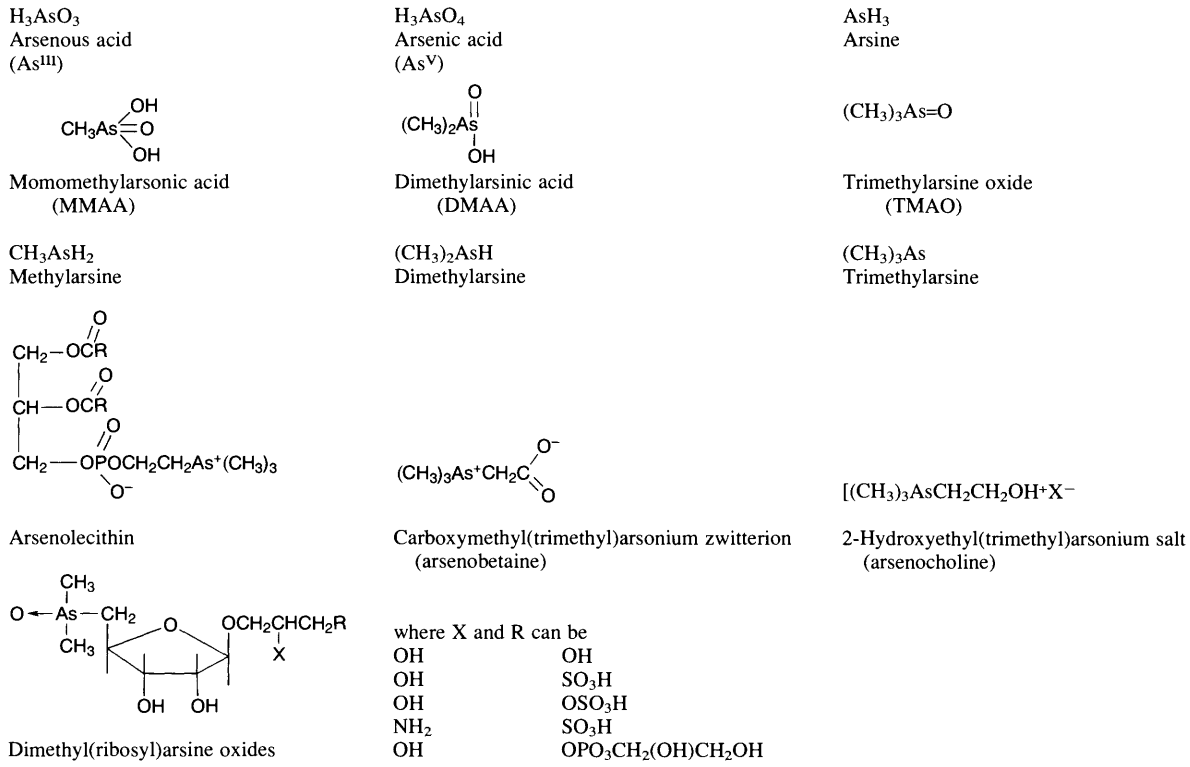

Fig. 2 Structures of arsenic species

the species since at low pH the formation of methylmercury is favoured in preference to the dimethylmercury. The organic forms of mercury are far more easily absorbed than the inorganic form so the methylated species constitute 60–90% of the total mercury in fish. However, the interconversion between the different forms of mercury is complex and it has now been suggested that various mercury cycles may operate in the environment. For example, in sediments the biomethylation of mercury is obviously important whereas in fish methylmercury may result from an atmospheric depositional flux of methylmercury.[3]

Due to the long established importance of mercury speciation, the element has received considerable attention. A review by Puk and Weber[22] has described many of the analytical approaches applied to mercury speciation. However, it is worth remembering that many analysts stabilise samples to be analysed for mercury with dichromate. Although this is extremely effective at preventing loss of total mercury, it will substantially affect the speciation, since the dichromate converts the volatile species to inorganic mercury. Various hyphenated techniques or derivatization methods may be employed successfully for the determination of mercury species although the use of CRMs (certified reference materials) is to be highly recommended.

11 Conclusions

Metal speciation is now well established in many areas of chemistry. Although a lot has been achieved in terms of increasing our knowledge of the behaviour of some elements in the environment, much still needs to be done. Whole areas of environmental chemistry can now be readdressed to provide more insight into the mobility of metals in the environment and how these pathways link with the bioavailability of the metals, bioaccumulation and possible toxicity. Certainly there is parallel interest in metal speciation in the area of clinical chemistry.

At the forefront of such developments is the analytical chemist who must find new ways of extracting the metal species from complex matrices and further develop analytical techniques to perform both qualitative and quantitative analysis at lower and lower levels. Thus, the whole area of metal speciation continues to provide an exciting and challenging arena for research in the future.

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