Speciation Analysis of Heavy Metals in Natural Waters: A Review

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Metal speciation in natural waters is of increasing interest and importance because toxicity, bioavailability, environmental mobility, biogeochemical behavior, and potential risk in general are strongly dependent on the chemical species of metals. This paper provides an overview of the need for speciation of heavy metals in natural waters, the chemical and toxicological aspects of speciation, and the analytical procedures for separation and the different techniques for final determination that are used today. The trends and developments of speciation are also discussed. Finally, the case of chromium (Cr) was selected for a detailed presentation because the speciation of this metal has attracted a great deal of interest in view of the toxic properties of Cr(VI).

Analytical methods for the determination of metals have been developed in the past for the total concentrations of these elements. However, knowledge of total concentrations gives only little or no information about toxicity, environmental mobility, biogeochemical behavior, bioavailability, and potential risk in general, which are strongly dependent on the chemical species of heavy metals (1–4).

Knowledge about metal speciation, i.e., the different physical or chemical forms in which a metal occurs, is widely regarded as crucial to the understanding and prediction of metal behavior and its impact on any environmental system (5). For all these reasons, the problem of element speciation in waters is of increasing interest and importance, and speciation analysis is currently performed routinely in many countries to control the quality of the environment. The importance of metal speciation is now incorporated in new legislative requirements that often list specific species.

Different definitions and various meanings apply to the term speciation. Chemical “speciation” may be defined as the determination of the individual concentrations of the various chemical forms of an element that together make up the total concentration of that element in a given matrix (6, 7). On this basis, it is possible to distinguish 2 different approaches: organometallic speciation, in which metals whose inorganic and organic forms are characterized by different toxicity, mobility, etc. (mainly Hg, Pb, Sn, As, and Se), and inorganic speciation, in which different oxidation states of a metal are characterized by different toxicities and mobilities (Cr, As, Se, and Sb). The need for the determination of individual chemical species occurs especially where these species are known to have a different impact and behavior (8).

The application of equilibrium speciation modeling in aquatic systems is most appropriate to conditions where the oxidation state is constant and where the interactions involve the reversible formation of solution or adsorption complexes. It is less useful in considering changes in oxidation state, the formation and dissolution of precipitates (oxides, carbonates, and sulfides), and the formation of nonlabile complexes (e.g., organometallic forms of Sn, Hg, and Pb) because each of these may be under kinetic and/or biological control (9).

Natural or anthropogenic changes in environmental conditions can strongly influence the behavior of these elements by altering the forms in which they occur. Some of the more important controlling factors are pH, redox potential, and availability of reactive species such as complexing ligands, particle surfaces for adsorption, and colloidal matter (10). 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 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 (11). The aim of speciation analysis is often to provide information about the bioavailability of the elements. Considerable evidence now exists for assuming that the free ionic forms of metals like Cu, Cd, and Al are usually the forms most toxic to aquatic biota and that complexation by natural ligands reduces the toxicity of these metal ions. However, the relationship between metal species and bioavailability can be rather complicated (2).

In speciation analysis, the analytical difficulties are related not only to the choice of relevant techniques for measuring the individual species, but also to the fact that the concentration of the element is often so low that even the total concentration is extremely difficult to determine. A further fractionation is then rarely possible unless preconcentration techniques that will not disturb the species distribution are available. A number of books and reviews have been published in recent years about different aspects of speciation (12–18).
Analytical Procedures

Many analytical techniques, both physical and chemical, are available to measure speciation (19, 20), although rarely can a complete distribution of all species be determined. A complementary approach is the application of speciation models, which estimate element distributions on the basis of known or postulated chemical reactions. In addition, a number of practical difficulties are also 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; 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 containers. Although the preservation of samples is frequently achieved by the addition of acid, as mentioned above, this may not be appropriate for speciation studies because it may lead to changes in oxidation state and alterations in the speciation. For that reason, procedures for sampling and preservation of water samples should necessarily be adopted so that they do not disturb the equilibria established among the species. It is recommended that samples be collected in polyethylene bottles, filtered through a 0.45 μm filter immediately after collection, and stored at 4°C.

In some cases, 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 aluminum. Sampling, separation, and preconcentration procedures must not disturb the equilibria between various species in solution (21, 22).

The determination of chemical species is possible in natural water, air, soil, and sediments, and in biological samples. No analytical method can be trusted to produce reliable results unless its accuracy has been controlled and documented by the use of representative certified reference material (CRM). Until now, very few reference materials have been available for metal speciation analysis in waters (23–25). The speciation of metals in waters is a difficult analytical problem, because the determination of the low concentrations of most metals requires analytical techniques with high sensitivity and selectivity. This problem is even more difficult for the determination of ultratrace levels of the individual species. One of the primary concerns is clean conditions in the working area, because atmospheric aerosols contain metals in the form of particulate matter.

Methods for Separation

It is often necessary to perform some kind of preconcentration procedure. A variety of methods are available including liquid–liquid extraction, freeze-drying, gentle evaporation, and preconcentration using an ion-exchange resin. The choice of the type of extractant is correlated with the nature of the metal, the chemical form of the metal, the matrix from which the compounds are to be extracted, and the analytical techniques available in the laboratory for the final determination. The pH, extraction time, and sample-to-solvent ratio have to be controlled for extraction efficiency (26). Of these different procedures, the liquid–liquid extractions probably yield the best preconcentration factors, with factors of >100 sometimes being possible. This procedure 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 to prevent analyte loss. More accurate results may be obtained if standard solutions of the analytes of interest are treated in an identical manner, because this should overcome many of the problems associated with different species of an analyte having different affinities for the extractant.

In organometallic speciation, the procedure is based on the simultaneous determination of all species. A suitable organic solvent or a mixture is used with or without the addition of complexing agents.

In inorganic speciation, the approach for the determination of different oxidation states is usually based on selective complex formation and subsequent extraction. This approach depends on the different abilities of different oxidation states to form stable complexes, with a complexing agent, at a well-controlled pH.

Most separation methods in use are based on the formation of metal dithiocarbamate complexes and their extraction into an organic solvent. There are different combinations of complexing agents and extractants such as ammonium tetrathiomethylene dithiocarbamate (ATDC), diethylidithiocarbamate (DDTC), or ammonium pyrrolidinedithiocarbamate (APDC) with methyl isobutyl ketone (MIBK) in chloroform, or liquid anion exchanger Amberlite LA-2 in MIBK.
In liquid–solid extraction (LSE), column methods require that the adsorption reaction be not only thermodynamically favorable but also kinetically fast. The eluate from the solid phase should be accurately checked for recoveries. Species can be separated by using cation- and anion-exchange resins in separate columns. In LSE, the preconcentration of the analytes can also be achieved by using columns packed with adsorbent material (e.g., alumina, silica gel, polymer gel, or fine-particle substrate coated with an organic compound). Organic and inorganic species (like Sn) or species with different oxidation forms (like As) can be separated. Chelating resins are simple to use and allow much higher preconcentration factors to be attained. Additionally, the sample is not contaminated with heavy metal impurities from buffers and organic reagents (27).

Methods for Determination

The rapidly growing interest in elemental speciation has brought about the development of a new generation of analytical techniques that enable discrimination among the different forms of a metal or metalloid. These species-specific techniques are shown schematically in Figure 1 (28, 29).

Chromatographic Methods

These methods are especially useful for determining chemical species (30). Liquid chromatography (LC) is generally more applicable than gas chromatography (GC) to speciation studies. Methods have been developed for the separation of most types of inorganic species, such as cations, anions, and metal complexes, in addition to organometallic compounds, by using as the stationary phase a large number of resin supports with anion- and cation-exchange, size-exclusion, chelating, and reversed-phase functionalities. LC is one of the most common methods used for the separation of nonvolatile analytes, and it has been extensively coupled with atomic spectroscopy for detection. A large number of resin supports may be used as the stationary phase with anion-exchange, cation-exchange, size-exclusion chelating, and reversed-phase functionalities. LC has also been interfaced with other detection techniques (e.g., spectrophotometry, fluorimetry, voltammetry, and conductance), but most provide information only on elution rate (31). The combination of LC with element-specific detection such as flame atomic absorption spectrometry (FAAS), graphite furnace AAS (GFAAS), inductively coupled plasma–atomic emission spectrometry (ICP–AES), and ICP mass spectrometry (ICPMS) represent powerful approaches for the determination of metal-containing species.

The LC/ICPMS combination has the particular advantage of an extremely sensitive detection system (32). This technique requires careful selection of the chromatographic conditions because organic solvents and high salt concentrations in buffers can significantly affect ICPMS performance (33, 34). Because the effluent flow rate of an LC column exactly matches the typical liquid flow rate of most commonly used nebulizers, the coupling of both components is quite straightforward, whereas as a result of the possibility of using reversed-phase, reversed-phase ion-pairing, ion-exchange, or size-exclusion chromatography, the application range of LC/ICPMS is wide (35, 36). Hydride generation AAS (HGAAS) has also been used for detection with LC (37, 38).

The use of GC for speciation of inorganic analytes is relatively limited, because most GC detectors are not element-specific. The coupling of GC with atomic spectrometry for detection has been used for analytes that are present in relatively high concentrations. For GC, electrothermal AAS (ETAAS) provides the most suitable detection, but FAAS and ICP–AES have also been used with success. GC requires species that are sufficiently volatile and thermally stable to elute from the GC column. GC has been used, in particular, for the determination of volatile organometallic compounds of Hg, Sn, and Pb. The first step in the analysis may involve derivatization based on the formation of volatile hydrides (39).

Besides LC, GC has also been coupled with ICPMS for speciation work. In comparison with LC/ICPMS, GC/ICPMS offers a higher resolving power and 100% introduction efficiency, allows a more stable plasma, and gives rise to fewer spectral interferences. Of course, GC/ICPMS can be used only for the separation and detection of volatile and thermally stable compounds or compounds that can be derivatized to a volatile form. GC/ICPMS has already been applied to the speciation of a number of elements and especially to the determination of organometallic compounds of Sn, Hg, and Pb in environmental samples (40). Supercritical fluid chromatography (SFC)/ICPMS combines the advantages of GC/ICPMS and LC/ICPMS. SFC/ICPMS shows a high chromatographic resolving power and 100% sample introduction efficiency, whereas—in contrast to GC/ICPMS—it can also be used for studying nonvolatile and thermally labile compounds of high molecular weight. SFC/ICPMS has already been used for the determination of organometallic compounds of As, Sn, Sb, Hg, and Pb (41, 42).

Recently, capillary electrophoresis (CE)/ICPMS has received considerable attention. CE/ICPMS permits a rapid and efficient separation of species, based on their charge and size, through application of a voltage gradient along a capillary (43).

Other Methods for Determination

Many other different analytical techniques have been applied to the determination of ultratrace levels of individual species. AAS, using either a flame or a graphite furnace for atomization, and AES are the most frequently used techniques for determination, and they are coupled with appropriate separation procedures. Concerning electrochemical methods, polarography and voltammetry offer some evident opportunities for speciation studies with techniques like differential pulse polarography (DPP), anodic stripping voltammetry (ASV), and cathodic stripping voltammetry (CSV). ASV is much more sensitive than DPP, but the number of elements that can be determined is more restricted. Higher sensitivity can be obtained with CSV if it is preceded by adsorptive collection of a complex of the metal (44–48). Isotope dilution mass spectrometry (IDMS), an analytical technique that combines high accuracy and precision with good sensitivity, is ap-
plicable to elements that have ≥2 stable isotopes available with an artificially altered isotopic composition (e.g., Cr). Although ICPMS is not species-specific, it offers the advantage of being a very sensitive technique that lends itself very well to coupling with chromatographic separation techniques. ICPMS excels by offering not only extremely low detection limits but also real multi-element capabilities and isotope information; the latter enables the application of isotope dilution techniques, which are unsurpassed for accuracy of quantitation. Considerable work has already been devoted to the performance enhancement of ICPMS for speciation analysis, with respect to detection power and also to ease of operation. Detection limits could be improved by preconcentration or by application of new sample introduction techniques, such as electrothermal vaporization, thermospray (TS), direct injection nebulization (DIN), ultrasonic nebulization (USN), hydraulic high-pressure nebulization (HHPN), and direct coupling of GC with ICPMS (49).

Flow injection analysis (FIA) is a very useful technique for speciation studies, but it is not a separation technique itself. Although FIA does not have the discriminating power of LC, it can be effectively applied to speciation studies because of its many possibilities as an automated, continuous, unsegmented methodology. The high versatility of FIA enables discrimination between different chemical forms in straightforward mixtures such as Cr(III) and Cr(VI), Fe(III) and Fe(II), As(V) and As(III), and Se(IV) and Se(VI), most of which involve inorganic species. However, FIA does not yet allow speciation of more complicated mixtures of metals and organometals. On the other hand, the association of FIA with LC opens up interesting new prospects in speciation studies (50).

**Chromium**

The case of Cr is particularly representative in this context because it demonstrates that speciation studies need a critical consideration of the system analyzed to perform the test most suited to obtain useful and accurate information. Chromium is ubiquitous in nature, occurring in various forms that are chemically, physically, and morphologically different. Most surface waters contain very low levels of Cr (from 1 to a few micrograms per liter), except for wastewaters coming from industries. Industries dealing with paints, pigments, dyes, mordant, rubber, plastics, ceramics, textiles, leather, and steel and its alloys, as well as welding activities, smelter works, and its adverse effects on lung, liver, and kidneys. Inhalation of Cr(VI) may cause bronchial carcinomas. The mechanisms of Cr metabolism, genotoxicity, and carcinogenicity are extensively described (54, 55). The Directive of the European Commission (EC) 80/778/EEC, L229/20, D48 (17) states that total Cr should not exceed 50 μg/L in drinking water. In occupational health, the occupational exposure limits (OELs) for soluble and certain water-insoluble compounds in indoor air are limited to 0.5 mg/m³ for Cr and 0.05 mg/m³ for Cr(VI), which reflect the different toxicities for both species.

Not only the toxicity but also the mobility and bioavailability of Cr depend fundamentally on its chemical form. Cr(VI) compounds are usually highly soluble, mobile, and bioavailable compared with sparingly soluble trivalent Cr species. Thus, a detailed knowledge of each species rather than the total Cr level is required to properly evaluate the physiological and toxicological effects of Cr, its chemical transformations in water, soil, and the atmosphere, as well as its distribution and transport in the environment.

The analytical chemistry of Cr has undergone considerable change in the last decade. Many different analytical techniques have been used in an effort to quantitate the various Cr forms present in the natural environment. Chromium speciation methods have been reviewed in several papers (56–58) in which, however, only a limited number of specified techniques are usually taken into account.

Sampling, separation, and preconcentration procedures may disturb the equilibrium between various species in solution to a significant degree. One of the greatest difficulties results from the redox equilibrium between Cr(III) and Cr(VI). Its great sensitivity to changes in pH and the addition of oxidizing or reducing agents must be considered in every step of the sample treatment until the species separation has been performed. In the case of Cr, acidification not only affects particle size distribution but also induces severe matrix effects in speciation analysis, especially by altering its oxidation state.

Concentrations of Cr in natural waters are very low. Thus, preconcentration of Cr is usually necessary. Some of the techniques used for preconcentration include coprecipitation, solvent extraction using a variety of reagents, ion exchange, and electrodeposition (59). Because of the lack of speciation standards containing Cr(III) and Cr(VI), sometimes the total Cr content of standard reference materials is used to check the accuracy of the procedures. For Cr speciation, however, strong extraction agents, enhanced temperature, and long extraction time should be avoided, because Cr(VI) is sensitive to reduction in strongly acidic media, whereas Cr(III) is sensitive to oxidation in strongly alkaline solution. One promising extraction technique that limits conversion of species is supercritical fluid extraction (SFE). The extractions performed at low temperatures and over short times may prove advantageous for thermally labile and easily oxidized Cr compounds (60).

An evident handicap in speciation analysis is that Cr in environmental compartments is commonly at trace or ultratrace levels and, consequently, its different chemical species are...
even more elusive. Consequently, Cr speciation must rely on superior separation and detection capabilities, using methods of guaranteed accuracy and precision. Today, a variety of techniques are available for Cr analysis. Among them are techniques that allow simultaneous determination of trivalent and hexavalent Cr as well as methods involving separation and detection of one of these forms and total Cr concentration. Usually, Cr speciation involves only differentiation between Cr(VI) and Cr(III) compounds.

The standardization and simplification of the speciation schemes, the development of controlled, reliable, and reproducible sampling, storage, and pretreatment procedures, and the study of CRMs are essential to achieving the goal of Cr speciation. To minimize perturbation of systems like anoxic waters, the sediment–water interface, and colloidal materials, some new analytical methods for the determination of Cr species in situ have to be developed.

Determination of low concentrations of Cr and trace levels of the individual species in samples of natural water requires analytical techniques with a sufficiently high sensitivity and selectivity such as FAAS, ETAAS, ICP–AES, direct current plasma AES (DCP–AES), ICPMS, IDMS, neutron activation analysis (NAA), spectrophotometry, and electrochemical procedures (61–69).

Recently, Kingston and Koropchak (70) presented an application of speciated isotope dilution MS (SIDMS) for Cr speciation in water samples. SIDMS is the first technique to evaluate the species conversion that may occur during sampling and pretreatment procedures. This technique improves accuracy in the determination of chemical species in samples, and it can be used to measure elements in their oxidation states as well as in organometallics or other molecular forms of species. Information about chromatographic techniques with off-line separation and preconcentration or about on-line procedures for Cr is available (71, 72).

The many varied techniques used today are not yet fully satisfactory for all needs, indicating that further development of new methods and approaches is still essential. For the FAAS technique, it has been clearly shown in the literature review that Cr speciation is strongly affected by interferences, and that for the ETAAS technique the conditions are more dependent on the sample matrix and the characteristics of the instrument used (73). These are the most frequently used techniques for the determination of Cr. The detection limits for simple aqueous Cr solutions are 3 and 0.08 μg/L with FAAS and ETAAS, respectively. These spectrometric techniques coupled with appropriate separation procedures allow the selective determination of trivalent and hexavalent Cr (74).

Hexavalent Cr in aqueous solution can be determined by ion chromatography with subsequent detection by ICPMS. The detection limit of this method is 1–2 ppb (75). In a comparison with the cost of equipment of other methods, the ICPMS method was the most expensive. Of the existing electrochemical techniques, voltammetry is the most popular for the determination of Cr. High sensitivity can be obtained by CSV protected by adsorptive collection of a complex of Cr(III) (76, 77). IDMS has proved to be a reliable technique for Cr speciation. Because of the necessary species separation, the amount of time consumed by a technique like thermal ionization IDMS is not much greater than that for a technique like GFAAS, which is normally much faster for total element analysis. The high accuracy linked with IDMS results makes it a technique of special interest. Another important topic in a developed IDMS method is that both species are directly determinable. This is a great advantage compared with the results of indirect determinations, in which only 1 species besides the total element content is determined (78).

Energy dispersive X-ray fluorescence spectrometry (EDXRF) is well suited for the separation of Cr(VI) and Cr(III) from each other and for the determination of their concentrations in aqueous solutions. This technique is simple and relatively rapid and provides results of good precision and accuracy, but it is not directly suitable for surface waters because of their low concentration of Cr.

**Trends and Developments in Speciation**

Interest in the speciation of metals continues to expand, as shown by the large number of reports published in many journals. A large number of analytical techniques have been used in the investigations. An important trend is the application of more complex and expensive instruments and equipment. This is justified mainly by the need for detection and quantitation of the very low concentrations of species in real samples. Selective extraction procedures have been used for many years to obtain fractions of samples for subsequent analysis. Coupled-instrument methods continue to gain importance. There is a large variety of such methods. Chromatographic methods of separation are the most important. Attention is being given also to the application of SFC to speciation studies. Details of some of the developments in analytical procedures have been reported for a number of elements such as Al, As, Hg, Pb, Sn, and Cr, and there has been increasing concern about the accuracy and precision of the analytical data. Validation of speciation methods is greatly needed because the use of these methods can lead to a high probability of errors as a result of the different analytical steps that are usually required (e.g., extraction, derivatization, separation, and detection).

Concerning the use of reference materials, it is well known that no analytical method can be trusted to produce reliable results unless its accuracy has been verified by the use of a representative CRM. Although many CRMs are available for analytical programs giving information on the total concentrations of selected elements, at the moment very few CRMs with species-specific data are available. For that reason, the production of a range of reference materials specifically for speciation work is now under way. CRMs for metal speciation analysis for Sn, Hg, As, Cr, Se, and Pb and for different matrixes are available or are currently being prepared. The main agencies that produce CRMs for speciation analysis are the National Research Council, Canada (NRCC), the National Institute for Environmental Studies, Japan (NIES), and the Bureau of Community Reference, EC, Europe (BCR). Aqueous CRMs should contain the species of the elements in concentrations rep-
Metal speciation is well-established today in many areas of chemistry, especially in environmental chemistry. Although a lot has been achieved in terms of increases in our knowledge of the behavior of some elements in the environment and in metal speciation measurement techniques over the past 25 years, much still needs to be done.

There is a need for fundamental research on the occurrence and fate of new species (toxicity, environmental pathways) and the development of new analytical techniques for their determination, as well as for increased efforts for the improvement, development, and validation of techniques for ligand and redox-state speciation. Development/improvement of techniques such as high resolution ICPMS (e.g., for Cr and Co) and sensitive detection techniques coupled with electrophoresis (e.g., for Ni speciation) must also be investigated (79, 80).

The methods that have been developed do not provide an absolute breakdown of metal species, but rather operationally defined classifications. Because of this operational nature, standardization of the procedures is essential, if different results are to be compared. Thus far, this has rarely been achieved (81).

It is also necessary to develop new in situ analytical methods for species determination, to study the role of living organisms in trace metal control, to develop chemical speciation schemes that can be directly related to measures of bioavailability, and to emphasize the need for experimental conditions to be as close as possible to those prevalent in the environment.

References
