GUIDELINES FOR TERMS RELATED TO CHEMICAL SPECIATION AND FRACTIONATION OF ELEMENTS. DEFINITIONS, STRUCTURAL ASPECTS, AND METHODOLOGICAL APPROACHES

INTRODUCTION

Cr(VI) ions are considered far more toxic than Cr(III). While both methylmercury and inorganic mercury are toxic, they show different patterns of toxicity. Often these different chemical forms of a particular element or its compounds are referred to as "species". The notion that the distribution among its various species will have a major effect on the behavior of a particular element has been accepted in such diverse fields as toxicology, clinical chemistry, geochemistry, and environmental chemistry. New developments in analytical instrumentation and methodology now often allow us to identify and measure the species present in a particular system.

DEFINITIONS OF TERMS RELATED TO SPECIATION AND FRACTIONATION

i. *Chemical species*. Chemical elements: specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure
ii. *Speciation analysis*. Analytical chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample
iii. *Speciation of an element; speciation*. Distribution of an element amongst defined chemical species in a system
iv. *Fractionation*. Process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties.

STRUCTURAL ASPECTS OF SPECIATION

Isotopic composition
The isotopic abundances of an element can vary for several reasons, including radioactive decay (radiogenic) and physical separation (anthropogenic or environmental separation). Anthropogenic differences in isotopic composition occur in addition to those arising from radiogenic and environmental factors.
Only for very light elements do primary kinetic isotope effects become sufficiently large that differential toxicity arises, and in toxicology, isotopic speciation analysis is mainly of interest for identifying sources of exposure. Therefore, for all but the lightest elements, biological interest in isotopic composition rests largely with tracer studies.

Electronic and oxidation states
The oxidation state of an element can profoundly affect its toxicity. Cr(III) may be an essential element, but Cr(VI) is genotoxic and carcinogenic. The case of chromium also illustrates how the oxidation state influences bioavailability. Cr(VI) is taken up by many cells as chromate (CrO_4^{2-}) utilizing anion transporters, whereas Cr(III) ions permeate the lipid membrane with difficulty. In contrast to chromium, the more reduced species of As are the more toxic: arsine (AsH_3) > arsenite (As(III)) > arsenate (As(V)).
The oxidation state can also affect the absorption and elimination of an element. The Fe(II) ion is soluble under physiological conditions and diffuses freely across membranes, whereas Fe(III) does not enter cells readily and is more prone to hydrolysis in aquatic and biological systems. The Hg(II) ion becomes trapped in cells, but some bacteria possess a mercuric reductase system that reduces Hg (II) to volatile Hg^0, which then diffuses from the cell.

Inorganic compounds and complexes

The distribution of an element among different inorganic compounds profoundly affects its transport and bioavailability by determining such properties as charge, solubility, and diffusion coefficient. The practical importance of inorganic speciation analysis is well illustrated by occupational exposure to Ni and its compounds. Nickel salts such as chloride and sulfate are water soluble and of low oral toxicity. Nickel oxides and sulfides are highly insoluble in water, but their bioavailability may be influenced by biological ligands. Trinickel disulfide (subsulfide) (Ni_3S_2) is a potent carcinogen in animals. Exposures are seldom to a single species of Ni; for example, in various refining operations, workers may encounter Ni_3S_2, NiO, Ni_0, Ni.Cu oxides, Ni.Fe oxides, $NiSO_4$, $NiCl_2$, and $NiCO_3$. Therefore, an air filter analyzed for exposure monitoring may contain Ni salts deposited from aerosols as well as other inorganic forms of the metal and particulates of different sizes. While a fractionation (for instance, determining soluble and insoluble forms or the particle size distribution) provides more information than measuring total Ni only, additional useful information on species of differing carcinogenic potential can be obtained by speciation analysis.

Complexation reactions with inorganic ligands produce coordination complexes with varying stabilities. The distribution of species in a system containing both metal and ligand will depend on factors such as concentrations, stoichiometry, pH, and ionic strength. This means that such species cannot normally be separated from each other without changes in distribution. However, the lability of inorganic complexes varies within very wide limits from complexes that form and dissociate rapidly, to systems in slow exchange, to complexes that, for most practical analytical purposes, can be considered inert. Hydrolysis is an important aspect of metal ion chemistry. For many metals, formation of hydroxides in aerobic aqueous environments at neutral pH is a key determinant of their solubility and bioavailability. The reaction $M^{n+} + nH_2O \leftrightarrow M(OH)_n\downarrow + nH^+$ frequently produces neutral metal hydroxides with extremely low solubility (e.g., $Fe(OH)_3$, $K_s \approx 10^{-38}$ M^4). Most metals, excluding alkali and alkaline earth elements, form one or more hydroxocomplexes under natural conditions.

The interplay between kinetic and thermodynamic control of speciation can give rise to surprising effects. In the United Kingdom and Norway it was found that the mixing of two waters, one acidic with high concentration of labile monomeric aluminum and the other with high pH, had serious effects on fish. In the actual mixing zone the water was considerably more toxic than any of the components on their own. The cause was found to be the formation of transient polymeric aluminum-hydroxo complexes with high toxicity. Minutes later the extreme toxicity was gone.

A large variety of inorganic complexes can be found in the environment. Anions such as SO_4^{2-} and Br^- generally form weak complexes which are not very important in open fresh waters. However, in certain waters such as well water or soil solutions and in marine waters they may be important. Other anions such as F^- and CO_3^{2-} are important for specific elements. The most commonly occurring type of inorganic complex by far is the hydroxocomplexes as discussed above.

Organic complexes

Complexation reactions with organic ligands produce coordination complexes with differing degrees of thermodynamic stability and lability, the latter term being related to the reactivity of the species. The distribution of species in a metal-ligand complex also depends on such factors as concentration, stoichiometry, pH, and ionic strength. Whereas some complexes may be *labile* during analysis, some organic metal chelates (e.g., ferrioxamine) are of sufficient thermodynamic stability to be isolated quantitatively under a number of conditions.

Unfortunately, the large number of organic ligands available compromises the predictive utility of stability constants; the "complete" species distribution is never known and predictions of toxicity or bioavailability are successful only when the picture is simplified.

Organometallic compounds

Organometallic compounds are defined as compounds containing a covalent bond between a carbon atom and a metal. The practice in nomenclature is to consider any element other than C, H, and the rare gases to be metals if this is useful. Some elements form organometallic compounds with a metal-carbon bond of strong covalent character. In toxicology, hydrophobicity and volatility are important. Toxicity often results from bioaccumulation in fatty tissues and penetration of membrane barriers.

In environmental alkylation, an important distinction can be made between biomethylation and addition of a larger alkyl chain. In general, metals undergo only biomethylation, important examples being Hg, Ge, and Sn. Biomethylation of metals is generally restricted to microorganisms in sediments and soils. Methylation of metals generally increases their toxicity.

In addition to the products of bioalkylation, manufactured organometallics find their way into the environment, and those of major interest in speciation analysis include compounds of As, Sn, Hg, and Pb (e.g. herbicides and fungicides).

Macromolecular compounds and complexes

The highest structural level at which speciation analysis is considered here is the macromolecular level. At this level, limits to the definition of unique chemical species must be set.

Natural organic matter (NOM) is a heterogeneous mixture of organic compounds derived from decomposition of biotic matter. NOM in the dissolved form is ubiquitous in natural waters, where levels commonly range from <0.5 to 40 mg C L^{-1}. In the environment, binding to dissolved natural organic matter (DNOM), such as the polyanions humic and fulvic acids, contributes to the pattern of transport and availability of elements such as Cd, Cu, and Pb and sorb organic micro pollutants enhancing thereby solubility and mobility in the environment.

METHODOLOGICAL APPROACHES FOR SPECIATION ANALYSIS

Isotopic composition

Isotope ratios can be measured by mass spectrometric techniques and can be used to determine the environmental origin of the element (e.g. Pb).
Thermal ionization mass spectrometry (TIMS)
Inductively coupled plasma quadrupole mass spectrometry (ICP-MS)

Electronic and oxidation states

Chromium is given as an example. Cr(VI) can be selectively determined spectrophotometrically after complex formation with 1,5-diphenylcarbazide. Separation of Cr(III) and Cr(VI) is frequently achieved by ion-exchange HPLC with detection by AAS.

Inorganic compounds and complexes

Hydrolysis of Al^{3+} is an example for inorganic complexes. While one would like to observe the individual products of hydrolysis of a metal ion directly with optical or magnetic spectroscopic techniques, hydrolysis reactions rarely lead to a sufficiently simple speciation to allow individual components to be observed. Generally, potentiometric determination of pH is used to deduce stoichiometries based on the reaction

$$xM^{z+} + yH_2O \leftrightarrow M_x(OH)_y^{(xz-y)+} + yH^+$$
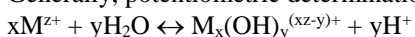
Supplementary methods are used to deduce the presence of polynuclear complexes, including solubility measurements, ultracentrifugation, and light scattering. In dilute solutions of Al salts, pH measurements have shown the formation of $AlOH^{2+}$, and competition with OH^- by colored organic ligands has further yielded stability constants for $Al(OH)_2^+$ and $Al(OH)_3$. In alkaline solution, solubility studies of gibbsite ($Al(OH)_3$) have given a stability constant for $Al(OH)_4^-$ and this tetrahedral species was observed by IR and Raman spectroscopies.

Organic complexes

Potentiometric data are widely used to derive stability constants of metal-ligand complexes that allow modeling of the species distribution as a function of pH. The complete set of stability constants $\beta_\lambda = [MH_vL_\lambda] / [M][H+]^v[L]^\lambda$ allows calculation of the concentration of each species as a function of pH, provided the total metal concentration, [M], is known.

When dissolved species are electroactive at the mercury electrode, electrochemical techniques such as anodic stripping voltammetry (ASV) and adsorptive cathodic stripping voltammetry (ACSV) are the most direct methods for studying trace metal speciation at very low concentrations ($10^{-8} - 10^{-11}$ M).

Organometallic compounds

Speciation analysis for organometallic compounds is usually carried out by hyphenated techniques based on the coupling of chromatography with element-selective detection.

Thermally stable and volatile species, and those that can be converted into such, are preferably separated by gas chromatography. Species not amenable to GC are separated by HPLC or capillary zone electrophoresis. ICP-MS is by far the most widely used detection technique.

Macromolecular compounds and complexes
Chromatographic techniques dominate the analysis for metal–organic macromolecular complexes. There is a wide variety of columns available for size-exclusion, ion-exchange, affinity, and reversed-phase chromatographies.

When using these techniques it is often assumed that the macromolecule and the metal that are found in the same fraction were associated with one another in the original sample. Before this can be concluded, it is necessary to ensure that dissociation of the metal from the bioligand has not occurred. Unfortunately, such dissociation can be affected by the buffer and the column packing, and this is the rule rather than the exception. When a complex sample such as humic matter is fractionated, each fraction will still contain numerous potential ligands, and the unequivocal association of a metal with a given component is by no means straightforward. The association must be supported by additional evidence, for instance, from mass spectrometry or affinity chromatography.

FRACTIONATION
For the reasons given briefly above, an assessment of the impact of an element cannot be made based solely on its total concentration. It is, however, often not possible to determine the concentrations of the different chemical species that sum up to the total concentration of an element in a given matrix. Often, chemical species present in a given sample are not stable enough to be determined as such. During the measurement process the partitioning of the element among its species may be changed. This behavior can be caused by, for example, a change in pH necessitated by the analytical procedure, or by intrinsic properties of measurement methods that affect the equilibrium between species. Very common but less obvious is the situation where the analyte must be transformed into a complex to be measured or separated from the sample matrix. Even in the idealized situation that the complexation reaction is highly selective, errors can occur if the sample contains other species that can be transformed into the target analyte within the time frame of the analysis.

In some cases, species are stable enough to be determined as such. In a given situation the determination of the concentration of such a species may be what is actually desired. However, this does not mean that the speciation of the element has been determined, only the concentration of one or a few species. The direct determination of labile species at their natural levels requires noninvasive methods such as direct spectroscopic or potentiometric measurements that do not perturb the sample.

In many cases the large numbers of individual species (e.g., in metal-humic acid complexes) will make it impossible to determine the speciation. The practice has been to identify various classes of species of an element and to determine the sum of its concentrations in each class. This practice is useful and will continue. Such fractionations can be based on many different properties of the chemical species, such as size, solubility, affinity, charge, and hydrophobicity. Fractionation may involve an actual physical separation (e.g., filtration, size-exclusion chromatography). In some instances, fractionation may be refined by supplementary speciation analysis. For example, for aluminum in natural waters a fractionation can be performed to determine organic and inorganic aluminum concentrations. With further analyses and calculations the inorganic fraction can be subdivided into individual species.

Many existing methods are claimed to give concentrations for "free" or "available" elements. Most of these methods are based on electrochemical and/or kinetic discrimination. In order to produce information on the chemical speciation of the elements the measured fraction is factored into chemical species concentrations using an equilibrium model. These attempts at indirect measurements of speciation basically suffer the same shortcomings as do fractionations according to size. The discrimination inherent in the method of measurement can be more or less selective but it is not absolute. Further, the equilibrium model applied is based on a choice of complexes and their stability constants, which makes the results questionable.

Although a direct determination of the speciation of an element is often not attainable, the available methods can still provide much useful information. An evaluation of the environmental impact of an element may sometimes be made without determining its speciation and even though the fractions are only operationally defined.

METHODOLOGICAL APPROACHES FOR FRACTIONATION

A typical example of an operationally defined fractionation procedure is that for aluminum in natural waters. The method is based on kinetic discrimination, measuring only those aluminum species that react within the timeframe defined by the analytical procedure. In order to achieve consistency between results obtained using different methods and by different analysts it is important to validate procedures as far as possible against solutions of relevant species with known concentrations and known labilities. The procedure for "reactive" aluminum can be validated based on the interaction between aluminum and fluoride in synthetic solutions.

Another example is fractionation using membranes of different pore sizes. The most obvious fractions are of dissolved species and particulates, but fractionations according to molecular size are also possible using appropriate membranes. However, one should keep in mind that the distinction between dissolved and particulate fractions is arbitrary and that the results obtained with a given set-up are critically dependent on operating conditions. For instance, the retention of iron colloid particles on a standard

0.45-µm membrane filter is strongly dependent on the filter load. When the filter clogs it starts retaining much smaller particles as well. Further, the molar mass cut-off given for a certain type of membrane is based on measurements using a certain set of calibrants, often with globular shape, and this cut-off does not necessarily apply to all the chemical species fractionated.