Recent developments in trace metal speciation in fresh water

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Abstract – Trace metal speciation, i.e., determination of individual concentrations of various physicochemical forms of a trace metal, is becoming increasingly important in the analysis of fresh waters. This paper concisely describes the present status and future prospects of this field, particular emphasis being laid on separation and concentration techniques. In the author's laboratory, centrifugation, flotation and sorption have been successfully applied to trace metal speciation as separation and concentration techniques for suspended particulate matter, inorganic colloids and humic substances.

INTRODUCTION

In fresh waters, trace metals may exist in various physicochemical forms, which include hydrated ions of different oxidation states, inorganic and organic complex ions, nonionic dissolved species and colloids. Trace metals are also frequently adsorbed on, occluded in, or included in, inorganic, organic or biological suspended particulate matter. TABLE 1 shows some examples for copper. The transport, behavior and fate in the environment as well as biological effects (bioavailability, toxicity, etc.) of a trace metal will differ greatly depending on its physicochemical forms. Therefore, information on the physicochemical forms of trace metals in fresh waters is very useful in studies of geochemistry, biological sciences, environmental problems and water treatment. From the standpoint of analytical chemistry, it is often quite dangerous to analyze fresh waters for trace metals and interpret the analytical results without paying attention to different behavior of each physicochemical form of a trace metal in separation and determination steps.

TABLE 1. Physicochemical forms of copper in fresh waters

Form	Example	Approximate size (nm)
Hydrated ions	$Cu(H_2O)_6{}^{2+}$	1
Other inorganic ions and complexes	$CuHCO_3{}^+$, $CuCO_3$, $CuOH^+$, $Cu(OH)_2$	1 – 3
Organic complexes	Cu-amino acid, Cu-fatty acid, Cu-fulvic acid	2 – 6
Associated with colloidal species	Fe_2O_3, MnO_2, clay, humic acid, humic acid-Fe_2O_3	10 – 500
Adsorbed on suspended particulate matter	clay, soil, microorganisms	>500

Consequently, in recent years, trace metal speciation, i.e., determination of individual concentrations of various physicochemical forms of a trace metal, has become one of the most important problems in water analysis (refs. 1-3). Two approaches are used in speciation studies. One is the calculation method, in which equilibrium concentrations of various species are calculated with the aid of computers by using pH, the redox potential, equilibrium constants, and measured total concentrations of trace metals and other constituents interacting with them (e.g., chelating agents). Application of this method is rather restricted because of inaccurate equilibrium constants, lack of equilibrium data, especially for interaction with particulate matter, and nonequilibrium conditions which may exist in fresh waters. In connection with this approach, studies are useful on interaction of metal ions with particulate matter, colloidal particles and chelating agents in artificial model solutions.

The other approach is the experimental one, in which a specified species of a trace metal is determined. Such determination techniques as atomic absorption spectrometry, atomic emission spectrometry and neutron activation analysis, however, are not capable of differentiating various species of a trace metal. Ion-selective electrodes can selectively detect some hydrated metal ions, but their sensitivity is not sufficient in most cases. Although spectrophotometry and voltammetry are species-specific to some extent, their selectivity is not satisfactory. In general, it is difficult to directly determine a specified species of a trace metal in fresh waters. Therefore, separation and concentration techniques are required prior to determinations in speciation studies. Combined with separation and concentration techniques, conversion of species (e.g., from adsorbed to soluble species, from organic to inorganic species) by acidification, ultraviolet photolysis, oxidation and reduction is also useful in trace metal speciation. So far, a number of measurement schemes for trace metal speciation in fresh waters have been proposed combining the available determination, separation and conversion techniques in different ways. Most of them, however, do not give data for well-defined species but so-called "operationally-defined" data which depend on various operating conditions.

SEPARATION AND CONCENTRATION TECHNIQUES FOR SPECIATION STUDIES

Various separation and concentration techniques proposed to date for speciation studies will be briefly described (refs. 4, 5). In addition to careful control of contamination and losses, which is generally required in trace element analysis, possible equilibrium shifts and physicochemical changes between various species during the separation steps must always be kept in mind. From these viewpoints, most of the existing techniques are more or less unsatisfactory.

Filtration

This technique is widely used for separation and size fractionation of particulate matter in water. For size fractionation, filtration through polycarbonate etched-track membrane filters (Nuclepore) of various nominal pore sizes ranging from 12 to 0.015 μm is most recommended using either positive or negative applied pressure. For smaller particles, ultrafiltration is used under higher applied pressures. Ultrafilters consist of a filtration membrane on a porous support with various nominal pore sizes ranging from 14 to 1.1 nm, which correspond to nominal molecular weight cutoffs of 3×10^5 to 500.

Dialysis

Diffusion through dialysis membranes of nominal pore sizes ranging from 5 to 1 nm, corresponding to molecular weight cutoffs of 10^4 - 10^3, is used to separate smaller species from colloidal species in water. In the _in situ_ dialysis technique, a dialysis bag filled with purified water is directly immersed in the water body of interest to collect smaller species exclusively. A long time is required to attain the equilibrium. The Donnan effect should also be considered, when nondialyzable anions are present. In conventional laboratory techniques, purified water is changed periodically or recycled through a chelating resin column to effect nearly perfect separations. In the Donnan dialysis technique, a cation exchange membrane, which is permeable to cations and impermeable to humic substances and anionic complexes, is used to concentrate hydrated metal ions in the water sample into a salt solution.

Gel filtration

This technique is based on inclusion and subsequent elution of solutes through a column containing porous polymeric gel (e.g., dextran gel Sephadex) as molecular sieve. It can be used to fractionate humic substances containing trace metals in water according to their molecular size or molecular weight differences, provided that an appropriate concentration technique is combined with it.

Centrifugation and Flotation

These two techniques will be discussed later in detail.

Carrier precipitation

Chromium(III) is coprecipitated with hydrated iron(III) oxide, leaving chromium(VI) in solution. Chromium(VI) is coprecipitated with barium sulfate after masking of chromium(III) with salicylic acid.

Electrodeposition

The species deposited on the mercury or solid cathode include hydrated metal ions and metal complexes which will dissociate in the diffusion layer to liberate metal ions, depending on the deposition potential, the electrode system and other operating conditions. This technique is usually combined with subsequent electrochemical stripping in anodic stripping voltammetry (ASV).

Sorption

A column of iminodiacetate chelating resin (Bio-Rad Chelex-100) sorbs hydrated metal ions and dissociable metal complexes. Inert metal complexes and most of metals associated with colloidal particles will not be retained on the column.

Chromium(III) is collected on preformed hydrated iron(III) oxide, leaving chromium(VI) in solution. Chromium(VI) is sorbed on a poly(dithiocarbamate) chelating resin column, while chromium(III) is not. Chromium(III) and chromium(VI) are converted into their tetramethylenedithiocarbamate complexes and separated from each other by reversed-phase high-performance liquid chromatography.

Methylmercury and mercury(II) sorbed on activated carbon are differentiated by desorption with 0.1 M nitric acid-95 % acetone.

Separation by sorption of heavy metals complexed with humic substances will be discussed later in detail.

Liquid–liquid extraction

Nonpolar organic species (organically associated metals) in water can be extracted with organic solvents such as chloroform and a hexane-butanol mixture. Chromium(VI) is selectively extracted as diethyldithiocarbamate or tetramethylenedithiocarbamate into isobutylmethyl ketone or chloroform, leaving chromium(III) in the aqueous phase.

Volatilization

Inorganic tin(IV) and organotin compounds are volatilized as hydrides by reduction with sodium tetrahydroborate, collected in a liquid nitrogen trap, and then separated from each other by fractional volatilization. Under proper conditions, inorganic mercury is selectively reduced to elemental mercury with tin(II) or tetrahydroborate in the presence of organomercury compounds and volatilized for cold-vapor atomic absorption spectrometry. Similarly, arylmercury compounds are separated from alkylmercury compounds.

Several separation and concentration techniques developed in the author's laboratory follow.

CENTRIFUGATION

This technique is useful to separate and concentrate particulates having densities different from that of water. A spherical solid particle of diameter d and density ρ in a liquid of density ρ_0 moves during the centrifugation time t according to the following equation:

$$\frac{x}{x_0} = \exp\left[\frac{d^2(\rho-\rho_0)\omega^2 t}{18\eta}\right] \tag{1}$$

where x_0 and x are the initial and final distances of the particle from the

rotation axis, ω is the angular rotation velocity, and η is the viscosity of the liquid. This equation is based on Stokes' law, which is valid under the following conditions: (1) The motion of the particle is slow. (2) The suspension is dilute. (3) The liquid is considered to be continuous compared with the particle diameter. (4) The particle is uncharged.

The application of centrifugation to speciation studies in fresh waters has so far been rather limited and not fully evaluated. Recent studies in the author's laboratory have revealed usefulness of this technique.

Separation of suspended particulate matter

Centrifugation is a concentration technique suitable for the determination of heavy metals associated with suspended particulate matter by microscale ASV (ref. 6). Less than 1 mg of suspended particulate matter in 50 ml of pond water was separated by centrifugation at 3500 rpm for 20 min and decomposed completely with 2 µl of 9 M perchloric acid and 10 µl each of 14 M nitric and 28 M hydrofluoric acids at 150° C for 1 h in a closed Teflon microvessel. The solution was then converted into 300 µl of 0.06 M perchloric acid solution by evaporating off nitric and hydrofluoric acids and then diluting with water, and copper, lead, cadmium and zinc were determined by microscale differential pulse anodic stripping voltammetry (DPASV) after pre-electrolysis on a hanging mercury drop electrode at -1.2 V vs. the SCE for 10 min. The procedure was simple and rapid and contamination and losses were minimized, because the single Teflon microvessel was used throughout the whole procedure —— from centrifugation to stripping. As shown in TABLE 2, the proposed technique gave sufficiently reproducible analytical results.

TABLE 2.   Heavy metals associated with suspended particulate matter in pond water

Sample[a]	Particle concentration (mg/l)	Aliquot No.	Metal (µg/l)			
			Cu	Pb	Cd	Zn
I	10	1	3.0	1.0	0.4	9.0
		2	2.3	1.0	0.4	8.0
		3	2.5	1.4	0.4	6.7
II	15	1	3.5	2.0	<0.1	6.5
		2	3.0	2.7	0.2	10.0
		3	3.8	1.9	<0.1	8.0

[a]Sampled on separate days.

Size fractionation of suspended particulate matter

Centrifugation and filtration were compared for the size fractionation of suspended particulate matter prior to selective leaching of the adsorbed heavy metals (ref. 7).

In the centrifugation technique, 40 ml of water was centrifuged in a 50-ml centrifuge tube using a swinging rotor for the quantitative sedimentation of 5.0- or 0.4-µm particles according to Eq. 1. After removal of the supernatant by suction, 300 µl of leaching solution (0.1 M hydrochloric acid or 1 M nitric acid) was added to the centrifuge tube, mixed by vibration, and then centrifuged at 3500 rpm for 15 min. Heavy metals in the leaching solution and the supernatant were determined by microscale DPASV under the following conditions: 300 µl of 1 M nitric acid-0.1 M hydrochloric acid electrolyte, pre-electrolysis on a hanging mercury drop electrode at -1.0 V vs. the SCE for 5 min.

In the filtration technique, 50 ml of water was filtered through a 5.0- or 0.4-µm Nuclepore membrane filter, which was then treated with 4 ml of the leaching solution in a 100-ml beaker under ultrasonic irradiation for 1 min. The solution was centrifuged at 3500 rpm for 15 min and delivered to DPASV using 3 ml of 1 M nitric acid-0.1 M hydrochloric acid electrolyte.

For size fractionation, centrifugation is inherently inferior to filtration from the following two facts: (1) The fractionation depends on $d^2(\rho-\rho_0)$ as shown in Eq. 1. Particles of different densities may exist in fresh waters. (2) Smaller particles near the bottom of a centrifuge tube inevitably sediment along with larger ones under the condition of perfect recovery of the latter, even if the density is the same. On the other hand, centrifuga- tion is superior to filtration in the following two respects: (1) It can directly evaluate sedimentation behavior of suspended particulate matter. (2) The detection limits are lower owing to less contamination hazards and smaller volumes of leaching solutions required.

The two techniques gave consistent results in the analysis of water containing 25 mg/l each of kaolin and bentonite (particle size 0.4 - 10 μm, density 2.5 g/cm^3) and 5 μg/l each of copper(II), lead and cadmium ions. Also, as shown in TABLE 3, the analytical results obtained by both techniques for two fresh water samples were in fairly good agreement with each other. The particle density, measured by the heavy liquid method with zinc bromide solutions, was 1.8 g/cm^3 in both samples, although small amounts of heavier particles were found in the river water. If the particle density varies between 1.4 and 2.5 g/cm^3, the corresponding particle diameters will change between 7.1 and 3.7 μm and between 0.6 and 0.3 μm, under the two conditions used in the centrifugation. The time required for an analysis was nearly the same (several hours) for the two techniques.

TABLE 3.   Selective leaching of adsorbed heavy metals after size
           fractionation of suspended particulate matter in fresh waters

| Particle size (μm) | Particle concentration (mg/l) | Technique | Metal (μg/l) desorbed from particles with | | | | | |
| | | | 0.1 M HCl | | | 1 M HNO$_3$ | | |
			Cu	Pb	Cd	Cu	Pb	Cd
River water								
>5.0	27	C	0.5	0.7	0.03	1.0	1.2	0.03
		F	0.6	0.5	0.02	1.0	0.9	0.04
>0.4	35	C	0.8	1.1	0.03	2.1	1.9	0.04
		F	1.1	0.7	0.04	1.8	2.0	0.04
<0.4		C	——	——	——	0.7	0.3	n.d.
		F	——	——	——	0.6	0.4	n.d.
Pond water								
>5.0	27	C	0.6	0.4	n.d.	1.1	1.3	0.05
		F	0.4	0.7	n.d.	1.2	1.1	0.03
>0.4	32	C	1.4	1.0	n.d.	1.8	2.0	0.1
		F	1.1	1.6	n.d.	2.2	2.6	0.05
<0.4		C	——	——	——	0.8	0.5	n.d.
		F	——	——	——	0.8	0.5	n.d.

C: Centrifugation (350 rpm, 7.7 min for 5.0 μm; 3500 rpm, 12.0 min for 0.4 μm), F: Filtration, n.d.: not detected.

Specimen preparation for electron microscopy of submicron particles

The scanning and transmission electron microscopes (SEM and TEM) are useful instruments for investigating the morphology of submicron particles in water samples. Furthermore, the analysis of individual submicron particles is possible for major elements by using the analytical electron microscope (AEM), a high resolution electron microscope equipped with an energy dispersive X-ray analyzer and an electron energy loss spectrometer. Reliable results can be obtained, in electron microscopical investigations, only by preparing specimens without morphological changes such as deformation, rupture and agglomeration of the particles.

Centrifugation is a promising technique for preparing specimens for electron microscopy (ref. 8). In the proposed technique, particles ranging from 0.1 to 1 μm in 5 ml of water were collected on a 10 - 20 nm carbon film on a 200-mesh gold or copper AEM specimen grid, placed on a special polycarbonate holder in a 7-ml centrifuge tube, by centrifugation in a swinging rotor at 10000 rpm for 18 min. The specimens thus prepared were reproducible and preserved the morphology of the particles satisfactorily. In the analysis of submicron particles in a pond water, the present technique was compared favorably with three conventional specimen preparation techniques; i.e.,
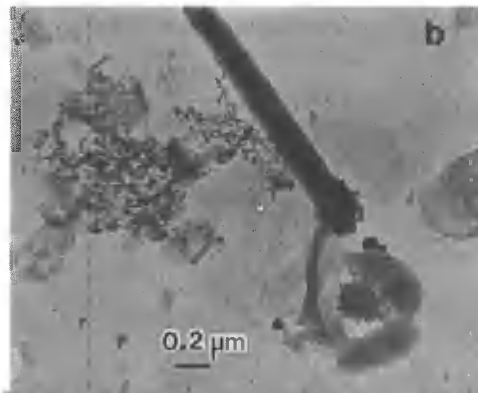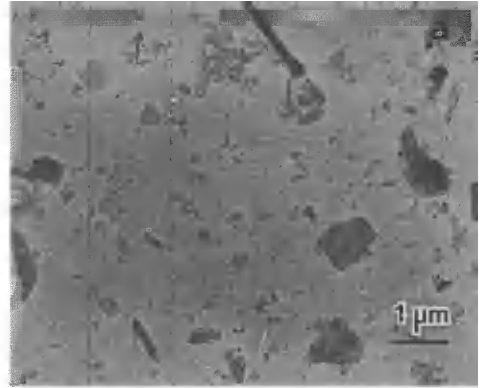
Fig. 1. SEM images of submicron particles in pond water. Specimens prepared by a) centrifugation and b) filtration

Fig. 2. TEM images of submicron particles in pond water. Specimen prepared by centrifugation

filtration, direct drying and spray drying. Although both centrifugation and filtration were superior to the other two techniques for preparing specimens for the SEM, the former gave slightly clearer images as shown in Fig. 1. Specimens prepared by centrifugation were most suitable for the TEM and AEM. The TEM images (Fig. 2) gave much more information than the SEM images (Fig. 1.a) even at the same magnification; inner structures were clearly visible with high resolution. X-ray spectra suggested, for example, the existence of clay particles associated with a small, varying amount of hydrated iron(III) oxide.

FLOTATION

Flotation is the process by which dispersed solid or liquid particles, precipitates, colloidal particles, and solutes in an aqueous solution are floated to the solution surface with the aid of a rising stream of gas bubbles (ref. 9). Generally, substances to be floated should be hydrophobic and therefore attachable to the bubbles. Since hydrophilic substances can be rendered hydrophobic easily with surfactants, the applicability of this technique is very wide. Flotation is especially useful as a rapid concentration technique in water analysis using large volumes of samples. Although flotation is usually carried out batchwise in simple flotation cells described below, flow systems are used for sample volumes greater than several liters (ref. 10).

Selective flotation of suspended particulate matter

Flotation offered a rapid and convenient concentration technique for the determination of traces of heavy metals adsorbed on suspended particulate matter in fresh waters (ref. 11).

A 500-ml water sample was magnetically stirred for 5 min with 2 ml of 5 % (w/v) sodium chloride solution and 3 ml of 1 mg/ml cationic surfactant solution (cetyldimethylbenzylammonium chloride in 70 % ethanol) to render
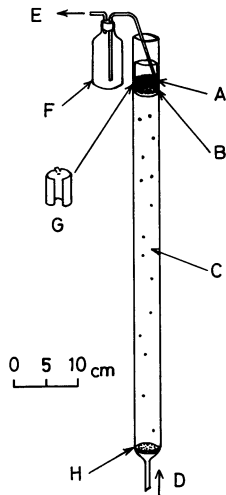
Fig. 3. Glass flotation cell
  A: Scum
  B: Foam layer
  C: Water sample
  D: Nitrogen
  E: Suction
  F: Sampling bottle
  G: Polyethylene insert
  H: Sintered-glass disk
      (5 - 10 µm pore)

negatively charged suspended particulate matter hydrophobic and to form flocs of 0.5 - 2 mm sizes. Flotation was carried out in a flotation cell (Fig. 3) by passing nitrogen at a flow rate of 1 - 1.5 ml cm^{-2} min^{-1} for 15 s. The flocs were rapidly floated by capturing tiny nitrogen bubbles, 0.1 - 0.5 mm in diameter, on the surfaces and in the interstitial spaces. Ethanol, used as solvent for the surfactant, was essential to produce a stream of tiny gas bubbles, because it prevented coalescence of bubbles which appeared from adjacent pores of the sintered-glass disk. The flocs were thus collected as the scum on the solution surface and on the polyethylene insert, and then transferred into the sampling bottle. Heavy metals were desorbed by ultrasound with 4 M nitric acid and, after separating the particulate matter by centrifugation, were determined by graphite furnace atomic absorption spectrometry (GFAAS).

Kaolin and bentonite (particle size 1 - 10 µm) were rapidly floated and recovered in greater than 96 % yields, whereas cations such as zinc and cadmium ions, anions such as chromate ions, colloidal hydrated iron(III) oxide, and iron(III)-humic acid complexes were not floated at all.

The proposed technique was compared with filtration through 0.45-µm pore Millipore HA membrane filters or centrifugation at 4000 rpm for 10 min, in the analysis of various water samples. In the analysis of artificial water samples containing 100 mg/l each of kaolin and bentonite and various heavy metals at the low µg/l levels, the three techniques gave consistent results, except for somewhat higher values for chromium(III) and lead obtained by the filtration technique. Radioactive tracer experiments were carried out by adding labeled chromium(III), manganese(II), zinc and cadmium to a pond water, with a good agreement among the results obtained by the three techniques. TABLE 4 shows the analytical results for three samples of fresh and waste waters, which are in good agreement with each other, except for higher values for manganese and lead obtained by the filtration technique. Flotation is more rapid and convenient than filtration and centrifugation.

Simultaneous concentration of traces of heavy metals existing in various physicochemical forms in fresh waters

The coprecipitation-flotation technique has been successfully applied (ref. 12). To a 500-ml water sample was added 0.5 ml of indium nitrate solution (100 mg indium/ml), and the pH was adjusted to between 9 and 9.5 with 5 ml of buffer solution (8.5 g of sodium carbonate and 10.0 g of sodium hydrogencarbonate in 200 ml) to form flocculent hydrated indium oxide precipitates for collecting traces of heavy metals, associated with suspended particulate matter, complexed with humic acid, and existing as inorganic colloids and cations. Then 3 ml of mixed anionic surfactant solution (100 mg of sodium oleate and 200 mg of sodium dodecyl sulfate in 300 ml of 70 % of ethanol) was added to render the precipitate surfaces hydrophobic. Flotation was carried out in the flotation cell (Fig. 3) by passing nitrogen for 30 s to collect the precipitates as the scum on the solution surface.

Greater than 95 % yields were obtained for the flotation of kaolin, bentonite, oxides and hydrated oxides of iron(III), aluminum, manganese(IV) and silicon, calcium carbonate, diatomaceous earth, river sediment, colloidal hydrated iron(III) oxide, humic substances and such cations as manganese(II), cobalt, nickel, copper(II), zinc, cadmium and lead.

TABLE 4. Traces of heavy metals adsorbed on suspended particulate matter
          in fresh and waste waters

Sample	Particle concentration (mg/l)	Metal	Found (µg/l) after separation by		
			Flotation	Centrifugation	Filtration
River water	42	Cr	1.6	1.4	2.0
		Mn	20.0	18.4	31.6
		Cu	0.4	0.6	0.6
		Cd	0.0	0.0	0.0
		Pb	7.8	7.8	13.6
Pond water	24	Cr	0.6	0.6	0.4
		Mn	80.8	78.6	85.2
		Cu	10.6	10.8	10.2
		Cd	0.2	0.2	0.2
		Pb	7.6	8.0	7.2
Industrial waste water	54	Cr	0.2	0.2	0.6
		Mn	22.4	25.8	42.6
		Cu	0.6	0.6	0.6
		Cd	0.0	0.0	0.0
		Pb	8.6	7.8	11.0

Concentration of humic acid

Humic substances, deriving from soils where they originate from biological
tissues, are the major organic constituents of fresh waters. Although
their chemical structure is still incompletely known, they can be described
as acidic, hydrophilic, aromatic polymers (molecular weight 3×10^2 to 3×10^4) containing carboxylic, phenolic hydroxyl and alcoholic hydroxyl groups.
Humic substances are commonly divided into three fractions based on their
solubility in acid and alkaline solutions: (1) fulvic acid, which is soluble
in both solutions; (2) humic acid, which is soluble in alkaline solutions but
insoluble in acid solutions; and (3) humin, which is insoluble in both
solutions. They play an important role in fresh waters, interacting with
metal ions and hydrated metal oxides as well as clay minerals and various
organic compounds.

We have recently devised a coprecipitation-flotation technique combined with
ultrafiltration for concentrating humic acid in fresh waters (ref. 13). To a
1-liter water sample was added 1 ml of iron(III) nitrate solution(10 mg
iron/ml), and the pH was adjusted to 7 with 0.1 M potassium hydroxide
solution to form hydrated iron(III) oxide precipitates for collecting humic
substances. Then 3 ml of mixed anionic surfactant solution (20 mg of sodium
oleate and 60 mg of sodium dodecyl sulfate in 100 ml of 70 % ethanol) was
added to render the precipitate surfaces hydrophobic. Flotation was carried
out in the flotation cell shown in Fig. 4 by passing nitrogen at a flow rate
of 30 - 40 ml/min for 2 min to collect the precipitates as the scum on the
solution surface. After completion of the flotation, a silicone rubber
stopper was fitted into the narrow, the upper part of the cell removed, and
10 ml of 2 M hydrochloric acid added to dissolve hydrated iron(III) oxide.
Humic acid was filtered off on an ultrafilter of nominal molecular weight
cutoff of 10^4, and then ultrasonically dissolved in 10 ml of 0.1 M potassium
hydroxide solution. For 0.01 to 5 mg/l of humic acid, recoveries greater
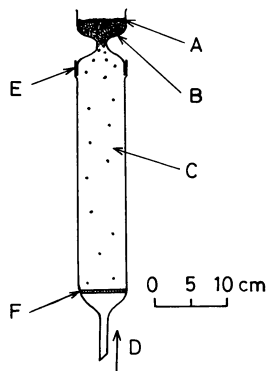than 95 % were obtained.

Fig. 4. Glass flotation cell
         A: Scum
         B: Foam layer
         C: Water sample
         D: Nitrogen
         E: Ground glass joint
         F: Sintered-glass disk
            (5 - 10 µm pore)

SORPTION OF HEAVY METALS COMPLEXED WITH HUMIC SUBSTANCES

Heavy metals complexed with humic substances can be selectively separated from other metal species by sorption on macroreticular anion exchangers or nonionic synthetic polymers.

Sorption on DEAE-Sephadex A-25

The following measurement scheme has been proposed using the macroreticular weak-base anion exchanger DEAE-Sephadex A-25, a cross-linked dextran gel with diethylaminoethyl groups (refs. 14, 15).

Fraction 1 (Humic substances and other negatively charged species): After removal of supended particulate matter by centrifugation followed by filtration through 0.4-μm Nuclepore membrane filters, 1000 ml of water was passed through a 16 mm diam. x 10 mm A-25 column (50 - 100 μm particle size) at a flow rate of 50 ml/min to sorb humic substances and other negatively charged species. The anion exchanger was ultrasonically treated with 5 ml of 4 M nitric acid for 1 min batchwise to desorb the heavy metals. After centrifugation, 40-μl aliquots of the supernatant were used to determine copper, lead and cadmium by GFAAS.

Fraction 2 (Positively charged inorganic colloids): The initial 100-ml fraction of the effluent from the A-25 column was filtered through a 0.015-μm Nuclepore membrane filter to collect positively charged colloids. The filter was ultrasonically treated for 10 min with 5 ml of 1 M nitric acid, which was then delivered to the determination of copper, lead and cadmium by DPASV after pre-electrolysis on a hanging mercury drop electrode at -1.2 V vs. the silver chloride electrode (3.3 M KCl) for 5 min.

Fraction 3 (Cations): Copper, lead and cadmium in a 5-ml aliquot of the filtrate through the 0.015-μm membrane filter were determined by DPASV in a 0.1 M potassium chloride-0.001 M hydrochloric acid electrolyte.
Heavy metals complexed with humic substances were quantitatively retained on the A-25 column between pH 3 and 9, whereas copper, lead and cadmium ions as well as colloidal hydrated oxides of aluminum and iron(III) passed through the column. The heavy metals adsorbed on the A-25 were desorbed quantitatively with 4 M nitric acid, leaving most of humic acid on the A-25. Adsorption of cations on the 0.015-μm membrane filter was negligible. The heavy metals associated with colloidal hydrated iron(III) oxide were completely liberated by ultrasonic irradiation in 1 M nitric acid. TABLE 5 shows the analytical results for three water samples.

Sorption of indium-treated Amberlite XAD-2

A nonionic macroreticular styrene-divinylbenzene copolymer, Amberlite XAD-2, is another useful sorbent for concentrating humic substances in fresh waters. Here low-energy physical sorption operates instead of anion exchange. However, separation of humic substances from inorganic species in fresh waters was not satisfactory, because significant amounts of cations, such as copper(II), cadmium and lead ions, were also sorbed on the XAD-2 presumably owing to small amounts of cation-exchangeable groups present. Pretreatment of the resin with indium ions easily solved this problem (ref. 16).

TABLE 5. Analysis of water samples

Sample	Metal	Found (μg/l) in		
		Fraction 1 (humic substances and other negatively charged species)	Fraction 2 (positively charged inorganic colloids)	Fraction 3 (cations)
River water	Cu	1.32	0.11	0.5
	Pb	0.19	0.05	<0.1
	Cd	0.04	<0.01	<0.1
Pond water	Cu	0.93	0.18	0.3
	Pb	0.10	0.07	<0.1
	Cd	<0.003	0.04	<0.1
Tap water	Cu	0.16	0.13	1.3
	Pb	0.04	0.02	<0.1
	Cd	<0.003	<0.01	<0.1

The commercial XAD-2 resin (0.2 - 0.8 mm particle size) was pulverized to 1 - 10 μm to improve the sorption, washed with methanol, 2 M nitric acid and water ultrasonically, and then treated with an indium nitrate solution (10 μg indium/ml) at pH 5 to saturate cation-exchangeable sites. To a 16 mm diam. x 5 mm column of the indium-treated XAD-2, 100 ml of water (pH 5) filtered through a 0.4-μm Nuclepore membrane filter was introduced at a flow rate of 2 ml/min to sorb humic substances. The heavy metals sorbed on the resin were ultrasonically desorbed with 5 ml of 0.5 M nitric acid batchwise, leaving the humic and fulvic acids on the resin, for the determination by GFAAS.

Heavy metals complexed with humic acid were quantitatively sorbed on the resin between pH 3 and 5, but the recovery gradually decreased with increasing pH (85 % at pH 7). Such cations as manganese(II), copper(II), cadmium and lead ions, anions such as chromate and EDTA complexes, and colloidal hydrated iron(III) oxides were not sorbed at all. The sorbed heavy metals were quantitatively desorbed with 0.5 M nitric acid, leaving most of the humic acid on the resin.

CONCLUSION AND FUTURE PROSPECTS

The present status of trace metal speciation in fresh waters has been briefly discussed, emphasis being put on separation and concentration techniques. In the future, the importance of trace metal speciation will increase more and more in the analysis of fresh waters. Simple total element analysis does not provide sufficient information for studying geochemical and biological cycling as well as pollution control and water treatment. Most of measurement schemes for trace metal speciation so far proposed, combining separation, conversion and determination techniques, give only data that depend on operating conditions, such as labile fractions with respect to spectrophotometry, ASV or sorption on chelating resins, although they are practically useful, for example, in correlation between ASV-labile measurements and toxicity to aquatic biota. To study behavior and effects of trace metals in aquatic systems more satisfactorily, schemes which can measure well-defined species, instead of operationally-defined data, will be necessary. To attain this goal, new selective separation and concentration techniques for inorganic, organic and hybrid colloidal particles should be developed and crosschecked. If possible, in situ techniques are preferable to minimize contamination, losses and physicochemical changes during transportation and storage of water samples. Another problem is morphological and chemical characterization of individual submicron particles. Experiments with artificial model systems, containing various trace metals, inorganic and organic colloids and complexing agents, may be useful to establish reliable speciation techniques.

REFERENCES

1. T.M. Florence and G.E. Batley, CRC Crit. Rev. Anal. Chem. 9, 219-296 (1980).
2. G.G. Leppard (ed.), Trace Element Speciation in Surface Waters, Plenum Press, New York (1983).
3. D.T.E. Hunt and A.L. Wilson, The Chemical Analysis of Water 2nd ed., p. 559, Royal Society of Chemistry, London (1986).
4. A. Mizuike, Enrichment Techniques for Inorganic Trace Analysis, p. 103, Springer-Verlag, Berlin (1983).
5 S.J. de Mora and R.M. Harrison, Water Res. 17, 723-733 (1983).
6. T. Miwa, L. Jin and A. Mizuike, Mikrochim. Acta [Wien] 1984 III, 259-264 (1984).
7. T. Miwa, S. Tillekeratne and A. Mizuike, Anal. Sci., in press.
8. T. Nomizu and A. Mizuike, Mikrochim. Acta [Wien], in press.
9. A. Mizuike and M. Hiraide, Pure & Appl. Chem. 54, 1555-1563 (1982).
10. A. Mizuike, M. Hiraide and K. Mizuno, Anal. Chim. Acta 148, 305-309 (1983).
11. M. Hiraide, J. Mizutani and A. Mizuike, Anal. Chim. Acta 151, 329-337 (1983).
12. M. Hiraide, J. Mizutani and A. Mizuike, Nippon Kagaku Kaishi 1981, 161-165 (1981).
13. M. Hiraide, F. Ren and A. Mizuike, Bunseki Kagaku, submitted.
14. M. Hiraide, S. Tillekeratne, K. Otsuka and A. Mizuike, Anal. Chim. Acta 172, 215-221 (1985).
15. S. Tillekeratne, T. Miwa and A. Mizuike, Mikrochim. Acta [Wien] 1985 III, 289-296 (1986).
16. M. Hiraide, Y. Arima and A. Mizuike, 47th Symp. Jpn. Soc. Anal. Chem., Abstracts, p. 63 (1986).