Special Issue
METAL SPECIATION
(Guest Editor: C.M.G. van den Berg)

ANALYTICA CHIMICA ACTA

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Special issue on Metal Speciation
  (Guest Editor: C.M.G. van den Berg)

Contents (continued)

SPECIAL ISSUE

METAL SPECIATION

PREFACE

Chemical speciation is the fundament of the geochemical, biological and biogeochemical variability of elements in natural systems. Speciation data can be used to predict the behaviour of metals in natural waters and to study biogeochemical processes. However, the study of metal speciation in natural waters is generally hampered by analytical difficulties arising from the low concentrations in which many elements occur and the instability of the complexed species during sample storage or sample treatment. Furthermore, the speciation is often altered as a result of the analytical procedures used for their detection. Where this alteration is carried out in a controlled manner the original speciation can subsequently be evaluated but it has to be kept in mind that unexpected alterations can be introduced.

Another problem lies in the complex nature of natural waters. Data obtained by methods determining organic complexation of metals in sea water, for instance, have been used to demonstrate complexation by a single complexing ligand, by two ligands, or by a large number of ligands. There is indeed no reason why there should not be a large number of complexing ligands in natural waters although it is conceivable that one or two ligands are predominant and dominate the experimental results. The presence of a large number of complexes of greatly varying stability would go some way to explain the large apparent variation in the organically complexed fraction of copper in natural waters depending on the analytical method. Thus far it has been very convenient to justify differences in complexation detected by different methods as being caused by differing sample compositions, but it is hoped that any differences in the future will begin to make sense with a better understanding of the analytical methods and of the natural system.

This issue of *Analytica Chimica Acta* is dedicated to the interesting topic of speciation to see how far we have advanced in solving some of the experimental and theoretical problems, to get a discussion going and to stimulate further work on chemical speciation of elements in natural waters and natural systems in general. The plan for this issue originated at a meeting on "Electroanalysis of speciation in natural waters" held in Liverpool in July, 1990. The proceedings (extended abstracts) of that meeting were published in *Analytical Proceedings* (Vol. 28, 1991). Because of the enthusiastic contributions of all participants of that meeting it was decided to attempt to produce a set of full papers of further experiments in *Analytica Chimica Acta* broadened by inviting other contributors known to be active in this field but not necessarily using electrochemical methods. This issue is the result. The area covered is not fully comprehensive (for instance metalloids are not included) and it represents to some extent a snapshot of work going on at the moment, of authors having time to contribute, and of work which happened to come to my attention.

Electrochemical methods are very suitable for speciation studies as the electrochemical response is directly species-related. It is therefore natural that electroanalytical methods are well represented in this volume. However, the species specific response is not unique to electrochemistry so it is therefore good to see that nine of the eighteen contributions to this issue are not electrochemical.

Definitions

I would like to use this opportunity to add to the definitions in the chemical vocabulary. The term *Speciation* benefits from further clarification. Chemical species has previously been defined as follows [1,2]: chemical species refers to a specific form (monoatomic or molecular) or configuration in which an element can occur, or a distinct group of atoms consistently present in different compounds or matrices.

The term speciation has been used in the literature for two quite different meanings: to indicate the *analysis of chemical species* or the

quantification of the chemical species; and secondly to indicate the *species distribution* [3]. It may be preferable to define speciation loosely as "the occurrence in different species". This definition seems sufficiently broad and simple to also encompass any future species, as yet unknown, as it does not define them. Such species may include inorganic complexes, organic complexes, organic compounds, redox species and colloidal species.

Additionally I would like to propose the following derived definitions:

Speculation: discussion of speciation.

Speculator: someone dabbling in speciation.

Spectacle: the activity of Speculators!

I would like to thank all contributors to this issue for their willingness to contribute their interesting manuscripts, and the referees for their assistance with the evaluation of the manuscripts.

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Automated voltammetric system for shipboard determination of metal speciation in sea water

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Abstract

An automated and semi-intelligent voltammetric system is described for trace metal analysis. The system consists of a voltammeter interfaced with a personal computer, a sample changer, 2 peristaltic pumps, a motor burette and a hanging mercury drop electrode. The system carries out fully automatically approximately 5 metal determinations per hour (including at least 3 repetitive scans and calibration by standard addition) at trace levels encountered in clean sea water. The computer program decides what level of standard addition to use and evaluates the data prior to switching to the next sample. Alternatively, the system can be used to carry out complexing ligand titrations with copper whilst recording the labile copper concentration; in this mode up to 8 full titrations are carried out per day. Depth profiles for chromium speciation in the Mediterranean Sea and a profile for copper complexing ligand concentrations in the North Atlantic Ocean measured on board-ship with the system are presented. The chromium speciation was determined using a new method to differentiate between Cr(III) and Cr(VI) utilizing adsorption of Cr(III) on silica particles.

Keywords: Voltammetry; Chromium; Copper; Metal speciation; Sea water; Shipboard determination; Waters

The redox and chemical speciation of trace metals in natural waters have important implications for the geochemical cycling and bioavailability of trace metals [1,2,3]. Electrochemical methods are very suitable for the study of speciation because of their high sensitivity and because the electrochemical response is species specific. Several studies on metal speciation, using electrochemical techniques, have been reported for estuarine [4,5], coastal [6] and oceanic environments [7,8].

Because of the low concentrations involved and the labour-intensity of the methods, the amount of data available on metal speciation is still limited. Furthermore, speciation measurements have to be performed quickly upon sampling, because of a possible change in the equilib-

ria during storage and transport of the samples [9,10]. This requirement and the number of measurements involved in determining trace metal speciation necessitate automated on-board measurements. Automation of the electrochemical methods for accurate determination of trace metals in ocean waters has, however, been hampered for a long time because of the low levels involved. A number of examples of flow system for electrochemical determinations can be found in the literature [11–16]. However, the systems described are in most cases not suitable for the concentration levels encountered in unpolluted ocean waters. Furthermore, in the case of voltammetric flow systems, a suitable method for accurate low level addition of metal standard solution has yet to be found.

This article describes an automated voltammetric system for determination of trace metals at the levels encountered in unpolluted ocean

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water, at a rate of approximately 5 and 7 determinations per hour, with and without calibration respectively. The system combines flow and batch operations and is applied here to determine the speciation of chromium and copper in sea water. Mixing of sample and reagent (for chromium measurements) takes place on-line; this mixture is pumped into a conventional voltammetric cell, which enables accurate determination of the metal concentration using a hanging mercury drop electrode (HMDE). The system was used on-board ship for speciation studies of copper and chromium using cathodic stripping voltammetry (CSV). Characterisation of the system and its field applications for trace metal determinations are explored in the following sections.

EXPERIMENTAL

Apparatus

A diagram of the automated electrochemical system is shown in Fig. 1. The system consisted of an HMDE (663 VA Stand, Metrohm; drop surface area: 0.38 mm^2), a sample changer (PSA 20.020, Chemlab), a motor burette (665 Dosimat, Metrohm) and 2 peristaltic pumps (Minipuls 3, Gilson; four channels). These devices were connected to the digital input/output ports (DIO 1 and DIO 2) of the voltammetric analyser (Auto-

Fig. 2. Detailed diagram of the electrical connections between computer, voltammeter and peripheral devices.

lab, Eco Chemie). An AT 80286 IBM compatible personal computer (Toshiba 3100) controlled the voltammetric analyser and the devices connected to this instrument.

The electrical connections are shown in more detail in Fig. 2. The personal computer (PC) was connected to the Autolab via a parallel interface card placed in an 8 bit slot of the computer. The HMDE was interfaced with the voltammeter via an interface box (IME, Ecochemie). The pumps and sample changer were TTL controlled via port DIO 1 of the voltammeter. The motor burette was controlled in a serial way via port DIO 2.

The original computer program (EAS 1.0; written in Quick Basic 3.0) provided by the manufacturer of the Autolab voltammeter to control the electrode, potential scans and data treatment, was altered in order to control the pumps and sample changer. Further changes have been made to make the program self-decisive and intelligent (e.g. to reject failed scans and to perform additional scans when required). A dot matrix printer (IBM Proprinter) was used to print the results of the analysis.

The inner diameter of the tubing (Santoprene, Altec) used in the peristaltic pumps was 1.85 mm for sample delivery and 0.38 mm for chromium reagent delivery. PTFE tubing (1.0 mm. i.d.) was used for sample transport from pump to voltammetric cell. Ultraviolet irradiation was carried out for 4 h by using a 100-W high pressure mercury vapour lamp (Hanovia). The sample bottles (20)

Fig. 1. Diagram of the automated voltammetric system with narrow lines representing electrical connections and wider lines representing sample and reagent flows.

of the sample changer were replaced by 30-ml FEP bottles to minimise adsorption effects.

Reagents

Water purified by reverse osmosis (Milli-RO, Millipore) followed by deionisation (Milli-Q, Millipore) was used for rinsing and to prepare reagents. All reagents were purchased from BDH (AnalaR quality), unless indicated differently.

An aqueous solution containing 0.025 M diethylenetriamine pentaacetic acid (DTPA, Sigma), 5 M $NaNO_3$ and 0.5 M CH_3COONa was prepared to determine chromium. Chromium contamination [comprising Cr(VI) and Cr(III)] was removed from a solution containing 5 M $NaNO_3$ and 0.5 M CH_3COONa by co-precipitation with hydrated iron(III) oxide by addition of iron (II) chloride (10^{-4} M) prior to the addition of the DTPA. The precipitate was removed from the solution by filtration using a 0.45 μm cellulose acetate membrane filter [10]. Addition of 0.625 ml of the chromium reagent to 10 ml sea water gave the required pH (pH 5.3 in sea water) and reagent concentration, whereas Cr(VI) contamination due to the addition was < 0.01 nM. A suspension of 100 g l^{-1} LiChrosorb Si 60 silica (particle size 5 μm, surface area 490 m^2 g^{-1}; Merck) was used to remove Cr(III) from sea water prior to the determination of Cr(VI). A quantity of 100 μl of this suspension was added to 25 ml of each sample at least 20 min before analysis. Cr(III) contamination was removed from the LiChrosorb silica by soaking overnight in 5 M HCl, followed by rinsing with deionised water and four-fold UV irradiation. The UV irradiation oxidised Cr(III) to Cr(VI), which in turn desorbed from the silica. Between the UV irradiation steps the LiChrosorb silica was centrifuged, and subsequently rinsed with deionised water. Cr(VI) contamination due to addition of the silica at a level of 0.4 g l^{-1} was < 0.01 nM.

Standard solutions of Cr(VI) were prepared by dissolving K_2CrO_4 in water. Standard solutions of Cr(III) were prepared by dilution of an atomic absorption standard solution and were acidified to pH 2.5 using 6 M HCl (quartz-distilled).

A stock solution of 0.5 M tropolone (Aldrich) was prepared in methanol (quartz doubly-distilled). The pH buffer contained 1 M boric acid–0.35 M NH_4OH (both Aristar grade), giving a pH of 8.35 upon 100-fold dilution with sea water. Standard solutions of copper were prepared by dilution of an atomic absorption standard solution and were acidified to pH 2.5 using 6 M HCl (quartz-distilled).

Sample collection and treatment

Chromium speciation was determined on-board ship (FS Valdivia) during a cruise on the Mediterranean Sea in February 1992 as part of the EROS 2000 program. The data presented here were obtained in samples collected at station 10 (36°N, 5°W).

Copper complexing ligand concentrations were measured on-board ship during RRS Challenger Cruise 76 (March 1991) on the North Atlantic Ocean. The data shown were obtained in samples collected at station 3 (48°N, 20°W).

Samples were collected using PTFE-coated Go-Flo bottles (General Oceanics). The samples were nitrogen-pressure filtered through 0.4-μm polycarbonate membrane filters (acid soaked, Nuclepore), immediately upon collection. A 30 ml aliquot of the filtrate coming out of the Go-Flo bottle was sub sampled directly into a silica tube (30 ml) and subjected to UV digestion prior to the determination of total chromium. This procedure prevented loss of the Cr(III) fraction by adsorption on the walls of intermediate sample bottles [10]. All sample handling took place in a laminar flow hood.

Automated CSV determination of chromium speciation sea water

The speciation of chromium was determined by CSV using a new method to differentiate between Cr(VI) and Cr(III). Separation was achieved by utilising the known adsorption of Cr(III) onto silica particles [17], to remove Cr(III) from sea water prior to determination of Cr(VI). Cr(VI) was determined after addition of 100 μl of a suspension containing 100 g l^{-1} LiChrosorb silica into PTFE sample bottles placed in the sample changer, followed by 25 ml sea water giving a final silica concentration of 0.4 g l^{-1}. The automated measurements were initiated after a reaction time of at least 20 min.

Total chromium was determined as Cr(VI) after UV irradiation (4 h) of the untreated samples to convert all Cr(III) to Cr(VI) [10]. The Cr(III) fraction was then calculated from the difference between total chromium and Cr(VI). The samples to be analysed for total and labile chromium on-board ship were placed on the same sample tray, thus minimising day to day variability of the measurements.

The procedure to determine Cr(VI) automatically was as follows: from a sample bottle 10 ml of sea water was pumped into the voltammetric cell by peristaltic pump 1 at a rate of ca. 8.5 ml min^{-1} (0.6% R.S.D., $n = 10$). The sample flow was mixed with that of the chromium reagent at a ratio of 16:1 (sample–reagent) giving a DTPA concentration of 20 μM. The pumping time of the peristaltic pump was calibrated three times a day to give precisely 10.0 ml, and the difference in pumping efficiency was normally less than 0.3%. A 10-ml aliquot of each sample was used to rinse the tubing, voltammetric cell and the electrode, whereas a second aliquot was used for analysis. The voltammetric cell was deaerated for 3 min using water-saturated N_2, 2 new mercury drops were made and after the extrusion of a third drop the potential of the HMDE was set to -1 V whilst the solution was stirred. Adsorption of the Cr(III)–DTPA$_2$ complex on the HMDE was carried out for a period of 30 s, the stirrer was stopped and a quiescence period of 8 s was allowed. Then a potential scan was carried out using the square wave modulation at a frequency of 100 Hz, a modulation amplitude of 25 mV, and a step height of 2.4 mV. The scan direction was towards more negative potentials, and the reduction peak corresponding with chromium appeared at -1.2 V.

Fig. 3 shows a flow diagram of the subsequent steps made by the program. After a series of scans the relative standard deviation (R.S.D.) of the peak heights of 3 consecutive chromium determinations was calculated; a fourth determination (loop 1) was carried out if the R.S.D. was greater than a pre-set value (5%). In case the requirement of R.S.D. < 5% was still not met, determinations differing by more than 5% from the mean value were discarded and another scan
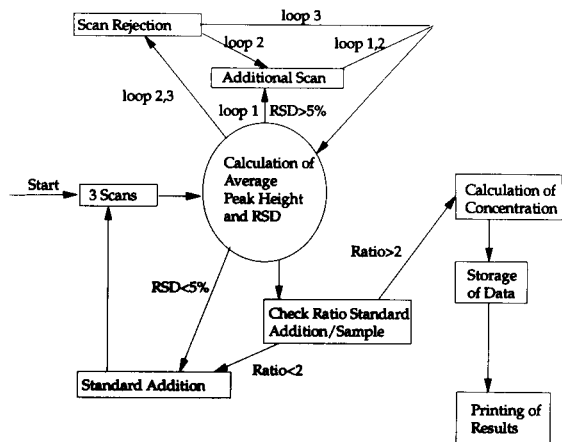
Fig. 3. Diagram showing the subsequent steps made by the computer program for the determination of metal concentrations in natural waters.

was carried out (loop 2). Loop 3 was executed in case after the second additional scan the value of the R.S.D. still exceeded 5%. During loop 3 only scan rejection took place.

After 3 (or maximally 5) scans, a standard chromium addition as Cr(VI) was made using the motor burette and the chromium signal was determined as before. Subsequently, the ratio of the peak height of the sample with and without added chromium standard was evaluated to verify whether the standard addition was sufficiently large. More chromium was added if the ratio was below 2, the added amount being estimated from the ratio. This test was carried out once more, and the sensitivity (nA nM^{-1}) was then calculated from the increase in the peak height. Subsequently, the chromium concentration in the sample was calculated from the mean peak height of the sample without standard addition and the sensitivity. The scans were then stored digitally for possible further data treatment and the results of the analysis, together with the standard deviation, were printed. A printed message was given stating that the chromium determination was imprecise in case the R.S.D. had exceeded 5% in any series of scans. The voltammetric cell was then emptied by pump 2 and the next sample was pumped into the cell by pump 1.

The procedure to determine copper complexing ligand concentrations

The concentration of copper complexing ligands was determined on-board ship using CSV with tropolone as the copper binding ligand [18]. The following procedure was used for the complexing ligand titrations with copper: sea water (25 ml) to which 0.01 M borate buffer and 0.4 mM tropolone was added was pipetted into PTFE bottles. Copper was added to give an added concentration range between 0 and 20 nM in 10 steps. The sample aliquots were allowed to equilibrate for 8–10 h with the added metal and tropolone. Then the PTFE bottles were placed in the tray of the sample changer (in order of increasing copper addition) and the automated analysis was started. Labile copper concentrations of 2 sample titrations could be measured in a single run, as the tray held 20 bottles.

The sequence of events of the automated determination of labile copper was initiated by pumping a 10-ml sample aliquot into the voltammetric cell. Similar to the chromium determination this aliquot was used for rinsing, whereas a second aliquot was used for the analysis. The voltammetric cell was deaerated for 5 min using N_2, subsequently 2 mercury drops were discarded and 3 s after the extrusion of the third mercury drop the adsorption period was initiated. Adsorption of the Cu–tropolone complex on the HMDE was carried out for 40 s, whilst stirring the solution with the potential set at -0.075 V. Then the stirrer was stopped and a quiescence period of 8 s was allowed, followed by the potential scan carried out using the square wave modulation at a frequency of 200 Hz, a modulation amplitude of 25 mV and a step height of 2.4 mV. The scan direction was towards more negative potentials, and the reduction peak corresponding with copper appeared at -0.225 V. The potential was set briefly (1 s) to -0.6 V between the accumulation and quiescence period in order to desorb interfering organic compounds.

The computer program performed three repetitive scans and checked the standard deviation of the peak height. A tangent along a plot of the peak height versus the copper concentration in the equilibrated aliquots (see Fig. 6a) was used to determine the sensitivity. Furthermore, a standard addition of copper was made to an aliquot with a high concentration of added copper to corroborate the sensitivity obtained from the tangent, and to verify that the endpoint of the titration had been reached. The initial copper concentration in the sample was measured after UV irradiation of 30 ml of an acidified aliquot of the sample (pH 2.5).

RESULTS AND DISCUSSION

Reproducibility and accuracy of the automated system

The accuracy of the automated electrochemical system was tested by determination of total dissolved nickel in certified sea water [BCR reference Southern North Sea water (CRM 403)] using in-line UV irradiation [19]. Acidified sea water sample aliquots (pH ~ 2.5) were pumped at a speed of ca. 0.7 ml min^{-1} through a silica coil (3 m × 0.5 mm i.d.) placed in the aluminium housing (home-built) of an UV irradiation unit (100 W Hanovia high pressure mercury vapour lamp). Mixing of the sea water and nickel reagent (aqueous mixture of 3.2 mM DMG and 0.15 M NH_3) flows at a ratio of 16:1 (sample–reagent) took place beyond the UV lamp to give a final concentration of 0.2 mM DMG and pH 9.0. All other sample transport procedures and program routines used were similar as described for the Cr(VI) determination. Electrochemical procedures used were: adsorption for 60 s at a potential of -0.8 V; potential scan using square wave at a frequency of 300 Hz, a modulation amplitude of 25 mV, and a step height of 2.4 mV. The scan direction was towards more negative potentials, and the reduction peak corresponding with nickel appeared at -1.0 V.

A total nickel concentration of 3.90 ± 0.03 nM ($n = 4$) was found in the certified sea water (BCR CRM 403) using the automatic voltammetric system. A concentration of 4.03 ± 0.17 nM ($n = 2$) was found by fully manual determinations of total nickel (i.e. batch-wise UV irradiation and manual sample handling). These concentrations compare

well with the certified nickel concentration in the BCR sea water of 4.23 ± 0.34 nM [20].

Additional experiments were carried out to compare automated and manual determinations of chromium. Sea water aliquots originating from the Menai Straits were analysed in order to verify the precision and reproducibility. An R.S.D. of 3.4% was obtained from a series of 8 automated total dissolved chromium determinations (in different aliquots of the same sample), whereas the standard deviation of 10 manual determinations was 4.7%. The mean concentrations determined by the 2 techniques were in very good agreement {Automated: [Cr] = 20.6 nM (n = 8); Manual: [Cr] = 20.5 nM (n = 10)}. Using the automated determination of chromium, the calculated standard deviation of the Cr(III) concentration is ±0.15 nM in the presence of 2.0 nM Cr(VI) and 2.5 nM of total chromium, as the Cr(III) concentration is obtained by difference. Determination by CSV of chromium in sea water in which the metal concentration had been verified by other techniques has previously been shown to produce accurate results [10].

Determination of Cr(VI)

The optimum addition of silica particles for removal of Cr(III) was determined using UV irradiated Atlantic Sea water [sampled at station 8 (30°N, 24°W) during the RRS Challenger Cruise in 1991]. Cr(III) was added to give a concentration of 1.0 nM, in the presence 2.00 ± 0.05 nM (n = 10; manual determination) chromium as Cr(VI). According to Fig. 4a, a concentration of 0.4 g l^{-1} LiChrosorb silica was sufficient for the removal of the added Cr(III). Concentration levels of silica below 0.2 g l^{-1} did not take out all the added Cr(III), whereas levels over 1.2 gl^{-1} were found to interfere with the electrochemical Cr(VI) determination.

The reaction time was varied to investigate the kinetics of the removal of Cr(III) by the LiChrosorb silica at a concentration of 0.4 g l^{-1} in UV treated Atlantic Sea water (see Fig. 4b). The time on the x-axis of Fig. 4b refers to the reaction time between the silica particles and added Cr(III) prior to addition of chromium reagent. Most of the Cr(III) (ca. 80%) was re-
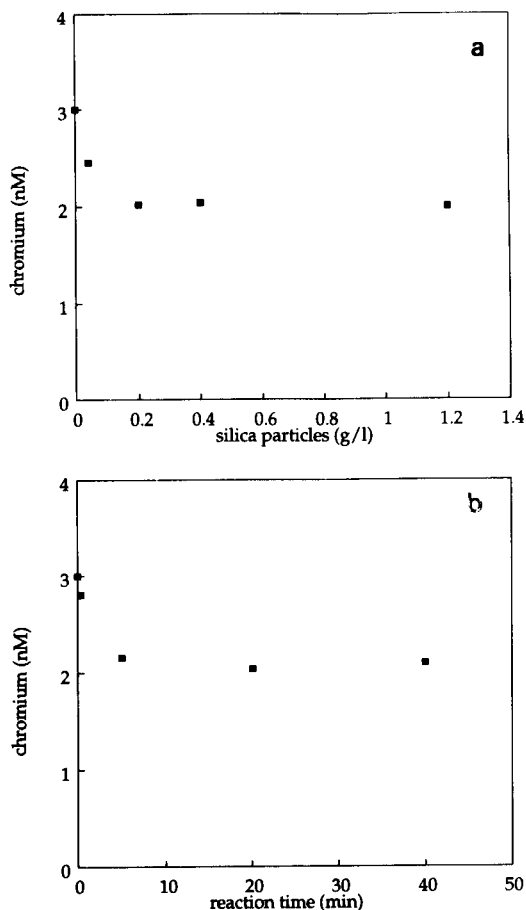
Fig. 4. (a) Chromium versus added silica particle concentrations for sea water aliquots with 1.0 nM added Cr(III). Initial chromium concentration [as Cr(VI)] in UV treated sea water sample, originating from the North Atlantic Ocean, was 2.00 ± 0.05 nM (n = 10). A reaction time of at least 20 min was applied. (b) Chromium concentration in UV treated sea water aliquots plotted against reaction time between 0.4 g l^{-1} silica particles and 1.0 nM Cr(III). Initial chromium concentration [as Cr(VI)] was 2.00 ± 0.05 nM (n = 10). Reaction time is taken from the moment of addition of silica and Cr(III) until addition of chromium reagent.

moved from the solution within a reaction time of 5 min with the silica, but a reaction time > 20 min was used to ensure complete removal of all Cr(III).

The speciation of chromium in the Mediterranean

The automated system was tested by determination of the redox speciation of chromium in the
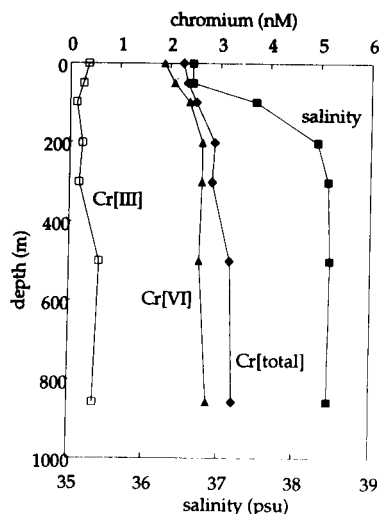
Fig. 5. Vertical depth profile of Cr(III), Cr(VI), Cr(total) and salinity for station 10 (36°N, 5°W) in the Mediterranean. Cr(total) was measured after UV treatment of the samples, Cr(VI) after addition of 0.4 g l^{-1} silica particles prior to a reaction time of minimally 20 min, and Cr(III) as the difference between Cr(total) and Cr(VI). The standard deviations for Cr(total), Cr(VI) and Cr(III) are, respectively, ~ 0.08 nM, ~ 0.07 nM and ~ 0.15 nM.

Mediterranean. Fig. 5 shows the vertical distribution of the different species of chromium in the water column of the Mediterranean Sea at station 10. The Cr(VI) concentration was found to increase slightly with depth from ~ 2 nM at the surface to ~ 2.8 nM below 200 m. The deeper water mass, with its higher salinity, represents the Mediterranean Sea water (salinity between ca. 37–39 p.s.u.). The upper water mass has a lower salinity and originates from the North Atlantic Ocean. The Cr(VI) concentrations determined in the deeper water mass during this EROS 2000 cruise are in very good agreement with other data on the Mediterranean [21]. The Cr(VI) concentrations in the upper part of the profile in Fig. 5 are similar to the concentrations in the North Atlantic Ocean reported elsewhere [17]. The concentration of Cr(III) is much lower than that of Cr(VI). Few data are available on Cr(III) in unpolluted sea water. However, concentrations of Cr(III) similar to those shown in Fig. 5 have been reported for the North Atlantic Ocean [17], the

Mediterranean [21] and the Eastern Pacific Ocean [22].

Even though the difference between Cr(VI) and total chromium for individual samples is small (and sometimes within the standard deviation of the analysis), the fact that there is a systematic positive difference between the total dissolved chromium and Cr(VI) concentrations for all the samples indicates that the Cr(III) concentrations found can be treated with confidence.

Natural surface active organic material in the sea water affected the sensitivity of the Cr(VI) determinations, causing the sensitivity to vary between 9 and 13 nA nM^{-1} in samples from different origin at a constant 30 s adsorption period. This variation in the sensitivity illustrates the necessity to use the standard addition technique for calibration of the CSV sensitivity for each sample.

Copper complexation in the Atlantic Ocean

The result of a typical complexing ligand titration is shown in Fig. 6a.: a plot of the CSV labile copper concentration as a function of the total copper concentration in a sample originating from a depth of 100 m in the North Atlantic Ocean. Curvature indicates that not all ligands were saturated by the copper initially present in the sample and that some of the added copper was bound by the complexing matter. The Van den Berg–Ruzic plot [1] is straight (Fig. 6b), indicating that only one competing ligand is predominant in the present conditions, as was the case for all samples determined. Fig. 7 shows a depth profile for station 3 of the copper complexing ligand concentration. The ligand concentration was ca. 7 nM in the surface waters, a maximum of ca. 12 nM was reached at 200 m and the concentration decreased to ca. 5 nM towards greater depth. The higher ligand concentration in the upper water column is probably attributable to metal complexation by soluble exudates of phytoplankton [23] or residues of algal cells damaged by predation. The conditional stability constants of the copper–natural ligand complexes range between 12.0 and 13.0 (log values) at the detection window (log $\alpha_{CuTrop} = 3.29$) used. This means that relatively weak copper binding ligands have been
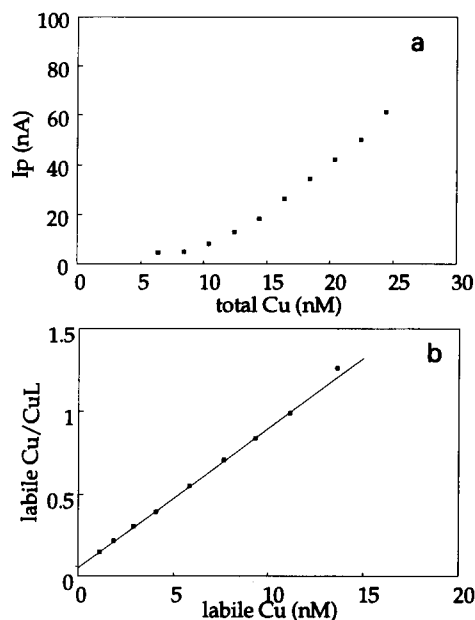
Fig. 6. (a) Complexing capacity titrations: plot of the peak height of the CSV labile copper as a function of the total dissolved copper concentration for a sample from station 3 (48°N, 20°W) in the North Atlantic Ocean. (b) Linear transformation of titration curve data of (a), showing a plot of [CSV labile Cu]/[CuL] as a function of CSV labile copper. CuL is the concentration of Cu^{2+} complexed by natural organic ligand L.

determined for this profile when compared with for example a North Sea sample determined with 0.193 mM catechol resulting in a log $\alpha_{CuCat} = 6.24$ and ligand concentration of 7.9 nM with a conditional stability constant of 15.6 (log value) [2].

Conclusions

Reliable data concerning metal speciation in natural waters are scarce. The problems involved obtaining speciation data are numerous. Important aspects concerning speciation measurements are that the samples have to be measured immediately upon collection and with a minimum of disturbance of the equilibria involved. Use of automated cathodic stripping voltammetry onboard ship suits these requirements to a great extent. Furthermore, the risk of contamination by sample handling is reduced greatly by automated determination. Our data shows that the use of computer controlled equipment results in good

reproducibility. Accurate delivery of sample by peristaltic pump and metal standard by burette, and furthermore precise timing of the subsequent electrochemical analysis steps greatly increase the reliability of the data obtained. The good reproducibility of the titrations shown in Fig. 6a and b is hard to achieve by manual sample handling. This good reproducibility of the data is necessary for accurate calculation of ligand concentrations and binding constants in the case of complexing ligand titrations. Furthermore, precise determination of Cr(VI) and total dissolved chromium are necessary to calculate Cr(III) from the difference.

The accuracy of metal determinations using the automated system was shown by measurements of total dissolved nickel in certified sea water. The use of in-line UV irradiation for the determination of total nickel further reduced the risk of contamination caused by sample handling.

The described automated system has proved to be reliable in the field. The system has enabled
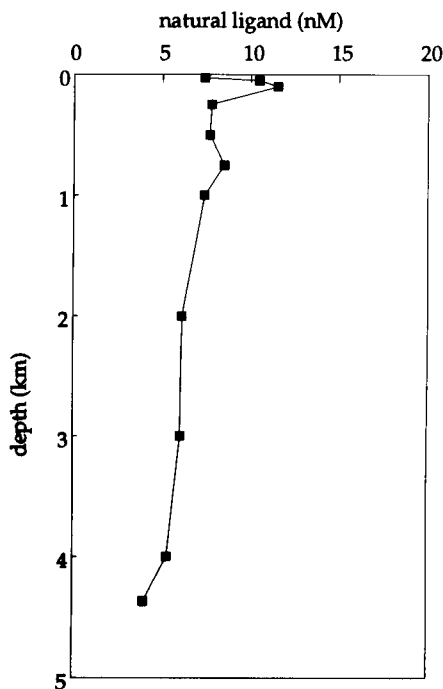
Fig. 7. Vertical depth profile of the concentration of natural ligands for station 3 (48°N, 20°W) in the North Atlantic Ocean.

us to measure metal speciation automatically on-board ship. The built-in intelligence has assured full stand-alone performance of the system which is very convenient in case of sea sickness of the human operator. The automatic rejections of low quality scans, such as caused by ship movement and engine vibrations, appeared to be very convenient. The automated system increased greatly the number of measurements that could be performed per day. During the Mediterranean cruise 15 depth profiles for chromium speciation were obtained (among other metals) within 10 days. On the Atlantic Ocean, up to 80 aliquots for copper speciation were measured per day, compared to at most 30 manually. This way, 16 depth profiles of copper complexing capacity were determined comprising approximately 1600 labile copper determinations, in a period of 4 weeks.

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Speciation of manganese in Chesapeake Bay waters by voltammetric methods

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Abstract

The Mn(II) to Mn(0) reduction wave (peak) at a mercury electrode was investigated for its analytical usefulness in anoxic Chesapeake Bay waters which contain significant quantities of dissolved and particulate organic matter. The Mn(II) to Mn(0) reduction is characteristic for all Mn(II), Mn(III) and Mn(IV) complexes and thus represents total dissolved Mn. It does not provide information on only the Mn(II) oxidation state as suggested previously. Inorganic Mn(II) and organic Mn(III) complexes were studied by sampled d.c. polarography, differential pulse polarography, cyclic voltammetry and square wave voltammetry. All methods show that the Mn(II) to Mn(0) reduction is quasi-reversible in sea water. Square wave voltammetry was used for analytical work on field samples. Both Mn(II) and Mn(III) give similar current versus concentration slopes for the Mn(II) to Mn(0) peak when added to Chesapeake Bay samples. The minimum detection limit is near 200 to 300 nM. Comparison of organic free and organic rich laboratory and field samples shows that E_p shifts to more negative potentials for the organic rich samples. Thus, a major finding of this voltammetric study is that manganese can be complexed by organic ligands in marine systems with zones characterized by high organic matter decomposition and low O_2 concentrations. Organic complexation of dissolved Mn may have important consequences for Mn chemistry in marine systems characterized by an oxic/anoxic interface.

Keywords: Voltammetry; Polarography; Chesapeake Bay waters; Manganese; Sea water; Waters

Manganese has been measured by differential pulse polarography at a dropping mercury electrode in estuarine [1] and in hypolimnetic waters [2–4]. In those studies as well as in laboratory studies monitoring the dissolution of solid state manganese(III,IV) phases [5], the peak for the reduction of Mn(II) to Mn(0) has been indicated to be specific for the measurement of Mn(II) in solution. However, the work of Magers et al. [6] clearly shows that all Mn(II), Mn(III) and Mn(IV) complexes give the same reduction potential within 50 mV for the Mn(II) to Mn(0) conversion. The reduction of Mn(III) to Mn(II) for organic complexes of form MnL_3 (where L is a bidentate ligand) varies over 900 mV from -0.3 to -1.2 V.

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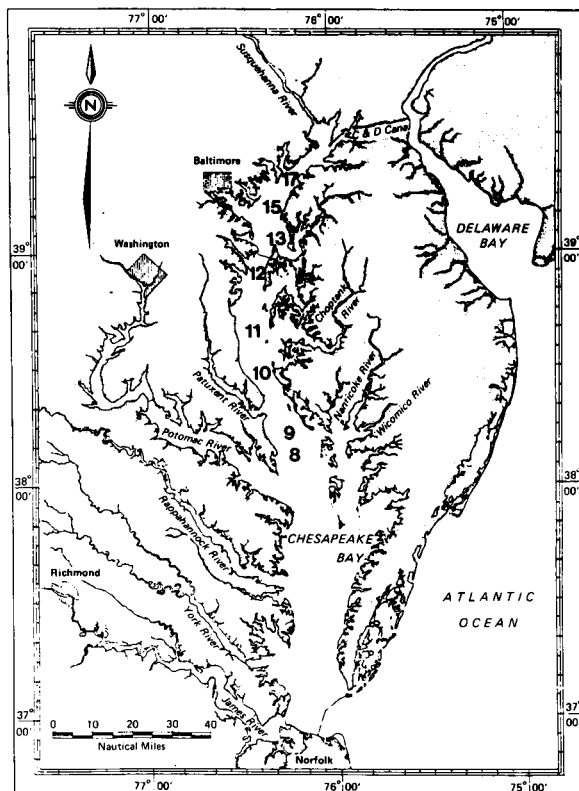
Fig. 1. Map of Chesapeake Bay with sampling locations.

in organic rich waters of estuaries. The complexation of manganese by organic ligands has not been previously documented in marine waters by voltammetric methods.

The Chesapeake Bay can develop extensive seasonal anoxia in the summer months in the northern section of the bay above the Potomac River [7]. A sill which separates the northern from the southern sections entrains deep waters in the northern section and creates a pycnocline. The vertical salinity gradient also results in a vertical redox gradient which is generally characterized by an oxic zone (upper 10–12 m), an anoxic zone where O_2 and H_2S do not coexist (12–26 m) and a sulfidic zone (bottom 2–4 m of a 30 m water column). The anoxic and sulfidic zones have high dissolved manganese concentrations (up to 10 μM). The anoxia develops from intense phytoplankton productivity. On death, the phytoplankton fall through the water column and are remineralized across the oxic/anoxic interface and in the bottom sediments. Primary productivity in the Chesapeake Bay is high. Based on published reports it is greater than that found in the world's largest anoxic basin, the Black Sea. In the Black Sea, organic material which can complex metals is present in the anoxic and sulfidic zones at the 100 μM level [8] which complexes some of the dissolved manganese present [9].

EXPERIMENTAL

Thus, the reduction of Mn(II) to Mn(0) which has been used to measure Mn in natural waters is not specific for the Mn(II) redox state. This reduction peak which occurs near -1.5 V measures total soluble manganese.

In this paper we report on the use of square wave voltammetry (SWV) at a hanging mercury drop electrode (HMDE) to monitor the Mn(II) to Mn(0) reduction peak for analytical purposes in the anoxic waters of the Chesapeake Bay (Fig. 1) from June 25 to June 27, 1991. This peak provides data on total soluble manganese not on Mn(II) only. In addition, the peak potential, E_p, and the slopes (nA/μM) of standard addition curves for this peak are compared for Chesapeake Bay waters and Sargasso Sea water. The comparison shows that the E_p is shifted to more negative values and that the slopes are decreased in Chesapeake Bay waters. These data indicate that manganese is complexed by organic ligands

From June 25 to June 27, 1991, Chesapeake Bay waters (oxic and anoxic) were sampled directly from Go-Flo bottles by pressure filtration through 0.40 μm nuclepore filters. The samples were collected via three way stopcocks into polypropylene or glass syringes. Air was excluded from all samples to minimize oxidation by pressurizing the top of the Go-Flo bottles with ultra high purity nitrogen. The sample was used to purge oxygen from the tubing which leads from the filter to the three way stopcock prior to drawing the sample into the syringe. The syringes were sealed with the stopcocks and stored in a glove bag under a nitrogen atmosphere until analyses were performed. All sampling equip-

ment was made trace metal clean by acid cleaning. Samples for voltammetry were typically run immediately after collection (three replicates with one standard addition series in triplicate can be performed in less than 20 min).

Square wave voltammetry at a hanging mercury drop was performed with an EG & G Princeton Applied Research Model 384B-4 voltammetric analyzer in conjunction with a Model 303A static drop mercury electrode (SDME). The reference electrode was a SCE reference electrode so that the Model 303A was modified slightly as discussed in Luther et al. [10]. We determined that optimal conditions for analysis in the square wave mode are 100 mV/s scan rate, 20 mV pulse height with a 50 Hz frequency. The minimum detection limit (MDL) is about 200 to 300 nM. The scan range typically used was from -1.2 to -1.7 V with the inorganic Mn peak near -1.500 V. Total manganese was determined by the method of standard additions for the marine samples.

Sampled d.c. (SDC) polarography and differential pulse polarography (DPP) were performed on oceanic sea water (pH 8.1) to which 0.1 mM inorganic Mn(II) and organic Mn(III) complexes had been added. The oceanic water was obtained from the northwest Atlantic Ocean east of the Gulf Stream (the Sargasso Sea) and is low in organic matter content. The water was filtered through 0.40 μm nuclepore filters. The scan rate was typically 2 or 4 mV/s for SDC and DPP, and the pulse height for DPP was varied 10–25 mV to determine the n value for electron transfer. Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were performed using scan rates from 5 to 100 mV/s. CV scans were performed with EG & G Princeton Applied Research equipment as above or an IBM Model EC 225-2A voltammetric analyzer with an EG & G Model 303 SDME.

Mn(III) complexes with tartrate and other organic ligands were made according to the method of Magers et al. [6]. A citrate complex of Mn(III) was made according to the method of Duke [11]. Mn(III) acetate from Alfa Products was the solid source of Mn(III) for our experiments. These yellow to brown colored solutions have low molar absorptivities (300–500) with maximum wave-

lengths of 420–450 nm. The complexes are stable at near neutral pH in the absence of oxygen and are stable to oxygen oxidation in 0.1 M NaOH for about one day. The citrate complex is stable for extended periods.

Oxygen was measured by the method of Culberson and Huang [12]. Salinity was measured with a Neil Brown CTD system.

RESULTS AND DISCUSSION

Sampled DC polarography (SDC), linear sweep voltammetry and cyclic voltammetry data indicate that the Mn(II) to Mn(0) reduction peak in all sea water solutions to which Mn(II) or Mn(III) complexes are added is quasi-reversible. The E_p increases slightly with scan rate and the i_p is proportional to the square root of the scan rate using both CV and LSV. The CV shows that both the cathodic and anodic current increase with concentration. However, the cathodic current is greater than the anodic current, and the anodic current peak is not as well defined as the cathodic current (Fig. 2). Thus, anodic stripping experiments should not greatly increase the detection limit for manganese in seawater. Our anodic stripping experiments confirm only a slight increase in the detection limit for manganese in full strength sea water.

0.10 μA
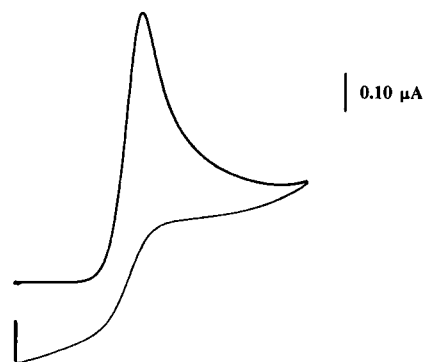
Fig. 2. Cyclic voltammogram of a 50 μM Mn(II) chloride solution in sea water. The cathodic current is greater than the anodic current indicating that the electrode reaction is quasi-reversible. The scan range and rate are -1.25 to -1.75 V and 50 mV/s, respectively.

Plots of $\ln[(i_d - i)/i)]$ versus potential from the SDC data were linear ($r > 0.992$). The half-wave potential, $E_{1/2}$, is -1.500 V for Mn(II) in sea water and -1.525 V for Mn(III) citrate in sea water. The negative shift in $E_{1/2}$ for Mn(III) versus Mn(II) solutions indicates organic complexation [13]. However, 1.56 electrons (not 2) are calculated for electron transfer at the electrode for the inorganic Mn(II) solutions indicating that there are kinetic constraints to electron transfer at the mercury electrode in sea water. For Mn(III) organic complexes, the n value determined was nearer to 1.40 in sea water. Analysis of the peak width at half-height, $W_{1/2}$, in DPP for both Mn(II) and Mn(III) solutions indicates that the number of electrons (n) is near 1.3 for both forms of manganese and not equal to 2.

Figure 3 shows the SDC polarogram of the Mn(III) tartrate complex in 0.3 M NaOH solution and demonstrates that the Mn(III) complex has a Mn(II) to Mn(0) reduction wave as do all Mn(II) salts as well as a Mn(III) to Mn(II) reduction wave. The current for the Mn(II) to Mn(0) wave is twice the Mn(III) to Mn(II) reduction wave as
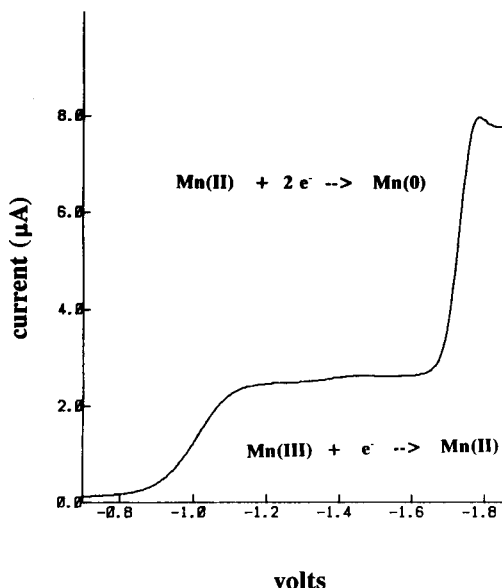
Fig. 4. Plots of current versus pulse height (×) at constant frequency (50 Hz), and versus frequency (○) at constant pulse height (20 mV) for 10 μM Mn(II) in Sargasso Sea water.

expected based on the number of electrons transferred. Inorganic Mn(II) solutions added to the tartrate solution result in a stoichiometric increase in the Mn(II) to Mn(0) wave but no increase for the Mn(III) to Mn(II) wave as expected based on the results of Magers et al. [6].

Figure 4 shows the variation of pulse height at constant frequency (50 Hz) and of frequency at constant pulse height (20 mV) versus current in the square wave voltammetry mode for 10 μM Mn(II) in Sargasso Sea water. The peaks increase significantly with pulse height and the optimum pulse height approaches 35 mV. A truly reversible electrode process should provide an optimum value at 25 mV [50/n (mV)]. Because the optimum pulse height approaches 35 mV, the square wave data show that the electrode process is quasi-reversible which is consistent with the other experiments described above. The peaks broaden significantly at pulse heights > 50 mV, and peak height measurements show poorer precision. The current response reaches a plateau at a square wave frequency of 75 Hz.

Based on the SWV results in Fig. 4, we investigated manganese voltage–current relationships in solutions of varying salinities using the SWV mode. Mn(II) was added to low organic content (DOC) Sargasso Sea water purged of O_2 by the method of standard additions. Figure 5 demonstrates the expected linearity ($r \geq 0.9997$) from the MDL (near 200–300 nM) to at least 80 μM. The current versus concentration slopes (nA/

Fig. 3. Sampled DC polarogram of the manganese(III) tartrate complex. Note that the reduction curve for Mn(III) to Mn(II) is half the height of the Mn(II) to Mn(0) curve as expected.
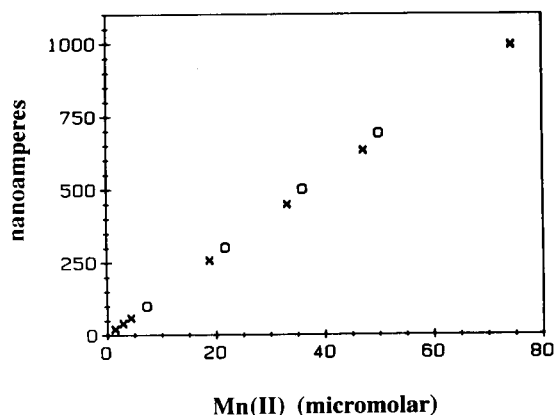
Fig. 5. Standard addition curves of Mn(II) added to Sargasso Sea water (×) and 0.565 M NaCl (○).

μM) for Mn(II) in Sargasso Sea water of 35‰ are near 13.0 nA/μM. Sargasso Sea water diluted to 17.5‰ yielded slopes near 14.0 nA/μM whereas sea water diluted to 3.18‰ gave slopes near 15.0 nA/μM. The range in salinity tested is identical to that found in the Chesapeake Bay. Sodium chloride solutions (0.565 M) gave slopes of 13.8 nA/μM. These data indicate a slight salinity affect on the Mn(II) to Mn(0) reduction peak. E_p is typically -1.49 to -1.50 V versus the SCE for the high salinity (chloride) solutions. Comparing the 3.18‰ sea water solution versus the higher salinity solutions, there is a $+15$ mV
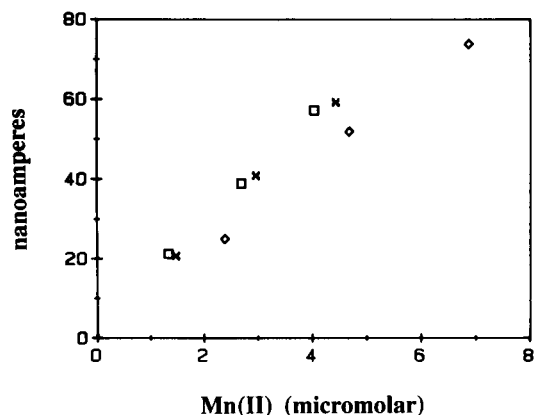
Fig. 6. Standard addition curves of Mn(II) added to Sargasso Sea water (×), Sargasso Sea water diluted to 3.18‰ (□), and Chesapeake Bay water (◇). Mn(III) complexes added to Chesapeake Bay waters give similar curves as the Mn(II) added to Chesapeake Bay waters.
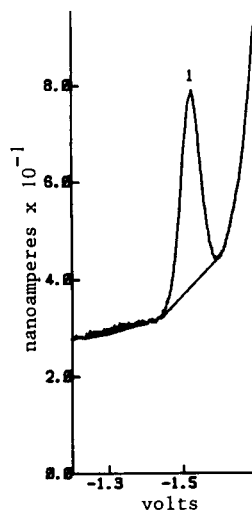
Fig. 7. Square wave voltammogram of manganese observed for Chesapeake Bay water samples. E_p occurs at -1.522 V with a current of 39.61 nA.

shift indicating less inorganic complexation on lower chloride content. This shift is consistent with Carpenter's ESR data which indicates that chloride is the major anion complexing Mn(II) in solutions of varying inorganic anion content [14].

Mn(II) added to the organic rich waters of the Chesapeake Bay which had a salinity range of 2–20‰ gave slopes of 10.5–11.0 nA/μM for all salinities. Figure 6 shows a comparison of the standard addition curves at low Mn(II) concentrations for Chesapeake Bay waters and manganese free Sargasso Sea water of 35‰ and 3.18‰. These data for Mn(II) standards added to low organic content Sargasso Sea waters appear more linear than those reported by Knox and Turner [1] and Davison [2] who used differential pulse polarography for estuarine and lake waters, respectively. Knox and Turner [1] noted a larger slope for manganese concentrations < 4 μM and a smaller slope for concentrations > 4 μM. However, Davison [2] showed linear curves in the SDC mode which is a less sensitive mode than DPP. In these Chesapeake Bay samples, standard addition curves were linear from the MDL to 40 μM. However, there were not enough samples of low manganese concentration for a more direct comparison with the results of Knox

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and Turner [1]. We do note some slight curvature in the Chesapeake Bay sample in Fig. 6 which is similar in shape but not magnitude as that noticed by Knox and Turner [1].

Figure 7 demonstrates a typical square wave voltammogram for manganese in Chesapeake Bay waters. E_p always ranges from -1.52 to -1.53 V in these anoxic Chesapeake Bay waters from 1991. For inorganic Mn(II) solutions added to Sargasso Sea water, E_p is typically near -1.50 V. The negative peak shift of 20 to 30 mV and the decrease in the current versus concentration slopes for these organic rich and anoxic Chesapeake Bay waters as compared to oceanic waters with low organic content indicates organic complexation of manganese [13]. In addition, for oxic surface samples, there was a shift in E_p of -30 mV from the low to high salinity samples indicating that organic complexation is important. This field data is similar to the SDC laboratory data on stock manganese solutions (see above).

Addition of Mn(III) complexes (1–5 μM in Mn and 100–500 μM in ligand after addition) to low DOC and deaerated Sargasso Sea water results in lower current versus concentration slopes approaching 10.5 nA/μM. However, the manganese eventually precipitates because of redistribution of the chelate to Mg and Ca. Addition of Mn(III) complexes to 0.565 M NaCl results in standard curves without precipitation of Mn(III). E_p is typically > -1.52 V in these NaCl solutions also and indicates that the Mn is complexed. Addition of Mn(III) complexes to Chesapeake Bay waters results in current versus concentration slopes that are similar to Mn(II) standard addition curves (within 5–10% as expected based on the determination of the n value for the peak). No manganese precipitation was noticed on addition of Mn(III) complexes to these Chesapeake Bay waters.

Table 1 presents representative manganese, oxygen and salinity data obtained from the suboxic/anoxic waters in Chesapeake Bay during June 25 to June 27, 1991. The sampling locations are given in Fig. 1. Although the manganese data reported in Table 1 have shifted E_p values indicating that the manganese is organically complexed, we cannot indicate the oxidation state of

TABLE 1

Representative data from the water column of the northern section of the Chesapeake Bay during June 25 to June 27, 1991. Several stations were sampled over the course of a day

Station	Depth (m)	Mn (μM)	O$_2$ (μM)	O$_2$ Sat. (%)	Salinity (‰)
9	35.0	1.98	2.2	0.9	20.815
10A	15.6	2.10	2.3	1.0	18.934
10A	25.0	2.56	1.2	0.5	19.914
10A	28.0	2.46	1.1	0.5	20.007
10B	11.9	2.85	32.3	13.4	17.465
10B	27.9	0.54	0.7	0.3	20.198
10C	28.9	2.30	0.7	0.3	20.234
11	24.9	3.18	0.7	0.3	19.879
12A	11.0	3.59	65.7	26.4	15.825
12A	30.7	5.70	0.7	0.3	18.693
12D	12.6	3.79	20.6	8.2	15.000
12D	29.9	6.10	2.9	1.2	18.475
12E	13.0	4.05	16.0	6.8	16.307
12E	30.2	7.08	2.9	1.2	18.412
13	12.4	3.99	88.6	35.4	13.296

the manganese because both Mn(II) and Mn(III) complexes added to these waters give identical current versus concentration slopes. This is consistent with the addition of Mn(II) chloride and Mn(III) citrate solutions to voltammetric cells containing 10 ml of Sargasso sea water and 500 μl of a citrate buffer [11]. E_p for both Mn(II) and Mn(III) solutions in this matrix is -1.54 V. The same E_p shows organic complexation of dissolved manganese but provides no information on manganese oxidation state.

Earlier work by Knox and Turner [1] and Davison [2] did not show evidence for significant organic complexation of manganese in natural waters partly because of the nonlinear nature of the DPP standard addition curves at lower concentrations. More recent freshwater studies [3,4] also show no organic complexation of manganese. The difference between those studies and this one may be related to differences between the environmental systems. Knox and Turner [1] performed their work in the Tarmar Estuary which is characterized by high suspended solid content.

The only samples that they analyzed were surface samples which contained significant oxygen concentrations. Davison and co-workers [2,3] and De Vitre et al. [4] sampled the anoxic hypolimnetic waters of productive English and Swiss lakes where dissolved Fe is typically higher in concentration than dissolved Mn. In this study the deeper mid-salinity waters of the Chesapeake Bay showed extensive anoxia due to the decomposition of the particulate organic matter (derived from phytoplankton production) to dissolved organic carbon. Oxygen concentrations were generally < 10 μM for the samples studied. Significant sulfide levels (≤ 5 μM) were detected only in the bottom waters of station CB12. Also, dissolved Mn is typically higher than dissolved Fe in Chesapeake Bay waters which is different from the lake studies [2–4].

In the recent oceanographic literature, there has been more emphasis on the interactions of trace metals with organic ligands. For example, Bruland and co-workers [15–17] have demonstrated that there are one or more ligand classes of unknown composition which bind to Cu and Zn as determined by voltammetric methods. Smith and Martell [18] indicate that Zn(II), Mn(II) and Fe(II) complexes of one to one stoichiometry with the same organic chelate have stability constants within one log unit. The order of strength for most amino acids for example is Zn(II) $\geq$ Fe(II) > Mn(II). Thus, Fe and Mn should also be complexed in sea water in environments rich in organic matter and particularly where O_2 does not reach significant concentrations. Recently, Lewis and Landing [9] using low pressure column chromatography methods followed by atomic absorption spectroscopy detection found significant Mn complexation with unspecified organic compounds in the Black Sea. The studies of Lewis and Landing [9] and our own in anoxic systems of similar redox chemistry indicate that Mn complexation may be more important than previously assumed. These studies are particularly important since the two groups use different instrumental techniques to determine organic complexation. Thus, we conclude that manganese is complexed with organic ligands under low O_2 conditions where intense remineralization of particulate organic matter to dissolved organic matter occurs. Mopper and Kieber [8] found a variety of carboxylic acids and sugars up to tens of μM in concentration in the interface region of the Black Sea. These are ideal ligands to complex Mn.

We hypothesize that organic complexation of Mn has important geochemical consequences and may be related to the formation of Mn(III) complexes at oxic/anoxic interfaces. Our data does not allow us to indicate the oxidation state(s) of the manganese. Because these waters are low in O_2 and high in organic matter content, we cannot rule out the possible existence of Mn(III) organic complexes which are known to be stable [6,11]. In fact, soluble Mn(III) complexes maybe formed as a consequence of organic matter decomposition at the interface. We present two possibilities.

First, MnO_2 and other Mn(III,IV) solid phases are potential oxidants for organic matter decomposition [19]. The higher oxidation state compounds can come from atmospheric sources and/or the oxidation of Mn(II) by O_2. {The Mn(II) can diffuse into the water column from bottom sediments because of sulfide reduction of Mn(III,IV) oxide phases [20].} These solid state manganese compounds will be reduced to Mn(II) or Mn(III) during organic matter decomposition. Not all of the organic matter is oxidized to CO_2 as much of it is degraded to soluble organic ligands [8] which can complex soluble manganese in either the +2 or +3 oxidation state.

Second, some of the dissolved organic matter produced during organic matter decomposition has unsaturation and is a potential oxidant for Mn(II). Recently, Richert et al. [21] demonstrated that organic ligands can undergo electrochemical oxidation. The Mn(II) bound to this oxidized organic ligand can transfer an electron directly on the bond axis to the oxidized ligand which in turn becomes reduced. The overall result is the formation of Mn(III) (reaction 1):

$$(R)-Mn^{II} \rightarrow (R)^{-}-Mn^{III} \qquad (1)$$
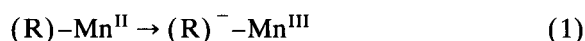
The oxidation of Mn(II) by oxidized lignin material to produce Mn(III) chelates has recently been documented for white rot fungi [22,23].

In sea water systems, any soluble Mn(III) complexes can then diffuse or be mixed by tides or

internal waves throughout the anoxic zone. These complexes may be a major missing component of the system's oxidizing capacity which can affect the anaerobic oxidation of sulfide that has been reported in both the Black Sea [24] and the Chesapeake Bay [25,26]. On reaching the sulfidic zone, sulfide which may also bind to the manganese is then oxidized by the Mn(III) complex (e.g., reaction 2):

$$(R)^- -Mn^{III}-S \rightarrow (R)^- -Mn^{II}-S^+ \rightarrow$$

$$\text{products} \left[\text{reduced organic matter, } S_8, SO_4^{2-} \right.$$

$$\left. + (R)^- -Mn(II) \right] \quad (2)$$
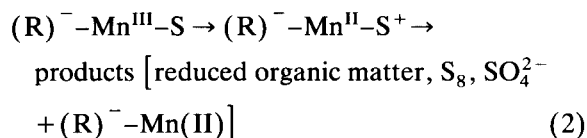
The Mn(II) produced may be cycled again for the oxidation of reduced sulfur. Because of low O_2 levels and because the Mn(II) and Mn(III) are dissolved and complexed, solid phase Mn(III,IV) compounds do not form in great amounts [9]. Tebo [27] showed that the anoxic zone of the Black Sea was characterized by Mn(II) oxidation rates which are "potentially" high. Because Mn(II) oxidation rates are measured by the actual precipitation and collection of MnO_2, oxidation of Mn(II) to soluble Mn(III) is not determined and total Mn(II) oxidation may be underestimated in these and other marine environments.

Recently, Boughriet et al. [28] have presented ESR data documenting the existence of Mn(III) in suspended particles of the Seine estuary. We plan to address the existence of dissolved Mn(III) by using an excess of a strong ligand to outcomplex the natural ligands for the manganese present in marine samples. The Mn(III) to Mn(II) reduction peak may then be monitored for analytical information on Mn(III), and the Mn(II) to Mn(0) peak will indicate total manganese as shown in this and other work [29]. A major finding of this voltammetric study is that manganese can be complexed by organic ligands in marine systems with zones characterized by high organic matter decomposition and low O_2 concentrations.

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Determination of copper complexation in sea water by cathodic stripping voltammetry and ligand competition with salicylaldoxime

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Abstract

Copper complexing ligands in sea water were determined by cathodic stripping voltammetry (CSV) with ligand competition using salicylaldoxime (SA) at a detection window intermediate to that and overlapping with those currently available using other electroactive ligands. The optimised condition to determine total dissolved copper in sea water by CSV using SA entails an SA concentration of 25 μM, a solution pH of 8.0–8.4, and a deposition potential of -1.1 V; the voltammetric scans were initiated at -0.15 V. The conditional stability constants for copper complexation by SA were calibrated by ligand competition against EDTA in sea water of salinities between 1 and 35. The following empirical relationships were found to hold between these conditional stability constants and the salinity (S, in psu): log $K'_{CuSA} = (10.12 \pm 0.03) - (0.37 \pm 0.02)$ log S, and log $\beta'_{Cu(SA)_2} = (15.78 \pm 0.08) - (0.53 \pm 0.07)$ log S. The centre of the detection window for detection of copper complexation in sea water using SA can be varied in the range of 3.6–5.8 by varying the SA concentration between 1 and 25 μM; this range lies between that covered by tropolone (2.5–4.5) and 8-quinolinol (5.0–8.4). Use of SA in CSV has the analytical advantage of high sensitivity for copper (3–4 fold greater than using catechol, 8-quinolinol or tropolone), reflected in a limit of detection of 0.1 nM copper at a deposition time of 1 min which can be lowered further by extending the deposition time. Comparative complexing ligand titrations indicated that an equilibration period of 6 h is required to attain equilibrium between the added copper, the natural complexing ligands and the added SA. The method was tested by determining copper complexation at several detection windows in samples from the North Sea, the NW Mediterranean Sea and the NE Atlantic Ocean, revealing the presence of several complexing ligands. At a constant detection window the ligand concentration in the upper water column of a station in the NE Atlantic was found to vary between 3 and 8 nM, with a maximum at 78 m depth.

Keywords: Stripping voltammetry; Complexation; Copper; Salicylaldoxime; Sea water;

Interest in the chemical speciation of trace metals in natural waters has led to the development of several techniques to determine interactions between uncomplexed metal ions and organic complexing ligands. Various techniques are based on equilibration with MnO_2 [1], adsorption on cation exchange resin with [2] and without [3]

ligand competition, adsorption on C_{18} cartridges [4], anodic stripping voltammetry (ASV) [5], cathodic stripping voltammetry (CSV) [6], and chemiluminescence [7]. Of these methods those based on equilibration with MnO_2, resin adsorption with ligand competition, chemiluminescence with ligand competition, and those utilising CSV with ligand competition, employ measurement after equilibrium has been established between an added ligand or adsorption site with the natural complexing ligands and metal ions in the sample.

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The resin adsorption, ASV, and chemilumines-
cence methods subsequently determine their re-
spective labile metal fractions relying on *kinetic*
stability of the non-labile fraction, whereas the
MnO_2 and CSV methods determine the labile
fraction without disturbing the established equi-
librium. The non-labile metal fraction of the lu-
minescence method [7] is that bound by the added
competing ligand EDTA, so the extent to which
other ligands are detectable depends on the con-
centration of added EDTA whereas ligands simi-
lar to EDTA would obviously be undetectable.

Investigations into the chemical speciation of
copper in sea water tend to agree that this metal
occurs mainly organically complexed. Differences
in the stability of the organic complexes which
are detected could be due to variations in the
water composition, to systematic errors in the
techniques used, or to a combination of the pres-
ence of perhaps many complexing ligands that
form complexes of a range of stabilities with a
bias of the analytical techniques for complex sta-
bilities falling within a limited range.

Recent studies have indicated that differences
in the degree of organic complexation detected
for copper and other metals cannot be simply
reconciled with the analyses having been carried
out in samples from different origin, or with
possible systematic errors of some the techniques.
Comparative determinations of copper complex-
ing ligand concentrations and conditional stability
constants have indicated that several complexing
ligands are present in estuarine waters [8], coastal
waters and samples of oceanic origin [9], and that
the ligand that is detected depends on the detec-
tion window of the analytical technique used be-
cause of the large spread of complex stabilities of
the natural complexes with values of conditional
stability constants spanning 5–6 decades. For this
reason it is useful to further investigate copper
complexation at carefully controlled detection
windows.

In CSV the stability of the surface active com-
plexes formed by the added ligand determines
the detection window [6,8,9]. Variation of the
concentration of the added ligand or selection of
a ligand that forms weaker or more stable com-
plexes causes therefore a shift in the location of

the detection window. The demonstrated ability
to vary the detection window over many orders of
magnitude is currently a unique property of CSV
although all methods utilising ligand competition
theoretically should be able to vary the detection
to some extent. Copper can be determined by
CSV at low levels in sea water using catechol [6],
8-quinolinol (oxine) [10], and tropolone [11]. The
stabilities of the copper complexes with catechol
span a large range but catechol cannot be equili-
brated for longer than a few minutes with the
natural complexing material due to its sensitivity
to oxidation by dissolved oxygen and recent ex-
periments have indicated that very long equilibra-
tion times (several hours) are necessary [9]; the
complexes with tropolone are comparatively weak
and are particularly suitable for complexation
studies at low detection windows [11], whereas
the complexes with oxine are very stable making
this ligand more suitable for the detection of very
stable natural complexes as weak complexes
would be dissociated fully by the added oxine.

The detection window of the existing CSV
procedures is centred on complexes with α-coef-
ficients in the range of (log values) 2.5–4.0
(tropolone) and 6.4–9.0 (oxine) leaving a gap for
complexes with α-coefficients between (log val-
ues) 4.0 and 6.4 in which copper complexation
cannot be determined accurately.

The application of salicylaldoxime (SA)
(salicylaldehyde oxime, 2-hydroxybenzaldehyde
oxime) to copper complexation studies is re-
ported here. The stability of the complexes of SA
with copper is intermediate to that of oxine and
tropolone, and a large range of complex stabili-
ties can be covered by variation of the SA con-
centration; a further advantage is that copper can
be determined in sea water by CSV using SA at
better sensitivity than using other ligands. The
optimisation of the CSV method to determine
total dissolved copper, labile copper, and com-
plexing ligand concentrations, is described in this
paper; the conditional stability constants for com-
plexation of copper by SA are calibrated against
EDTA in sea water at salinities between 1 and 35
psu, and a comparative complexation study is
carried out at several detection windows using SA
and other electroactive complexing ligands.

MATERIALS AND METHODS

Equipment

A Metrohm Model 646 VA-processor polarograph was interfaced to a Metrohm Model VA 675 automated hanging mercury drop electrode (HMDE) with associated sample changer for 10 voltammetric cells. The reference electrode was Ag/AgCl, saturated AgCl, 1 M KCl, whereas the counter electrode was a platinum wire. The drop surface area of the HMDE was 0.45 mm^2. Solutions were stirred during the deposition step by a rotating PTFE rod. Scans produced during complexing ligand determinations were transferred to an IBM-AT compatible (Opus) computer via a serial link and peak heights were evaluated using a program (GPES2) of a different voltammeter (Autolab from Ecochemie, Netherlands). Experiments involving cyclic voltammetry were carried out using an Autolab voltammeter connected to a Metrohm Model 663 VA automated HMDE.

All sample containers were cleaned by soaking 1 week in 50% HCl (GPR grade), followed by at least 1 week in 2 M HNO$_3$ (AnalaR grade), and were stored partially filled with 0.01 M HCl (Aristar grade). Voltammetric cells (glass and PTFE) used for total dissolved copper determinations were cleaned using 2 M HCl, and 0.01 M HCl. PTFE voltammetric cells were used for complexing ligand titrations. After a preliminary soak in acid these cells were rinsed with purified water between titrations.

pH measurements were calibrated against a pH 9 standard on the NBS pH scale. Either a 100-W UV system with 5 30-ml silica sample tubes or a 1-kW system with 8 100-ml silica sample tubes was used to irradiate sea water samples prior to determinations of total dissolved copper.

Sample collection and storage

Sea water from the NW Mediterranean Sea (a mixture of filtered samples from various depths at 40°03′N/01°51′E and 41°57′N/5°57′E, collected during the Cybele cruise, April 1990) and from the North Sea (53°34′N/0°58′E, 3 m depth) was used for preliminary experiments. The sea water from the Mediterranean was stored frozen,

whereas the North Sea water was stored at 4°C. Complexing ligand concentrations in filtered (0.4 μm membrane filters) sea water from the NE Atlantic were determined on-board ship within hours of sampling; the samples described here were collected during Challenger cruise 76/91, March 1991, at station 4 (42° : 16 : 28N/ 20° : 1 : 9W). Sea water used for total dissolved copper determinations was acidified to pH 2.2 by addition of 10 μl 6 M HCl (Aristar) per 10 ml of sea water.

Reagents

Water was purified by reverse osmosis using a Milli-RO system followed by deionisation using a Milli-Q system. Hydrochloric acid and ammonia (Aristar grade, Merck) were further purified by isothermal distillation. An aqueous stock solution containing 0.01 M SA was prepared in 0.1 M HCl and was found to be stable for at least 8 weeks at 4°C. Copper standard solutions were prepared by dissolution of an atomic absorption spectrometry standard solution (Merck, SpectrosoL grade) in 0.01 M HCl. A pH buffer containing 1 M boric acid (Aristar grade) and 0.35 M ammonia was UV-irradiated for 4 h to remove organic contaminants; 100 μl of this buffer in 10 ml sea water gave a pH of 8.35. The pH of an aqueous stock solution containing 0.1 M EDTA was adjusted to neutral using ammonia. Stock solutions of Triton-X-100 (Merck, GPR) were diluted with water from a stock solution containing 100 mg l^{-1}.

Procedure to determine total dissolved copper using CSV

Acidified sea water was UV-irradiated for 4 h prior to the copper determination whereas untreated sea water was used for labile copper determinations. An aliquot of 10 ml sea water was pipetted into the voltammetric cell; ammonia was used to approximately neutralise the pH of acidified sea water, and 100 μl borate pH buffer (final concentration 0.01 M) and 25 μl SA (final concentration 25 μM SA) were added. The solution was deaerated by purging (5 min) with water-saturated argon gas. The deposition potential was set to −1.1 V; four mercury drops were discarded before a new mercury drop was ex-

truded, and the solution was stirred for a preset period of 1–2 min (length depending on the copper concentration but normally 1 min was used). Then the stirrer was stopped and a quiescent period of 10 s was allowed before switching the potential to −0.15 V for a period of 15 s to re-oxidise the deposited and plated copper. Then the potential scan was initiated from −0.15 V and was terminated at −0.6 V. The scanning parameters were: differential pulse modulation, 5 pulses s^{-1}, scan rate 20 mV s^{-1}, pulse height 25 mV. The determination was repeated after addition of copper standard in order to calibrate the sensitivity.

Procedure to determine copper complexing ligand concentrations

Approximately 100 ml sea water was transferred to a PTFE bottle and 0.01 M borate buffer and 0.3–10 μM SA (actual concentration depending on the desired detection window) were added. Copper was added to 10 PTFE voltammetric cells giving a concentration range between 0 and 30 nM (in 10 ml sea water) in 10 steps; 10 ml aliquots of sea water were then pipetted into the voltammetric cells and the cells were covered with clean petridishes to equilibrate overnight (the equilibration time was 8 h for the Atlantic data). The labile copper concentration (i.e. that which reacted with the added SA) in each cell was then determined by CSV using 1 min deposition (2 min when [SA] < 1 μM); a deposition potential of −0.15 V was used to determine the labile copper concentration to eliminate dissociation of natural complexes of copper which has been shown to occur at potentials more negative than −0.65 V and which is likely to occur at potentials more negative than ca. −0.3 V [12].

The sensitivity was evaluated from the sensitivity at copper concentrations where the natural ligands had been saturated with copper; the sensitivity was further corroborated by comparison with the slope obtained by further additions of copper standard to two voltammetric cells at the high end of the copper titration where all organic complexing would be likely to saturated with copper (no equilibration time was allowed for these copper additions to prevent organic complexation

in case any uncomplexed organic ligands remained).

The voltammetric cells were rinsed with distilled water between titrations, and the same order of cells was maintained, in order to condition the cells with copper and eliminate adsorption onto the cell walls.

Determination of α_{CuSA}

North Sea water (35 psu) was UV-irradiated and diluted with UV-irradiated water to obtain salinities of 1, 10, 20 and 35 psu. EDTA (0.1–100 μM) was added to 4–6 PTFE voltammetric cells, and equilibrated overnight with sea water (10 ml) containing 0.02 M borate pH buffer and 25 nM copper, and in separate experiments with various concentrations of SA (1, 2 and 25 μM). A further 3 voltammetric cells contained the same solution without EDTA for each level of SA. Subsequently the labile copper concentration was determined using an adsorption potential of −0.15 V.

Theory: evaluation of complexing ligand titrations and calibration of the stability of copper complexes with SA

The linearisation procedure to evaluate complexing ligand concentrations and conditional stability constants from the titration data has been described before [1,11,13,14]. Briefly, the following relationship is used:

$$[\text{Cu}_{\text{labile}}]/[\text{CuL}]$$
$$= [\text{Cu}_{\text{labile}}]/C_{\text{L}} + \alpha'/(K'_{\text{CuL}}C_{\text{L}}) \qquad (1)$$

where [CuL] is the concentration of copper complexed by natural ligands L, [Cu$_{\text{labile}}$] is the labile copper concentration, C_{L} is the ligand concentration, and K'_{CuL} is the conditional stability constant for the formation of CuL in sea water. The labile copper concentration is defined by

$$[\text{Cu}_{\text{labile}}] = \Sigma[\text{Cu}(\text{SA})_x] + [\text{Cu}'] \qquad (2)$$

where [Cu(SA)$_x$] is the concentration of copper complexed by SA and [Cu'] is the concentration of inorganic copper (all copper not complexed by SA or natural organic complexing lig-

ands L). α' is the overall α-coefficient [15] of Cu^{2+} (excluding complexation by L),

$$\alpha' = (\alpha_{Cu'} + \alpha_{CuSA}) \tag{3}$$

where $\alpha_{Cu'}$ is the α-coefficient for inorganic complexation of Cu^{2+}:

$$\alpha_{Cu'} = 1 + \Sigma\left(\beta'_{CuX_i}[X]^i\right) + \Sigma\left(\beta'_{Cu(OH)_i}/[H^+]^i\right) \tag{4}$$

where β'_{CuX_i} is the stability constant for copper complexes with i major anions (X) in sea water and $\beta'_{Cu(OH)_i}$ the acidity constant for copper valid at the ionic strength of sea water, and where α_{CuSA} is the α-coefficient for complexation of copper by SA:

$$\alpha_{CuSA} = K'_{CuSA}[SA'] + \beta'_{Cu(SA)_2}[SA']^2 \tag{5}$$

where K'_{CuSA} and $\beta'_{Cu(SA)_2}$ are the conditional stability constants for the formation of CuSA and $Cu(SA)_2$ respectively, and [SA'] is the concentration of SA not complexed by copper (invariably [SA'] equalled the total SA concentration as this was much greater than the copper concentration).

The α-coefficients used here are slightly modified from those defined by Ringbom and Still [15] in that $[Cu^{2+}]$ is included only with the inorganic copper concentration (Eqn. 4) to avoid having to make corrections in other equations containing α-coefficients for complexes of copper (Eqn. 5 and later). This means for instance that α_{CuSA} (as defined in Eqn. 5) is α_{CuSA} (according to Ringbom and Still [15]) − 1.

Concentrations of CuL were calculated from $[CuL] = C_{Cu} - [Cu_{labile}]$, where C_{Cu} is the total dissolved copper concentration at each point of the titration. A value for C_L is obtained by least squares linear regression from the $slope^{-1}$ of a plot of $[Cu_{labile}]/[CuL]$ as a function of $[Cu_{labile}]$ (as shown in Fig. 6B), whereas a value for K'_{CuL} is obtained from $K'_{CuL} = \alpha' \times slope/Y$-axis intercept. Concentrations of Cu^{2+} were calculated from $[Cu^{2+}] = [Cu_{labile}]/\alpha'$.

Though it is possible to determine ligand concentrations without knowing a value for α_{CuSA}, a reasonably accurate value is required to calculate K'_{CuL} and Cu^{2+} for sea water conditions; values for α_{CuSA} were therefore determined by calibration against a known chelating agent (EDTA)

added to sea water diluted with purified water to several salinities between 1 and 35 psu. The ratio, X, of the reduction current in the presence of EDTA (i_p) over that in the absence of EDTA (i_{p0}) was used to calculate values for α_{CuSA} (analogous to [11,16]):

$$\alpha_{CuSA} = \left[(\alpha_{Cu'} + \alpha_{CuEDTA})X - \alpha_{Cu'}\right]/(1 - X) \tag{6}$$

RESULTS AND DISCUSSION

Cyclic voltammetry

Cyclic voltammetry (preceded by 60 s adsorption at −0.05 V and using a scan rate of 50 mV s^{-1}) of sea water to which 25 μM SA and 30 nM copper had been added revealed the presence of a reduction peak corresponding to the reduction of Cu(II) to Cu(0) (Fig. 1A). The peak potential for the copper peak was ca. −0.36 V, compared with a peak potential of −0.17 V for inorganic copper in sea water. The reduction potential of the copper(II)–SA complex is shifted towards this rather negative reduction potential compared to that of uncomplexed copper(II) (at approximately 0 V) and inorganic copper(I) (−0.17 V), which is stabilised by chloride complexation in sea water [6,17], by a combination of complexation stabilisation and adsorption stabilisation.

The shape of the cathodic peak was affected by a shoulder that was not present when a second scan was carried out immediately after the first without renewed adsorption. The shoulder in the first scan became more pronounced at faster scan rates, and the height of shoulder increased with the copper concentration. Assuming that the shoulder was due to adsorption stabilisation, which is known to produce this kind of phenomenon [18], the absence of the shoulder in the second scan suggests that the SA complex of copper may adsorb in two configurations, the more stable one (producing the shoulder) forming more slowly than the one representing the main peak.

The presence of an anodic peak (Fig. 1A) indicates that the electrochemical reaction is
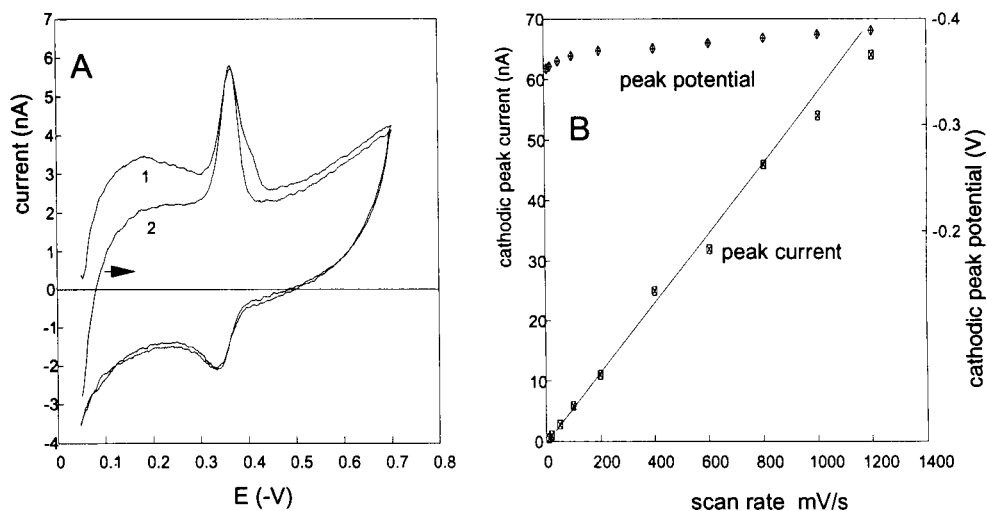
Fig. 1. (A) Cyclic voltammetry of sea water containing 30 nM copper, 0.01 M borate pH buffer (pH 8.4) and 25 μM SA; the scan rate was 50 mV s^{-1}. (B) Effect of varying the scan rate on the peak height and peak potential of the reduction peak of copper.

largely reversible; the lower height of the anodic peak, and a small shift of 19 mV towards a more positive potential, may be due to diffusion of reduced copper(0) into the mercury.

The height of the cathodic peak was found to increase approximately linearly with the scan rate when this was increased from 10 to 1200 mV s^{-1} (Fig. 1B) in line with the expected response for the reduction of an adsorbed compound, and indicating that the entire adsorbed layer of copper complexes is reduced at all scan rates tested. Further evidence of the apparently fast kinetics of the reduction reaction is the small shift of the peak potential of 0.036 V towards more negative potentials when the scan rate was increased from 10 to 1200 mV s^{-1} (Fig. 1B).

Variation of the SA concentration and the pH

The differential pulse modulation was used for analytical purposes as this was found to improve the sensitivity, and largely eliminated the shoulder on the cathodic peak presumably due to greater reversibility of the main reduction peak for copper. Variation of the SA concentration revealed that the height of the reduction peak for copper in sea water (pH 8.4) increased with the SA concentration at SA concentrations > 0.1 μM, levelling off at SA concentrations > 25 μM

(Fig. 2A). The increased sensitivity with increasing SA concentration was caused by increased stability of the adsorptive copper complexes with SA; this increase was reflected in a negative shift in the peak potential of the copper reduction peak (Fig. 2A). Apparently formation of the adsorptive complex was complete at SA concentrations ca. 25 μM in sea water of pH 8.4. This experiment was carried out at several salinities, and a SA concentration of 10–25 μM was found to be optimal (giving greatest peak height) for the determination of copper in fresh and sea water at salinities between 1 and 35.

The shift of ca. 0.33 V of the peak potential from that for uncomplexed copper(II) at ca. -0.05 V [6] to ca. -0.38 V by 25 μM SA is greater than can be accounted for by complexation alone as a shift of 0.33 V would suggest a value for log α_{CuSA} of ca. 11 (estimated using a ca. 29 mV shift/decade of α for a 2-electron reduction step), much greater than a value of 5.8 obtained by calibration against EDTA (see below). The large shift is therefore caused by a combination of complex stabilisation and adsorption stabilisation.

Variation of the solution pH showed that the sensitivity increased with the pH at pH values between 5 and 7.8, whereas maximum sensitivity
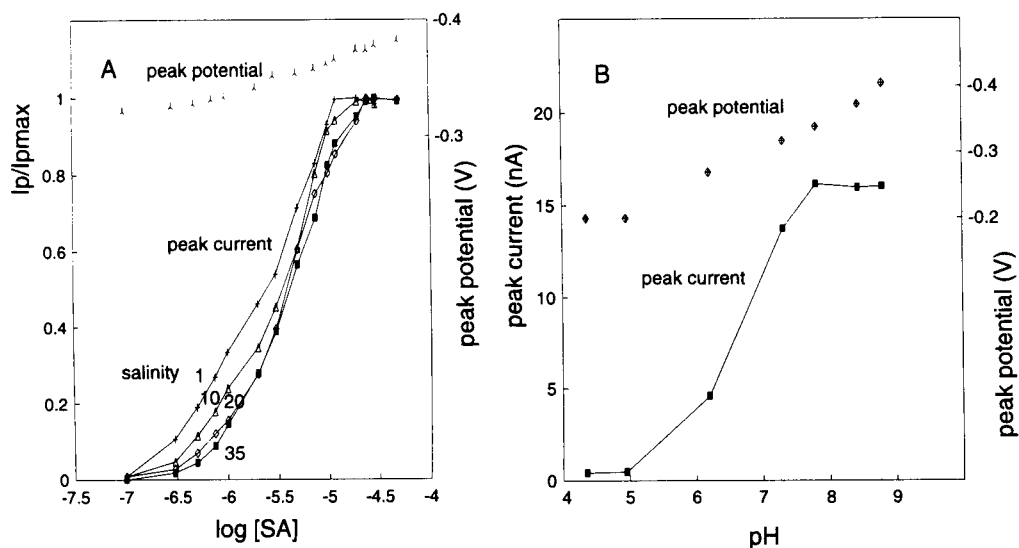
Fig. 2. The effect of varying the SA concentration (A) and the solution pH (B) on the CSV sensitivity and peak potential for copper. (A) The solution contained 0.02 M borate pH buffer (pH 8.4) and 17 nM of copper; the peak potentials shown are for sea water of a salinity of 35 psu; the deposition potential was −1.1 V; (B) 25 μM SA and 10 nM copper in sea water with a salinity of 35 psu.

was obtained at pH values between 7.8 and 8.8 (Fig. 2B). The increase with the pH presumably is a result of increased stability of the complexes of copper with SA, whereas at pH values above 7.8 this complex formation was increasingly affected by competition of hydrolysis of copper. The increased stability of the copper–SA complexes with the increase in the pH caused a negative shift in the peak potential of approximately 46 mV/pH unit at pH values between 5 and 8.8.

A pH buffer consisting of 1 M boric acid and 0.35 M ammonia (final pH 8.35 in sea water) was used for the speciation measurements in sea water as organic contaminants in this buffer are removed readily by UV-irradiation and the stability of its complexes with copper is very low [19]. However, a different pH buffer (HEPES for instance which gives a pH of ca. 7.7 in freshwater and sea water) may be preferred for the determination of dissolved copper or copper speciation in freshwater or estuarine water of low salinity as the borate pH buffer is not effective in such waters due the high value of its acidity constant (pK_{HB} is ca. 9.2 in freshwater).

Variation of the deposition potential and the deposition time

The deposition potential may affect the efficiency of the adsorption step as a result of Coulombic effects. The deposition potential was varied between −0.1 and −1.3 V; deposition was carried out with stirring followed by a quiescent period of 10 s whereafter the potential was set to −0.2 V for 15 s and the scan was initiated. In this manner the deposition process involved only adsorption at deposition potentials greater than the reduction potential of the copper complex with SA at −0.37 V, whereas the deposition step combined adsorption with plating at more negative deposition potentials. The plated copper was re-oxidised and re-adsorbed when the potential was switched to −0.2 V from more negative deposition potentials, whereafter it was reduced again during the subsequent potential scan. It was found that the sensitivity for copper (10 nM) in sea water containing 25 μM SA and at pH 8.4, increased gradually when the deposition potential was decreased from −0.1 to −0.8 V, whereas a stronger increase occurred at more negative po-

tentials, producing greatest sensitivity at deposition potentials between -1.1 and -1.3 V (Fig. 3A). Possible explanations for the large increase at these negative potentials could be a positive charge on the adsorbing copper complex of SA, or an elimination of interference caused by adsorption of a different metal complex of SA.

Variation of the deposition time caused a linear increase in the peak height for 1.6 nM copper with the deposition time (using a deposition potential of -0.2 V) until 7 min and non-linearly thereafter, whereas a shorter linear increase with the deposition time until 2 min was apparent at a higher copper concentration of 7 nM (Fig. 3B). The shorter linear increase with the deposition time at the higher copper concentration was probably caused by saturation of the drop surface by the adsorbed copper–SA complexes, the non-linear increase occurring at a coverage corresponding to a reduction current of 17–20 nA (differential pulse mode) in both cases. At low copper concentrations the sensitivity can therefore be increased readily by extending the adsorption time.

Interferences

Other metals can potentially interfere with the CSV determination of copper by forming com-

plexes competitively, or by forming electroactive complexes. Such interferences were evaluated by the addition of other metals (at levels well above those naturally occurring) to sea water containing 3 nM copper, 25 μM SA and 0.01 M borate buffer. Additions of 100 nM Cd, 200 nM Al, 100 nM Cr(VI), 120 nM As(V), 100 nM Mn(II), 50 nM Pd, 50 nM Te, 50 nM Mo, 50 nM Bi and 100 nM Se(IV) did not produce interfering peaks when the sea water was analysed using CSV using 1 min adsorption at -0.05 V. Peaks (peak potentials and sensitivities, $s = $ nA/nM, are given) were produced by the addition of 50 nM Co (-0.67 V; $s = 0.05$), 100 nM Pb (-0.43 V; $s = 0.002$), 200 nM Fe(III) (-0.74 V; $s = 0.05$), 125 nM Ni (-0.52 V; $s < 0.005$), 200 nM U (-0.66 V; $s = 0.003$), 200 nM V (-0.74 V; $s = 0.007$), 200 nM Zn (-1.09 V; $s < 0.005$), and 200 nM Ti (-1.26 V; $s = 0.007$), but the peaks did not overlap with that for copper, and their sensitivity was much lower than that for copper ($s = $ ca. 1–2), so no interference by these metals is to be expected in the analysis of natural waters.

Surface active substances can interfere by competitive adsorption on the HMDE and lowering the surface area available for adsorption of copper SA complexes. Interference by such compounds was tested by the addition of Triton X-100
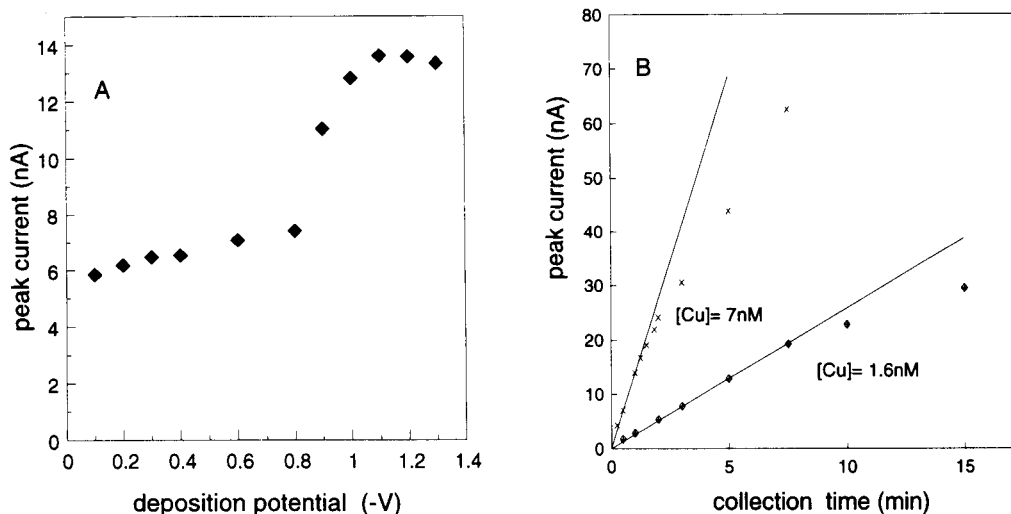

Fig. 3. The effect of varying the deposition potential (A) and the deposition time (B) on the voltammetric response for copper in sea water. (A) 10 nM copper and 25 μM SA; (B) 25 μM SA and 1.6 and 7 nM copper as indicated; each scan was initiated from -0.20 V.

to sea water and determining the CSV peak height for 6 nM copper in UV-irradiated sea water (1 min adsorption at −0.05 V). The peak height was diminished 11%, 18% and 88% by the addition of 0.5, 1 and 2 mg l^{-1} Triton X-100 respectively. This may be compared with observations of the presence of surfactants equivalent to Triton X-100 at levels between 0.2 and 3 mg l^{-1} in coastal waters [20,21]. It is therefore likely that interferences occur with the determination of copper in waters containing such organics by CSV using SA. Comparison of the CSV sensitivity for copper in UV-irradiated and untreated sea water samples from the NE Atlantic showed that the sensitivity was typically doubled as a result of the radiation treatment suggesting that the concentration of surface active organic matter in uncontaminated sea water from oceanic origin is sufficiently high to cause this effect.

Natural organic complexing compounds interfere with the determination of total dissolved copper by masking part of the dissolved copper. Advantage was taken of this effect in this study to determine the concentration of such compounds in sea water, and the stability of their complexes with copper. This interference and that of natural surface active materials can be eliminated by UV-irradiation of the sea water.

Linear range and limit of detection

The linear range of the CSV response for copper was determined in UV-irradiated sea water with a deposition time of 1 min at a deposition potential of −1.1 V. It was found that the peak height increased linearly with the copper concentration up to a peak height of 30 nA corresponding to a copper concentration of 35 nM. This linear range can be extended by using a shorter deposition time but this would be at a loss of sensitivity.

The limit of detection was determined in purified (by equilibration with 0.1 mM MnO_2 [6] and Chelex-100) and UV-irradiated sea water containing 0.27 nM copper, using a deposition time of 1 min at −1.1 V. The relative standard deviation of the peak height of repeated scans of copper was 12.5% ($n = 10$) from which a limit of detection can be calculated (3σ) of 0.1 nM. This limit could be lowered by extending the deposition time as illustrated in Fig. 3B.

The limit of detection for copper by CSV preceded by adsorptive collection of complexes with SA (0.1 nM) is lower than using catechol (0.2 nM [6]), tropolone (0.4 nM [11]), or oxine (0.2 nM [10]) (all normalised to 1 min deposition). However, differences in sensitivity occur due to differences in instrumentation. For this reason CSV
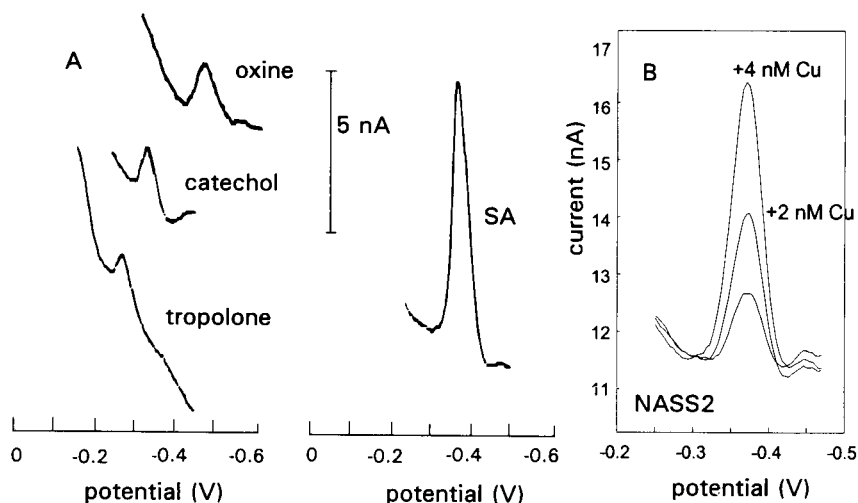

Fig. 4. (A) Comparative scans obtained using DPCSV for 3.1 nM copper in sea water using 20 μM oxine, 1 mM catechol, 0.7 mM tropolone and 25 μM SA. The deposition time was 1 min at −1.1 V. (B) CSV scans for copper in NASS2 water containing 25 μM SA and 0.01 M borate buffer (pH 8.4) and for two sequential additions of 2 nM copper each.

scans obtained for 3.1 nM copper in sea water using SA can be compared in Fig. 4A with those obtained using catechol, oxine and tropolone at constant instrument parameters (Metrohm polarograph, 1 min deposition, differential pulse modulation of 25 mV amplitude and 0.2 s interval); it can be seen that the sensitivity for copper using SA is actually 2–3 times greater than using catechol or oxine and 4 times greater than using tropolone. The good sensitivity for copper is convenient when labile copper concentrations are determined in the presence of competing complexing ligands.

The accuracy of the method to determine total dissolved copper in sea water was tested by determination of copper in reference sea water from two sources, NASS2 which is of open oceanic origin [22], and certified sea water from the BCR which originates from the North Sea [23]. Copper concentrations of 1.77 ± 0.22 nM ($n = 4$) and 1.71 ± 0.21 ($n = 3$) were found on two separate occasions in the NASS2 reference material (analysed without UV-irradiation), in good agreement with a certified value of 1.72 ± 0.17 nM, whereas a value of 2.89 ± 0.12 nM ($n = 5$) was found in the BCR water without UV-treatment, and 3.50 ± 0.31 nM ($n = 4$) after UV-irradiation of the sample; the latter value is in reasonable agreement with the accepted value of 4.00 ± 0.38 nM (a sea water density of 1.025 g cm^{-3} was used to con-

vert the BCR value to the nM scale). It is interesting to note the marked difference in the results for the UV-irradiated and untreated BCR sea water suggesting the presence of a strongly bound copper fraction in this sample. The scans obtained for copper in NASS2 water and for 2 additions of copper standard to this material are shown in Fig. 4B.

Calibration of K'_{CuSA} and $\beta'_{Cu(SA)_2}$

The ratio, X, of the peak current for copper in the presence and absence of EDTA ($X = i_p/i_{p0}$), can be used as a measure of the comparative stability of copper complexes with SA and EDTA [9,16]. X was determined in sea water of several salinities and at several concentrations of EDTA in order to determine values for α_{CuSA}. The decrease in X with increasing concentration of EDTA is shown in Fig. 5A, where it can be seen that the stability of the copper complexes with SA is greater than with EDTA in sea water as 50% peak suppression occurs when the EDTA concentration is approximately four times higher than that of SA; the stability of the EDTA complexes with copper was approximately two times greater than those of SA at the lowest salinity (1 psu). Values for α_{CuSA} were determined at several SA concentrations at a salinity of 35 psu, and at one SA concentration (25 μM) at salinities of 20, 10 and 1 psu. The stability of the EDTA complexes
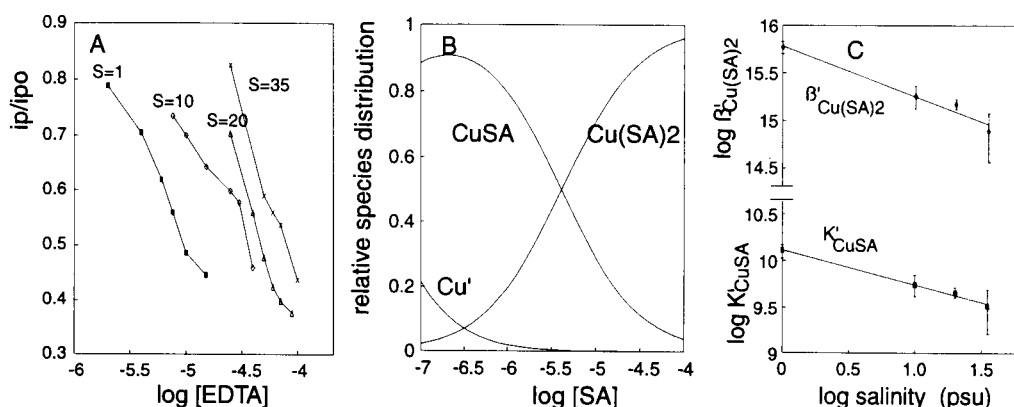
Fig. 5. Calibration of α_{CuSA} by ligand competition with EDTA in sea water at several salinities. (A) The depression of the peak current as a function of the EDTA concentration for 25 μM SA. (B) Model calculation of the chemical speciation of copper in sea water as a function of the SA concentration. (C) Variation of log K'_{CuSA} and log $\beta'_{Cu(SA)_2}$ as a function of log S. The straight lines shown are the result of linear regressions through the data; the results of the regressions are given in the text.

of copper was calculated using stability constants valid for an ionic strength of 0.1 M [24] corrected for the side-reactions of EDTA with the major ions by an ion-pairing model. It can be seen in Table 1 that at a given concentration of SA (25 μM) the value for α_{CuSA} increased with decreasing salinity presumably due to diminished major cation competition.

It is necessary to calculate values for the conditional stability constants, K'_{CuSA} and $\beta'_{Cu(SA)_2}$ in order to calculate values for α_{CuSA} at concentrations of SA differing from those used for the calibrations. During preliminary calculations only the formation of a 2:1 ($Cu(SA)_2$) complex was assumed and values were calculated for $\beta'_{Cu(SA)_2}$. However, the data did not fit the model well as there were systematic trends in $\beta'_{Cu(SA)_2}$. Furthermore, the high stability of the copper complexes with SA compared with those formed with EDTA suggests that most copper in UV-irradiated sea water should be complexed by nanomolar levels of SA, which is not in agreement with the comparatively high SA concentration ($\approx$ 10 μM) required to obtain maximum sensitivity in sea water (Fig. 2A). For this reason it is likely that formation of a surface active (polynuclear) complex ($Cu(SA)_2$) is preceded by the formation of a 1:1 (CuSA) complex (which does not adsorb on the HMDE) at low concentrations of SA. This scenario would explain the appearance of the copper peak at higher SA concentrations (> 0.1 μM) as well as the high stability of the copper complexes with SA. The formation of a 2:1 complex has previously been shown to be similarly related to

the formation of a surface active complex in the case of copper complexation by catechol [6].

The following procedure was therefore used to calculate first $\beta'_{Cu(SA)_2}$ and subsequently K'_{CuSA}: the increase in the peak height with increasing concentration of SA was used as a measure of the formation of $Cu(SA)_2$. The following relationship was used between the ratio of i_p/i_{pmax} (i_{pmax} is the maximum peak current obtained with increasing concentrations of SA; at this concentration of SA all copper is assumed to be present as $Cu(SA)_2$) and the distribution of copper complexes:

$$i_p/i_{pmax} = \alpha_{Cu(SA)_2}/\alpha' \qquad (7)$$

where $\alpha_{Cu(SA)_2}$ is the α-coefficient for $Cu(SA)_2$:

$$\alpha_{Cu(SA)_2} = \beta'_{Cu(SA)_2}[SA']^2 \qquad (8)$$

and α' is the overall α-coefficient for complexation of copper (Eqn. 2).

Values for α' were calculated from Eqn. 2 after calibration of the overall α-coefficient for copper complexation by SA (α_{CuSA}) against EDTA; values for $\alpha_{Cu(SA)_2}$ were calculated indepently from the SA titrations using Eqn. 6 (Fig. 2A), and were used to calculate values for $\beta'_{Cu(SA)_2}$ using Eqn. 8. Finally values for K'_{CuSA} were calculated from the difference between α_{CuSA} (obtained from the calibration against EDTA) and $\alpha_{Cu(SA)_2}$ using Eqn. 3. The values determined for α_{CuSA}, K'_{CuSA}, and $\beta'_{Cu(SA)_2}$ are shown in Table 1. The values for $\beta'_{Cu(SA)_2}$ obtained here are much greater than a previously published value of $10^{10.0}$ (calculated from $\beta'_{Cu(SA)_2} = 10^{17.5}$ [24] valid at an

TABLE 1

Values for α_{CuSA}, K'_{CuSA} and $\beta'_{Cu(SA)_2}$ determined by ligand competition with EDTA in sea water at several salinities and at 20°C (each value consists of 4–6 data pairs)

Salinity (psu)	[SA] μM	log α_{CuSA}	log K'_{CuSA}	log $\beta'_{Cu(SA)_2}$
35	1	3.68 ± 0.08	9.60 ± 0.08	14.95 ± 0.08
35	2	3.85 ± 0.20	9.41 ± 0.20	14.70 ± 0.19
35	25 [a]	5.83 ± 0.20	9.55 ± 0.20	14.98 ± 0.20
35	Averaged data [b]		9.52 ± 0.31	14.89 ± 0.33
20	25	6.02 ± 0.05	9.67 ± 0.05	15.18 ± 0.05
10	25	6.11 ± 0.13	9.75 ± 0.13	15.26 ± 0.13
1	25	6.60 ± 0.07	10.10 ± 0.07	15.77 ± 0.07

[a] Average of 5 repeated determinations (26 data pairs) using 25 μM SA. [b] Average of all data (35 data pairs) obtained at a salinity of 35 psu at three SA concentrations.

ionic strength of 0.1 M after correction for proton competition using $pK_1 = 11.46$ and $pK_2 = 8.84$ valid at an ionic strength of 0.5 M [25]). However, the differences in the solution composition (the presence of the major cations is likely to further lower the conditional stability constant calculated from the literature value) and the ionic strength make it difficult to compare the literature value with those obtained here.

The complexation of copper by SA in sea water of a salinity of 35 psu was calculated as function of the SA concentration using the stability constants estimated from the data; it can be seen in Fig. 5B that the electroactive $Cu(SA)_2$ complex is formed progressively at SA concentrations above 0.1 μM in agreement with the appearance of the reduction peak at these levels (Fig. 2A).

Plots of log K'_{CuSA} and log $\beta'_{Cu(SA)_2}$ as a function of log salinity were linear for salinities between 1 and 35 (Fig. 5C) illustrating that a linear equation can be used to interpolate values for these stability constants for salinities in this range. The following empirical relationships were found by linear least squares regression of the data:

$$\log K'_{CuSA} = (10.12 \pm 0.03) - (0.37 \pm 0.02) \log S$$

$$\log \beta'_{Cu(SA)_2} = (15.78 \pm 0.08)$$
$$- (0.53 \pm 0.07) \log S$$

The results of the linear regressions are shown in Fig. 5C.

The detection window for the determination of complexing ligands in sea water which in CSV with ligand competition is centred by the α-coefficient of the added competitive ligand (in this case α_{CuSA}) [8,9] is situated between (log values) 3.7 and 5.8 when the SA concentration is varied between 1 and 25 μM (see Table 1). This range of detection windows is conveniently situated between those for tropolone (2.5–4.0) and oxine (6.4–9.0). There is some overlap between the detection windows as they stretch to about a decade on either side of each centre [8,9]; it is therefore possible to carry out comparative complexing ligand studies using overlapping detection windows but with different added electroactive ligands.

Variation of the equilibration time of copper, SA and natural complexing ligands

Previous experiments using oxine as added competitive ligand in CSV indicated that copper complexed by natural complexing ligands in sea water was released only slowly requiring more than an hour to reach equilibrium with the added oxine [9] indicating slow kinetics of dissociation of the natural copper complex. To investigate possible effects of slow kinetics of complex formation the complexing ligand concentration in a sample originating from the North Sea was determined after several equilibration periods of between 10 min and 24 h. Copper was thereto added to 11 voltammetric cells giving a concentration range of 1–25 nM added copper, and 10 ml sea water (previously mixed with 2 μM SA and 0.01 M borate buffer) was pipetted into each cell 10 min prior to the CSV measurement of labile copper. Mercury drops accumulating in the voltammetric cells from the repeated determinations were removed after 6 h to prevent possible interfering effects resulting from adsorption. A separate titration was carried out which was analysed only once after an equilibration period of 24 h. The results (Table 2) indicate that the apparent concentration of complexing ligands increased gradually from 6.0 nM (after 10 min equilibration) to 13.8 nM (after 6 h equilibration) whereafter no further change occurred. The ligand concentration obtained after 24 h equilibration was confirmed by the separate titration indicating that the repeated CSV determinations had not caused

TABLE 2

Concentration of natural complexing ligands concentration detected after different equilibration periods (conditions: 2 μM SA and pH 8.35; the sea water originated from the North Sea and the initial copper concentration was 3.7 nM)

Titration	Equilibration time	C_L (nM)	log K'_{CuL}
1	10 min	6.05 ± 0.19	14.29 ± 0.96
	1 h	6.73 ± 0.15	13.75 ± 0.40
	3 h	10.79 ± 0.22	13.14 ± 0.18
	6 h	13.79 ± 0.29	13.29 ± 0.21
	24 h	13.74 ± 0.56	12.79 ± 0.28
2	24 h	13.04 ± 0.64	12.99 ± 0.22

significant changes to the sample aliquots. The standard deviations of the stability constants calculated from the shorter period equilibration data were comparatively high; nevertheless it appears that the stability of the complexes detected after 10 min was greater than after longer equilibration periods suggesting that the stronger ligands had been detected before the higher concentration of weaker complexing ligands. This perhaps unexpected result was due to the sea water containing a small but significant (3.7 nM) concentration of copper which was already strongly complexed prior to the beginning of the experiment and contributing greatly to the detected concentration of complexing ligands. The apparent increase in the concentration of complexing ligands with time was caused by slow complex formation by the free ligands in the sample. The cause of the slow reaction may lie in the low concentration of free copper (most added copper is initially complexed by SA which is present in excess) and in possible side-reactions of the natural complexing ligands with the major cations in the sea water.

These equilibration experiments indicate that a period of 6 h is sufficient to attain equilibrium between the added copper, the natural complexing material and the added competitive ligand.

Determination of copper complexing ligands in sea water

The CSV method for copper complexation studies using ligand competition with SA was tested in preliminary experiments by determining the concentration of complexing ligands in samples from the NW Mediterranean and the NE Atlantic at several detection windows; the detection window was varied by carrying out the analysis at several SA concentrations (0.3, 0.5, 1, 2 and 10 μM SA). Comparative determinations were carried out using other electroactive chelating agents (oxine and catechol) at different as well as comparable detection windows. The ligand concentrations and conditional stability constants can be compared in Table 3. Ligand concentrations between 3 and 14 nM were detected in the sample from the Mediterranean forming very stable complexes with conditional stability constants of $10^{13.0}$ to $10^{14.8}$. The reproducibility of the ligand concentration was good when the titration was repeated using the same concentration of SA, which is understandable as the ligand concentration is calculated from the slope of the linearised data which is comparably insensitive to variations in individual analyses of the labile copper concentration and produced small standard deviations.
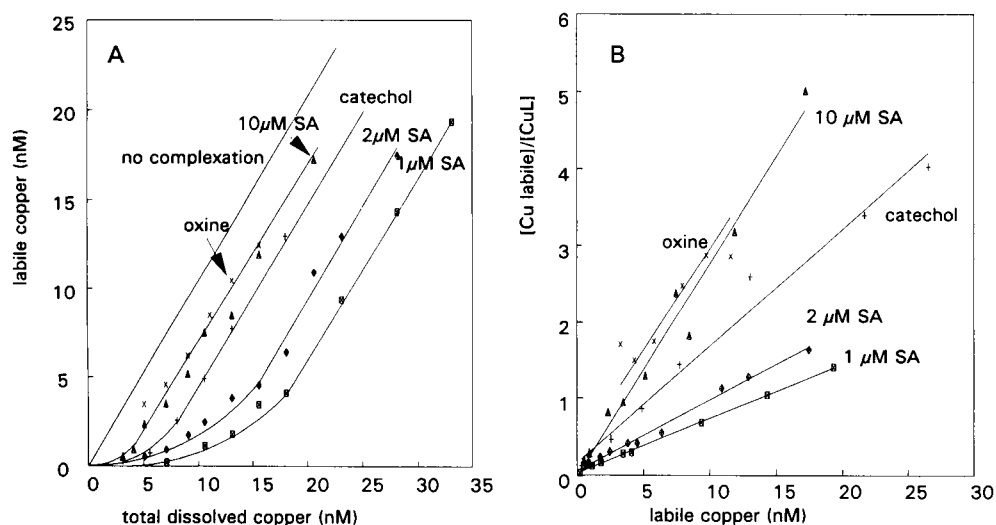

Fig. 6. Complexing ligand titrations of sea water from the Mediterranean at various detection windows. The results are shown in Table 3. (A) Labile copper as a function of total dissolved copper; (B) linearisation of the data.

TABLE 3

Comparison of ligand concentrations and conditional stability constants for complexes of copper in Mediterranean sea water (containing 3.1 nM copper) and in a sample from the NE Atlantic (Challenger Cruise 76/91, Station 4, 300 m depth, containing 1.55 nM copper) obtained with different added competing ligands and at several detection windows (the centre of each detection window is determined by log α_{CuAL})

Added ligand	log α_{CuAL}	C_L (nM)	log K'_{CuL}
Mediterranean sample			
1 μM SA	3.61	14.4 ± 0.2	13.08 ± 0.19
2 μM SA	3.99	10.8 ± 0.3	13.30 ± 0.04
2 μM SA	3.99	10.2 ± 0.3	13.03 ± 0.31
10 μM SA	5.04	3.9 ± 0.3	14.5 ± 0.5
1 μM oxine	5.02	3.2 ± 0.3	14.26 ± 0.18
100 μM catechol	5.75	6.5 ± 0.3	14.8 ± 0.4
Atlantic sample			
0.3 μM SA	3.03	12.8 ± 0.6	12.2 ± 0.3
0.5 μM SA	3.27	8.1 ± 0.4	12.7 ± 0.4
2 μM SA	3.99	4.9 ± 0.3	13.1 ± 0.3

Five complexing ligand titrations using different concentrations of SA are shown in Fig. 6A, and the linearisation of the results is shown in Fig. 6B. It can be seen that the labile copper concentrations increase at constant total dissolved copper concentrations with increasing levels of SA due to the enhanced competition of the added SA. Comparison of the ligand concentrations (C_L) and the conditional stability constants (K'_{CuL}) in Table 3 reveals that the ligand concentrations decreased and the conditional stability constants increased with increasing detection windows due to the presence of several natural ligands: the stability constants of the complexes varied between 13.0 and 14.8, the ligand concentrations diminishing with increasing complex stability in line with previous findings [8,9] except when catechol was used as the added competitive ligand. The catechol differs from the other ligands in that it is subject to oxidation by dissolved oxygen and is therefore not equilibrated along with the added copper and the natural ligands in the sample. The slow dissociation of stable natural copper complexes in sea water [9] therefore prevented that equilibrium was attained between the natural complexing material and the added catechol causing the apparent overestimation of the concentration of L in line with the higher

than expected ligand concentration (higher than that found at a lower detection window) detected using catechol.

The calibrated value of the α-coefficient for copper complexation with 10 μM SA (5.04) (log value) is approximately the same as that for 1 μM oxine (5.02); similar ligand concentrations (3.9 and 3.2 nM) and values for log K'_{CuL} (14.5 and 14.3) were obtained using 10 μM SA and 1 μM oxine in accordance with the similar detection windows. The detection window at this level of SA and oxine is very high and the added ligands largely outcompete the natural complexing ligands in view of the very low detected ligand concentrations (3.2 and 3.9 nM), only slightly greater than the dissolved copper concentration (3.1 nM) in this sample. Even so it is surprising that there are complexing ligands in the sea water which form copper complexes with values for log K'_{CuL} of 14.5–14.8, more than 4 decades more stable than copper complexes with EDTA in sea water (log K'_{CuEDTA} = 10.06).

Similar results were obtained when the detection window was varied for a sample from the NE Atlantic (see Table 3): the detected ligand concentration decreased with increasing detection window indicating the presence of several complexing ligands all present at concentrations greater than the dissolved copper concentration (1.55 nM). The stability of the complexes was high (log K'_{CuL} between 12.2 and 13.1).

A study of copper complexation in the NE Atlantic and the Mediterranean is currently being carried out using the method presented here. Preliminary results indicate the presence of organic complexing ligands at all depths of the NE Atlantic ocean. Ligand concentrations and conditional stability constants for the upper water column of a station in the NE Atlantic (Challenger cruise 76/91, station 4, 42°16′N/20°1′W) determined using ligand competition with 2 μM SA (equivalent to a detection window for α_{CuL} centred at $10^{4.0}$) are shown in Fig. 7. The ligand concentrations were always greater than the copper concentrations. It can be seen that the ligand concentrations varied between 3 and 8 nM, with a maximum at 78 m depth. The maximum in the upper water column is indicative of a biological
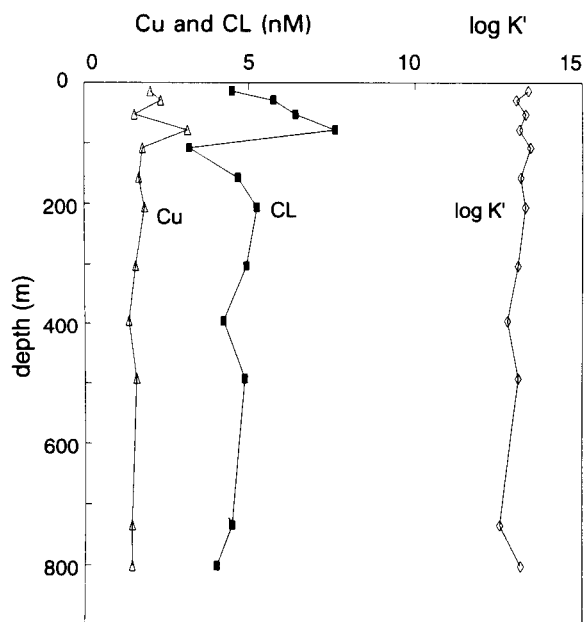
Cu and CL (nM)   log K'

Fig. 7. The concentration of copper complexing ligands and of dissolved copper is shown along the values for log K'_{CuL} in the NE Atlantic (Challenger Cruise 76/91, Station 4, 42°16′N/20°1′W). The CSV measurements were carried out using ligand competition with 2 μM SA.

origin of this organic complexing material. The ligand concentrations may be compared with the dissolved copper concentrations which were also determined in these waters by CSV (with 25 μM SA) after UV-treatment of acidified sample aliquots: the copper concentration in these waters (1.3–3.1 nM) is low but in the concentration range expected for these waters (see for instance [26]). The stability of the complexes was high with values for the conditional stability constants (log values) of ca. 13. Copper is therefore fully complexed with an α-coefficient (log value) of ca. 4 by the dissolved organic material in these waters. These preliminary results indicate that ligand competition using SA is a suitable approach to investigate the complexation of copper in sea water.

Conclusions

The optimised conditions for the determination of dissolved copper in sea water using CSV with SA as added surface active ligand include an SA concentration of 25 μM and a pH of 8.0–8.5,

and deposition at -1.1 V with reoxidation prior to the scan at -0.15 V. The sensitivity of CSV using SA is higher than with other added ligands, and consequently the limit of detection has been lowered to 0.1 nM using 1 min deposition which facilitates the determination of low copper levels in unpolluted natural waters.

The optimised condition for the study of copper complexation utilises an SA concentration of 1–10 μM suitable for the detection of copper complexes of intermediate stability (lower than can be detected using oxine but higher than using tropolone as added competing ligand). The detection window for copper complexation by organic material can be moved to more stable complexes by increasing the concentration of SA further; however, the detected ligand concentrations become very low in this condition. The centre of the detection window can be varied between 3.6–5.8 by varying the SA concentration between 1 and 25 μM; this range lies between that covered by tropolone (2.5–4.5) and oxine (5.0–8.4). It is not advisable to lower the detection window further by using SA concentrations much below 1 μM as side-reactions of SA with other trace elements in sea water could then be significant and would be difficult to take into account.

The comparative determinations of copper complexation at different detection windows have confirmed the operational relationship between the detection window, the detected concentration of complexing ligands and the stability of the complexes. Investigations into copper complexation using several detection windows have to be carried out to fully investigate this phenomenon which is caused by the presence of several complexing ligands (or several complexing sites on each ligand) in the sea water.

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Investigation of metal sulfide complexes in sea water using cathodic stripping square wave voltammetry

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Abstract

Cathodic stripping square wave voltammetry has been used to detect H_2S in sea water over a wide range of concentrations (nM to mM). The addition of metal ions to the solutions was found to depress the signal. This depression was attributed to the formation of metal sulfide complexes [MHS^+, $M(HS)_2$]. Stability constants for the formation of sulfide complexes with Cd^{2+}, Cu^{2+}, Cu^+, Pb^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+} have been estimated in sea water at pH 8.0 and 25°C using this method. The stability constants of cadmium sulfide complexes (log $\beta_{CdHS} = 6.3$ and log $\beta_{Cd(HS)_2} = 12.7$) were found to be in reasonable agreement with previous measurements. The values of log $\beta_{CuHS} = 7.0$ and log $\beta_{Cu(HS)_2} = 13.0$; log $\beta_{PbHS} = 7.1$ and log $\beta_{Pb(HS)_2} = 13.5$; log $\beta_{ZnHS} = 6.0$ and log $\beta_{Zn(HS)_2} = 13.7$, log $\beta_{FeHS} = $ log $\beta_{CoHS} = $ log $\beta_{NiHS} = 5.3$ and log $\beta_{MnHS} = 6.7$ were also obtained. Correction for the formation of strong chloro complexes for Hg^{2+} and Cu^+ in sea water gave measured values close to the literature values. The results have been used to determine the speciation of HS^- in surface sea water and of a number of metal ions in various anoxic basins.

Keywords: Stripping voltammetry; Metal sulfide complexes; Sea water; Waters

Metal sulfide complexes are responsible for controlling the solubilities of trace metals in anoxic waters and are thought to be important for the stabilization of hydrogen sulfide in surface oxic waters. Cutter and co-workers [1,2] have found H_2S between 0.1 to 1.1 nM in Atlantic surface waters. These measurements and the more recent results of Luther and Tsamakis [3] confirm the presence of H_2S in oxygenated sea water. The stability of H_2S in oxygenated waters has been attributed to the formation of copper sulfides that are resistant to oxidation [3–5]. The kinetic results indicated that the copper sulfide complex is easier to be oxidized than HS^- and

zinc sulfide may be responsible for the stability of H_2S in surface oxic sea water [6].

To elucidate the cause of the stability of H_2S in oxic waters it is necessary to have reliable stability constants for the complexation of metals with sulfide. The stability constants for metal sulfide complexes have only been experimentally determined for Cd and Hg complexes. The main difficulty in studying the metal sulfide complexation is the low solubility of metal sulfides. The values of stability constants of metal sulfide complexes have been estimated from metal dithizone extraction constants [7] and sulfide solubility products using a linear free energy relationship [4]. It would be desirable to have stability constants determined experimentally. In this paper we examine the use of the cathodic stripping square wave voltammetric technique introduced

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by Luther and Tsamakis [3] to investigate the stability constants for the formation of metal sulfide complexes at low levels of sulfide. By using this technique we are able to avoid the precipitation of metal sulfide and study the complexation in solution phase.

EXPERIMENTAL

The stock solutions of NaHS were prepared from reagent grade $Na_2S \cdot 9H_2O$ crystals which were washed with distilled water to remove any oxidized surface layer. The solutions were standardized using iodometric titrations. The metal standard solutions were obtained from Sigma. The sea water used was Gulf Stream water, collected 10 miles off the coast of Miami and filtered through 0.45-μm Millipore filters. The salinity was determined with a Guildline Autosal conductance bridge, using the Practical Salinity Scale. The pH of sea water was measured using a Ross glass electrode, a double junction Ag/AgCl reference electrode and a Metrohm pH meter. The system was calibrated with 0.005 M Tris buffers in artificial sea water on the total pH scale.

The stability constants of metal sulfide complexes were determined by cathodic stripping square wave voltammetry. The sea water with added metal was titrated with sulfide, and the stripping current of free sulfide deposited on the hanging mercury drop electrode was measured. The sulfide was analyzed by cathodic stripping square wave voltammetry using an EG & G Princeton Applied Research Model 384B polarographic analyzer with a Model 303 static mercury drop electrode (SMDE) in the hanging mercury drop electrode (HMDE) mode. The medium size of mercury drop was used in all experiments. A platinum wire served as the auxiliary electrode. The Ag/AgCl reference electrode supplied with the instrument was not used in order to avoid the interference of sulfide with the Ag/AgCl. The silver wire was sealed in a glass tube to prevent contact with solution. A double junction saturated calomel electrode (SCE) from EG & G Princeton Applied Research was used as the reference electrode. Deposition of sulfide was set at a potential of -0.10 V (versus the saturated calomel electrode (SCE)) for 60 s. A 2 mV s^{-1} scan increment with a 100 Hz square wave pulse and 50 mV square wave pulse height was used to strip the sulfide from the mercury drop. The potential scan was carried out in a negative direction ranging from -0.1 to -0.9 V using square wave modulation. Calibration of the technique gave a linear relationship between current and concentration of sulfide with a correlation coefficient of 0.998 ($n = 5$).

An aliquot of 10 ml of sea water was pipetted into the polarographic cell. The sea water was purged for 4 min with oxygen-free, water saturated high purity argon gas to remove the oxygen. An appropriate quantity of metal standard solution was then added to the sea water. The concentration of total metal in sea water ranged from 500 to 1500 nM. Titrations were conducted by successive standard additions in the range of 250 to 500 nM sulfide. After each sulfide addition the solution was allowed to equilibrate for 1 min by stirring the solution without purging before the next deposition stripping cycle was repeated. Titrations were carried out to ten-fold excess of sulfide over the metal concentration.

RESULTS AND DISCUSSION

A voltammogram of sulfide (1000 nM) in sea water is shown in Fig. 1. Sulfide gives a wave at about -0.60 V versus the saturated calomel electrode (SCE). The polarographic behavior of sulfide ion has been investigated by a number of workers [8,9]. The electrode process is a two electron oxidation of mercury and the formation of the insoluble HgS.

$$HS^- + Hg \rightleftharpoons HgS + H^+ + 2e^- \qquad (1)$$
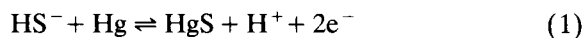
At the deposition potential the hanging mercury drop electrode (HMDE) was oxidized to produce Hg^{2+}. In the presence of HS$^-$ in the solution the hanging mercury drop electrode was covered by a monomolecular layer of HgS. The amounts of HgS formed on the HMDE are proportional to the deposition time and concentration of sulfide

in the solution. During the cathodic stripping process a negative direction scan was applied to the HMDE, a reverse of reaction 1 took place and the resulting current was measured. The square wave voltammetric waveform combines a large-amplitude square wave modulation with a staircase waveform. One of advantages of square wave voltammetry is the rapid scan rates. The peak-shaped voltammograms obtained display excellent sensitivity and rejection of background currents [10].

The effect of the addition of 500 nM of Cd^{2+} to sea water containing 1000 nM of HS^- was measured by square wave voltammetry. The resulting voltammogram is shown in Fig. 2. At these low concentrations of metal and sulfide, no precipitation of metal sulfide was observed and the peak current of HS^- decreased. We have attributed this decrease to the formation of cadmium sulfide complexes which are electrochemically inactive species. Only the free sulfide can give a response to the square wave voltammetric technique. The other metals which form complexes with sulfide behave in a similar manner.

Fig. 2. Effect of addition of Cd^{2+} on the polarographic response of sulfide from square wave voltammetry.

The titration of sea water with added metal with sulfide can be used to determine the stability constant for the formation of the metal sulfide complex.

The dominant species of sulfide in sea water at pH 8. 0 is HS^- [11]. When metal cations, M^{n+}, and hydrogensulfide anions, HS^-, coexist in the solution, metal sulfide complexes, $MHS^{(n-1)+}$ and $M(HS)_2^{(n-2)+}$, are formed:

$$M^{n+} + HS^- \rightleftharpoons MHS^{(n-1)+} \qquad (2)$$

$$M^{n+} + 2HS^- \rightleftharpoons M(HS)_2^{(n-2)+} \qquad (3)$$

The stepwise conditional stability constants for these complexation reactions are given by:

$$\beta_1 = [MHS^{(n-1)+}] / [M^{n+}]_F [HS^-]_F \qquad (4)$$

$$\beta_2 = [M(HS)_2^{(n-2)+}] / [M^{n+}]_F [HS^-]_F^2 \qquad (5)$$

where $[M^{n+}]_F$ is the concentration of free metal in solution, $[HS^-]_F$ the concentration of free HS^- which is a electroactive species and $[MHS^{(n-1)+}]$ and $[M(HS)_2^{(n-2)+}]$ are the concentrations of the metal sulfide complexes which are assumed to be
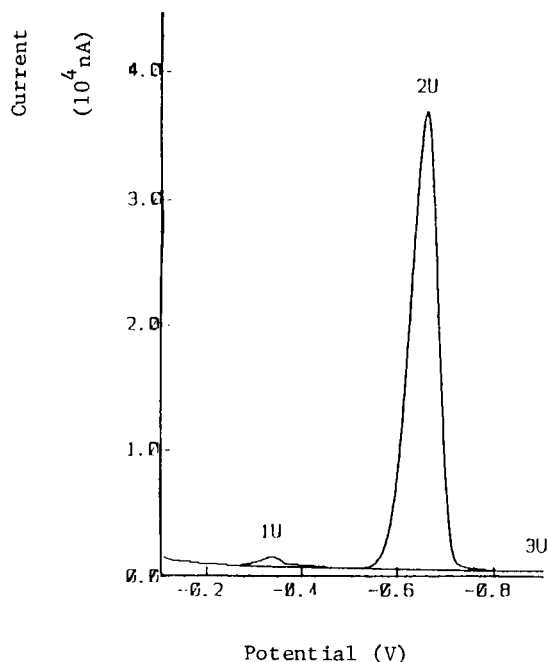

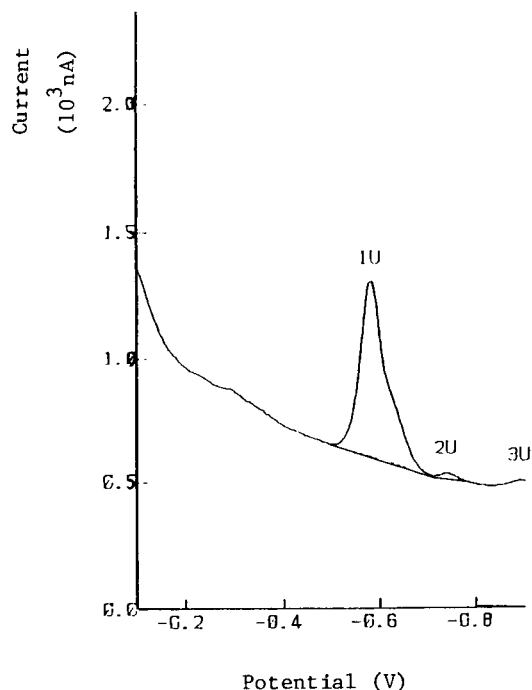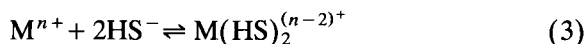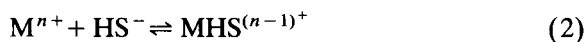Fig. 1. Voltammogram of sulfide in sea water from cathodic stripping square wave voltammetric method.

electrochemically inactive species. The values β_1 and β_2 are the conditional stability constants of the metal sulfide complexes measured under specific conditions, e.g., ionic strength, temperature and solution composition.

The concentration of total sulfide, $[HS^-]_T$, is the sum of the concentration of free sulfide, $[HS^-]_F$, and metal sulfide complexes:

$$[HS^-]_T = [HS^-]_F + [MHS^{(n-1)+}]$$
$$+ 2[M(HS)_2^{(n-2)+}] \qquad (6)$$

Combining Eqns. 4 and 5 with Eqn. 6 yields:

$$[HS^-]_T = [HS^-]_F(1 + \beta_1[M^{n+}]_F$$
$$+ 2\beta_2[M^{n+}]_F[HS^-]_F) \qquad (7)$$

The concentration of total metal, $[M^{n+}]_T$, is the sum of the concentration of free metal, $[M^{n+}]_F$ and metal sulfide complexes:

$$[M^{n+}]_T = [M^{n+}]_F + [MHS^{(n-1)+}]$$
$$+ [M(HS)_2^{(n-2)+}] \qquad (8)$$

Combining Eqns. 4 and 5 with Eqn. 8 yields:

$$[M^{n+}]_T = [M^{n+}]_F(1 + \beta_1[HS^-]_F + \beta_2[HS^-]_F^2) \qquad (9)$$

Rearranging Eqn. 9 gives:

$$[M^{n+}]_F = [M^{n+}]_T / (1 + \beta_1[HS^-]_F + \beta_2[HS^-]_F^2) \qquad (10)$$

The substitution of Eqn. 10 into Eqn. 7 yields:

$$([HS^-]_T/[HS^-]_F - 1)/[M^{n+}]_T$$
$$= (\beta_1 + 2\beta_2[HS^-]_F)/(1 + \beta_1[HS^-]_F$$
$$+ \beta_2[HS^-]_F^2) \qquad (11)$$

The total concentration of metal, $[M^{n+}]_T$, and sulfide, $[HS^-]_T$, are known from the standard additions, and the concentration of free sulfide are determined polarographically at each titration point. The values of β_1 and β_2 can be determined from Eqn. 11 by knowing $[HS^-]_T$, $[HS^-]_F$ and $[M^{n+}]_T$. A non-linear regression analysis of experimental data (about ten points) yields values of the stability constants, β_1 and β_2, for metal sulfide complexes.
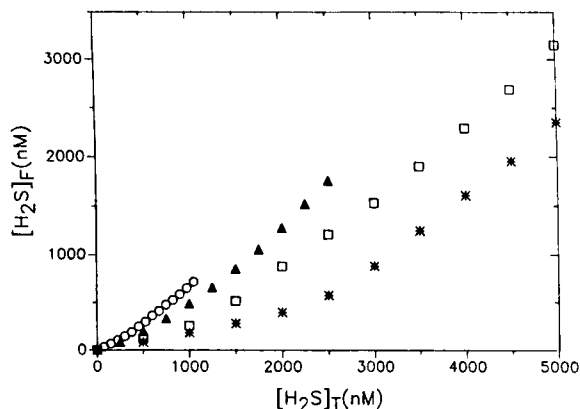
Fig. 3. Titration of sulfide on Cd^{2+} at different concentrations in sea water: (○) 0.250, (▲) 500, (□) 1000 and (∗) 1500 nM.

The accuracy of the approach used here was first evaluated by titrating Cd^{2+} in sea water with sulfide. The concentration of Cd^{2+} added to sea water was varied from 250 to 1500 nM. The titration curves are shown in Fig. 3. The stability constants of cadmium sulfide calculated from these titrations using Eqn. 11 are given in Table 1. Independent titrations with different concentrations of Cd^{2+} reproduced the stability constants within 0.2 in log β. The average stability constants, log $\beta_1 = 6.3$ and log $\beta_2 = 12.7$, are in reasonable agreement with literature values determined by solubility methods [12]. Dyrssen [13] has refitted the cadmium sulfide solubility data of Ste-Marie et al. [12] and gave values of log $\beta_1 = 6.4$ and log $\beta_2 = 13.8$ for the formation of $CdHS^+$ and $Cd(HS)_2$ complexes. Since the stability constants of Ste-Marie et al. [12] were determined in 1.0 M $NaClO_4$ solution, the slightly lower values

TABLE 1

Stability constants of cadmium sulfide complexes determined at various concentration of cadmium added to sea water ($S = 35$, $T = 25°C$)

$[Cd^{2+}]$ (nM)	log β_1	log β_2
250	6.5 ± 0.1	12.9 ± 0.1
500	6.4 ± 0.1	12.5 ± 0.1
1000	6.1 ± 0.1	12.5 ± 0.1
1500	6.3 ± 0.1	12.8 ± 0.1
Average	6.3 ± 0.2	12.7 ± 0.2
Lit. [13]	6.4	13.8

obtained in this study are expected due to the formation of cadmium chloro complexes in sea water.

The same technique as for cadmium has been used to determine the stability constants of Cu^{2+}, Pb^{2+}, Zn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+} and Hg^{2+} sulfide complexes in sea water. The titration curves for different metals are similar to Cd^{2+}. The resulting stability constants are listed in Table 2. The results of Cu^{2+} and Pb^{2+} titrations gave similar values for $\log \beta_1$ and $\log \beta_2$. The stability constants for lead sulfide complexes are larger than the values estimated from dithizone extraction constants by Dyrssen [7], while those for copper are smaller.

The stability constant for mercury sulfide complexes ($\log \beta_2 = 12.8$) determined in sea water is significantly lower than the literature value of 37.7 [14]. The value of $\log \beta_1$ (7.8) is also lower than the calculated value of 30.1 from metal dithizone extraction constants [13] and the calculated value of 20.0 from a linear relationship between $\log \beta_1$ and $\log \beta_2$ for various Hg^{2+} complexes [15]. These differences are largely due to the effect of the different media used. The stability constants reported in this paper are conditional stability constants in the sea water medium. Care has to be taken to compare the conditional stability constants determined in different media. In sea water the chloro complexes

can cause the conditional stability constants to be significantly lower than the thermodynamic stability constants for some metals such as Hg^{2+}, Cu^+ and Ag^+, which form strong complexes with Cl^- in sea water. The difference between the thermodynamic stability constants, β_i(HgHS, therm), and conditional stability constants in sea water, β_i(HgHS, SW), can be calculated from the stability constants of metal chloro complexes, β_i(HgCl), and the concentration of chloride, $[Cl^-]$, in sea water.

$$\beta_i(\text{HgHS, therm.})/\beta_i(\text{HgHS, SW})$$

$$= [Hg^{2+}]_T/[Hg^{2+}]_F$$

$$= 1 + \sum_{i=1}^{2} \beta_i(\text{HgCl})[Cl^-]^i \qquad (12)$$

Using the values of 6.72 for β_1(HgCl$^+$) and 13.23 for β_2(HgCl$_2^0$) [16] and the chloride concentration of sea water, the difference of 12.84 in $\log \beta$ between the thermodynamic stability constants and conditional stability constants in sea water can be obtained. This gives a value of 20.6 for the measured stability constant ($\log \beta_1$) with the chloride correction, which is in good agreement with the estimated value of 20.0 [15]. The correction for chloride complexes in sea water will give a value of 25.6 for $\log \beta_2$ of Hg(HS)$_2$ complex. This value is still significantly lower than the literature value of 37.7, which had been corrected

TABLE 2

Stability constants of metal sulfide complexes determined in sea water at 25°C

Metal ion	This study		Literature		
	$\log \beta_1$	$\log \beta_2$	$\log \beta_1$	$\log \beta_2$	Ref.
Cd^{2+}	6.3 ± 0.2	12.7 ± 0.2	6.4 [a]	13.8 [a]	12
Cu^{2+}	7.0 ± 0.2	13.0 ± 0.2	14.1	21.6	13
Pb^{2+}	7.1 ± 0.5	13.5 ± 0.4	5.0	12.5	13
Zn^{2+}	6.0 ± 0.3	13.7 ± 0.1	6.5	14.0	13
Fe^{2+}	5.3 ± 0.1	–	1.4	8.9	13
Co^{2+}	5.3 ± 0.1	–	4.7	12.2	13
Ni^{2+}	5.3 ± 0.1	–	3.8	11.4	13
Mn^{2+}	6.7 ± 0.1	–	−0.5	7.0	13
Hg^{2+}	7.8 ± 0.1	12.8 ± 0.1			
	20.6 [b]	25.6 [b]	20.0	37.7 [a]	13,14
Cu^+	6.8 ± 0.6	12.6 ± 0.6			
	11.8 [b]	17.6 [b]	13.3	17.2	13
Ag^+	$\geq 9.5 \pm 0.2$	$\geq 15.3 \pm 0.1$	13.3 [a]	17.2 [a]	16

[a] Constants experimentally determined. [b] Corrected for strong chloro complexes in sea water.

for $HgCl_x$ complexes in 1 M KCl from solubility measurements [14].

The stability constants for copper(I) sulfide complexes (log $\beta_1 = 6.8$, log $\beta_2 = 12.6$) determined in sea water are lower than the estimated values (log $\beta_1 = 13.3$, log $\beta_2 = 17.2$) using a linear correlation of Cu(I) and Ag(I) complexes [13]. Like Hg^{2+}, Cu^+ forms strong complexes with chloride in sea water, which causes a lower value of the conditional stability constants for copper(I) sulfide complexes. Using the values of 2.7 for log $\beta_1(CuCl^0)$ and 5.5 for log $\beta_2(CuCl_2^-)$ [16] and chloride concentration of sea water, a difference of 5.04 in log β was obtained from the correction for chloro complexes in sea water. This gives a value of 11.8 for our measured stability constant with the chloride correction (log β_1), which is close to the estimated value of 13.3 [13]. The correction for chloride complexes in sea water gives a value of 17.6 for log β_2 of $Cu(HS)_2^-$, which is in good agreement with the estimated value of 17.2 [13].

The stability constants for silver had to be determined in 0.7 M $NaClO_4$ solution to avoid the precipitation of AgCl from sea water. The titration results confirm that Ag^+ forms strong complexes with sulfide. After the first addition of HS^- the remaining HS^- in solution is below the detection limit. Thus, the stability constants for silver sulfide complexes (Table 2) are only the lower limits. More sensitive techniques are needed to determine a reliable stability constant for silver sulfide complexes.

Fe^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} were found to have weak complexation with sulfide. The titration results for Fe^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} could be fit by assuming that only the 1:1 complexes were formed. The stability constants of Fe^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} sulfide complexes determined in sea water are also summarized in Table 2. The stability constants for Fe^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} estimated from metal dithizone extraction constants yield a weak 1:1 complex and a strong 1:2 complex [13]. It was found that a negative value of β_2 would be obtained from the fit of titration data for these weak metal sulfide complexes if both 1:1 and 1:2 complexes was assumed to form in sea water.

Although the present results are in general agreement with literature values there are some potential limitations of this estimation which need further discussion. It has been assumed that the metal sulfide complexes are non-labile to the electrode process. However, the metal sulfide complexes are in rapid equilibrium with metal ion and ligand, HS^-,

$$M^{2+} + HS^- \underset{k_d}{\overset{k_f}{\rightleftharpoons}} MHS^+ \qquad (13)$$
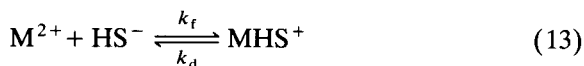
The rate constant of formation, k_f, and rate constant of dissociation, k_d, of metal sulfide complexes are related to the stability constant, β, via

$$\beta = \frac{k_f}{k_d} \qquad (14)$$

For metal sulfide complexes to be non-labile, the rate constants for the dissociation of metal sulfide complexes have to be small so that the effect of dissociation can be neglected. This assumption was made based on the magnitude of the stability constants. This assumption might not be valid. The metal sulfide complexes might be able to dissociate to release free sulfide ion within the diffusion layer of HMDE and contribute to the measured current. The contribution from this kinetic current depends on the diffusion layer thickness, rate constant of dissociation, k_d, stability constant, β, of metal sulfide complexes and the diffusion coefficients of the various species [17]. The diffusion layer thickness is dependent on the rate of stirring in the cell of the HMDE, which was the same for different experiments. Although it is reasonable to assume that the diffusion coefficients of HS^- and MHS^- are of the same order of magnitude, the rate constants of dissociation, k_d, are different for different metal complexes. This contribution from kinetic current might cause an underestimation of the stability constants for some metal sulfide complexes such as $Hg(HS)_2$.

Application to the sulfide speciation in surface sea water

The concentrations of sulfide in surface sea water have been determined to be 0.1–2 nM with an average of 0.51 ± 0.07 nM [1,3]. The measure-

ments using different techniques confirm the presence of H_2S in oxygenated sea water. Metal sulfide complexes have been proposed to be the dominant species of sulfide in surface sea water and cause the stability of sulfide in oxic surface sea water against the oxidation [4,5]. This contention is a result of the calculation based on the estimated stability constants for metal sulfide complexes. Our kinetic studies indicate that most metals (except Zn) increased the rate of oxidation of H_2S [6]. Thus, the stability of sulfide in surface sea water is unlikely to be due to the formation of copper sulfide complexes.

Using the stability constants determined in this study, a speciation calculation was made with the SURFALL program. The concentration of metals in surface sea water were taken from the measured values in the open ocean [18,19]. The percentages of metal sulfide complexes of the total sulfide in surface water obtained from the calculation are summarized in Table 3 along with the total concentrations of metals used in the calculation. The percentage of three dominant sulfide species in surface sea water is shown in Fig. 4. The hydrogensulfide ion, HS^-, is the dominant species accounting for 92.8% of total sulfide. The next important species is H_2S which accounts for 5.1% of total sulfide. This implies that a significant amount of sulfide is available for the air–sea exchange. $CuHS^+$ only accounts for 1.2% of total sulfide in surface sea water. The other metal sulfide complexes only account for less then 0.9% of total sulfide in sea water. Most of the mercury

Fig 4. The percentage of three dominant sulfide species in surface sea water at total sulfide concentration of 0.51 nM.

(98%) was bound as $HgHS^+$ and the remainder is $Hg(HS)_2$ in surface sea water. Although mercury has a strong tendency to complex with sulfide, its concentration is too low to influence the speciation of sulfide in surface sea water. The low concentration of sulfide in surface sea water is the net result of production and removal processes. The hydrolysis of carbonyl sulfide has been proposed to produce the H_2S in the open ocean sea water [20]. Bacterial activity in the micro anoxic environments of the biogenic particulate matters could also produce H_2S in the surface sea waters.

Application to the speciation of metals in anoxic waters

Sulfide levels can reach high concentrations in anoxic waters like the Black sea (425 μM), the Cariaco Trench (58 μM) and the Framvaren Fjord (6 mM) [21–23]. The stability constants determined in this study can be used to determine the speciation of metals in these waters. The fraction of free metal at various levels of sulfide can be estimated from

$$[M]_F/[M]_T = 1/\left(1 + \beta_1[HS^-] + \beta_2[HS^-]^2\right) \tag{15}$$

The fraction of free metals in various anoxic waters have been estimated from Eqn. 15 and the results are given in Table 4. As it is quite apparent most of the metals are complexed with sulfide in anoxic waters. The total metal in these solu-

TABLE 3

Speciation of some trace metal sulfide complexes in surface sea water with a concentration of total sulfide of 0.51 nM

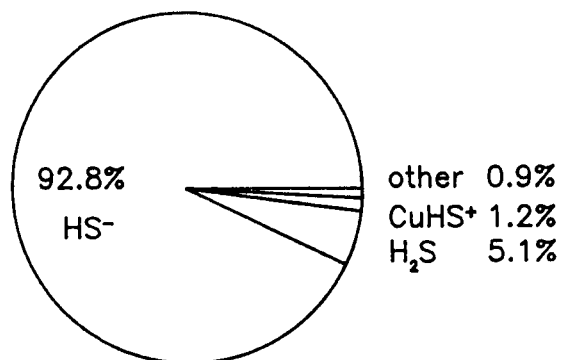| Metal ion	Total conc. (nM)	[MHS]/[S]$_T$ (%)	[M(HS)$_2$]/[S]$_T$ (%)
Cu^{2+}	1.2	1.184	0.001117
Hg^{2+}	0.002	0.3853	0.0145
Cu^+	0.4	0.2441	0.00013
Pb^{2+}	0.17	0.1795	0.0005
Zn^{2+}	0.05	0.0055	0.0002
Cd^{2+}	0.002	0.0005	0 [a]
Ni^{2+}	2.0	0.0387	0 [a]
Co^{2+}	0.01	0.0002	0 [a]

[a] Too small to measure.

TABLE 4

Fraction of free metal ions in various anoxic environments

Metal	Cariaco	Black Sea	Framvaren
Fe^{2+}	7.95×10^{-2}	1.17×10^{-2}	8.35×10^{-4}
Cu^{2+}	2.92×10^{-5}	5.52×10^{-7}	2.78×10^{-9}
Mn^{2+}	3.43×10^{-3}	4.69×10^{-4}	3.33×10^{-5}
Cd^{2+}	5.89×10^{-5}	1.10×10^{-6}	5.54×10^{-9}
Ni^{2+}	7.95×10^{-2}	1.16×10^{-2}	8.35×10^{-4}
Co^{2+}	7.95×10^{-2}	1.16×10^{-2}	8.35×10^{-4}

tions are controlled by the levels of sulfide if equilibrium is reached. The results shown in Fig. 5 indicate that this may be a reasonable hypothesis for some metals. Further studies are needed to determine the effect of pH, temperature and ionic strength on the stability constants by the methods outlined in this paper.
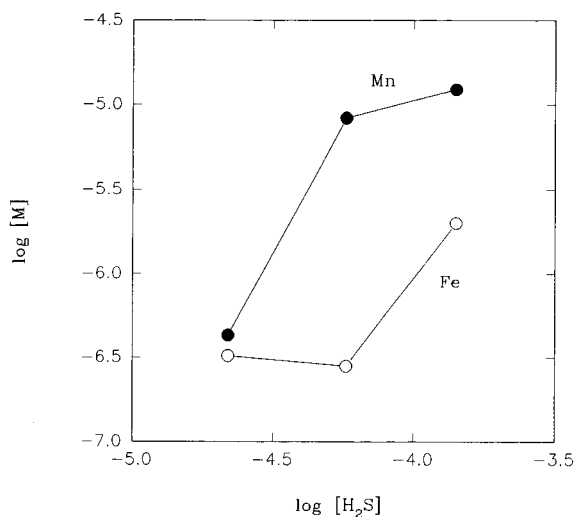
Fig. 5. Comparison of total sulfide concentration and Fe and Mn concentration in the anoxic environments.

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Zinc speciation in lake waters and its determination by ligand exchange with EDTA and differential pulse anodic stripping voltammetry

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Abstract

A technique to determine free zinc ion concentration and zinc speciation in lake water was developed. The technique involves ligand exchange of added EDTA with natural organic ligands and measurement by differential pulse anodic stripping voltammetry (DPASV); pretreatment of samples and separation are not necessary. The validity of the ligand exchange approach was demonstrated in model waters containing glycine and CDTA (or EDTA), as representatives of weak and strong organic ligands. The ligand exchange and DPASV measurements were applied to water samples from Lake Greifen, a small eutrophic lake in Switzerland. The average fractions of dissolved zinc species in this lake were 7.8% free zinc ions, 33.5% weak organic complexes and 50.5% strong organic complexes. Total dissolved Zn in the lake water column ranged between 18 to 25 nM, free zinc ion concentrations 1.3–1.8 nM, with an average pZn of 8.8 and an average ratio of total Zn to $[Zn^{2+}] \approx 13$. The extent of complexation of Zn in the lake was much less than in some sea waters (ratios 60–100 in the North Pacific), probably due to higher ratios of total Zn concentration relative to organic ligands in the lake.

Zinc is generally considered as an essential microelement for organisms, since it is involved in several enzyme systems. Its biological availability has been shown in algal cultures to be controlled by its free ion concentration [1,2]. The role of Zn in limiting phytoplankton growth in some oceanic waters, in which total Zn is in the subnanomolar range, and as a selective factor on the species distribution of marine phytoplankton, is currently being discussed [2,3]. Total dissolved Zn concentrations that have been observed in

some lakes are 1–2 orders of magnitude higher than those in open sea [4,5]. In a eutrophic lake, Zn appears to be removed from the water column by sedimentation together with biological material, with a maximum sedimentation rate during summer stratification [5]. However, Zn speciation in fresh water has not been extensively investigated. In order to understand the mutual feedback interactions between zinc and phytoplankton in fresh water, studies on its speciation are needed. Determination of the concentration of free metal ions is very significant for studies of biological effects.

Zn speciation in sea water has been studied using ligand exchange with EDTA and XAD-2 resin separation [6], MnO_2 equilibration [7], up-

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take kinetics of Chelex-100 ion-exchange resin [8], differential pulse anodic stripping voltammetry (DPASV) [4,9,10] and differential pulse cathodic stripping voltammetry (DPCSV) of Zn–pyrrolidine dithiocarbamate (PDC) complexes [10,11]. Voltammetric techniques have been widely used to speciate metals without preseparation. However, application of direct voltammetric methods allows one only to differentiate between electrochemically labile and non-labile species; the presence of ligands forming labile complexes may remain undetected. Labile species may not be generally identified with bioavailable species.

Using ligand-exchange techniques, metal ions bound to exchangeable ligands can be determined and free metal ion concentrations can be calculated at the low levels of total metal concentrations in natural waters. Ligand-exchange techniques are based on the competition for metal complexation between natural ligands and added known ligands, and the subsequent specific determination of these complexes. For example, a ligand-exchange technique of cathodic stripping voltammetry of zinc–PDC complex ions has recently been used to speciate Zn in sea water [10,11]. But this method could not be applied to waters containing higher concentrations of Zn because coverage of these surfactant complexes on the mercury electrode was too high.

It was intended in this study to develop an EDTA ligand-exchange technique followed by DPASV measurement to determine Zn speciation in fresh waters with relatively high Zn concentrations. Zn speciation and free zinc ion concentrations in a eutrophic lake, Lake Greifen, Switzerland, are presented and discussed.

THEORY

Total dissolved Zn in lake waters includes voltammetrically labile and inert species. The inert Zn species are non-reducible complexes at the electrode surface or not dissociated complexes in the boundary layer at the chosen potential. They may include strong organic complexes and Zn adsorbed on colloidal particles. The labile species include free aquo ions, inorganic complexes and

weak organic complexes. With the assumptions that the contribution to the electrolysis current is entirely due to fully labile complexes and that the diffusion coefficients of labile complexes (organic and inorganic) equal that of the free zinc ion, the peak current, i_p, may be regarded as a measure of the sum of all labile zinc species. The total Zn is distributed in an original lake water as the following:

$$T[Zn] = [Zn]_o^{lab} + [ZnL_1] = [Zn^{2+}]_o + [Zn]_{in}$$
$$+ [ZnL_2] + [ZnL_1] \qquad (1)$$

where $[Zn]_o^{lab}$ represents the concentration of labile Zn, and $[Zn^{2+}]_o$, $[Zn]_{in}$, $[ZnL_2]$ and $[ZnL_1]$ are the concentrations of free zinc ion, inorganic, labile (weak) organic complexes and non-labile organic complexes, respectively. The inorganic complexes can be estimated from the inorganic complexing coefficient α_{in} with complex formation constants β_i and concentrations of the inorganic species i:

$$\alpha_{in} = \beta'_{OH}[OH^-] + \beta''_{OH}[OH^-]^2 + \beta_{CO_3}[CO_3^{2-}]$$
$$+ \beta_{HCO_3}[CO_3^{2-}][H^+] \qquad (2)$$

The ZnEDTA complex is non-labile in terms of DPASV (see below). When EDTA is added to lake water under the condition:

$$K_2[L_2] \leq K_{ZnEDTA}[EDTA^{4-}] < K_1[L_1]$$

(where $K_2[L_2]$ and $K_1[L_1]$ are the complexing coefficients of labile organic ligands and non-labile ligands, K_{ZnEDTA} is the stability constant of the Zn–EDTA complex and $K_{ZnEDTA}[EDTA^{4-}]$ represents the complexing coefficient of EDTA), it can be assumed that the added EDTA competes with inorganic and labile organic ligands for Zn and that the non-labile complexes do not exchange with added EDTA. In that case, the initial concentration of labile Zn redistributes between EDTA and labile species:

$$[Zn]_o^{lab} = [ZnEDTA] + [Zn]^{lab}$$
$$= [ZnEDTA] + [Zn^{2+}]$$
$$+ [Zn]_{in} + [ZnL_2] \qquad (3)$$

The concentration of ZnEDTA complex is determined from the difference between the peak cur-

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rent in the original sample and that after equilibration with added EDTA:

$$[ZnEDTA^{2-}] = S(i_p^o - i_p^e) \qquad (4)$$

where i_p^o and i_p^e are, respectively, the peak currents in the absence and presence of EDTA, S is the sensitivity in A M^{-1}. The concentration of free zinc ion in the presence of EDTA can then be calculated from the equilibrum with EDTA:

$$[Zn^{2+}] = [ZnEDTA^{2-}]/(K_{ZnEDTA}[EDTA^{4-}]) \qquad (5)$$

The free EDTA ligand concentration in the Eqn. 5 is obtained from the mass balance of total EDTA,

$$\begin{aligned} T[EDTA] = [EDTA^{4-}]\big(1 + K'[H^+] \\ + K'K''[H^+]^2 + K_{Ca}[Ca^{2+}] \\ + K_{Mg}[Mg^{2+}]\big) + [ZnEDTA^{2-}] \end{aligned} \qquad (6)$$

where K', K'', K_{Ca} and K_{Mg} are, respectively, the first and second protonation constants of EDTA^{4-}, and the stability constants of CaEDTA^{2-} and MgEDTA^{2-}. Only the major cations Ca^{2+} and Mg^{2+} were assumed to compete with Zn^{2+} and H$^+$ for EDTA (other cation concentrations are negligible, see below); only the protonated species HEDTA^{3-} and H$_2$EDTA^{2-} are included at neutral pH.

Substituting [Zn^{2+}] obtained from Eqn. 5 into the mass balance equation of labile Zn at the given EDTA concentration (Eqn. 3), the complexing coefficient $K_2[L_2]$ of weak organic complexes is obtained,

$$K_2[L_2] = ([Zn]^{lab}/[Zn^{2+}]) - (1 + \alpha_{in}) \qquad (7)$$

The coefficient is the product of the average conditional stability constant of weak organic complexes and weak ligand concentration. Using this coefficient, the concentrations of free zinc ions, inorganic and weak organic complexes in the original sample without EDTA can be evaluated from the mass balance of initially labile Zn:

$$[Zn^{2+}]_o = [Zn]_o^{lab}/(1 + \alpha_{in} + K_2[L_2]) \qquad (8)$$

EXPERIMENTAL

Water samples were collected from the water column of Lake Greifen, Switzerland, at its deepest point, and at one main inlet (Aabach, surface sample). The lake is highly eutrophic and has a surface area of 8.5 km^2 and a volume of 150×10^6 m^3. Its average depth is 17.7 m, with a maximum of 32.2 m. The tributaries of the lake are highly loaded with nutrients and pollutants from sewage and agriculture. Go-Flo sampling bottles (General Oceanics, 5 l) were used in order to collect samples from different depths. Under N$_2$ pressure, the samples were transferred from the Go-Flo to polyethylene bottles [12].

Samples were filtered and further treated just after transport to the laboratory. The filtration device and filtering membranes (0.45 μm) were washed with 0.01 M HNO$_3$ and rinsed with bidistilled water.

Total dissolved Zn was determined in acidified samples by flame atomic absorption spectrometry (AAS) after preconcentration using the extraction of 8-quinolinol complexes on Sep-Pak C$_{18}$ resins [13], in a procedure modified from Ref. 14. Recovery of added Zn (10–40 nM) to lake water samples was 90–100%.

Dissolved organic carbon (DOC) was determined with a Shimadzu TOC 500 analyzer. Calcium and magnesium were measured by inductively coupled plasma emission spectrometry (ICP–AES). Total inorganic carbon and carbonate concentrations were calculated from alkalinity (measured by Gran-plot titration) and pH measurements in situ.

DPASV measurements of labile Zn were performed with a hanging mercury drop electrode, an Ag/AgCl reference electrode and a graphite counter electrode in a Metrohm VA 663 stand combined with a Metrohm E506 polarecord. After 10 min purging with suprapure N$_2$, a new Hg drop was made and the stirrer was simultaneously switched on. Labile Zn was deposited at -1.2 V for a period of 2 min under stirring followed by a 15 s rest, then scanning was initiated. The scanning parameters were: initial potential -1.2 V (vs. the Ag/AgCl reference electrode); pulse height 50 mV; scan rate 5 mV s^{-1}. The reduction

peak potential of Zn in Lake Greifen water was -1012 ± 20 mV. The measurements were performed within a maximum interval of one week, during which the samples were stored in a dark room at 4°C. Samples were buffered at pH 8.0 with 6×10^{-3} M HEPES (N-2-hydroxyethyl-piperazine N'-2-ethanesulphonic acid; the stock solution contains 1 M HEPES and 0.5 M NaOH). This buffer has very weak complexing properties [15]. High density polyethylene beakers (50 ml) were used as polarographic cells. Adsorption of zinc onto the cell walls was not detected, because labile Zn in lake water without added EDTA or Zn is reproducible over 24 h using these polyethylene beakers.

The kinetics of the formation of Zn–EDTA complexes were experimentally studied under lake water conditions. A given concentration of EDTA, at the same order of magnitude as the concentration of initially labile Zn, was added to 25 ml of a lake water sample (buffered with HEPES). Labile Zn in the sample was monitored over time by DPASV. The decrease of labile Zn (initially 11.1 nM) was very slow after addition of 20 nM EDTA; the measured concentration was stable after about 15 h (Fig. 1a). All experiments for Zn speciation were thus equilibrated overnight for 20–24 h. For comparison, a kinetic experiment was also performed in a solution containing 0.01 M NaCl, 2 mM Ca, 61.3 nM Zn and 100 nM EDTA at pH 8;

the results indicated a similar decrease over time and similar equilibration time (Fig. 1b).

The peak current of Zn is proportional to deposition time (1–3 min under stirring and 15 s rest) with the cell constant of 0.047 A M^{-1} min^{-1} in a solution of 0.01 M NaCl and 6×10^{-3} M HEPES buffer. The sensitivity is calibrated for each lake water by titration with standard Zn solution in the absence and presence of EDTA. 25 ml aliquots of lake water were pipetted into two series of beakers, with 6×10^{-3} M HEPES buffer at pH 8.0 ± 0.1. Zinc was added to all beakers but one for each series, giving a concentration range of added Zn between 3–153 nM in 8–9 steps. A given amount of EDTA, in most cases 20 nM, was added to one series of beakers. All beakers were kept in the dark overnight for 20–24 h until DPASV measurement.

Free zinc ion concentration and zinc speciation were calculated from the labile Zn measurement at different concentrations of EDTA; [ZnEDTA] is calculated as the difference between initially labile Zn and measured labile Zn after addition of EDTA (Eqn. 4). Lake water samples were thus titrated with EDTA in the same concentration range as initially labile Zn. 25 ml aliquots of lake water were pipetted into a series of beakers, 6×10^{-3} M of HEPES buffer (pH 8) was added, and the aliquots were spiked with different concentrations of EDTA (10–60

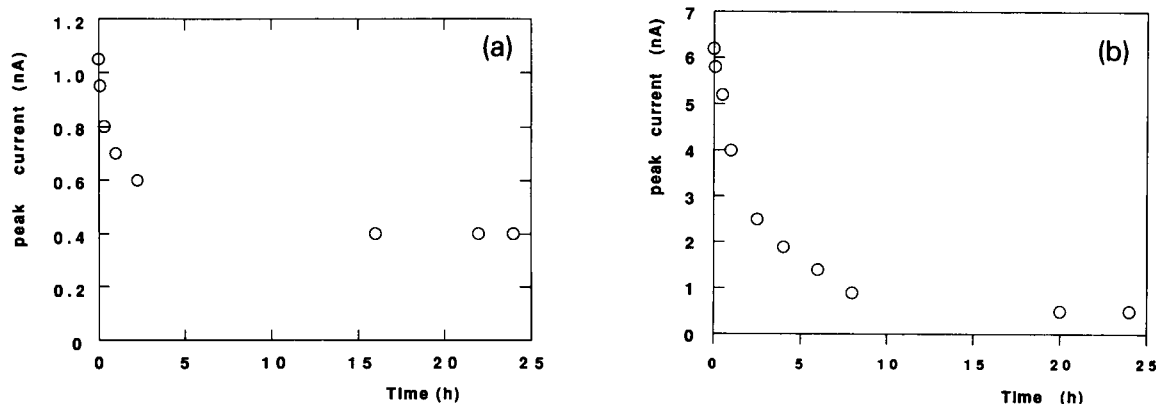Fig. 1. Variations of DPASV peak current as function of time after addition of EDTA to a Lake Greifen water sample (a) and a prepared solution (b). The water sample was collected on 16 Dec. 1991, at 30 m depth, T[Zn] = 24.5 nM, initially labile [Zn] = 11.6 nM and [EDTA]$_{added}$ = 20 nM, pH = 8.0. The solution was prepared with NaCl 0.01 M, Ca 2 mM and Zn 61.3 nM at pH 8, and [EDTA]$_{added}$ = 0.1 μM.

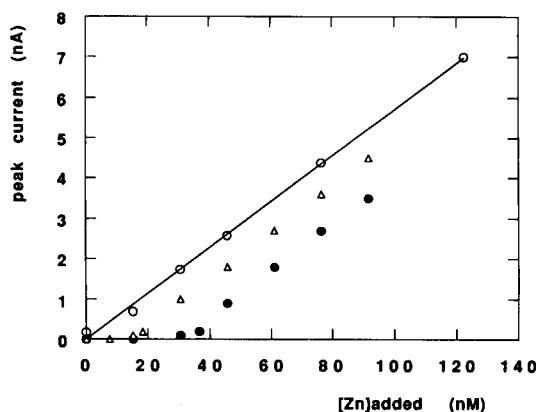H.B. Xue and L. Sigg / Anal. Chim. Acta 284 (1994) 505–515

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Fig. 2. Titration curves of (○) glycine, (●) EDTA and (△) CDTA solutions with Zn. [Glycine] = 50 μM, [EDTA] = 40 nM and [CDTA] = 20 nM, pH 8.0 buffered by HEPES.

buffered at pH 8 by HEPES, to which standard Zn solutions were added, step by step; labile Zn was measured by DPASV after each addition and 5 min mixing. Equilibrium was approached within 3–5 min in these solutions of glycine, EDTA or/and CDTA without calcium. This also provides evidence that slow equilibration in lake water was not due to adsorption of the added zinc onto the walls of the polyethylene beakers.

The resulting titration curves are plotted in Fig. 2. The titrations of EDTA and of CDTA showed that no labile Zn was detectable when the added Zn concentration was smaller than EDTA or CDTA. This means that Zn–EDTA and Zn–CDTA complexes are definitely non-labile in terms of the DPASV time scale. In contrast, Zn–glycine complexes are completely labile, giving a linear titration curve, which passes nearly through the zero point. No complexing effect of the HEPES buffer could be detected in these titration curves.

Two stock solutions (1 l) containing Ca (2 mM), initial Zn (30.6 nM), glycine (1.34 mM) and CDTA (15 nM, solution A) or initial EDTA (30 nM, solution B) were prepared. NaCl standard solution was added to each stock solution (final 10^{-2} M) to make the ionic strength similar to that of lake water. The pH of the stock solutions was kept constant at 8.00 ± 0.05 by adding NaOH (1.9 mM) and HEPES (6 mM). The solutions

nM) in 5–7 steps. All the prepared samples were allowed to equilibrate overnight in the dark before DPASV measurement.

In order to examine our hypothesis, labile Zn was measured in model systems containing weak or/and strong organic ligands. Glycine (aminoacetic acid, log K of its Zn complex 5.3) and CDTA (trans-1,2-diaminocyclohexanetetraacetic acid, log K = 19.3) were taken as representatives of weak and strong organic ligands, respectively. Glycine (50 μM), EDTA (40 nM) and CDTA (20 nM) solutions were first separately titrated with Zn. 25 ml of these solutions in 0.01 M NaCl were
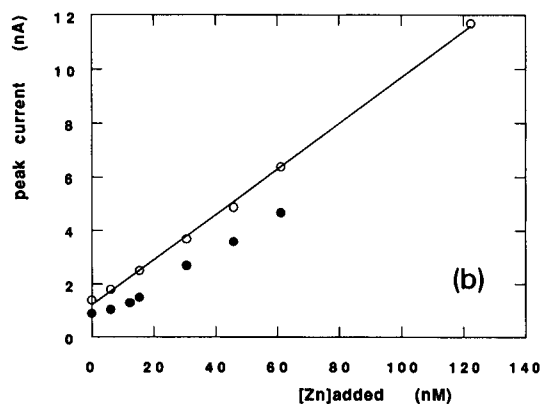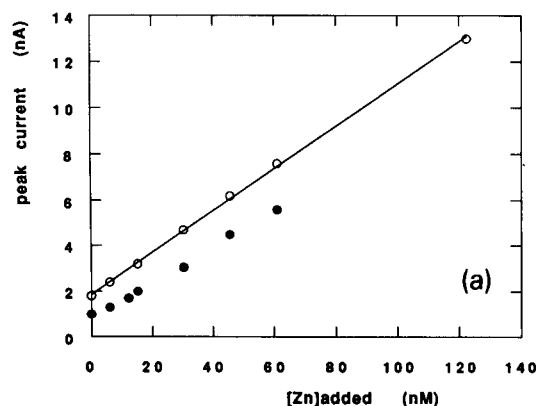

Fig. 3. Titration curves of model water A (a) and model water B (b) with Zn in the absence and presence of additional EDTA. Composition of the model waters is shown in Table 1; the hollow points represent the titration in original model waters and the solid points in the presence of additional EDTA (30 nM).

were kept overnight to equilibrate and considered as model waters A and B. CDTA was a strong organic ligand in the model water A, as well as EDTA (initially 30 nM) in the model water B. The model waters were then titrated with Zn to calibrate DPASV sensitivities, and titrated with EDTA to compare measured labile Zn and complexing coefficients of the Zn–glycine complex with those computed by an equilibrium program. Series for titrations were prepared like those of natural lake waters. 25 ml aliquots of the model waters were pipetted into two series of 50 ml solid polyethylene beakers. Then zinc was added to all beakers except one for each series. A given amount (30 nM) of EDTA was pipetted to one of the series. 25 ml aliquots of the model solutions were pipetted into a third series of the beakers, spiked with different concentrations of EDTA in the range 10–60 nM. All the samples were allowed to equilibrate overnight in the dark before DPASV measurement.

RESULTS

As shown in Fig. 2, complexes with the strong organic ligands EDTA and CDTA are definitely non-labile, and complexes with the weak organic

TABLE 1

Zinc speciation computed by the Microql program for model waters containing weak and strong organic ligands at pH 8.0

Component or species	Model water A	Model water B
Ca	2 mM	2 mM
Initial Zn	30.6 nM	30.6 nM
Glycine	1.34 mM	1.34 mM
CDTA	15 nM	–
EDTA	–	30 nM
$[Zn^{2+}]$	3.15 nM	2.74 nM
[Zn–Glycine]	16.1 nM	13.4 nM
[Zn]lab	19.9 nM	16.7 nM
[Zn–EDTA]	–	13.9 nM
[Zn–CDTA]	10.7 nM	–
Free [glycine] (deprotonated)	24.5 μM	24.5 μM

ligand glycine are completely labile. Figure 3 a and b gives titration curves of the model waters A and B with Zn in the absence and presence of EDTA (additional for B). Linear titration curves in the absence of additional EDTA with a positive intercept in the y-axis show that the water samples contain labile Zn and that strong organic ligands (CDTA or EDTA) were saturated. Titration points in the presence of additional EDTA exhibit a concave section in the lower part and a
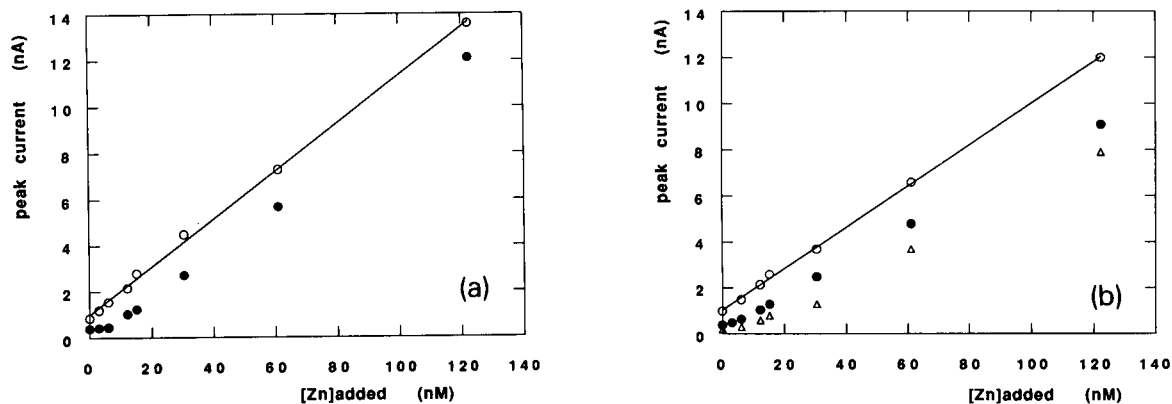
Fig. 4. Titration curves of Lake Greifen water samples with Zn in the presence of different concentrations of EDTA. (a) The sample was collected on 14 April 1992 at 5 m depth, the hollow circles represent the titration in the original water sample and the solid circles in the presence of EDTA (20 nM). (b) The sample was collected on 16 Dec. 1991 at 30 m depth, the hollow circles show the titration in the original water, the solid circles in the presence of 20 nM EDTA and the hollow triangles in the presence of 50 nM EDTA.

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TABLE 2

Chemical composition of Lake Greifen water

	Station				
	30 m	0 m	5 m	20 m	Aabach
	Sampling date				
	16 Dec.	14 April	14 April	14 April	14 April
pH	7.88	8.47	8.40	8.17	8.47
Alk. [a] (mM)	3.94	3.92	3.94	3.89	4.25
DOC (mg l^{-1})	3.4	3.4	3.2	3.2	4.6
Ca (mM)	1.65	1.73	1.79	1.80	2.05
Mg (mM)	0.70	0.71	0.73	0.73	0.78
Zn (nM)	24.5	21.4	19.9	18.4	74.9

[a] Alk. = alkalinity.

linear portion in the upper part. The shift of the titration curves reflects complexation of Zn with additional EDTA.

Table 1 lists composition and speciation of the model waters computed by the Microql equilibrium program [16] with the stability constants from [17]. The measured labile Zn was 19.6 nM for water A [computed 19.9 nM (65%)]; for water B, measured labile Zn was 16.6 nM [computed 16.7 nM (55%)]; good agreement is thus found between measured and calculated values (error < 5%). Free zinc ion concentrations and speciation of initially labile zinc in the model waters were also determined from Eqns. 2–8 and from the labile Zn measurements (as an average in the presence of different concentrations of additional EDTA). The determined free zinc concentrations were 3.2 nM for water A (computed 3.15 nM) and 2.2 nM for water B (computed 2.74 nM); good agreement is thus also obtained for free $[Zn^{2+}]$ (error < 20%). The complexing coefficient of Zn–glycine ($K \times$ free [glycine]) was determined as 5.9 by DPASV (average value), which compares well with the calculated value 4.9 for the conditions of these model waters (error < 20%). The agreement between labile Zn, free Zn ions and the complexing coefficient of Zn–glycine from DPASV determination and those from the computed equilibrium composition demonstrates the validity of our hypothesis for the determination of weak and strong organic ligands.

Figure 4 exemplifies titration curves of lake water with Zn in the absence and presence of EDTA. The titration curves exhibit patterns similar to those of model waters. A linear titration curve in the absence of EDTA with a positive intercept shows that the original lake water sample contains labile Zn in excess of strong organic ligands. Titration points in the presence of EDTA exhibit a shift, a concave section in the lower part and a linear section in the upper part. The linear section is nearly parallel to the titration curve in the absence of EDTA. The shift of titration curves reflects the complexation of Zn with added EDTA.

TABLE 3

The speciation of Zn in lake water samples

		Lake Greifen					Lake Lucerne
		30 m, 16 Dec.	0 m 14 April	5 m 14 April	20 m 14 April	Aabach 14 April	5 m 21 Oct.
T [Zn] diss.	(nM)	24.5	21.4	19.9	18.4	74.9	10.7
Initially labile Zn	(nM)	11.1	12.4	8.2	9.8	37.1	4.0
	(% of T[Zn])	45.6	57.9	41.2	53.3	49.5	37.4
Non-labile [ZnL$_1$]	(nM)	13.4	9.0	11.7	8.6	37.8	6.7
	(% of T[Zn])	54.4	42.1	58.8	46.7	50.5	62.6
Weak [ZnL$_2$]$_{org}$	(nM)	8.1	8.2	5.6	6.3	25.4	2.7
	(% of T[Zn])	33.1	38.4	27.9	34.2	33.9	25.3
Inorganic complexes	(nM)	1.41	2.29	1.33	1.77	6.03	0.49
	(% of T[Zn])	5.8	10.7	6.7	9.6	8.1	4.6
Free ion [Zn^{2+}]	(nM) [a]	1.65 ± 0.32	1.85 ± 0.40	1.32 ± 0.46	1.77 ± 0.34	5.63 ± 1.44	0.79 ± 0.04
	(% of T[Zn])	6.7	8.7	6.6	9.7	7.5	7.4

[a] The average values were obtained from 5–7 measurements with different concentrations of EDTA.

The DPASV proportionality constants of lake water samples are given by the slopes of the linear part of the titration graphs; they lie in the range 0.045–0.052 A M^{-1} min^{-1} and are thus similar to those in the corresponding ionic medium. The constant in the sample from Aabach Inlet is lower (0.032 A M^{-1} min^{-1}), probably due to higher concentration of organic matter. Similar slopes are also obtained for the linear portion of the titration curves in the presence of EDTA, indicating that there is no obvious change in DPASV sensitivity after addition of EDTA.

Table 2 gives the general composition of water samples from Lake Greifen and in an inlet (Aabach). The complexing coefficients of weak organic ligands in Greifen lake water were calculated from labile Zn measurements in the presence of EDTA using Eqn. 7; the average value from different samples is $K_2[L_2] = 4.3 \pm 0.5$. The complexing coefficients for major inorganic species were calculated as $\alpha_{in} = 0.86$–1.07 with stability constants from Ref. 16. The free zinc ion concentrations (mean with standard deviation) and the resulting Zn speciation in Lake Greifen are presented in Table 3. Consistent values were obtained from 5–7 measurements with different EDTA concentrations. Free zinc ion concentrations are between 1–2 nM at the deepest station. The inorganic complexes have the same range of concentrations as that of free ions. At the deepest point, the concentrations of weak organic complexes range between 6–8 nM, the total labile Zn 8–12 nM and non-labile Zn 9–13 nM. The fraction of free zinc ion concentration is about 7–10% of total dissolved concentrations with an average value of 7.8%. The average fractions of other species are, respectively, 8.2% for inorganic complexes, 33.5% for weak organic ligands and 50.5% for non-labile species by DPASV. These results are similar to those in the model waters. Total dissolved Zn in lake water is always higher than labile Zn by DPASV; this indicates the presence of non-labile species which may be strong organic–Zn complexes or possibly colloidal species. Some losses due to adsorption of Zn to the beaker walls at pH 8 may also occur. Up to 50% of total dissolved Zn may thus be present in form of strong organic complexes.

DISCUSSION

Complexation of a strong ligand like EDTA with trace metals is intrinsically fast and predominantly controlled by water loss from the inner coordination sphere of metals [18]. In the presence of an excess of Ca and Mg, however, the reaction rates have been shown to be significantly lower [19]. These slow complexation kinetics are observed in the lake water samples (Fig. 1a). A kinetic experiment in a model solution containing Zn, EDTA and Ca in excess at pH 8 indicated this kind of slow reaction; a (pseudo-first-order) half-life for labile Zn reacting with EDTA was calculated as ≈ 3 h in this solution (Fig. 1b). Similar results were also obtained in a river water sample (Glatt River) with a higher Zn concentration (100 nM) (unpublished results). These results are comparable with a previous report in Lake Ontario water [20]. Thus, we must pay more attention to equilibration time in titrations of natural waters with metal ions or ligands and in applications of ligand exchange techniques. Equilibrium of the ligand exchange reaction between added EDTA and weak organic ligands must be attained for a reliable determination of free zinc ion concentration by DPASV. If the equilibrium was not established, labile Zn and the complexing coefficient of weak ligands would be overestimated. Consequently, free zinc ion concentration would be underestimated and the extent of the underestimation would decrease with increasing EDTA concentration. However, such systematic variation of $K_2[L_2]$ and $[Zn^{2+}]$ was not found. The kinetic experiment showed that 20–24 h equilibration are sufficient.

Reliable results for $[Zn^{2+}]$ in lake water samples should theoretically be independent of the EDTA concentration added. Practically, the applicable range of EDTA concentrations was chosen in the same order of magnitude as originally labile Zn in order to perform precise measurements of labile Zn in the presence of different concentrations of EDTA; this range is also limited by the sensitivity of DPASV. On the other hand, an excess of EDTA may compete for Zn complexed with strong organic ligands and would lead to greater error, since [ZnEDTA] may then

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be underestimated. Under our working conditions, the results from 5–7 different concentrations of EDTA for each sample are close, with about 20% relative deviation in $[Zn^{2+}]$ or about 0.1 of pZn (Table 3). This is tolerable at such low levels. In addition, consistent speciation was obtained for different samples from Lake Greifen (Table 3).

Electrochemically labile zinc was detectable in all lake water samples examined, indicating that total dissolved Zn was in excess of ligands forming non-labile complexes. Using the ligand exchange method, information about the labile complexes is obtained. The weak labile complexes may behave in a similar way as glycine and may consist of fulvic acids and some low-molecular-weight organic compounds. The complexing coefficient $K_2[L_2]$ remains constant for different additions of EDTA if the total concentration of weak ligands is higher than the total Zn concentration, and the free $[L_2]$ concentration is constant due to buffering by protonation or by complexation with other metal ions. If the concentration of available weak organic ligands was not in excess of Zn, EDTA at different concentrations would obviously cause changes in free ligand concentrations and its complexing coefficient; this was however not the case for our lake water

samples. The complexing coefficients, 4.3 ± 0.5, are close for each sample and consistent for different lake waters. Competition between Zn and other metals in complexing with weak organic ligands may lead to underestimates of free zinc ion concentration, because concentrations of the other metals bound to weak organic ligands would decrease when they are complexed with added EDTA. Hence the free weak organic ligand concentration would increase. The constant complexing coefficient and free zinc ion concentration at different concentrations of EDTA indicate that the above biases were not a problem.

Reaction between EDTA and metals other than Zn may decrease the available EDTA for Zn. In our calculation, the major cations Ca and Mg have already been taken into account. Other trace metals (Cu, Pb) are present at lower concentrations than Zn and are thus not significant. Iron forms strong EDTA complexes, but calculations including iron(III) in the form of solid iron oxide at pH 8 indicate that the competition of Fe for EDTA and its influence on Zn speciation at equilibrium are negligible.

A critical point concerns the nature of the non-labile species and the assumption that they are not significant in exchanging with EDTA. The occurrence of strong organic ligands for Zn
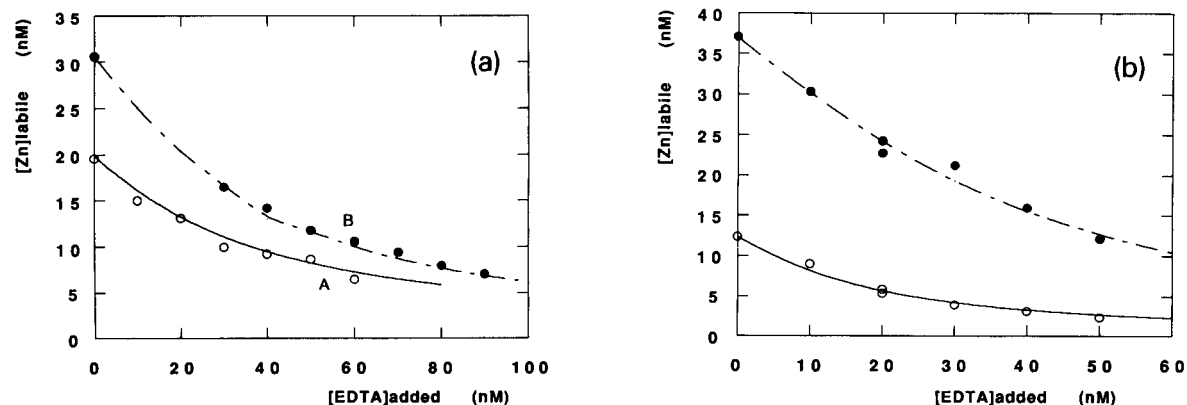
Fig. 5. Measured and computed labile Zn in the presence of different concentrations of EDTA in the model waters (a) and in Lake Greifen water samples (b). All the points represent measured values and the curves computed values. In (a) the hollow circles and continuous curve are for the model water A, the solid circles and the broken line for the model water B (Table 1). In (b) the hollow circles and the continuous curve are for the sample collected at 5 m depth on 14 April 1992, the solid circles and the broken line for the sample of Aabach Inlet on 14 April 1992. Computed labile Zn included free ion, inorganic and weak organic (or Zn–glycine) complexes, calculated by the Microql equilibrium program.

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in lake water is possible as in sea water. Relatively high DOC concentrations are found in these different samples. The non-labile Zn species are not well understood, including on the one hand some strong organic ligands, like EDTA and CDTA, and on the other hand, some colloidal Zn, in a size range < 0.45 μm (organic or inorganic colloids). Under our working conditions (pH 8, [Ca] $\approx$ 2 mM and [Mg] $\approx$ 0.5 mM, EDTA = 10–100 nM), the product $K_{EDTA} \times [EDTA^{4-}]$ ≈ 1–25. This means that EDTA in this system can only compete efficiently with ligands that yield a product $K_i \times [L_i]$ in a lower or similar range. This is also the case for strong ligands present at low concentrations. Thus, we should say that the voltammetrically labile Zn may not be the same as the exchangeable one; ligand exchange with colloidal species is difficult to evaluate. The stability constant of strong organic complexes in the lake waters, if they occur in concentrations of about 10 nM, would be in the range log $K > 9$; this is analogous to reported values for strong ligands with Zn in sea water. These ligands are probably heterogeneous ligands, which would include a range of different stability constants; our study does however not give any detailed information about these different ligands. The total concentration of these ligands appears to be smaller than the Zn concentration, so that the free ligand concentration may be very low. Higher DOC was measured in the Aabach Inlet water (DOC 4.6 mg l^{-1}) than in the samples from the water column (3.2–3.4 mg l^{-1}); a higher concentration of non-labile species is also found, which may correspond to strong organic ligands (Table 3).

The speciation of Zn in the presence of different concentrations of additional EDTA was computed by the Microql equilibrium program, in order to verify the assumptions (Fig. 5). The components for computation of the speciation in Lake Greifen water included Ca, Mg, Zn, carbonate, EDTA, and weak and strong organic ligands with total concentrations measured for each sample. The composition of the model waters A and B were taken as prepared. The stability constants were taken from Martell and Smith [17], except for the weak organic complexes in Greifen lake

waters. As regard to Zn–weak organic complexes in the natural waters, the determined complexing coefficients for each sample were matched with appropriate combinations of stability constants and concentrations. For the strong organic ligands, the concentration was taken as equal to the non-labile Zn species and stability constants in the range log $K = 9$–11 were assumed. In the case of a strong organic ligand with a stability constant log $K = 11$ the concentration of the Zn complex changes by less than 1% upon addition of EDTA in the range used; in the case of log $K = 9$, the concentration of the Zn complex would change by less than 20%. Computed labile Zn from the sum of free zinc ion, inorganic complexes and weak organic complexes were plotted as titration curves with EDTA. Measured labile Zn fit the computed curves very well as shown in Fig. 5a for the model waters and Fig. 5b for Lake Greifen water samples (with log $K = 11$ for the strong organic ligands). The accordance of measured with computed labile Zn and the similarity of Lake Greifen water with the model waters indicate the validity of this approach to determine free zinc ion concentration and Zn speciation in the lake water.

Our results here are in agreement with a preliminary study by Gonçalves et al. [21] on the complexation of zinc in Lake Greifen water, in which a combination of separation of hydrophobic complexes on Sep-Pak C$_{18}$ resins and DPASV measurement was used. By this method ca. 20% of total dissolved zinc was free ion and inorganic complexes and ca. 80% was organically complexed; about 10% was retained in hydrophobic complexes on the C$_{18}$ resins.

The extent of complexation of zinc by strong organic ligands, $\approx$ 50% of total dissolved Zn in lake water, is much less than those ($> 95\%$) in northeast and central North Pacific sea water samples reported by Donat and Bruland [4,10]. This may be partially explained by the concentration ratio of strong organic ligands to total zinc. It appears that the concentrations of strong organic ligands must be lower than total Zn in the lake waters examined here, so that zinc partially complexes with weak organic ligands (30%) and about 10% of free zinc ion with pZn 8.8 actually exists

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in the water column of Lake Greifen (total Zn 18–25 nM). In comparison, pZn = 11.7–12.7 were measured in the Pacific, with total Zn = 0.1–0.2 nM [4,10]; pZn = 9.2 in the Irish Sea with total Zn = 5 nM [11]; and pZn = 8.5–10.6 in the Gulf Stream with 2–6.5 nM total Zn [8]. In all these cases, the total concentration of strong organic ligands was higher than the total Zn concentration; conditional stability constants for these ligands are reported in the range log K = 8.4–11. It is difficult to find data on Zn speciation in fresh water in the literature. In addition to Lake Greifen samples, we determined Zn speciation in a water sample from Lake Lucerne, an oligotrophic lake, Switzerland, and list also these results in Table 3 for comparison. The DOC (1–1.5 mg l^{-1}) in Lake Lucerne was less than half of that in Lake Greifen. The total Zn and free zinc ion concentrations in Lake Lucerne are nearly half of those in Lake Greifen with a similar speciation.

Unlike the Zn complexation in Lake Greifen, Cu complexation by organic ligands is very strong [22], free copper ion concentration (pCu 14.9) are 6–7 orders of magnitude less than total copper, owing to high concentrations (40–90 nM) and high conditional stability constants (log K 14.3) of organic ligands. It appears that Zn could not compete with Cu for the specific organic ligands. The dynamics of pCu in the lake exhibit a seasonal pattern similar to that for algal productivity, thus the Cu complexing ligands are probably produced by algae. Although zinc in Lake Greifen is sedimented together with biological material and Zn to P ratios (0.03 mol mol^{-1}) in settling particles during summer stratification may correspond to the ratios in phytoplankton [5], evaluation of the possible sources of strong organic ligands and seasonal variations of free zinc ion concentration remain to be further clarified. In comparison to sea water, the higher total and free zinc and lower complexation of zinc in lake water may lead to different ecological effects [2]. More work is needed on Zn speciation and its interactions with phytoplankton in fresh water, that will be facilitated by the technique presented in this study.

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Applicability of chemically modified electrodes for determination
of copper species in natural waters

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Abstract

A procedure based on the stripping voltammetric determination of labile copper after its chemical accumulation on a graphite electrode modified with a complexation agent and a Nafion film is described. The method is sufficiently sensitive for the copper determination in the nM to μM range with a relative standard deviation of 1 to 6%. The detection window of the proposed technique is similar to that of conventional differential pulse polarography. Results for the copper speciation and the copper complexation capacity in model samples and in river waters are presented. A mathematical description of competitive complexation equilibria is discussed.

Keywords: Differential pulse polarography; Stripping voltammetry; Voltammetry; Copper; Waters

In the study of metal complexation in natural waters an investigation over a broad range of complex stabilities using analytical methods with different detection windows has been shown to be important [1]. Among voltammetric techniques based on the direct reduction of a labile metal fraction polarography and anodic stripping voltammetry (ASV) are most widely used for this purpose [2]. The voltammetric signal is controlled by association/dissociation rate parameters as well as diffusion parameters of metal species [3]. Other methods are based on a ligand competition. Utilizing this approach adsorptive cathodic stripping voltammetry has been successfully used to determine labile metal concentration [1]. Recently, voltammetry using chemically modified electrodes (CMEs) with immobilized complexing agent has been suggested for metal speciation studies [4–7]. CMEs represent sufficiently sensitive and selective voltammetric sensors and it is assumed that they allow the determination of free metal ions as well as those gradually released from kinetically labile complexes by dissociation during the accumulation step [8]. A perturbation necessary to produce the analytical signal concerns only a reaction layer in the vicinity of the electrode. However, using CMEs, some problems might also occur due to the two-phase reaction zone and the adsorption or penetration of complexes or ligands from the solution into the modifying layer.

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For the speciation studies an important prospect is offered by perm-selective properties of electrode coating [8]. Using a Nafion and a cellulose acetate/Nafion-coated mercury film electrode negatively charged and macromolecular metal-containing species were separated and excluded from the direct electrochemical determination [9,10]. Moreover, incorporation of a complexation agent into the Nafion film improves the stability and enhances the response of the CME [7].

Previous studies [5,6] dealing with the utilization of CMEs for the copper complexation study needed the reduction of copper(II) to copper(I) in the analyzed solution (by hydroxylamine hydrochloride) before the determination. Generally, a change of chemical composition of the sample means some complication in the speciation analysis.

In the present report, three types of CMEs were prepared and used in the investigation of complexation equilibria of copper. The derivatives of 4-acylpyrazolone (1-phenyl-3-methyl-4-octanoylpyrazol-5-on, ligand I; and 1-phenyl-3-methyl-4-stearoylpyrazol-5-on, ligand II) which react specifically with copper in a weak acidic medium [11] and oxine were used as the electrode modifiers. The electrodes were employed for the speciation of copper in river waters.

The procedure is based on the non-electrolytic accumulation of labile copper within a ligand/Nafion film at the electrode surface. After a medium exchange, the accumulated copper(II) complex with the modifier is determined by the anodic stripping technique. The variation of the detection window is possible due to a change of complexing ability of the electrode modifier.

EXPERIMENTAL

Apparatus and reagents

The voltammetric measurements were carried out by using a PA-4 polarographic analyzer with an *X–Y* recorder (Laboratorní přístroje, Prague). The experiments were performed in a one-compartment three-electrode cell with a modified graphite working electrode, a platinum auxiliary electrode and a saturated calomel reference electrode with a salt bridge containing 0.1 M KNO_3. For differential pulse (DP) polarography a static mercury drop electrode (SMDE) was used as the working electrode.

1-Phenyl-3-methyl-4-octanoylpyrazol-5-on (ligand I) and 1-phenyl-3-methyl-4-stearoylpyrazol-5-on (ligand II) were prepared as described elsewhere [12]. Nafion (DuPont) was used as a 1% solution in the mixture ethanol–water (1:1). Other chemicals were of analytical reagent grade. For preparing all solutions deionized and doubly distilled water was used.

Electrode preparation

The basal plane of a graphite rod (5.5 mm in diameter) impregnated with paraphine was cleaned by abrasive paper and polished on filter paper. For the electrode coating 3 µl of 0.37 M ligand solution in chloroform was transferred by a microsyringe onto the exposed area, and allowed to dry. Then 2 µl of 1% Nafion solution was applied in the same way.

Procedures

Samples of river water were passed through a 0.45-µm filter and stored in polyethylene bottles at 4°C (without acidification). Total copper was determined after ultraviolet irradiation of the samples (20 ml) in the presence of 30% hydrogen peroxide (20 µl) and concentrated nitric acid (50 µl, Suprapur, Merck) by a 500-W mercury vapour lamp for 4 h.

Voltammetry with CMEs

The electrode was immersed into 30 ml of a stirred sample for 60–300 s depending on the expected copper concentration (the accumulation time) at an open circuit. After being taken out and rinsed thoroughly with water, the electrode was transferred into the voltammetric cell containing 10 ml of 0.02 M acetate buffer (pH 3.5) and a constant potential of -0.35 V was held for 180 s (the reduction time). Finally the anodic stripping scan was made in DC mode at a scan rate of 0.1 V s^{-1}. The copper peak appeared between 0 and 0.1 V. The copper concentration was evaluated from a calibration graph. After

each Cu^{2+} determination the electrode was renewed by placing into a stirred solution of 1×10^{-3} M EDTA for 30 s.

DP polarography

A 10-ml aliquot of water sample was pipetted into the voltammetric cell and 0.1 ml of 2 M sodium acetate was added. After deaeration with argon for 5 min, the DP polarogram was recorded under the following conditions: starting potential 0.15 V, scan range 1.0 V, scan rate 5 mV s^{-1}, drop time 2 s, pulse amplitude 25 mV, pulse time 0.1 s.

RESULTS

Optimization of experimental conditions

The cyclic voltammograms obtained for electrodes modified with all three types of ligands after the accumulation of copper in model solutions (acetate buffer solutions of different pH) exhibited an asymmetric and a poorly quantifiable cathodic peak and a sharp anodic stripping peak (Fig. 1). Hence, the technique of ASV was chosen for analytical measurements. On repeat-
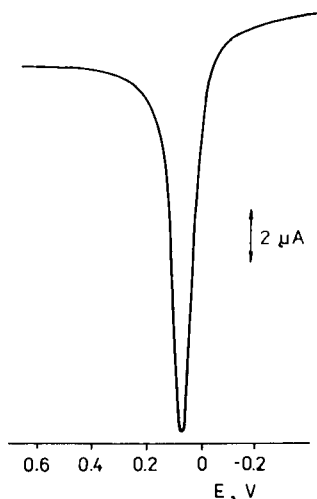

Fig. 1. Typical anodic stripping voltammogram recorded at a ligand II/Nafion modified graphite electrode for 2.7×10^{-5} M copper. Accumulation in 0.02 M acetate buffer (pH 7.5) for 180 s, reduction (at -0.35 V for 180 s) and stripping in 0.02 M acetate buffer (pH 3.5).

ing the ASV procedure for the CME once contacted with copper solution a stripping peak of lower intensity was found to remain. Therefore, chemical regeneration of the CMEs was necessary before the repeated determination of copper. For this purpose the chemical reaction of accumulated copper(II) with EDTA was employed and treatment of CMEs in a stirred solution of 1×10^{-3} M EDTA for 30–120 s (depending on the amount of copper uptaken on the CME surface) was sufficient.

The experimental parameters for copper determination have been investigated at a fixed copper concentration of 2.7×10^{-5} M. Four buffer systems [acetate (pH 3.5; pH 7.5), phosphate (pH 7.5) and borate (pH 8.3)] have been tried as media for the accumulation and ASV measurement. The effect of the composition of solution used for the accumulation on the electrode response was not significant. On the other hand, the medium for the voltammetric measurement was found to have a strong effect on the shape and the height of the stripping peak. The greatest signal was observed after employing the acetate buffer of pH 3.5 for the ASV procedure.

The peak height is strongly affected by the accumulation time as well as the potential and time of the copper(II) reduction. The dependence of the stripping peak current on preconcentration time increases rapidly and almost linearly until saturation of the CME is reached (Fig. 2a). Whereas the CME containg ligand I exhibits saturation after about 240 s (for 2.7×10^{-5} M Cu^{2+}), in the case of the CME containing ligand II this time is only about 120 s.

Pseudopolarograms (plots of the stripping peak current vs. the reduction potential used during the ASV procedure) exhibit maxima the potential values of -0.35 V for the CME containing ligand I and -0.45 V for the CMEs containing ligand II and oxine. These potentials correspond to the values typical for the copper complexes with N and O donor ligands. To avoid the reduction of other accumulated metals (lead, cadmium) the potential of -0.35 V was applied during the determination of copper in waters using all three types of CMEs. Fig. 2b shows the dependence of the CME response on the reduction time. It can
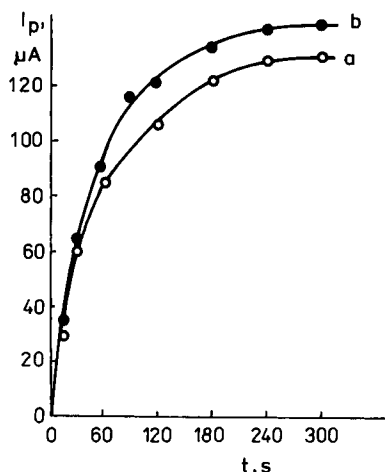
Fig. 2. Dependence of the stripping peak current for a CME containing ligand I/Nafion on the accumulation time (a) and the reduction time (b). Conditions: 2.7×10^{-5} M copper, accumulation, reduction and stripping in 0.02 M acetate buffer (pH 3.5), accumulation time 180 s (b), reduction time 180 s (a).

per by about 0, 40 and 20% and a 10-fold excess of lead by about 20, 70 and 25% for the electrode modified with ligand I, ligand II and oxine, respectively. A similar interference of cadmium was found. As a result of the reduction of accumulated copper, lead and cadmium at the potential values more negative than -0.6 V the stripping peaks of the interfering metals occurred also on the voltammograms.

Effects of model ligands

Among interfering ligands in solution, the competitive effects of two model agents (EDTA and oxalate) were examined. The aim of this investigation was to test the applicability of our CMEs for complexation studies in natural waters.

The titrations of 2.7×10^{-5} M copper with EDTA in acetate media of pH 3.5 and pH 7.5 were performed and the copper was determined immediately after each addition of an EDTA aliquot. These titrations led to a linear decrease of the CME response which was proportional to the amount of EDTA. The titration curve obtained using the CME with oxine can be seen on Fig. 3a. The CMEs with the derivatives of 4-acylpyrazolone gave similar results for 20, 60 as well as 180 s accumulation. In all cases the equivalent point corresponding to the theoretical value of the Cu to EDTA ratio (1:1) was achieved. The
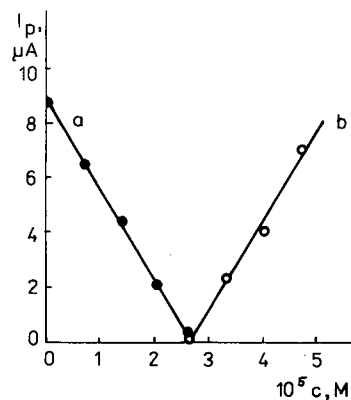
be concluded that the reduction of copper(II) bonded with modifying ligand is a slow process and, therefore, a rather long time (180 s) is necessary to obtain a measurable signal.

The calibration graphs for copper were linear from 1×10^{-6} M to 3×10^{-5} M ($r \geq 0.980$, $n = 6$) with 20–60 s accumulation and 180 s reduction. At higher copper concentrations or for a longer accumulation time the graphs have a curve shape due to the saturation. The detection limits (3σ) for all three CMEs under investigation were 1×10^{-6} M and 2×10^{-8} M after the accumulation time of 60 and 300 s, respectively. The precision was characterized by the relative standard deviation of 1 to 3% (for 2.7×10^{-5} M Cu^{2+}, $n = 8$) depending on the CME used.

The set of experiments was performed with the 2.6×10^{-5} M copper solutions (acetate buffer of pH 3.5) in order to examine the effect of other metals, mainly that of lead and cadmium. In the presence of these metal ions in solution to be analysed only the stripping peak of copper was observed after reduction at -0.35 V. The copper response diminished with increasing Pb:Cu, as well as Cd:Cu, ratio in the solution. A 2-fold molar excess of lead suppressed the peak of cop-

Fig. 3. Titration curves for model titrations of 2.7×10^{-5} M copper with EDTA (a) and 2.7×10^{-5} M EDTA with copper (b). Conditions: CME modified with oxine/Nafion film, 20 s accumulation in 0.02 M acetate buffer (pH 7.5), 180 s reduction in buffer solution of pH 3.5.

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same value was indicated at the titration of EDTA with copper solution (Fig. 3b). The copper–EDTA titrations carried out in phosphate (pH 7.5) or borate (pH 8.3) media yielded curves of a smooth concave shape. The equivalent point was shifted to the values of a Cu to EDTA molar ratio of 1 : 1.15 (phosphate) and 1 : 1.25 (borate).

The DP polarography at the SMDE performed in all media mentioned above allowed to distinguish two separated reduction peaks corresponding to Cu^{2+} (-0.06 V) and Cu(EDTA) complex (-0.23 to -0.38 V). The evaluation of the copper titration with EDTA yielded an equivalent point at a Cu to EDTA molar ratio between 1 : 1.0 and 1 : 1.1. Thus, the application of the CMEs as well as DP polarography on the Cu–EDTA system confirmed the relative kinetic inertness (or very slow dissociation) of Cu(EDTA) [9,13,14].

Figure 4 shows the titration curves obtained with the CME containing ligand I for the titration of copper with oxalate. The CMEs modified with ligand II and oxine exhibited similar pictures. The course of the titration curves indicates a quasi-labile character for copper oxalates, i.e., their dissociation within the diffusion layer during the accumulation step. Surprisingly, higher signals were obtained after a shorter accumulation time (20 s). On the DP polarograms recorded
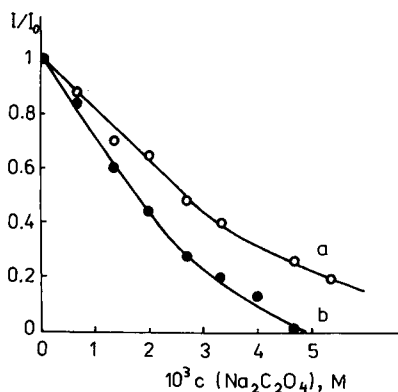
TABLE 1

Results of the labile copper determination in natural waters

Sample	Labile copper (μM) found by			Total copper (μM)
	CME (ligand I)	CME (oxine)	DPP	
Chvojnica (15 April 1992)	0.19	0.18	0.20	0.35
Myjava (15 April 1992)	0.15	0.15	0.16	0.32
(24 May 1992)	0.05	0.05	0.05	0.27

with SMDE in the individual titration points only one reduction peak of free copper ions (with a constant peak height) occurred at -0.10 V. This confirms the lability of the Cu–oxalate system.

Copper complexation in natural waters

The method described above was applied to the determination of labile copper in the samples of two river waters, the Chvojnica (pH 8.6) and the Myjava (pH 8.2). An intercomparison of the copper determination by voltammetry using the CMEs with ligand I and oxine and by DP polarography was used to evaluate the detection window of the proposed method. The use of conventional ASV with the hanging mercury drop electrode was unsuccessful because of a poor

Fig. 4. Effect of oxalate on the relative response, i/i_0, of 2.7×10^{-5} M copper for a CME containing ligand I/Nafion. Conditions: 0.02 M acetate buffer (pH 3.5), accumulation time of 20 s (a) and 180 s (b), 180 s reduction; i and i_0 denote the electrode response in the presence and in the absence of oxalate, respectively.
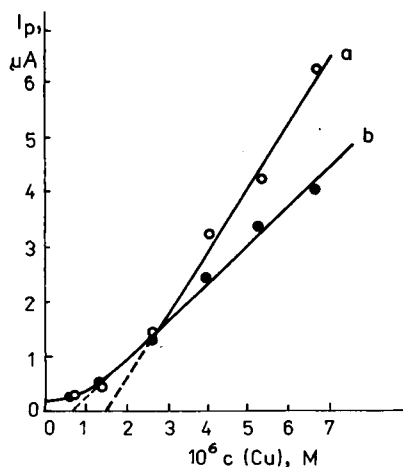

Fig. 5. Complexing ligand titrations of water samples from the Danube (a) and the Strkovec lake (b) obtained using a CME containing ligand I/Nafion. Conditions: 60 s accumulation, 180 s reduction in acetate buffer of pH 3.5.

potential separation of the reduction peak of labile copper. The results are summarized in Table 1. The mean concentrations of labile copper in river waters were found to be in the nM range.

The samples of fresh water from the Danube and the Strkovec lake (10 January 1992) were also investigated and no labile copper content was found when the samples were analyzed as received. Using 1×10^{-4} M copper solution as titrant, the copper complexing ligand titrations were carried out (10 min for the equilibration) and the ligand concentration, c_L, called the "complexation capacity" was determined by an extrapolation of the linear part of the titration curves to the CME signal equaled to zero (Fig. 5). Such an evaluation procedure is possible only in the case of inert complexes [15]. As we have no information about ligands present in the water samples, the values of c_L represent only approximate data.

DISCUSSION

At the application of CMEs to the determination and speciation of metals, the electrode function and its control are important. A decisive role occurs in the preconcentration step. In our case, the accumulation of copper on the CME with the immobilized ligand L and the Nafion film is based on the complexation reaction

$$Cu^{2+} + L, Nafion \rightleftharpoons CuL^{2+}, Nafion \qquad (1)$$
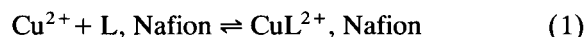
Nafion stabilizes an attachment of L on the graphite surface as well as enhances the electrode response due to the shift of equilibrium 1 to the right side.

The preconcentration process can be characterized by the hypothetical two-phase stability constant

$$K = \frac{[CuL^{2+}]_s}{[Cu^{2+}]_l[L]_s} \qquad (2)$$

where l and s denote the liquid phase and the solid phase, respectively. After expressing the concentration of the uncomplexed ligand, $[L]_s$,

from the total concentration of ligand, $[L]_{s,t}$, and the concentration of the complexed ligand, $[CuL^{2+}]_s$,

$$[L]_{s,t} = [CuL^{2+}]_s + [L]_s \qquad (3)$$

and using Eqn. 2, the concentration of the copper complex with modifier can be obtained

$$[CuL^{2+}]_s = \frac{K[Cu^{2+}]_l[L]_{s,t}}{1 + K[Cu^{2+}]_l} \qquad (4)$$

This concentration represents the result of the accumulation step and determines the CME response. Due to a heterogeneous character of the process, the value of $[CuL^{2+}]_s$ depends significantly also on the accumulation time (Fig. 2). At high values of $[Cu^{2+}]_l$, saturation of CME takes place and the signal reaches a limited value. For trace copper concentrations, Eqn. 4 can be simplified to the form

$$[CuL^{2+}]_s = K[Cu^{2+}]_l[L]_{s,t} \qquad (4a)$$

which explains the observed proportionality of the signal and the concentration of copper ions in solution (the calibration graph).

A competing complexation agent X (e.g., EDTA), present in solution (a water sample) causes a decrease in the signal which is proportional to the concentration of X (Figs. 3 and 4). Eqn. 4a is still valid, however, the value of K must be substituted by the conditional stability constant

$$K' = K / \left(1 + \sum_i \beta_i' [X]_l^i \right) \qquad (5)$$

where β_i' is the conditional stability constant for the copper complex with the complexing agent (agents) present in the water sample. In the case of kinetically labile complexes CuX_i, the value of the signal is higher than that expected for the given concentration of the ligand X in solution, $[X]_l$ (Fig. 4). The signal depends on the kinetics of dissociation and/or on the diffusion transport of the complex particles CuX_i.

The difference between the CME response (Fig. 4) and the constant value of the DP polarographic current at the titration of copper with oxalate can be explained probably by an exclusion

effect of the negatively charged Nafion film on the labile complex molecules and a thin diffusion layer at the modified electrode.

Conclusions

The comparison of the labile copper fraction found in the model as well as natural water samples by the CME–ASV and DP polarographic methods indicates that there is generally a good agreement. The present investigation showed that the CME–ASV procedure gives satisfactory analytical results for the determination of labile copper in river water matrices and represents a new alternative to the conventional DP polarographic determination. The methods have similar detection windows. The CME–ASV technique is sufficiently sensitive for the determination of labile copper in the nM to μM range.

A systematic investigation of the effects of the modifying ligand amount, $[L]_{s,t}$, stability of copper-modifier complex (K) and other parameters of the modifier layer as well as the diffusion layer on the values of both, the detection window and the water complexation capacity is now in progress.

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Complexation study of humic acids with cadmium(II) and lead(II)

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Abstract

A set of experiments on the complexation of cadmium and lead with humic acids were performed at pH 5.0 using normal- and reverse-pulse polarography and d.c. and differential-pulse anodic stripping voltammetry. The concentration of deprotonated groups at pH 7.0, the average stability parameter, $\overline{K}$, the differential equilibrium function, $f(K_{DEF})$, and a parameter, Γ, proportional to the metal buffer capacity of the system and related to the heterogeneity of the ligand, were calculated. It was concluded that the complexes are labile within the experimental error for the time scale of the techniques; that lead complexes are more stable than cadmium complexes and that the ligand has a more heterogeneous behaviour with respect to lead than to cadmium.

Keywords: Polarography; Stripping voltammetry; Cadmium; Complexation; Humic acids; Lead; Waters

Complexation by humic acids (HAs) is of great interest in environmental studies, as the interaction of these ligands with heavy metals determines to a large extent their bioavailability, toxicity and mobility [1]. Humic matter is a complex mixture formed by random condensation of degradation products of plants and animals. According to the origin of HA they can be classified in aquogenic humic materials (aquatic source) or pedogenic humic materials (terrestrial source). Pedogenic humic substances arise largely from the decomposition of lignite, rich in aromaticity, whereas aquogenic humic substances come mainly from the decomposition of plankton, with a large percentage of linear chains but poor in aromatics [2].

Pedogenic humic matter in natural waters (pH 5–9) is formed by anionic heterogeneous ligands, having a large number of aromatic rings with many carboxylic and phenolic groups, whose real composition depends on the extraction source. HAs present polyfunctional, polyelectrostatic and conformational effects, due to different functional groups, to a high charge density and to stereochemistry, respectively [2]. Each complexing site (with a specific chemical group and/or specific surroundings) acts differently in the complexation of the metal. The polyelectrostatic effect depends on the HA structure, the pH and the macroconstituents present in solution. Owing to the heterogeneity of the ligand, a continuous variation of the complexing parameters should be assumed in complexation studies of HAs [2–4].

A critical review of literature data on HA complexation with heavy metals shows that complexation has been studied using different analytical methods, in particular potentiometry with ion-selective electrodes [5–8] and voltammetric methods [9–12]. However, the results presented are of limited value because the macromolecular

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behaviour of these ligands was often not considered, concentrations of heavy metals and/or ligands higher than those found in natural waters were generally used and usually the influence on the voltammetric signal of the adsorption of HAs on the mercury interface was not considered.

The purpose of this work was to study the complexation of cadmium and lead with HAs in natural waters. Voltammetric titration of the ligand with the metal ion was done at pH 5.0 considering a continuous variation of the complexing stability parameter. Concentrations of HAs close to those present in natural waters were chosen, and the lowest concentrations used for Cd(II) and Pb(II) were 1×10^{-7} and 2×10^{-8} M, respectively. An excess of ligand in relation to the metal was used, as found in the environment including ground waters of soils. For these reasons techniques with very low detection limits were applied, such as anodic stripping voltammetry, using the d.c. or differential-pulse mode (DCASV and DPASV, respectively). In order to establish the influence on the results of the adsorption of HAs on the mercury interface, experiments with normal- and reverse-pulse polarography (NPP and RPP, respectively) were done.

It should be noted that the results obtained in this work cannot be generalized to other types of HA without some restrictions, as the physicochemical properties of these ligands depend on their composition.

THEORY

Definition of complexing parameters $\bar{K}$ and K_{DEF}

In the formation of a complex of the ML type, the affinity between the metal ion M and ligand L is measured by the overall stability constant K_{ML} if the ligand is homogeneous. For heterogeneous ligands with a large number of different functional groups in one molecule, the average stability parameter $\bar{K}$ should replace K:

$$\bar{K} = \frac{\sum\limits_{i}^{n} [\mathrm{ML}_i]}{[\mathrm{M}] \sum\limits_{i}^{n} [\mathrm{L}_i]} = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{L}]} \qquad (1)$$

where L_i represents the complexing site i of the macromolecule and n is the total number of different complexing sites in solution. In the determination of $\bar{K}$ all the sites have equal weights. Also,

$$\sum\limits_{i} [\mathrm{L}_i] = C_\mathrm{L} - \sum\limits_{i} [\mathrm{ML}_i] \qquad (2)$$

i.e., $\sum[\mathrm{L}_i]$ represent all the ligand not bound to the metal M. In this context $\bar{K}$ will represent a conditional parameter, valid for the experimental conditions of constant pH and macroconstituents, if they are present.

In order to simplify the notation of Eqn. 1, and because the different sites are not being distinguished, L and ML will replace $\sum[\mathrm{L}_i]$ and $\sum[\mathrm{ML}_i]$ in this paper. If two or more groups are bound to a metal ion, all these groups are taken as one coordination site, and so only complexes of the 1:1 type are considered.

Owing to the heterogeneity of the ligand, the strongest complexing sites are first titrated, followed by the others with lower affinity for the metal ion. In this context the average stability parameter $\bar{K}$, which is the mean of the stability of all complexing sites titrated, should decrease with increase in the number of occupation sites of the ligand.

In most instances the dissociation rate constant $k_{\mathrm{d}i}$ varies inversely with K_i ($K_i = k_{\mathrm{f}i}/k_{\mathrm{d}i}$), where $k_{\mathrm{f}i}$ is assumed to be constant, as the slow step in the formation process is in general the dehydration of M [13]. In this context the ligand might present an inert behaviour for $C_\mathrm{L} \gg C_\mathrm{M}$, if K_i is large enough, becoming more labile with the increase in C_M.

The interpretation of the titration data for highly polyfunctional natural complexant systems [3,14–16] led to the definition of a differential equilibrium parameter K_{DEF} given by a weighted arithmetic mean of the conditional equilibrium constants K_j for all sites of type j involved in the complexation [15,16]:

$$K_{\mathrm{DEF}} = \frac{\sum\limits_{j=1}^{m} K_j \theta_j (1 - \theta_j) \Delta x_j}{\sum\limits_{j=1}^{m} \theta_j (1 - \theta_j) \Delta x_j} \qquad (3)$$

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where θ_j is the degree of occupation by M of type j sites $(\theta_j = [ML_j]/C_{Lj})$, Δx_j is the molar fraction of the ligand type j sites $(\Delta x_j = C_{Lj}/C_L)$ and m is the number of different types of site involved in the complexation.

The parameter K_{DEF} depends on the complexing site composition through the K_j and Δx_j values, and on the weighting factor $\theta_j(1 - \theta_j)$. This factor implies that K_{DEF} is controlled mainly by the sites of type j that are nearly half-saturated at a given titration point. In this context, the differential equilibrium parameter is more specific for a range of sites than $\bar{K}$. However, because K_{DEF} always incorporates some influence from all sites present, it cannot be associated with only a single site with a K_j value.

From Eqn. 3, an expression based on experimental data [15,16] can be deduced for K_{DEF}:

$$K_{DEF} = -\frac{\alpha^2}{C_M}\left\{\frac{1-\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{d\ln C_L}{d\ln\alpha}\right)}{1+(\alpha-1)\left[\frac{d\ln(C_M/C_L)}{d\ln\alpha}\right]}\right\} \qquad (4)$$

where $\alpha = C_M/[M]$. C_L can be expressed in any units directly related to the total site concentration. This is another important advantage of K_{DEF} compared with $\bar{K}$, because for $\bar{K}$ the total molar site concentration should be known, which is usually a very difficult parameter to measure in natural waters.

Experimental determination of $\bar{K}$ and K_{DEF}

In order to determine $\bar{K}$ and K_{DEF} (Eqns. 1 and 4), the concentrations of M and ML should be known. They can be determined from the values of the potential or current obtained in the presence and absence of ligand, using voltammetric techniques. For labile complexes the variation in current is due to different diffusion coefficients for the metal ion (D_M) and the complex (D_{ML}).

Determinations of $\bar{K}$ and K_{DEF} from current values. Assuming that the process is diffusion controlled and that the complexes are labile, the current obtained is

$$i_p = |B|\bar{D}^r C_M \qquad (5)$$

where i_p is the peak current in DPASV and DCASV and the limiting current in NPP and RPP, $\bar{D}$ is the mean diffusion coefficient, r is a parameter equal to $1/2$ in polarographic measurements and between $2/3$ and $1/2$ in stripping techniques owing to the dependence of the diffusion layer thickness on the stirring rate, C_M is the total bulk concentration of metal and B is a constant depending on the number of electrons involved in the redox process and on the experimental conditions (such as stirring rate, radius of the drop and deposition time in stripping methods and on the drop time and flow-rate of mercury in polarography), being the signal related to the anodic or cathodic nature of the process. This parameter can be experimentally determined from the slope of a calibration graph, in the absence of ligand, using D_M values from the literature [17].

The mean diffusion coefficient $\bar{D}$ is the average of the diffusion coefficients of the metal and the complex, weighted by their relative proportion to C_M [18,19]:

$$\bar{D} = (D_M[M] + D_{ML}[ML])/C_M \qquad (6)$$

The experimental value of $\bar{D}$ was obtained for each C_M and C_L concentration from Eqn. 5, using the B parameter previously determined.

From Eqn. 6 and the mass balance for the metal,

$$C_M = [ML] + [M] \qquad (7)$$

the values of [ML] and [M] can be calculated if D_{ML} is known. This parameter can be determined from the slope of Eqn. 5 in the presence of a large excess of ligand such that $\bar{D} = D_{ML}$. In order to obtain [M] and [ML] with small errors, [M] should be in the range 10–90% of C_M. On the other hand, an excess of ligand should be present so that $C_L \approx [L]$ because $\bar{D}$ must be constant in the diffusion layer [18].

The value of [L] can be determined from the mass balance of the ligand (Eqn. 2) if the total concentration (molarity) of the ligand (C_L) is known.

The parameter $\bar{K}$ can be determined from [M], [ML] and [L] (Eqn. 1), and K_{DEF} from [M], C_M and C_L (Eqn. 4), where the units of C_L are not necessarily molarity.

Determinations of $\bar{K}$ and K_{DEF} from potential values. For labile complexes the potential shift in the presence and absence of ligand is given by the DeFord–Hume equation [20]:

$$E_{p_{ML}} - E_{p_M} = \Delta E_p = \frac{RT}{nF} \ln\left(\frac{D_M}{\bar{D}}\right)^r - \frac{RT}{nF} \ln(\alpha^0) \tag{8}$$

where $\alpha = C_M/[M] = 1 + \bar{K}[L]$ and superscript 0 represents the concentration at the electrode surface. This expression should be used in the presence of an excess of ligand so that $[L]^0 \approx [L]$, i.e., $\alpha^0 \approx \alpha$.

For polarographic measurements, where $[L]^0$ tends to increase compared with [L] owing to the reduction of the dissociated metal, [ML] should be lower than 5% of C_L, which corresponds, for strong complexes, to $C_L/C_M > 20$. This value decreases with decrease in the complexing stability parameter.

In stripping voltammetry C_{M^0} is usually larger than C_M in the stripping step, so a larger excess of ligand should be present compared with polarographic techniques, in order to have $[L]^0 \approx [L]$ (L^0 tends to decrease in the stripping step owing to its complexation with the oxidized metal ion). The ratio C_L/C_M should therefore be replaced with C_L/C_{M^0} in stripping voltammetry. In previous work [21], under the experimental conditions of stirring and drop radius used, it was found that $C_{M^0} = 15\ C_M t_d$ (min).

From Eqn. 8, α can be experimentally determined and then K_{DEF} can be obtained from Eqn. 4. On the other hand, as $\alpha = 1 + \bar{K}[L]$, the value of $\bar{K}$ can be calculated for $[L] \approx C_L$.

It should be emphasized that the stability parameters (K_{DEF} or $\bar{K}$) can be determined either from the current, if $D_{ML} < D_M$, or from the potential shift. The choice of one of these approaches depends on the value of D_{ML}. In order to determine α accurately it is necessary for the difference between ΔE_p and the first term on the

right-hand side of Eqn. 8 to be significant. If this difference is small, but i_p^{L+M} is different from i_p^M, speciation should be done from current measurements. When D_{ML} is not too different from D_M, the reverse situation holds and calculation from the potential shift gives more accurate values.

Degree of site occupation

The next step is to define a function that allows results obtained with different sets of concentrations of ligand and/or metal to be compared. Owing to the heterogeneity of the ligand during the titration with metal, different groups with less favourable affinity and concentration are being successively saturated, so it is a good choice to plot the stability parameter (K_{DEF} or $\bar{K}$) versus the degree of occupation of ligand sites θ ($\theta = [ML]/C_L$).

For many natural heterogeneous complexants, the differential equilibrium function $f(K_{DEF})$, defined by log K_{DEF} vs. log θ, presents a more or less linear trend when $[L] \gg [ML]$, which agrees with the assumption of the Freundlich isotherm [22]:

$$\log \theta = \Gamma \log K_{DEF}^0 - \Gamma \log K_{DEF} \tag{9}$$

where K_{DEF}^0 is the value of K_{DEF} for $\theta = 1$ and Γ is the heterogeneity parameter (equal to unity for a simple ligand, decreasing to zero with increasing heterogeneity), proportional to the metal buffer concentration of the system, b, according to the expression [22]

$$b = 2.3\Gamma C_M \tag{10}$$

EXPERIMENTAL

Chemicals and instrumentation

NPP and RPP experiments were carried out using an EG&G PAR Model 174A polarograph connected to an IBM PC computer with the Labtech Notebook program (Laboratory Technology), and ASV experiments using an EG&G PAR Model 273A polarograph with a hanging mercury drop electrode (HMDE). Data acquisition was performed by an IBM PS2/30-286 com-

puter with the Headstart program from EG&G PAR.

Three electrodes, a dropping mercury electrode (DME) for NPP and RPP or HMDE for DPASV and DCASV, together with a saturated calomel electrode and a platinum counter electrode, were used as working, reference and auxiliary electrodes, respectively. The HMDE and DME (Models 6.0335.000 and 6.1230.010, respectively) were obtained from Metrohm. A drop radius of 0.042 cm was formed for each experiment with the HMDE in the titration vessel. The pH was measured with an Orion Model 91-03 SC semimicro electrode.

The humic acids used were extracted from an Irish moss peat and separated from fulvic acids with 6 M HCl. The elemental composition determined on a freeze-dried sample was C 52.07, H 5.07, N 2.37, S 0.57 and O 39.93% [23]. The HAs were saturated with hydrogen ions [23]. The content of deprotonated groups in HAs up to the inflection point (pH 7.0) determined by potentiometric titration with KOH in 0.1 M KNO_3 was 1.5×10^{-2} M.

Dissolved organic carbon analysis gave 3.0 g l^{-1} of carbon. This means that there are eighteen carbon atoms for each deprotonated group and the equivalent molecular mass is 370, in agreement with that of Fluka humics of the order of 300.

The mercury used was of Suprapur grade from Merck and all the other reagents were of analytical-reagent grade and were used as received. Water was distilled and passed through a Milli-Q water-purification system (Millipore).

The glass polarographic cell was decontaminated each day with HNO_3 (1:2) for at least 12 h, and different cells were reserved for lead and cadmium, as it is very difficult to eliminate traces of lead adsorbed on the walls of the vessel.

Dissolved oxygen was removed from solutions in the polarographic cell by bubbling 99.9995% purity nitrogen through them. All experiments were carried out at $25.0 \pm 0.1°C$. Reproducible stirring was achieved using a magnetic bar with a length appropriate to the internal diameter of the bottom of the cell to prevent a lateral movement.

In the anodic stripping techniques the deposition time with stirring was 60 s, followed by 30 s without stirring before the potential scan.

Titration curves

A fixed-concentration solution of ligand in 0.02 M KNO_3 was titrated at pH 5 with a stock solution of metal ion under the experimental conditions presented in Table 1. pH 5 was chosen because it was a reasonable compromise between the values in the environment and the buffer capacity of the humics, it not being necessary to use another buffer; 0.02 M KNO_3 medium was chosen to simulate the ionic strength of a ground water, where a pH value of 5 is acceptable.

The pH value was measured after each addition of metal and corrected with 0.1 M KOH solution, if necessary, as the metal solution was acidic. With these additions the ionic strength did not change within an error of 3%.

Each set of experiments consisted of three consecutive steps: measuring the titration curve of the metal in the presence of the ligand; decontamination of the cell with HNO_3 (1:2) for at least 2 h; and measurement of the titration curve of the metal in the absence of ligand. The metal concentration range used in these studies depended on the voltammetric technique used, according to Table 1.

RESULTS AND DISCUSSION

Determination of D_{ML}

A value of D_{ML} of 5×10^{-8} cm^2 s^{-1} (Table 2) was determined from the slope of the plot of i_p vs. C_M using the first points of the titration of HA with lead, where $D_M[M] \ll D_{ML}[ML]$ and the value of B obtained from the slope of the calibration graph of the metal without ligand assuming that the diffusion coefficient of lead in 0.02 M KNO_3 is 8×10^{-6} cm^2 s^{-1} [17]. From light-scattering measurements at pH close to 5.0, a value of the same order of magnitude was obtained for D_{ML} $[(2 \pm 1) \times 10^{-8}$ cm^2 $s^{-1}]$. On the other hand, the agreement between the results at different angles of measurement showed that in solution the molecule has a spherical conformation.

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TABLE 1

Experimental conditions

Ion	Mode	C_{HA} (deprotonated groups M)	C_M (M)	E_{init} (V)	E_{final} (V)	t_d (s)	Scan rate (mV s^{-1})
Cd^{2+}	NPP	5.4×10^{-4}	5×10^{-6}–1×10^{-3}	-0.3	-0.9	2	2
	RPP	5.4×10^{-4}	5×10^{-6}–1×10^{-3}	-0.9	-0.4	2	2

				Deposition		Stripping	
				E_d (V)	t_d (s)	Potential range (V)	Scan rate (mV s^{-1})
	DPASV	2.9×10^{-4}	1×10^{-7}–7×10^{-5}	-0.9	60	-0.9 -0.2	5
	DCASV	2.9×10^{-4}	1×10^{-7}–7×10^{-5}	-0.9	60	-0.9 -0.2	20
Pb^{2+}	DCASV	2.9×10^{-4}	1×10^{-6}–7×10^{-5}	-0.7	60	-0.7 -0.2	20
	DCASV	1.5×10^{-4}	1×10^{-7}–1×10^{-5}	-0.7	60	-0.7 -0.2	20
	DPASV	1.2×10^{-5}	2×10^{-8}–1×10^{-6}	-0.7	60	-0.7 -0.2	5
	DPASV	5.6×10^{-6}	2×10^{-8}–1×10^{-5}	-0.7	60	-0.7 -0.2	5

With highly charged ligands, the polyelectrolytic effect can change the usual spherical form of the molecule in solution, as the increase in charge in the molecule creates an internal repulsion that promotes its stretching and might affect the diffusion coefficient of the complex. This may
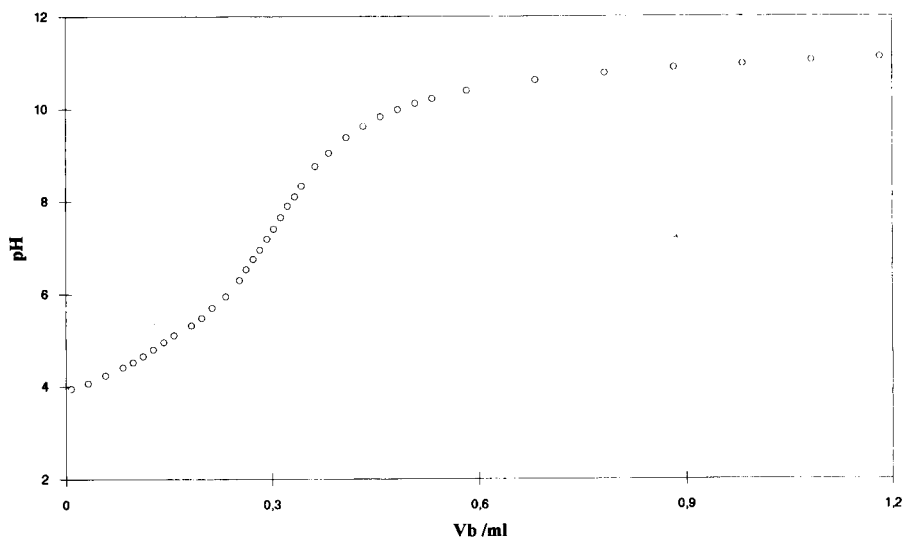
Fig. 1. Potentiometric titration of a dilute solution (1:20) of humic acid with 0.1 M KOH, in 0.1 M KNO$_3$. V_b = volume of KOH solution added.

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TABLE 2

Values for D_{ML} obtained experimentally

C_L (deprotonated groups, M)	C_{Pb} range (M)	D_{ML} (cm^2 s^{-1})
1.5×10^{-4}	$(1.0-3.0) \times 10^{-7}$	5.0×10^{-8}
2.9×10^{-4}	$(1.0-3.0) \times 10^{-6}$	5.1×10^{-8}
2.9×10^{-4}	$(5.0-8.0) \times 10^{-7}$	4.4×10^{-8}

be caused by increasing the pH or lowering the supporting electrolyte concentration.

For Cd^{2+} it was not possible to determine D_{ML}, as $D_M[M]$ is never negligible compared with $D_{ML}[ML]$ owing to the low complexing stability of HAs with this metal ion. A value equal to that obtained for Pb^{2+} was assumed, considering that the diffusion coefficient is mainly due to the ligand and that the sizes and conformations of the lead and cadmium complexes are similar.

Determination of C_L

In order to determine $\bar{K}$, the concentration (molarity) of the total ligand C_L has to be obtained, which is not easy owing to the heterogeneity of the HAs. In fact, the determination of the number of sites of a heterogeneous ligand is always difficult and never accurate, owing to the large variety of groups and the fact that they

respond in different ways to different metal ions; therefore for the same ligand the number of sites available depends on the metal ion used and its concentration range.

The C_L value used in these calculations is the concentration of deprotonated groups at pH 7.0 obtained from a potentiometric titration of the stock solution of humic acid (Fig. 1). A value of $C_L = 1.5 \times 10^{-2}$ M deprotonated groups per litre of the stock solution was found.

It should be mentioned that the experimental value of C_L may show a shift compared with the real value that should be used: the potentiometric titration gives mainly the carboxylic and not the phenolic groups (generally 10–20% of the total groups); however, some of these groups may also act as complexing sites for the metal ion at pH 5, which will increase the value of C_L. In addition, two or more carboxylic groups in suitable positions might form a bidentate chelate. As they should be considered as only one ligand for the heavy metal, according to the assumption of 1:1 complexes, the latter behaviour will decrease the experimental value of C_L.

Determination of [M] and [ML] at each experimental point
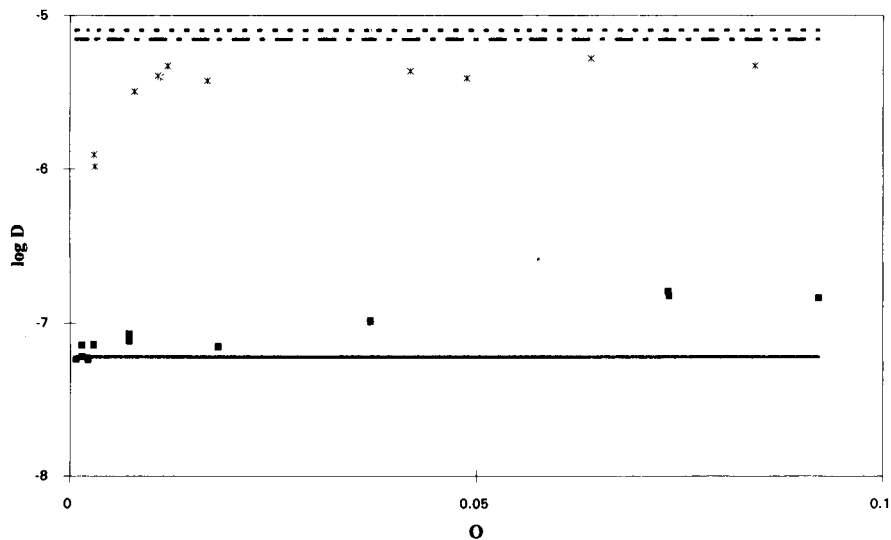
Figure 2 shows an increase in $\bar{D}$ values with

Fig. 2. Variation of $\bar{D}$ with θ in 0.02 M KNO$_3$ (pH 5) for (*) cadmium in the presence of 2.9×10^{-4} M deprotonated groups and (■) lead in the presence of 1.5×10^{-4} M deprotonated groups. The lines represent the values for the diffusion coefficient for (– — · — –) cadmium, (- - - - - -) lead and (———) ML.

addition of the metal; for cadmium the mean diffusion coefficient increases almost up to D_M ($C_M \approx$ [M]), but for lead $\overline{D}$ is much lower than D_M in the θ range considered, owing to the larger extent of complexation of lead compared with cadmium.

The values of [M] and [ML] were determined, according to Eqns. 6 and 7, for each experimental point of the titration, using for the diffusion coefficients of the metal ions $D_{Pb} = 8 \times 10^{-6}$ and $D_{Cd} = 7 \times 10^{-6}$ cm^2 s^{-1} [17] and for that of the complex $D_{ML} = 5 \times 10^{-8}$ cm^2 s^{-1}.

Average stability constant, $\overline{K}$

The complexation constant K represents a thermodynamic value for a simple ligand, but with a heterogeneous ligand its value changes with the degree of coverage θ. Hence K is redefined as $\overline{K}$ (Eqn. 1), an average stability parameter changing from point to point, yet its significance is lost because it no longer has a thermodynamic meaning. Its greatest fault is that the heterogeneous character is not considered in the ligand, all sites being treated as homogeneous dissolved entities and using a concentration of "free" ligand [L] that accounts for all the free sites and not, as it should, for the concentration

of each specific group that is actually complexing the metal.

In this work $\overline{K}$ was determined from the potential shift (Eqn. 8) considering $n = 2$, when there is an excess of ligand ($C_L/C_M > 10$, i.e., [ML] < 0.1 C_L), and from i_p values (Eqns. 1, 2 and 5–7) for $C_L/C_M > 2$ and 0.10 $C_M <$ [M] < 0.90 C_M.

The plot of log $\overline{K}$ versus log θ for cadmium and lead (Fig. 3) shows the decrease in $\overline{K}$ with increase in $\theta = $ [ML]/C_L. This was expected for a heterogeneous ligand because the metal chooses first the sites with greater affinity, the complexing stability of the site decreasing with increase in θ.

Another result from Fig. 3 is that the $\overline{K}$ values for lead are larger than those for cadmium, which corroborates the stronger reactivity of lead for oxygenated ligands [24].

Cleven and Van Leeuwen [18] performed a set of conductimetric experiments which showed that the binding of cadmium to HAs has a significant electrostatic contribution, whereas for lead there is a higher level of covalence in the binding.

Differential equilibrium function, $f(K_{DEF})$

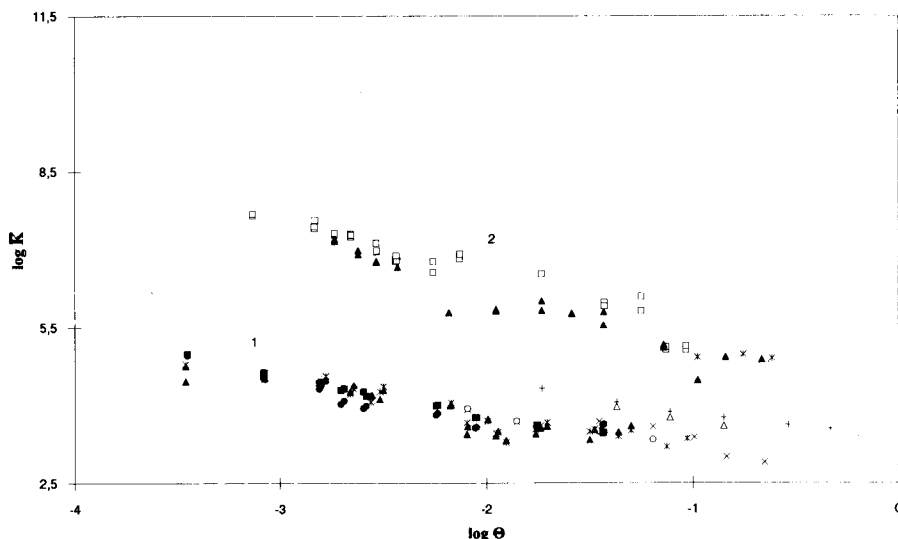In a titration of HAs with metal ions the concentration of ligand is constant and so is the

Fig. 3. Plots of log $\overline{K}$ vs. log θ. For cadmium in 0.02 M KNO$_3$ (pH 5) by: NPP, 5.4×10^{-4} M deprotonated groups from ($\times$) i_p or ($\circ$) ΔE_p; RPP, 5.4×10^{-4} M deprotonated groups from ($+$) i_p or ($\triangle$) ΔE_p; DCASV, 2.9×10^{-4} M deprotonated groups from ($*$) i_p or ($\blacktriangle$) ΔE_p; DPASV, 2.9×10^{-4} M deprotonated groups from ($\bullet$) i_p or ($\blacksquare$) ΔE_p. For lead, in 0.02 M KNO$_3$ (pH 5) by: DCASV, 2.9×10^{-4} M deprotonated groups from ($*$) i_p or ($\triangle$) ΔE_p; DCASV, 1.5×10^{-4} M deprotonated groups from ($\square$) ΔE_p.
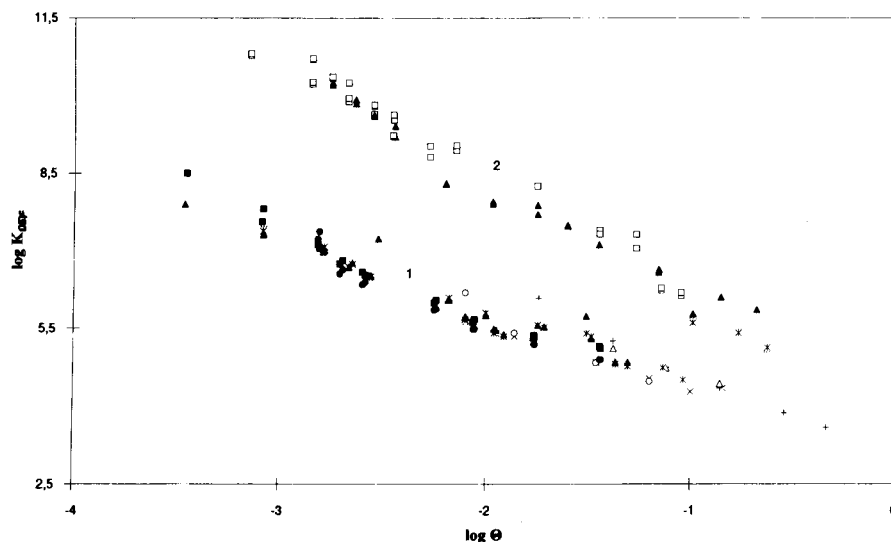
Fig. 4. Plots of $f(K_{\rm DEF})$. For cadmium in 0.02 M KNO$_3$ (pH 5) by: NPP, 5.4×10^{-4} M deprotonated groups HA from ($\times$) $i_{\rm p}$ or (○) $\Delta E_{\rm p}$; RPP, 5.4×10^{-4} M deprotonated groups from ($+$) $i_{\rm p}$ or ($\triangle$) $\Delta E_{\rm p}$; DCASV, 2.9×10^{-4} M deprotonated groups from ($*$) $i_{\rm p}$ or ($\blacktriangle$) $\Delta E_{\rm p}$; DPASV, 2.9×10^{-4} M deprotonated groups from (●) $i_{\rm p}$ or (■) $\Delta E_{\rm p}$. For lead in 0.02 M KNO$_3$ (pH 5) by: DCASV, 2.9×10^{-4} M deprotonated groups from ($*$) $i_{\rm p}$ or ($\blacktriangle$) $\Delta E_{\rm p}$; DCASV, 1.5×10^{-4} M deprotonated groups from (□) $\Delta E_{\rm p}$.

number of complexing sites; then, from Eqn. 4, the following expression for $K_{\rm DEF}$ can be derived:

$$K_{\rm DEF} = -\frac{\alpha^2}{C_{\rm M}}\left[\frac{1}{1 + (\alpha - 1)\left(\dfrac{{\rm d}\ln C_{\rm M}}{{\rm d}\ln \alpha}\right)}\right] \quad (11)$$

The major problem in the calculation of $K_{\rm DEF}$ is the derivative, because there is no analytical expression relating $C_{\rm M}$ to α. Hence the fitting of ln $C_{\rm M}$ vs. ln α was done with a polynomial (in this work only first- or second-degree polynomials were used), and then the derivative of the polynomial was used in Eqn. 11.

Comparing the results of the differential equilibrium function (log $K_{\rm DEF}$ vs. log θ) for cadmium and lead (Fig. 4), it can be seen that lead has a much greater affinity than cadmium for HAs, as previously discussed for $\bar{K}$ values, and covers a larger range of $K_{\rm DEF}$ values for the same range of degree of occupation of sites (log $K_{\rm DEF}$ of cadmium changes from 7.5 to 5 for variation of $C_{\rm L}/C_{\rm M}$ from 500 to 10 and that of lead changes from 10 to 6 for the same range of $C_{\rm L}/C_{\rm M}$).

Comparing the results for $f(K_{\rm DEF})$ (Fig. 4) with those for log $\bar{K}$ vs. log θ (Fig. 3), it can be seen that for high values of θ the values of $K_{\rm DEF}$ and $\bar{K}$ are close to each other, and for low values of θ the values of $K_{\rm DEF}$ are larger than those of $\bar{K}$. For high $C_{\rm L}/C_{\rm M}$ ratios (low θ values), $K_{\rm DEF}$ is much larger than $\bar{K}$ because the metal is complexed to the strongest sites and $K_{\rm DEF}$ includes a smaller range of ligand groups than $\bar{K}$, resulting in a higher value for the constant $K_{\rm DEF}$.

For higher θ values ($\theta > 0.3$), the fact that $K_{\rm DEF}$ is similar to $\bar{K}$ should reflect the titration of a large number of sites i of the same type, predominating over the other sites.

The comparison of the results for $\bar{K}$ with those for $K_{\rm DEF}$ shows that for these systems and for high $C_{\rm L}/C_{\rm M}$ ratios the utilization of differential parameters in the study of a heterogeneous ligand is better than using the average stability parameters that underestimate the results. On the other hand, it should be emphasized that in such a comparison the estimation of $C_{\rm L}$ is very critical when calculating $\bar{K}$, $K_{\rm DEF}$ being a much more reliable parameter for studies in systems where the calculation of $C_{\rm L}$ is difficult.
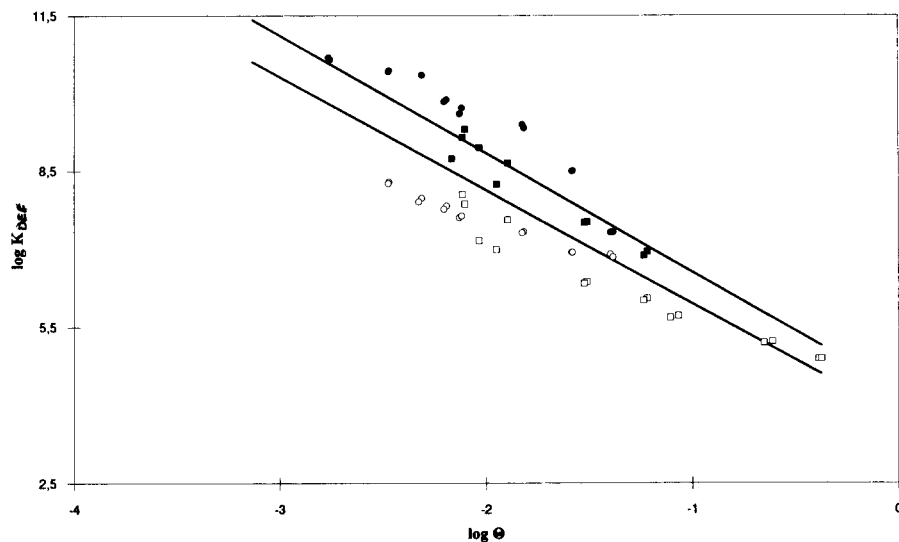
Fig. 5. Plots of $f(K_{DEF})$, for lead in 0.02 M KNO$_3$, (pH 5) by: DPASV, 5.6×10^{-6} M deprotonated groups from ($\square$) i_p or ($\blacksquare$) ΔE_p; DPASV, 1.2×10^{-5} M deprotonated groups from ($\bigcirc$) i_p or ($\bullet$) ΔE_p. The straight lines represent the range of results obtained for $C_{HA} > 1.5 \times 10^{-4}$ M deprotonated groups (Fig. 4, lead) with the associated error.

Comparison of $f(K_{DEF})$ under different experimental conditions

The results obtained for lead from ΔE_p and i_p using DPASV and DCASV agree within experimental error for $C_L > 1.5 \times 10^{-4}$ M deprotonated groups (Fig. 4). In this ligand concentration range, a.c. experiments showed that the electrode is almost saturated with adsorbed HA molecules
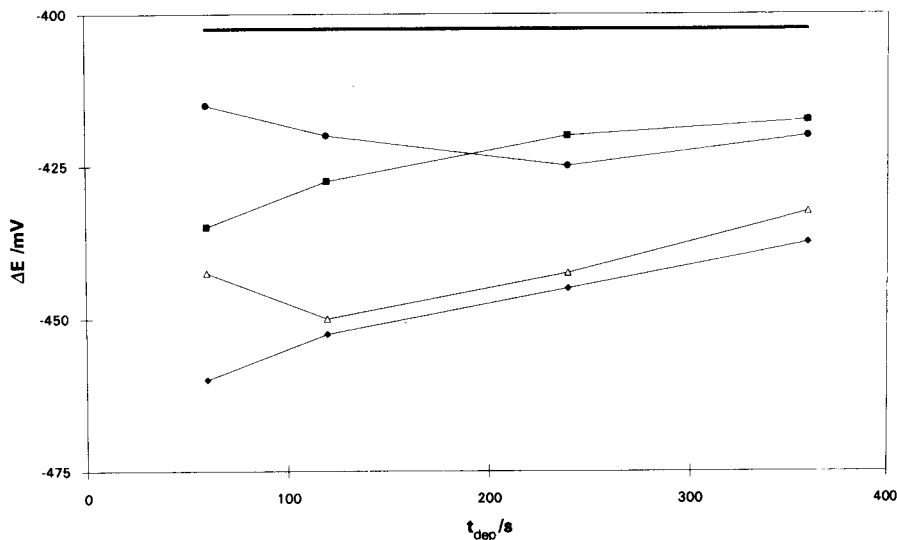
Fig. 6. Plots of ΔE_p vs. t_{dep} for lead in 0.02 M KNO$_3$ (pH 5) by DPASV: $\bullet = 2.9 \times 10^{-6}$ M deprotonated groups, 6×10^{-8} M Pb^{2+}; $\triangle = 5.6 \times 10^{-6}$ M deprotonated groups, 6×10^{-8} M Cd^{2+}; $\blacklozenge = 1.2 \times 10^{-5}$ M deprotonated groups, 6×10^{-8} M Cd^{2+}; $\blacksquare = 1.2 \times 10^{-5}$ M deprotonated groups, 3×10^{-7} M Cd^{2+}. Thick line: Cd^{2+}.

[24] for the experimental conditions (time, stirring rate, potential) used in voltammetric measurements.

For $C_L = 5.6 \times 10^{-6}$ and 1.2×10^{-5} M deprotonated groups, the results obtained with lead using DCASV or DPASV do not agree with the previous values (Fig. 5). Adsorption studies [25] showed that under these experimental conditions saturation is not attained. Hence it seems that adsorption always acts in the same way if saturation is attained, regardless of the voltammetric technique and/or C_L values used, but interferes with the results if saturation is not reached.

To explore this point further, some preliminary experiments were done with lead, varying the deposition time for the same C_L and C_M values. The results showed that increasing the deposition time from 30 to 360 s, for $C_L/C_M > 20$ and $C_L < 1.2 \times 10^{-5}$ M deprotonated groups, the peak potential begins to shift to more negative values (Fig. 6). However, the increase in the deposition time increases C_{M^0}, and a shift of the potential to more positive values should be observed in the absence of other effects.

The behaviour presented here for low values of C_L might be due to the increase in adsorption with deposition time, which increases the ligand concentration at the interface leading to an increase in ΔE_p. This strongly suggests that adsorption affects ΔE_p and therefore K_{DEF} values, giving different values of K_{DEF} for the same θ if different degrees of coverage of the electrode with adsorbed molecules are attained.

If under the experimental conditions saturation is observed and C_L is sufficiently high that the variation at the interface due to adsorption is negligible, the results might become independent of adsorption. In Fig. 5 it can also be seen that K_{DEF} values determined from i_p values do not agree with the straight line obtained for higher concentrations of ligand. This suggests that adsorption influences not only the peak potential but also the peak current.

In studies of cadmium using NPP and DCASV with different concentrations of humic acids (Fig. 3), similar results were obtained over a wide range of log θ. The results from RPP in terms of log K_{DEF} were systematically slightly higher than those of NPP, although they agreed within the experimental error. This might be due to the fact that at the initial potential of NPP (-0.3 V) the electrode is saturated with HAs in the concentration range used ($C_L \geqslant 5 \times 10^{-4}$ M), but at the initial potential of RPP (-0.9 V) [25], and during the drop time ($t_d = 2$ s), saturation is not attained. In DCASV the deposition step is also at -0.9 V, but it lasts 60 s with stirring, allowing saturation to be reached.

Finally, one must be aware that the results obtained in stripping voltammetry can be influenced not only by adsorption and heterogeneous ligand effects [22] but also by the surface effect [21]. In this work this effect is not clear, as the values of $f(K_{DEF})$ and log $\bar{K}$ vs. log θ, determined with ΔE_p for $200 < C_L/C_M < 10$ and with i_p, fit the same curve. Different voltammetric techniques were used not only to increase the metal concentration range but also to compare the results obtained using different techniques.

It is also important to check whether the complexes are completely labile or not. In the concentration range where speciation can be performed from i_p and ΔE_p measurements, the system seems to show labile behaviour as indicated by the good agreement between both sets of results. However, for high C_L and low C_M values ([M] $< 0.1 C_M$ for the lead system), log K_{DEF} cannot be determinated from i_p measurements, so no conclusions can be drawn about the lability of the lead complexes in this range.

The heterogeneity of the system produces a splitting of the stripping peak; in our results an increase of the width up to 30% has been observed.

Degree of heterogeneity

In order to establish the degree of heterogeneity of the ligand, in terms of the different sites of HAs present in solution, the Freundlich isotherm (Eqn. 9) was used for the linear range of $f(K_{DEF})$ (Fig. 4). This isotherm is generally found in natural systems for a certain range of θ [22].

As referred to in the Introduction, K_{DEF}^0 is the value of K_{DEF} for $\theta = 1$, but the relevant parameter is Γ, which is a measure of the degree of heterogeneity of the ligand. For a simple ligand

the value of Γ would be 1, and when the heterogeneity of the ligand increases the value of Γ decreases. The value $\Gamma = 0$ corresponds to an ideally heterogeneous ligand where an infinitely broad distribution of K_{DEF} is observed. For a natural system Γ values usually lie between 0.3 and 0.7 [22].

The same ligand may present different heterogeneity, depending on the metal present in solution, owing to (a) different complexing sites available to each metal ion and (b) the difference between the stabilities of the strongest and weakest sites titrated with the same metal.

The value of Γ obtained by linear regression was 0.69 ± 0.02 for cadmium, which was higher than that obtained for lead (0.43 ± 0.01).

The parameter Γ is also important because it is related to the metal buffer capacity of the ligand (Eqn. 10), which provides information on how much free metal the system can hold when the total amount of metal changes.

Conclusions

Values of the distribution functions of the average stability constant ($\bar{K}$) and differential equilibrium parameter (K_{DEF}) versus the degree of occupation of the ligand (θ) were determined for lead and cadmium complexes with humic acids (Figs. 3 and 4). From these plots the following conclusions were drawn. The lead complexes are more stable than the cadmium complexes for the same θ value. For speciation studies K_{DEF} should be used rather than $\bar{K}$, and K_{DEF} values obtained under different experimental conditions should be compared for the same θ value. It should be emphasized that in a titration of a constant ligand concentration with metal, K_{DEF} is independent of C_L.

The values of $f(K_{DEF})$ obtained from ΔE_p and i_p for each metal ion and $C_L > 1.5 \times 10^{-4}$ M deprotonated groups agree within an error of ± 0.2 over all of the θ range studied. When saturation of the electrode by adsorbed organic molecules occurs, the results obtained by different techniques (RPP, NPP, DCASV, DPASV) and for different ligand concentrations agree within an error of ± 0.3 in log K_{DEF} for cadmium and lead. Different K_{DEF} values are ob-

tained if saturation of the mercury electrode by adsorbed molecules is not attained. The degree of heterogeneity is higher for lead ($\Gamma = 0.43$) than for cadmium ($\Gamma = 0.69$).

SYMBOLS AND ABBREVIATIONS

α	$C_M/[M]$
K	stability constant
$\bar{K}$	average stability parameter
Γ	parameter proportional to the buffer capacity of the heavy metal and related to the heterogeneity of the ligand
ΔE_p	difference between the peak potentials of the complex and metal
θ	$[ML]/C_L$, degree of occupation of sites
B	constant (from the slope of i versus C_M)
b	buffer capacity of the ligand for the heavy metal
C_L	total concentration of complexing sites
C_M	total concentration of metal
$\bar{D}$	average diffusion coefficient
DCASV	direct current anodic stripping voltammetry
D_M	diffusion coefficient of the metal
DME	dropping mercury electrode
D_{ML}	diffusion coefficient of the complex
DPASV	differential-pulse anodic stripping voltammetry
E_p	peak (or half-wave) potential
$f(K_{DEF})$	differential equilibrium function
HMDE	hanging mercury drop electrode
HA	humic acid
i_p	peak (or limiting) current
K_{DEF}	differential equilibrium parameter
K_{DEF}^0	value of K_{DEF} for $\theta = 1$
[M]	concentration of free metal ion
M.W.	molecular weight
NPP	normal-pulse polarography
RPP	reverse-pulse polarography
t_d	drop time for the DME
t_{dep}	deposition time for stripping techniques

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Influence of surface-active substances on the redox processes of metal ions: a contribution to the speciation analysis of metals in aquatic systems

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Abstract

The cathodic (reduction) and anodic (oxidation) waves of different heavy metal ions (Cu, Cd, In, Pb, Tl) were investigated in the presence of the non-ionic surfactant Triton X-100 and humic acid (HA) by electrochemical methods (differential-pulse voltammetry and a.c. voltammetry). The adsorption and complexation properties of the organic substances were also examined. Conclusions about the possible influences of organic matter on the speciation analysis of heavy metal ions in the bulk solution and on the mercury/water interface are drawn. For Cu the predominant reaction with HA will be complexation even at lower pH (1.6). In neutral and alkaline solutions Cd and Pb ions will be complexed either in the bulk of solution or at the electrode surface. At lower pH inhibition of oxidoreduction processes occurred owing to surface coverage of the electrode. Indium will be complexed with HA present at higher pH (3.84), whereas at lower pH the hydrophobic/hydrophilic effects play an important role in the interfacial region. Owing to the low tendency to form complexes and the small size of thallium ion, its oxidoreduction processes are not influenced by the presence of either Triton X-100 or HA.

Keywords: Stripping voltammetry; Voltammetry; Metal ions; Speciation; Surfactants; Waters

The speciation of heavy metal ions in natural waters is governed by the presence of inorganic and organic ligands and particulate matter [1]. The presence of organic ligands, especially those with surface-active properties, is very important as they influence the distribution between heavy metal species in the bulk of solution and their fractionation at different phase boundaries. In natural waters the greatest amount of organic matter is present in the form of poorly defined humic material, which can be classified into fulvic, humic and humin fractions on the basis of their solubility at different pH values. Some metal ions (Cu, Fe, Al, Hg) form very stable complexes with humic substances, which are often the main species of these heavy metal ions present [2]. There is evidence that humic acid (HA) diminishes the toxicity of heavy metal ions [3], the degree of which can be ascertained by means of anodic-stripping voltammetric (ASV) measurements, considering the ASV-labile metal ion fraction at natural pH as the toxic form. There is also evidence that HA can enhance the toxicity of metal ions. The presence of HA increased the toxicity of cadmium for Daphnia [4]. For mercury there is clear evidence of a correlation between the content of humic matter and the biological uptake of mercury (due to methylation) [5].

Powell and Town [6] investigated the interaction of stable hydrophobic copper(II) complexes

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with HA and FA. The apparent lability of copper(II) complexes decreased in the presence of humic matter and the formation of an HS–Cu–L ternary complex was proposed.

Synthetic surface-active substances (SAS), although not present in high concentrations in natural waters, may influence the behaviour of trace metal ions by changing the properties of natural phase boundaries [7].

In this work, the influence of HA and Triton X-100 on the speciation analysis of metal ions on the mercury/water interface and in the bulk of solution was studied by following the anodic (oxidation) and cathodic (reduction) waves of metal ions by differential-pulse (DP) voltammetry [8]. Triton X-100 is a typical non-ionic surface active substance which adsorbs strongly at the mercury electrode and affects charge-transfer processes at the electrode/electrolyte interface. HA is at the same time a surface-active and complexing agent. The complexing capacity was measured in the bulk of solution by direct titration with heavy metal ions [9,10] by means of DPASV and calculated by the van den Berg–Ružić method [11,12]. The work was aimed at improving the knowledge of the possible effects of surface-active substances on the speciation analysis of trace metals in the bulk phase and in the interfacial region under conditions similar to those in natural aquatic systems.

EXPERIMENTAL

Voltammetric measurements were performed with a PAR-174A polarographic analyser connected to a Hewlett-Packard Model 7045A or Kipp and Zonen BD90 x–y recorder. Measurements were performed in the differential-pulse mode with a pulse duration of 57 ms. For a.c. measurements an ECP-100 polarograph connected to an ECP-110 unit (Modular Research Polarograph, EDT Research, London) was used. A.c. voltammograms were recorded with an amplitude of 10 mV and a frequency of 77.5 Hz. All measurements were performed in a 100-ml quartz cell equipped with a three-electrode system consisting of a hanging mercury drop electrode

(HMDE) (Metrohm, Herisau) as the working electrode, a coiled platinum wire as the counter electrode and an Ag/AgCl electrode as the reference electrode.

For the cathodic waves of Cd and In the initial potential was −0.4 V whereas for Pb and Tl it was −0.2 V. The time of accumulation was 1 min, after which during the scan in the negative direction the reduction of metal ion on the mercury electrode was observed. The mercury electrode was covered with a layer of adsorbed organic material formed during the preceding accumulation period. For Cu only the anodic wave was investigated. The deposition potentials of the anodic waves were −0.6 V for Cu, Pb, Tl and −0.75 V for Cd and In. The oxidation wave was recorded during the scan in the positive direction after a 1-min accumulation period.

All chemicals were of analytical-reagent grade. Non-ionic surfactant Triton X-100 was obtained from Rohm and Haas and HA from Aldrich (previously EGA-Chemie) in the sodium salt form [8,13,14]. Saturated NaCl solution was prepared from NaCl that had been purified by prolonged heating at 723 K; the solution was further treated with active carbon, which was filtered off.

A Milli-Q water-purification system (Millipore) was used throughout. The laboratory vessels were cleaned with dichromate solution, redistilled water, HNO_3 (1:1) and several times with Milli-Q-purified water.

RESULTS AND DISCUSSION

The influence of HA and other SAS on the heavy metal ions depends on the nature of both the organic molecules and heavy metal ions present. In electrochemical measurements the potential and time of deposition/adsorption are the key factors that influence the interaction of the metal ion and organic substances [2,15,16]. In order to obtain well defined voltammetric waves, the concentrations of metal ions were in the range 10^{-6}–10^{-4} mol l^{-1}, which is higher than actual concentrations in natural waters. It was checked earlier that the influence of SAS on the electrode processes of metal ions does not de-
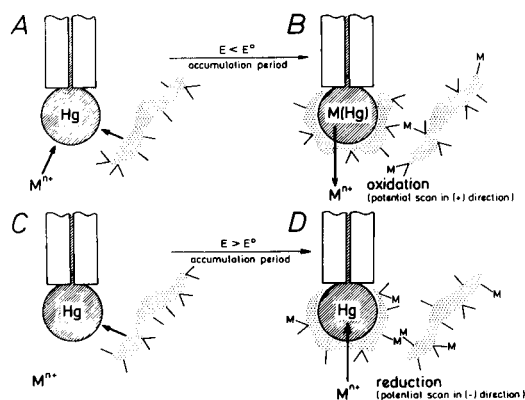
Fig. 1. Schematic representation of the processes occurring during the recording of the anodic wave (A and B) and cathodic wave (C and D) of metal ions in the presence of humic acid presented by its hydrophobic (indicated as a cloud) and hydrophilic moiety (i.e. OH, COOH, NH_2, SH; indicated by short lines on the hydrophobic moiety).

pend on the metal ion concentration [17], except in complexation [9]. The influence of SAS on the cathodic and anodic processes was examined on the well defined electrochemically reversible waves of cadmium, lead, thallium and indium. All four metals have standard redox potentials in the potential range that is most favourable for adsorption of organic molecules (near -0.6 V vs. Ag/AgCl). A general scheme of the applied procedure is presented in Fig. 1.

For studying reduction processes, the electrode is pretreated at a potential more positive than the standard redox potential to form an adsorbed layer of organic molecules. After the adsorption period the reduction process of the metal ion is measured at the modified electrode surface during a potential scan in the negative direction. For studying oxidation processes the electrode is kept at a potential more negative than the standard redox potential while simultaneously adsorption of organic molecules, reduction of metal ions and formation of amalgam occur. After the accumulation period the reoxidation of the metal is measured during the potential scan in the positive direction. This process takes place at the electrode which is modified by the adsorbed layer of organic molecules.

Typical effects of surface-active substances on the oxidoreduction processes at the mercury elec-

trode in the case of cadmium and Triton X-100 and cadmium and HA in acidic solution are illustrated in Fig. 2. At low pH (1.6) both the cathodic and anodic waves of Cd decrease with increasing concentration of organic substance and follow the adsorption isotherm of the SAS (HA or Triton X-100). The influence of the SAS on the cathodic wave of Cd was examined over wide concentration ranges of both Cd ions and Triton X-100 [17] and it was concluded that the rate constant for this reaction depends strongly on the inhibitor concentration at the completely covered surface ($\theta \approx 1$), which is a characteristic of the electrodeposition type of the mechanism in comparison with other oxidoreduction processes representing simple charge transfer. Since in an acidic medium functional groups of humic acid molecules are protonated, their adsorption behaviour at the mercury electrode is very similar to that observed for non-ionic surface-active substances such as Triton X-100. The effect of humic acid on the oxidoreduction processes of cadmium in acidic solution is similar to that of Triton X-100, as shown in Fig. 2. It is important to note that no difference was observed between the effects of the investigated surface-active substances on the reduction and oxidation processes of cadmium in acidic medium.

In neutral and alkaline solution the adsorption effects of humic acid on the redox processes of cadmium are completely different from those of
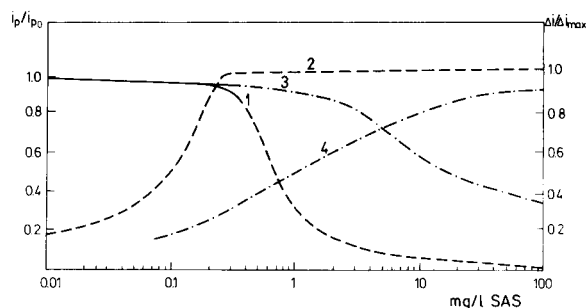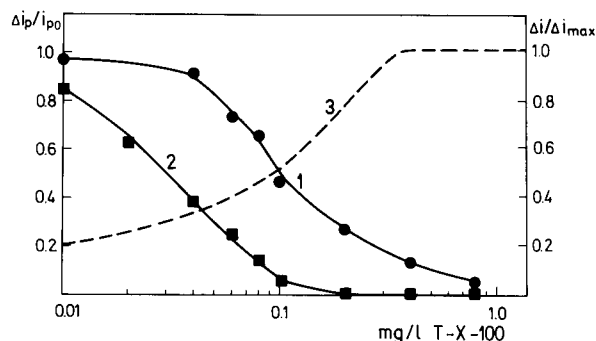

Fig. 2. Dependence of the relative height (i_p/i_{p0}) of the anodic and cathodic waves of cadmium (1×10^{-6} mol l^{-1}) on the concentration of Triton X-100 (1) or HA (3) and the adsorption isotherms for Triton X-100 (2) and HA (4) at pH 1.6 in 0.5 mol l^{-1} NaCl. i_{p0} = anodic or cathodic wave height in the absence of SAS.

neutral molecules of Triton X-100, especially with regard to the differences between reduction and oxidation processes. As most functional groups of humic acid are deprotonated at higher pH and thus negatively charged, the positively charged cadmium ions can be attached by electrostatic forces to the adsorbed layer of humic acid at the electrode surface. The adsorbed cadmium complexes cause an increase in the reduction wave of cadmium in the presence of humic acid. At the same time the anodic wave of cadmium gradually decreases with additions of HA owing to the complexation reaction in the bulk of solution [8]. The behaviour of cadmium is similar to that observed previously for oxidoreduction processes of lead [13].

The behaviour of a metal ion with a great tendency to form complexes with organic ligands, including also humic substances, can be shown typically with copper as a model. Depending on the copper concentration, the anodic wave is decreased to a different extent with additions of SAS [18]. At lower concentrations of copper ions the main interaction with humic acid is complexa-

tion in solution whereas at higher concentrations the anodic wave is diminished owing to surface inhibition of the electrode.

In addition to divalent cations, the influence of surface-active substances (humic acid and Triton X-100) on the processes of monovalent thallium and trivalent indium cations was also studied. Tl^+ ions are known to form complexes with low stability constants [19]. As an example, of all the metal ions investigated, Tl^+ ions have the lowest stability constant with EDTA. The log K values (at 293 K and $I = 0.1$ mol l^{-1}) of Tl^+, Cd^{2+}, Pb^{2+}, Cu^{2+} and In^{3+} with EDTA are 6.54, 16.46, 18.04, 18.80 and 25.0, respectively [19]. Generally organic substances have almost no effect on electrochemical measurements of Tl^+ [20,21]. The present experiments with Triton X-100 and HA showed only a decrease in the anodic wave of thallium, which could be due to the possible effect of different diffusion coefficients (Fig. 3). The diffusion coefficient for Tl^+ ion at infinite dilution and 298 K is 2.00×10^{-5} cm^2 s^{-1} whereas that for thallium in mercury from the anodic diffusion current is 0.99×10^{-5} cm^2 s^{-1} [20],

TABLE 1

Electrochemical and thermodynamic data and complexing capacity values (L_T) for HA with corresponding apparent stability constants (log K_{HA}) for Tl^+, Cu^{2+}, Cd^{2+}, Pb^{2+} and In^{3+},[a]

Metal ion	Log $[k^{-H_2O}$ (s^{-1})] [b]	$-\Delta G_h$ (kJ mol^{-1}) [c]	L_T for 10 mg l^{-1} HA in 0.5 mol l^{-1} NaCl	Log $[K_{HA}$ (l mol^{-1})]	k_c (cm s^{-1}) [d]	X_M^{amal}(sat) [e]
Tl^+	$-$ [f]	$-$ [f]	Undetectable (this work)	$-$	1 M KCl; 22°C; Hg (DME) 0.15 [27]	0.43
Cu^{2+}	8–10	2087	pH 6.4 [18] 9.3×10^{-7} mol l^{-1}	6.46 [18]	1 M KNO$_3$; 25°C; Hg (DME) 0.019 [27]	0.000061
Cd^{2+}	8.5	1801	pH 9.0 [7] 3.9×10^{-7} mol l^{-1}	6.68 [7]	0.5 M KCl; 25°C; Hg (DME) 1.5 [27] Sea water; 25°C; HMDE 0.11 [17]	0.086
Pb^{2+}	10	1497	pH 6.4 [13] 3.1×10^{-7} mol l^{-1}	6.23 [13]	1 M NaCl; 25°C; Hg (DME) 0.17–0.18 [27]	0.0134
In^{3+}	6.3	4072	pH 3.84 (this work) 2×10^{-7} mol l^{-1}	6.77 (this work)	1 M NaCl; 25°C; Hg (DME) 0.034 [27]	0.691

[a] All data from [26] except where indicated. [b] k^{-H_2O} = Rate constant for the exchange of water in first coordination sphere for metal ion M^{n+}_{aq}. [c] ΔG_h = standard Gibbs energy of hydration. [d] k_c = Conditional rate constant for the reaction $M^{n+}_{aq} + ne(Hg) \rightleftharpoons M(Hg)$; $E_c(M^{n+}/M(Hg))$ (indicated medium, temperature, type of electrode: dropping mercury electrode (DME) or hanging mercury drop electrode (HMDE). [e] $(X_M^{amal})_{sat}$ = solubility of M in Hg in mol l^{-1} concentration scale. [f] Tl^+ ions are hydrated but its hydration is minimal [28].

which means that the diffusion of thallium from amalgam could be the rate-determining step (see the solubility of thallium in mercury, Table 1). The physical hindrance due to the adsorption of Triton X-100 or HA also contributes to the lowering of the thallium anodic peak. The peak height of thallium was not influenced by the presence of SAS to a greater extent at higher pH, regardless of the thallium concentration.

Electrochemical processes of indium are strongly dependent on the concentration of chloride ions in solution [22]. Whereas the hydrated form of indium, $[In(H_2O)_6]^{3+}$, is reduced only with a high overvoltage, i.e., at very negative potentials, the presence of chloride ions and the formation of more reducible indium chloro complexes facilitate the oxidoreduction processes. The rate constants of the reduction process increase with increasing concentration of chloride ions in
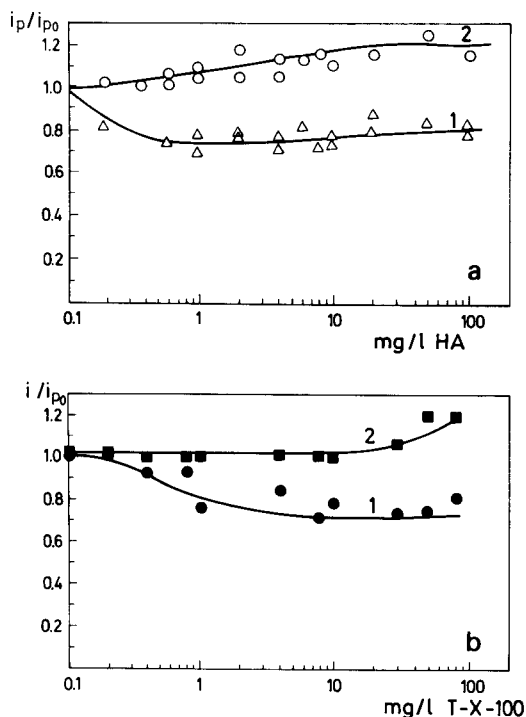
Fig. 4. Dependence of the relative height (i_p/i_{p0}) of (1) the anodic and (2) the cathodic wave of indium $(1 \times 10^{-4}$ mol $l^{-1})$ at pH 1.6 in 0.5 mol l^{-1} NaCl on the concentration of Triton X-100 and (3) the adsorption isotherm of Triton X-100.

solution, which results in an increased wave height in both differential-pulse voltammetry and a.c. voltammetry.

In the presence of surface-active substances such as Triton X-100 (Fig. 4), the anodic and cathodic peaks of indium decrease with increasing surface coverage of the electrode. Finally, with the surface completely covered by adsorbed organic molecules blockage of the reduction process of indium occurs (curve 2). At the same time the inhibitory effect of Triton X-100 on the anodic dissolution of indium from the amalgam (curve 1) is much lower than in the cathodic process. This was checked also with the following experiment. Indium amalgam was formed from the pure electrolyte solution in the absence of surface-active substances. Then 1 mg l^{-1} of Triton X-100 was added to the stirred solution and allowed to cover the mercury drop with an adsorbed layer. Anodic dissolution of indium was subsequently investigated in the presence of the adsorbed organic layer. The anodic current was decreased in comparison with the value obtained in pure electrolyte without surface-active substances, but not fully depressed. This means that there is an inhibitory effect of adsorbed Triton X-100, but not complete blockage of the anodic dissolution of indium. In contrast, the organic layer is impermeable to the charge transfer from the solution side, i.e., the reduction process cannot take place at the fully covered electrode. Indium, like thallium, has a great affinity for

Fig. 3. Dependence of the relative height (i_p/i_{p0}) of (1) the anodic and (2) the cathodic wave of Tl^+ ions at pH 2.0 in 0.5 mol l^{-1} NaCl on the concentration of (a) HA and (b) Triton X-100. Concentration of Tl^+: (a) 5×10^{-6} and (b) 1×10^{-4} mol l^{-1}.
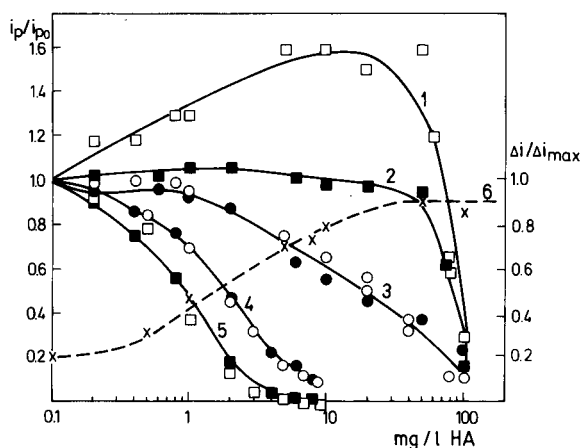
Fig. 5. Dependence of the relative height (i_p/i_{p0}) of (1,2,5) the cathodic and (3,4) the anodic waves of In^{3+} on the concentration of HA at (1,2,3) pH 3.84 and (4,5) pH 1.6 in 0.5 mol l^{-1} NaCl. Concentration of In^{3+}: (1,3,4,5, open symbols) 1×10^{-6} and (2,3,4,5, closed symbols) 1×10^{-4} mol l^{-1}. (6) Adsorption isotherm of HA obtained (at pH 6.0, in 0.5 mol l^{-1} NaCl) at a potential of -0.6 V vs. Ag/AgCl.

mercury (Table 1). The difference is that thallium ions are negligibly hydrated whereas hydration of indium ions is high. Dehydration step and hydrophilic/hydrophobic effects at the interfaces play the most important role in the charge-transfer processes of indium ions at the electrode.

In acidic medium the influence of HA on the electrochemical processes of indium fits well the general scheme for Triton X-100 (compare curves 1 and 2 in Fig. 4 with curves 4 and 5 in Fig. 5). At higher pH (3.84 here) in the presence of HA, oxidoreduction processes of indium are influenced by possible electrostatic attraction between positively charged cations and negatively charged functional groups of humic acid, both in the bulk solution and in the adsorbed layer at the electrode surface. The results presented in Fig. 5 (curves 1, 2 and 3) are in agreement with the previously reported data for the interaction of other metal ions (Cd, Pb) with the same humic acid at higher pH [8,13]. The complexing capacity value of 10 mg l^{-1} HA in 0.5 mol l^{-1} NaCl for indium at pH 3.84 was estimated to be 2×10^{-7} mol In^{3+} l^{-1} with an apparent stability constant log $K = 6.77$ mol l^{-1}. The usual titration procedure [9,10] with an increasing amount of indium

ions in the concentration range 1×10^{-8}–1×10^{-6} mol l^{-1} was applied first to the 0.5 mol l^{-1} NaCl solution and then to NaCl solution to which 10 mg l^{-1} of HA has been added. The results were calculated by the van den Berg–Ružić method [11,12]. These values must be treated with caution as different effects can influence the complexing capacity measurements of In ions at pH 3.84 (blockage of the electrode together with complexation in the bulk of solution). At higher pH the precipitation of indium hydroxide will occur [23].

In Table 1 some data on the complexation properties of Cu, Cd, Pb, Tl and In ions towards HA are presented. Cu and In have the highest complexing capacity values and also the highest log K values. Cd and Pb follow, which also reflects the specific nature of this HA (a high sulphur content, more fulvic character, low molecular weight). Table 1 also gives some other important parameters that influence the charge-transfer processes at the electrode/electrolyte interface. The reaction rates of electrochemical deposition and dissolution reactions of aquometal ions (M^{n+}_{aq}) at the mercury electrode are different for different metals. Some metals, such as cadmium, lead and thallium, have high reaction rates [k_c (Table 1)] whereas, for example, the rates for transition metals are extremely slow. It is known that the cathodic reduction of M^{n+}_{aq} to M(Hg) consist of the following processes: dehydration of the aquometal ion, charge (electron) transfer and dissolution of the deposited metal in mercury (amalgamation). As the charge transfer itself is believed to proceed very rapidly, the dehydration and/or amalgamation processes are expected to control the rate of the overall reaction. The dehydration constant of In is the lowest (Table 1), reflecting the fact that dehydration is the slowest step in the electrode reactions of In [20,24,25] and that the process that influences this step is the most important in the behaviour of In (protecting layer of HA or Triton X-100 on the electrode). The other metal ions have comparable dehydration constants. The results from Tamamushi [26] suggest that the amalgamation process plays an important role in determining the electrochemical reaction rate of the M^{n+}_{aq}

/M(Hg) system. Of all the metal ions investigated, In has the highest affinity of forming an amalgam, followed by Tl. All these properties have implications for the behaviour of heavy metal ions in electrochemical measurements.

Conclusions

By relatively simple electrochemical methods, following the influence of different organic substances on the oxidoreduction processes of different heavy metal ions and the adsorption properties of natural and synthetic organic matter, some conclusions regarding heavy metal ion speciation (preference for complexation in the bulk of solution, enrichment on the phase boundaries) and the nature of the organic matter (adsorbability at the electrode, interaction in the bulk of solution, preference for certain metal ions) can be drawn.

The anodic wave for a metal is a model for the behaviour of heavy metal ions trapped in a particle (in this instance in the hydrophobic drop of mercury) covered by a layer of organic molecules through which they have to pass in a more hydrophilic surrounding: natural water or electrolyte solution being oxidized to a higher oxidation state. The anodic wave is diminished owing to complexation reactions in the solution (Cu). By following the cathodic wave one can gain insight into what is happening when a metal ion has to cross into the particle, through the adsorbed layer of organic substances. This process can lead either to the hindering of the reduction process of metal ion due to the surface coverage of the electrode (Cd at low pH), or to the enrichment of metal ions due to the formation of a surface complex on the electrode owing to the higher concentration of the ligand on the electrode. Also, the reduction process may not be influenced by SAS at all, as with Tl^+ ions.

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Speciation of dissolved copper and nickel in South San Francisco Bay: a multi-method approach

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Abstract

A multi-method approach was used to investigate the chemical speciation of dissolved copper and nickel in South San Francisco Bay. Dissolved copper speciation was determined by four different analytical approaches: competitive ligand equilibration–cathodic stripping voltammetry [CLE–CSV], differential pulse anodic stripping voltammetry using a thin mercury film rotating glassy carbon disk electrode [DPASV(TMF-RGCDE)], DPASV using a hanging mercury drop electrode [DPASV(HMDE)], and chelating resin column partitioning–graphite furnace atomic absorption spectrometry [CRCP–GFAAS]. Dissolved nickel speciation was determined by CLE–CSV and CRCP–GFAAS. Each of the methods employed provided useful insight into the chemical speciation of dissolved copper and nickel. CLE–CSV provided the best characterization of the stronger (but less predominant) of the two organic copper-complexing ligands detected, while DPASV(TMF-RGCDE) provided the truest measurement of inorganic copper, and therefore, the best characterization of the weaker organic copper-complexing ligand which exerted the strongest influence on dissolved copper speciation. These methods provide complementary results which can be combined to provide a more complete understanding of the chemical speciation of copper. DPASV(HMDE) and CRCP–GFAAS both provide an operational measurement of labile copper which includes some fraction of labile organic complexes in addition to inorganic copper species. CLE–CSV and CRCP–GFAAS both yielded similar results for nickel speciation indicating the presence of a single class of extremely strong nickel-complexing ligands. The dissolved copper in South San Francisco Bay is found to exist predominantly as organic complexes (80–92%). About 27% of dissolved copper was complexed with the stronger (L_1; $[CuL_1] \approx 13$ nM, log $K'_{CuL_1} > 13.5$) of the copper-complexing ligands detected, while copper complexed with the weaker ligand L_2 comprised the greatest fraction of total dissolved copper species (52–65%; $[CuL_2] \approx 20$–30 nM; log $K'_{CuL_2} = 9.0$–9.6). A third to a half of total dissolved nickel was complexed by one, extremely strong class of organic ligands ($[NiL] \approx 17$–28 nM; log $K'_{NiL} > 17$); the remaining total dissolved nickel existed as inorganic or labile forms.

Much of the uncertainty about the relationship between total metal concentrations and their toxicity to aquatic organisms results from lack of

definitive knowledge of the chemical forms of these metals in natural waters. Trace metals such as copper and nickel can exist in a variety of dissolved and particulate chemical forms and/or species in natural waters. The dissolved forms can include the hydrated cations, Cu^{2+} and Ni^{2+}, complexes with inorganic ligands (e.g., CO_3^{2-},

OH^-, and Cl^-), and complexes with various naturally-present (e.g., phytoplankton metabolites or terrestrial humic substances) and/or anthropogenically-introduced organic ligands (e.g., EDTA, NTA). Particulate forms can range from copper and nickel associated with colloids, to those fractions adsorbed onto or incorporated into large particles resuspended from the bottom sediments by episodic events such as storm activity or tidal flushing. Measurements of the concentrations of total copper and nickel can include all of these various chemical forms, some of which are not toxic nor biologically available.

A number of studies using different organisms and different metals have found that the toxicity and availability of divalent, cationic, trace metals such as copper and nickel are controlled by their free metal ion concentrations, rather than by total metal concentrations as, for example, in the toxicity of copper and cadmium to phytoplankton [1,2] cadmium to grass shrimp [3], and the availability of the nutrient metals Fe, Mn, and Zn to phytoplankton [4]. Complexation of a metal cation by organic ligands can decrease its toxicity by decreasing the metal's free ion concentration. Organic complexation also appears to be important in controlling the concentrations and geochemical behavior of dissolved metals during estuarine mixing [5].

Excellent summaries of the inorganic speciation of copper and nickel exist [6,7]. Computer models containing the thermodynamic data to predict the degree of complexation of these divalent cations with various inorganic ligands are readily available. For example, under the pH and salinity conditions of South San Francisco Bay, free Cu^{2+} comprises approximately 5–10% of the inorganic forms of copper ($CuCO_3^0$ is reported to be the dominant inorganic species), while free Ni^{2+} is the dominant inorganic species of nickel, comprising $\approx$ 67% of its inorganic forms.

Complexation by organic ligands has recently been demonstrated to have an important influence on the speciation of dissolved copper and nickel in various estuarine, coastal, and oceanic waters (Cu: [5,8–15]; Ni: [16–19]. These studies, performed by a variety of investigators using various methods, have determined that more than 80% (and usually as much as 99%) of dissolved copper in surface waters is organically complexed. This level of organic complexation in estuarine waters can markedly lower the free Cu^{2+} activity relative to total dissolved copper. Only a few studies have determined the importance of organic complexation to the speciation of dissolved nickel in marine waters [16–19]. The fraction of dissolved nickel existing in organic complexes has been observed to range from about 30 to 70% in estuaries and coastal waters.

Flegal et al. [20] have recently reported the dissolved concentrations of six trace metals, including copper and nickel, in San Francisco Bay. They measured elevated concentrations of copper and nickel in South San Francisco Bay which exceed the United States' Environmental Protection Agency's present national saltwater quality criteria. Although the concentrations of copper- and nickel-complexing organic ligands within San Francisco Bay were unknown at the time, Flegal et al. [20] argued that among the trace metals they examined, copper and nickel are the most likely to be affected by organic complexation. Kuwabara et al. [21] observed a correlation between surface water dissolved organic carbon (DOC) and copper concentrations in South San Francisco Bay and suggested the trend was a result of organic complexation. However, until now no data have ever been presented directly demonstrating such complexation in San Francisco Bay nor the extent to which it occurs there.

A variety of analytical methods have been used to determine dissolved metal speciation in marine waters. In general, these methods involve isolation or detection of one of the metal fractions (i.e., free ion, inorganic complex, or organic complex) naturally present in the sample, or of a metal fraction created for the speciation determination (e.g., metal complexed with an added competing ligand). It has recently been recommended [10,15] that, in order to obtain the maximum amount of information on the speciation of a dissolved metal in a given natural water sample, speciation measurements should be made at different "analytical detection windows" because a spectrum of metal-complexing organic ligands of different strengths and concentrations may be

present, and because all analytical methods have limited detection windows. The analytical detection window for a metal speciation method is framed on one extreme by the ability of the analytical method to determine a decrease in the labile metal signal due to complexation of the metal by a natural ligand, and on the other extreme by the method's detection limit [10,15]. The analytical detection window may be varied by using the same analytical method but different analytical conditions, or by using different analytical methods having different analytical detection windows. Thus, one analytical approach may provide metal speciation information that could not be obtained by another approach.

In this study, we used different analytical methods in a concerted, multi-method approach to determine the speciation of dissolved copper and nickel in South San Francisco Bay. We report here the results of this multi-method approach and show that the different methods used provide complementary speciation results which, when interpreted in combination, provide a more complete understanding of the chemical speciation of copper and nickel. We also qualitatively define the fractions of dissolved copper and nickel determined by the methods used.

ANALYTICAL TECHNIQUES USED TO DETERMINE DISSOLVED COPPER AND NICKEL SPECIATION

Dissolved copper speciation was determined by four different analytical approaches:

(i) Competitive ligand equilibration–cathodic stripping voltammetry (CLE–CSV);

(ii) Differential pulse anodic stripping voltammetry (DPASV) using a thin mercury film rotating glassy carbon disk electrode (TMF-RGCDE);

(iii) DPASV using a hanging mercury drop electrode (HMDE);

(iv) Chelating resin column partitioning–graphite furnace atomic absorption spectrometry (CRCP–GFAAS).

Dissolved nickel speciation was determined by two different approaches: CLE–CSV and CRCP–GFAAS.

In the CLE–CSV method, a competitive equilibrium is established for the metal cation between the natural organic metal-complexing ligands and a well-characterized competing ligand added to the sample in a set concentration. The amount of metal complexed by the added competing ligand, which is related to both the concentrations and strengths of the added competing ligand and the natural metal-complexing ligands, is then measured by CSV. The DPASV techniques and the CRCP–GFAAS method measure the equilibrium concentrations of free metal ions and labile metal complexes that dissociate to free metal ions during the analytical time scale of these methods. The metal fractions detected by the DPASV techniques and CRCP–GFAAS include the inorganic metal fraction (i.e., free ions and inorganic complexes) and, depending upon the analytical timescale of the particular technique, may include a fraction of the relatively labile organic complexes as well. The theory underlying application of CLE–CSV, DPASV-(TMF-RGCDE), DPASV(HMDE), and CRCP–GFAAS to the determination of metal speciation in sea water has been described in detail in other references (CLE–CSV: Cu, [10,14,15,22]; CLE–CSV: Ni, [16,18]; DPASV: Cu, [12]; CRCP–GFAAS: Cu, [23]; Cu and Ni, [24]). A brief synopsis of each technique is given below.

CLE–CSV

In this method, the sample is titrated with copper or nickel in the presence of a well characterized ligand [8-quinolinol, 8-hydroxyquinoline (8-HQ) for copper; dimethylglyoxime (DMG) for nickel] which is added to establish a competing equilibrium with the natural ligands for copper or nickel. The copper or nickel concentration complexed by the added competing ligand ([MAL], where M is copper or nickel and AL is the added ligand) is then determined by CSV at each concentration of copper or nickel added during the titration. The copper or nickel complexed by the added competing ligand includes hydrated Cu^{2+} or Ni^{2+}, copper or nickel bound in inorganic complexes, and some fraction of the copper or nickel bound in natural organic complexes. [MAL] is a function of the concentration of the competing ligand added, the strength of the copper- or nickel-added ligand complex, the concentration

of the natural copper- or nickel-complexing lig-
ands in the sample (L_T), and the strength (i.e.,
the conditional stability constant, K'_{ML}) of the
copper- or nickel-natural ligand complexes. By
measuring [MAL], and by knowing both the con-
centration of the added competing ligand and the
conditional stability constant of its complexes with
M, both L_T and K'_{ML} can be determined. From
these values, the speciation of copper and nickel
in the natural sample can be estimated.

The CSV determination of [MAL] involves ad-
sorbing the MAL complexes in each of a set of
sample aliquots, which contain identical 8-HQ or
DMG concentrations but increasing concentra-
tions of copper or nickel, to the surface of a
hanging mercury drop electrode (HMDE) held at
a specific potential for a precisely controlled time
period. Then the current produced by reduction
of copper or nickel adsorbed onto the HMDE
from each aliquot is measured as the potential on
the HMDE is ramped negative. The peak current
values, which are proportional to [Cu(8-HQ)$_2^0$] or
[Ni(DMG)$_2^0$], are then plotted against the total
copper or nickel concentration (Cu$_T$ or Ni$_T$) in
each aliquot producing a titration curve. The
titration curve data are linearly transformed using
the following equation [22,25] which is derived
from appropriate mass balance and conditional
stability constant relationships:

$$[M^{2+}]/[ML] = [M^{2+}]/L_T + 1/(K'_{ML,M^{2+}}L_T) \tag{1}$$

where $[M^{2+}]$ is the concentration of hydrated
Cu^{2+} or Ni^{2+}, [ML] is the concentration of cop-
per or nickel complexed by organic ligand L, L_T
is total concentration of L detected, and $K'_{ML,M^{2+}}$
is the conditional stability constant (with respect
to M^{2+}) of the natural copper or nickel com-
plexes. $[M^{2+}]$ and [ML] are calculated from the
analytical measurement of [MAL] as follows:

$$[M^{2+}] = [MAL]/\alpha' = i_p/(S\alpha') \tag{2}$$

$$[ML] = M_T - [MAL] = M_T - i_p/S \tag{3}$$

where i_p is the M^{2+} reduction peak current, S is
the slope of the linear portion of the titration
curve at M_T concentrations exceeding $[L_T]$, M_T
is the concentration of total dissolved copper or

nickel, and α' is the overall side reaction coeffi-
cient for M:

$$\alpha' = \alpha_{M'} + \alpha_{MAL} \tag{4}$$

$\alpha_{M'}$ is the inorganic side reaction coefficient for
copper ($\alpha_{Cu'}$) or nickel ($\alpha_{Ni'}$); α_{MAL} is the side
reaction coefficient for complexation of M by AL:

$$\alpha_{MAL} = 1 + \Sigma\left(\beta'_{M(AL)_n}[AL]^n\right) \tag{5}$$

$\beta'_{M(AL)_n}$ is the overall conditional stability con-
stant (with respect to free M^{n+}) of the complexes
formed between the metal and the added com-
peting ligand, and [AL] is the concentration of
the added competing ligand. The values of both
$\alpha_{M'}$ and α_{MAL} depend upon the salinity and pH
of the sample.

Values for L_T and $K'_{ML,M^{2+}}$ are obtained from
a linear regression of a plot of $[M^{2+}]/[ML]$ as a
function of $[M^{2+}]$ (see Eqn. 1). Then, using these
values, the original concentrations of M^{2+} and
ML present in the sample can be estimated.

The analytical detection window for a CLE–
CSV speciation technique is determined primar-
ily by α_{MAL}. L_T and K'_{ML} can be characterized
for metal–organic complexes (ML) in natural wa-
ters whose α_{ML} value (i.e., $1 + (K'_{ML}[L'])$, where
$[L'] = L_T - [ML]$) lies within 1–2 decades above
and below α_{MAL}. Weaker organic ligands (i.e.,
those whose $\alpha_{ML} \ll \alpha_{MAL}$) are outcompeted by
AL, and the metal originally complexed by them
would be detected as if it had been free. For
organic ligands forming extremely strong metal
complexes (i.e., those whose $\alpha_{ML} \gg \alpha_{MAL}$), esti-
mates of L_T can be made along with a minimum
estimate of K'_{ML}.

DPASV

DPASV copper speciation determinations in-
volve titrating a sample (containing natural cop-
per-complexing organic ligands) with copper, and
measuring the oxidation current of copper de-
posited in a TMF-RGCDE or HMDE, as a func-
tion of added copper. During the DPASV deposi-
tion step, Cu^{2+} is reduced to elemental Cu^0 at
the mercury electrode surface, where Cu^0 will
then amalgamate with the mercury electrode. Af-
ter the deposition period, the potential on the
mercury electrode is ramped positive, and the

current resulting from oxidation of the amalgamated copper is measured. These measurements of peak current are plotted against the total copper concentration in the sample at the point in the titration at which the peak current was measured producing a titration curve. The titration curve data are linearly transformed using the following equation [26] which is derived from appropriate mass balance and conditional stability constant relationships:

$$[Cu']/[CuL] = [Cu']/L_T + 1/(K'_{CuL,Cu'}L_T)$$

(6)

where $[Cu']$ is the concentration of inorganic copper (i.e., the sum of the concentrations of hydrated Cu^{2+} and of inorganically complexed copper), $[CuL]$ is the concentration of copper complexed with strong natural organic ligand L, L_T is the copper complexing ligand concentration detected and $K'_{CuL,Cu'}$ is the conditional stability constant (with respect to Cu') of the natural copper complexes. $[Cu^{2+}]$ and $[CuL]$ are calculated from the analytical measurements as follows:

$$[Cu^{2+}] = i_p/S$$

(7)

$$[CuL] = Cu_T - [Cu']$$

(8)

where i_p is the DPASV peak current, S is the sensitivity of the inorganic copper response (see the Results section), and Cu_T is the total dissolved copper concentration. Values for L_T and $K'_{CuL,Cu'}$, are obtained from a linear regression of a plot of $[Cu']/[CuL]$ as a function of $[Cu']$ (see Eqn. 6). Then, using these values, the original concentrations of organically-complexed copper, inorganically-complexed copper, and free Cu^{2+} originally present in the sample can be calculated. Conditional stability constants $K'_{CuL,Cu'}$ were converted to conditional stability constants expressed with respect to $Cu^{2+}(K'_{CuL,Cu^{2+}})$ by multiplying $K'_{CuL,Cu'}$ by the inorganic side reaction coefficient for copper ($\alpha_{Cu'}$), appropriate for the salinity and pH of the sample.

The DPASV(TMF-RGCDE) technique has been developed and optimized to provide a short analytical time scale for its measurements so that only hydrated metal ions or rapidly dissociating metal complexes are detected. When this technique is used at a TMF-RGCDE rotation rate of 5000 rpm, strong copper–organic complexes (CuL) are not detected because these complexes are kinetically inert with respect to dissociation within the diffusion layer surrounding the TMF-RGCDE [12]. However, Cu' is detected by DPASV(TMF-RGCDE) because inorganic copper species are either directly electroactive themselves (hydrated Cu^{2+}) or they are inorganic complexes (e.g., $CuCO_3^0$, $CuCO_3OH^-$, $CuOH^+$) whose dissociation kinetics are so rapid, relative to their residence times in the TMF-RGCDE diffusion layer, that they are kinetically labile and detected as electroactive.

The DPASV speciation technique using a HMDE is not as sensitive as with a TMF-RGCDE. In addition, because a sample must be stirred much more slowly (less than about 700 rpm) to avoid dislodging the HMDE, the diffusion layer surrounding the HMDE is wider than that surrounding a TMF-RGCDE. Thus, copper complexes have longer residence times in the wider HMDE diffusion layer, and a greater probability exists that labile copper–organic complexes will dissociate and be detected as electroactive by DPASV(HMDE). Copper species detected by DPASV(HMDE) could include not only inorganic copper species (i.e., Cu'), but also some fraction of labile copper–organic complexes. The copper fraction detected by DPASV(HMDE) is henceforth referred to as "labile copper" (Cu_{lab}), and $[Cu_{lab}]$ and $K'_{CuL,Cu-lab}$ (the conditional stability constant for CuL expressed with respect to labile copper) would replace $[Cu']$ and $K'_{CuL,Cu'}$, respectively, in Eqn. 6.

The analytical detection window for the DPASV copper speciation techniques is centered around $\alpha_{Cu'}$ (≈ 10–20). The concentrations and conditional stability constants of copper–organic complexes in seawater whose α_{CuL} values ($\alpha_{CuL} = 1 + K'_{CuL,Cu'}[L']$) are within 1–2 decades above and below $\alpha_{Cu'}$ can be accurately determined by DPASV.

CRCP–GFAAS

CRCP–GFAAS also relies on the differences in the dissociation kinetics between inorganic

metal complexes and strong organic metal complexes. In this method, the sample is pumped through a chelating, ion-exchange resin (Chelex-100) which strongly binds free Cu^{2+} and Ni^{2+}, while letting most of the CuL and NiL forms pass. The analytical time scale of this method is determined by the contact time of the sample with the chelating resin. Thus, the fraction of copper or nickel detected as labile by this method (henceforth called "labile copper" Cu_{lab} or "labile nickel" Ni_{lab}) will depend on the sample flow-rate through the column. We have examined the flow-rate dependence of the method and attempted to use the fastest flow-rates possible to isolate [Cu'] and [Ni'], but to allow the bulk of the CuL and NiL to pass through the resin column without dissociating and partitioning onto the resin. Cu_{lab} or Ni_{lab} retained by the chelating resin is eluted with acid and determined by GFAAS.

The CRCP–GFAAS titration data are linearized and interpreted in a manner analogous to that described above for the DPASV techniques. In addition, the analytical detection window of this method is similar to that of the DPASV techniques.

EXPERIMENTAL

Sample collection

The sampling site in South San Francisco Bay (approx. 37°29'N, 122°7'W; in the central channel just south of the Dumbarton Bridge) was occupied twice: 30th May 1991 (SFB-1) and 17th October 1991 (SFB-2). Samples were collected using a peristaltic pump (Masterflex, Cole-Parmer) fitted with C-Flex tubing in the pump head. Water was drawn from a depth of about 1 m through acid-cleaned FEP-Teflon tubing attached to an aluminum pole which was oriented 5 m upstream of the boat's drift. The water was filtered as it was collected by pumping it through an acid-cleaned 0.45 μm pore-size in-line cartridge filter (MSI) directly into 20 l, acid-cleaned Teflon bags contained in dark brown, high-density polyethylene bottles (NOWPACK Bag-in-a-Bottle, Berghoff America). The bottles were placed in plastic bags, placed in ice, returned to

UCSC, and kept in a dark coldroom. Subsamples for analysis were withdrawn from these 20-l bottles using a peristaltic pump system consisting of FEP-Teflon tubing connected to C-Flex tubing in the pump head. All speciation analyses of these samples were completed within four weeks of collection.

Equipment and instrumentation

All analyses were conducted at room temperature ($\approx 25°C$) at vertical flow, Class-100 filtered air, clean benches in clean labs supplied with Class-100 filtered air. Eppendorf single-volume pipets fitted with acid-cleaned polyethylene tips were used for microliter additions of reagents and metal standards.

CLE–CSV and DPASV(HMDE). The instrumentation used for the DPASV(HMDE) technique is the same as that used for the CLE–CSV technique, and has been described previously [14,15]. Briefly, a Princeton Applied Research (PAR) 174A voltammetric analyzer (modified to increase the pulse frequency to 8 s^{-1} and to decrease the delay time prior to the current sampling period during a pulse from 40 to 13 ms) was used with a PAR 303A hanging mercury drop electrode and an *X–Y* recorder (Houston Instruments). The working electrode was a "large" mercury drop, the reference electrode was Ag/saturated AgCl, saturated KCl, and the counter electrode was a platinum wire. Samples contained in FEP-Teflon voltammetric cell cups were deoxygenated with oxygen-free nitrogen presaturated with water vapor, and were stirred with a PTFE-coated stirring star driven by a PAR Model 305 magnetic stirrer. An Eppendorf Maxipettor fitted with acid-cleaned polyethylene tips was used to deliver 10 ml sample aliquots.

For total dissolved copper and nickel concentration determinations by DPCSV, acidified (pH 2) water sample aliquots (70–90 ml) contained in PTFE-Teflon beakers were UV-irradiated by a 1200 W mercury arc lamp (Hanovia) for 5 h.

DPASV(TMF-RGCDE). The custom-made DPASV(TMF-RGCDE) apparatus has been described elsewhere [12,27]. Briefly, this apparatus consists of a PAR 174A voltammetric analyzer (modified to decrease the delay time prior to the

current sampling period during a pulse from 40 to 13 ms) connected to an electrochemical cell housed in a plexiglass cell stand, and to a strip chart recorder. The electrochemical cell consists of a 60-ml PTFE-Teflon sample cup, a rotating glassy-carbon disk working electrode (RGCDE) onto which a thin mercury film (TMF) is deposited, a Teflon-sheathed platinum wire counter electrode, and a Teflon-sheathed Ag/saturated AgCl, saturated KCl reference electrode. Similar systems are commercially available through PAR. Samples were deoxygenated with oxygen-free nitrogen.

CRCP–GFAAS. Samples were pumped through FEP-Teflon tubing into the resin columns using a peristaltic pumping system (Masterflex, Cole-Parmer). The resin columns were custom-made from PTFE-Teflon tubing (9 mm i.d.) fitted with PTFE-Teflon end caps and fritted polyethylene disks. GFAAS quantitation was performed using a Perkin Elmer Model 5000 atomic absorption spectrophotometer equipped with an HGA-500 graphite furnace atomizer and an AS-40 auto-sampler. L'Vov platforms were used with manufacturer-recommended drying, ashing, and atomization conditions.

Reagents

All aqueous solutions described below were prepared in ultrapure water (Milli-Q; Millipore) unless noted otherwise.

CLE–CSV. A 0.1 M 8-quinolinol (8-HQ, G.F. Smith) stock solution was prepared in 0.2 M subboiling quartz-distilled hydrochloric acid (QHCl). A 0.1 M dimethylglyoxime (DMG, G.F. Smith) stock solution was prepared in HPLC-grade methanol (Fisher); a 0.01 M DMG solution was prepared by diluting the 0.1 M stock solution with Milli-Q water. The buffers used (pH 7.8 and 8.1) were 1 M HEPPS (*N*-2-hydroxyethylpiperazine-*N'*-3-propanesulfonic acid; Research Organics) and ultraclean ammonia solution (Q-ammonia). The Q-ammonia solution was prepared by bubbling NH_3 gas through Milli-Q water. Copper and nickel standard solutions were prepared by dilution of 1000 ppm atomic absorption standard solutions (Baker Analyzed) with Milli-Q water and acidified to pH 3 with QHCl.

DPASV. Thin mercury films were plated onto the RGCDEs from solutions composed of Milli-Q water, 0.1 M KCl (G.F. Smith), and ca. 10 μg ml^{-1} Hg^{2+} (Bethlehem Apparatus triple distilled Hg dissolved in dilute ultra-pure nitric acid). Copper standard solutions were prepared as described above.

CRCP–GFAAS. The resin used was Chelex-100, a chelating ion-exchange resin (BioRad 100–200 mesh). A 1 M ultraclean ammonium acetate buffer solution was prepared and adjusted to pH 5.8. Metals were eluted from the Chelex-100 resin using 2.5 M nitric acid (Fisher, Trace Metal Grade; TM-HNO$_3$).

Total dissolved copper and nickel determinations

The total dissolved copper and nickel concentrations in South San Francisco Bay sample SFB-1 (May 1991) were determined by both GFAAS preceded by preconcentration via dithiocarbamate complexation and solvent extraction (DC–SE–GFAAS), and by CSV. Total dissolved copper and nickel concentrations in SFB-2 (October 1991) were determined by DC–SE–GFAAS and by GFAAS preceded by preconcentration using Chelex-100 (Chelex–GFAAS).

In the DC–SE–GFAAS technique, sample preconcentration is performed by adjusting the pH of samples stored acidified (pH 1.5) to between 4 and 4.5, adding a relatively high concentration of the strong Cu- and Ni-chelators 1-pyrrolidinedithiocarbamate and diethyldithiocarbamate (PDC–DDC), extracting the metal complexes into chloroform, evaporating the chloroform, and reconstituting the concentrated residues in weak QHNO$_3$. Copper and nickel concentrations were determined by GFAAS as described by Bruland et al. [27].

The CSV total dissolved copper and nickel determinations were performed using the method of standard additions following slight modifications of procedures described previously (Cu: [14,28]; Ni: [17,29]). Briefly, 10 ml acidified and UV-irradiated sample aliquots were pipetted into a FEP-Teflon cell cup, neutralized with 0.5 M Q-ammonia solution, and buffered to pH 8.3 (100 μl of 1 M boric acid (G.F. Smith)–0.35 M Q-am-

monia solution). For the copper determinations, 8-HQ was added to the buffered sample aliquot to give a concentration of 10^{-5} M. Adsorption was carried out at -0.1 V for 1 min with no stirring. During the stripping step, the potential was scanned at 20 mV s^{-1} in the differential pulse mode with a pulse amplitude of 25 mV and a pulse frequency of 8 s^{-1}. For the nickel determinations, DMG was added to the buffered sample aliquot to yield a concentration of 10^{-4} M. Adsorption was carried out at -0.7 V for 1 min with no stirring. Stripping was performed using linear scan at 50 mV s^{-1}.

The Chelex–GFAAS determinations of total dissolved copper and nickel were performed on acidified (pH 1.5) aliquots by adjusting the pH to 5.5–6.0 with the ammonium acetate buffer, and pumping them through a Chelex-100 resin column (volume = 5 ml) at a flow-rate of 0.5 ml min^{-1}. The resin was rinsed with twelve 2-ml portions of ammonium acetate buffer, twelve 1-ml portions of Milli-Q water, and eluted with 25 ml of 2.5 M TM-HNO$_3$. Copper and nickel in the acid eluates were determined by GFAAS. An overall collection and elution efficiency of 95% was used to correct the raw data.

Copper and nickel speciation determinations

Copper speciation in the South San Francisco Bay sample was determined by the CLE–CSV, DPASV(TMF-RGCDE), DPASV(HMDE), and CRCP–GFAAS methods. Nickel speciation was determined by CLE–CSV and CRCP–GFAAS.

CLE–CSV. Copper- and nickel-complexing ligand titrations were performed with slight modifications of procedures described previously (Cu: [15,22]; Ni: [16]). Stock HEPPS buffer solutions (1500 μl) were added to 150 ml of the water sample to hold the sample pH at its ambient value (pH: 7.8[SFB-1], 8.1[SFB-2]) and the mixture was shaken well. Ten milliliter aliquots of this solution were pipetted into each of 12 FEP-Teflon cell cups. Copper or nickel (in separate titrations) was added to 11 of the 12 aliquots providing an incrementally increasing concentration of the metal added. The buffered, copper- and nickel-spiked sample aliquots were then left

for 6–8 h in individual airtight containers to allow the added metal to equilibrate with the natural ligands. After this initial equilibration period either 1.32 μM 8-HQ for the copper analyses or 87 to 91 μM DMG for the nickel analyses was added to each of the 12 aliquots, which were then left to equilibrate further (overnight, 10–12 h) in individual airtight containers.

The next day each aliquot was purged for 8 min with water-vapor-saturated oxygen-free nitrogen. Then, the Cu(8HQ)$_2^0$ or Ni(DMG)$_2^0$ complexes in each aliquot were adsorbed onto a fresh mercury drop at an applied potential of -0.2 V for copper and -0.7 V for nickel (vs. Ag/sat. AgCl, sat. KCl) for an accurately controlled adsorption time ranging from 1 to 2 min (the same adsorption time was used for all aliquots in a given titration). During the adsorption step, sample aliquots were stirred as follows: Cu,SFB1-"fast" (700 rpm); Cu,SFB2-not stirred; Ni,SFB1 and SFB2-"slow" (400 rpm). After the adsorption period, the stirrer, if used, was switched off. Fifteen seconds later, the potential was scanned in the negative direction: the differential pulse mode was used for copper (scan rate 20 mV s^{-1}, pulse amplitude 25 mV, pulse frequency 8 s^{-1}), while the linear scan mode was used for nickel (scan rate 50 mV s^{-1}) and the respective reduction currents (Cu^{2+} at -0.35 V; Ni^{2+} at -0.98 V) were recorded.

From the measurement of the copper reduction peak current at each copper concentration added, a titration plot of peak current vs. total copper concentration (sum of copper concentration added + ambient copper concentration) was constructed. From these measurements, the concentrations of Cu^{2+}, and organically complexed copper (CuL) were calculated at every total copper concentration used (using Eqns. 2–5). The titration data were linearized by plotting [Cu^{2+}]/[CuL] vs. [Cu^{2+}] (see Eqn. 1). The concentration of the copper-complexing ligand class detected and the conditional stability constant of its complex were obtained from the slope and intercept of the linear least-squares regression of the linearization plot. A procedure identically analogous to that just described was used to obtain the nickel-complexing ligand concentra-

tion and the conditional stability constant of the nickel complex.

In order to minimize potential effects of adsorption of copper, nickel, or the $Cu(8HQ)_2^0$ or $Ni(DMG)_2^0$ complexes onto the FEP-Teflon cell cup walls, the copper, nickel, 8-HQ, and DMG concentrations of the spiked sample aliquot held by a given Teflon cell cup were kept approximately constant for all titrations (thereby conditioning the cell cup walls to the copper, nickel, 8-HQ, and DMG concentrations they contained), and the cups were rinsed only with Milli-Q water and stored dry between analyses.

DPASV(TMF-RGCDE). The procedure followed for the DPASV(TMF-RGCDE) copper speciation analyses was essentially that presented by Coale and Bruland [12]. Prior to sample analysis, the RGCDE was polished with 0.05 μm alumina at a rotation rate of a few hundred rpm, then thoroughly rinsed with dilute Q-HCl. The TMF was deposited onto the RGCDE by immersing it into 50 ml of de-oxygenated Milli-Q water containing 200 μl of a saturated solution of ultrapure KCl and 100 μl of a 5000 μg ml^{-1} Hg^{2+} solution. The rotation rate of the RGCDE was set to 5000 rpm, and its potential was held at -0.65 V with respect to the Ag/sat. AgCl, sat. KCl reference electrode for 15 min to deposit the TMF. After this 15 min deposition period, the TMF-RGCDE rotation was stopped, and the TMF formation solution was allowed to become quiescent for 30 s. The TMF-RGCDE potential was then ramped positive at 10 mV s^{-1} in the differential pulse mode (50 mV pulse amplitude, 5 pulses s^{-1}) to strip any deposited metals out of the TMF. The resulting oxidation current was recorded as a function of potential on a strip-chart recorder yielding a voltammogram. If the resulting "blank" voltammogram showed low or undetectable metal levels and a satisfactorily low background current, sample analysis proceeded. The TMF-RGCDE was rinsed with a de-oxygenated aliquot of the sample, and then a fresh, deoxygenated sample aliquot (50–60 ml) was mounted to the TMF-RGCDE. Copper in the sample was deposited into the TMF-RGCDE during a 10 min deposition step at -0.65 V and 5000 rpm. Rotation of the TMF-RGCDE was

then stopped, the sample allowed to become quiescent for 30 s, after which the potential was ramped positive in the differential pulse model with the oxidation current recorded as described above. The TMF-RGCDE potential was held at -0.15 V for 1 min while rotating, in order to strip the TMF completely of residual metals. Then the selector switch on the PAR 174A was flipped to the "OFF" position. The sample was spiked with a standard copper solution and the added copper was allowed to equilibrate with the sample for 15 min with the TMF-RGCDE rotating to enhance mixing, but with no potential applied. After the spike equilibration period, the deposition/stripping/recording cycle was repeated.

From the measurement of the copper oxidation peak height at each copper concentration added, a titration plot of peak current vs. total copper concentration (sum of concentrations of copper added + ambient copper) was constructed. From these measurements, the concentrations of inorganic copper ([Cu′]), and organically complexed copper ([CuL]) were calculated at every total copper concentration used (using Eqns. 7 and 8). The titration data were linearized by plotting [Cu′]/[CuL] vs. [Cu′] (see Eqn. 6). The concentration of the copper-complexing ligand class detected and the conditional stability constant of its copper complexes were obtained from the slope and intercept of the linear least-squares regression of the linearization plot.

The Cu′ response of the DPASV(TMF-RGCDE) technique (i.e., S in Eqn. 7) was estimated two ways. First, by measuring the slope of the analytical response (signal/metal concentration) during an actual sample titration at copper concentrations exceeding the copper-complexing organic ligand concentration. This was checked on some samples by inserting an aliquot of UV-irradiated seawater from which trace metals had been removed (UVSW: [17]) on the same thin mercury film after completing a sample titration, and then performing a copper titration on the UVSW aliquot to determine the peak current/[Cu′] response. The linear Cu′ response measured in UVSW was within 5–10% of the Cu′ response measured in the SFB samples at high copper concentrations exceeding [L$_2$]. Thus, if

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weaker classes of copper-complexing ligands were present in the samples, they could not account for more than 5–10% of additional copper complexation.

DPASV(HMDE). For the DPASV(HMDE) analyses a "large" HMDE and the "fast" (700 rpm) stirring speed on the PAR Model 305 stirring motor were used. The deposition step was performed for 10 min at a potential of -0.65 V. After a 30-s quiescent period, the potential was ramped positive at 10 mV s^{-1} in the differential pulse mode (50 mV pulse amplitude, 8 pulses s^{-1}). The copper titrations were carried out and interpreted in a similar fashion to the DPASV(TMF-RGCDE) technique with the exception that 10 ml sample aliquots were contained in FEP-Teflon voltammetric cell cups.

CRCP–GFAAS. For the CRCP–GFAAS copper and nickel speciation determinations, eight of twelve 400 ml aliquots of the sample (in Teflon bottles) were spiked with incrementally increasing copper or nickel concentrations; the remaining four 400 ml sample aliquots received no added copper or nickel. The aliquots were left overnight for the added metal to equilibrate with the natural organic ligands. The next morning the samples were pumped through the Chelex-100 resin column (2 ml resin, 9 mm i.d. column) at a flow-rate of 14–15 ml min^{-1}. The resins were then rinsed with five 2-ml portions of ammonium acetate buffer to remove high concentrations of alkali and alkaline earth metals from the resin, five 1-ml portions of Milli-Q water, and eluted with ten 1-ml portions of 2.5 M TM-HNO$_3$.

Copper and nickel concentrations in the eluates were determined by GFAAS and represent labile copper and nickel in the samples concentrated approximately 40-fold. Both standards in 2.5 M HNO$_3$ and standard additions on sample aliquots were analyzed to check for any matrix effects. Matrix effects were minimal because of the clean-up steps used in the column technique.

Copper and nickel concentrations in the eluates were corrected for their respective concentration factors and then plotted against the total copper or nickel concentrations used in the titrations. The slope at high copper or nickel concentrations (in excess of the concentrations of natural complexing ligands) was used to determine the column efficiency at these high flow-rates (efficiencies were close to 90%). Efficiencies on the order of 90% were also observed in titrations of UVSW. The raw data were corrected for this efficiency and concentrations of [Cu'] or [Ni'] and [CuL] or [NiL] were calculated. The titration data were linearized and the concentration of copper- or nickel-complexing organic ligands and their conditional stability constants calculated as described above for the DPASV techniques.

RESULTS

pH, temperature and salinity measurements
The pH, temperatures and salinities of the two samples were: SFB-1: 7.86, 18.0°C, 27.80–28.31 p.s.u.; SFB-2: 8.12, 19.6°C, and 31.62–31.65 p.s.u.

Total dissolved copper and nickel concentrations
The measurements of the concentrations of total dissolved copper and nickel for the two station occupations are presented in Table 1. The agreement in total dissolved copper and nickel concentrations determined by the various methods was excellent, especially considering that the comparisons were made on separate subsamples

TABLE 1

Concentrations of total dissolved copper and nickel (nM) in South San Francisco Bay

	SFB-1	SFB-2
Copper		
Dithiocarbamate complexation– solvent extraction–GFAAS	45.0	49.1 ± 1.4
CSV(8-HQ)	45.6 ± 0.7	
CRCP–GFAAS		47.0 ± 1.1
Mean [a]	45.4 ± 0.6	48.1 ± 1.8
Nickel		
Dithiocarbamate complexation– solvent extraction–GFAAS	49.0	57.6 ± 0.7
CSV(DMG)	50.9 ± 0.7	
CRCP–GFAAS		57.9 ± 2.2
Mean [a]	50.3 ± 1.2	57.7 ± 2.3

[a] Mean ± s.d. ($n = 4$).

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collected sequentially while on station, rather than on aliquots of an homogenized sample.

Dissolved copper speciation

The "dissolved" fraction of a metal has traditionally been defined as that which passes through a 0.4-μm Nuclepore or a 0.45-μm Millipore membrane filter. This operational separation made with conventional membrane filters is relatively routine. However, the so-called "dissolved" fraction can no longer be assumed to be composed of only "truly dissolved" species such as free hydrated metal ions, inorganic complexes and metals complexed by dissolved organic ligands. Trace metals associated with colloids may also make up a significant component of the "dissolved" fraction. Recently, the potentially important role of colloids influencing the trace metal chemistry of the "dissolved" fraction has received increasing attention [30]. However, the extent to which trace metals are associated with colloids in South San Francisco Bay is presently unknown. Thus, in this paper, any copper and nickel associated with organic colloids is operationally included in the dissolved metal–organic complex component.

Throughout this section, copper- and nickel-complexing ligand concentrations are presented in units of nanomoles of copper or nickel complexed by the ligands per liter of sample. For the CLE–CSV and DPASV(TMF-RGCDE) results, conditional stability constants are reported with respect to Cu^{2+} or Ni^{2+}. For the CRCP–GFAAS and DPASV(HMDE) results, the conditional stability constants calculated from the titration data (i.e., $K'_{ML,M\text{-lab}}$) were converted to, and are expressed in this section as, apparent conditional stability constants with respect to "free Cu^{2+} or Ni^{2+}" by multiplying the $K'_{ML,M\text{-lab}}$ values by $\alpha_{Cu'}$ or $\alpha_{Ni'}$. Using our measurements of pH, temperature, and salinity, and interpolating the data presented by Byrne et al. [7], for SFB-1: $\alpha_{Cu'} = 12$ and $\alpha_{Ni'} = 1.6$; for SFB-2: $\alpha_{Cu'} = 21$ and $\alpha_{Ni'} = 1.8$.

CLE–CSV. CLE–CSV, using 8-hydroxyquinoline (8-HQ) as an added competing ligand, characterized a very strong class of copper-complexing ligands (hereafter called L_1) in the South San Francisco Bay samples. Duplicate analyses of SFB-1 yielded an average L_1 concentration of 12.8 ± 3.7 nM with a conditional stability constant, $K'_{CuL_1} \geq 10^{13.8}$ M^{-1}. Duplicate analyses of SFB-2 yielded an average L_1 concentration of 13.2 ± 0.4 nM with $K'_{CuL_1} \geq 10^{13.5}$ M^{-1}. The concentration of L_1 is substantially less than the ambient concentration of dissolved copper, and because its affinity for copper is so strong, L_1 is already totally bound with copper and no more L_1 is available to bind additional copper. This concentration of very strongly complexed copper, which is constant between the spring (SFB-1) and autumn (SFB-2) sampling trips, comprises an average of 28% of the total dissolved copper.

The copper complexation characteristics of L_1 were able to be determined only by setting up a strong competing equilibrium with 8-HQ. The side reaction coefficient of free Cu^{2+} with respect to forming complexes with 8-HQ is $\approx 9.3 \times 10^4$ (i.e., $\alpha_{Cu\text{-8HQ}} = [Cu\text{-8HQ}]/[Cu^{2+}] \approx 9.3 \times 10^4$). 8-HQ outcompeted all weaker classes of copper-complexing ligands, including the weaker class, L_2, determined by the other analytical methods.

A representative titration plot of the peak reduction current versus total dissolved copper for the SFB-2 sample is presented in Fig. 1. The first filled symbol at the ambient total dissolved copper concentration of 48.1 nM corresponds to a labile-Cu value of 36 nM and $[CuL_1] = 12.9$ nM. In this case the labile-Cu value includes $[Cu']$ and the concentrations of any CuL_2, since 8-HQ would outcompete these forms. It can be seen in Fig. 1 that the 8-HQ does not outcompete the CuL_1 complexes due to their high conditional stability constant ($K'_{CuL_1} \geq 10^{13.8}$ M^{-1}).

DPASV(TMF-RGCDE). Historically, much of the DPASV speciation research has been performed with hanging mercury drop electrodes (HMDEs). Results of metal speciation research with HMDEs have prompted a number of criticisms of the DPASV approach to studying metal–organic ligand interactions in natural waters [12]. These criticisms center on three main concerns: (1) possible overestimation of $[Cu']$ due to direct electrochemical reduction of copper–organic complexes during the depositon step,

which would produce an apparent "inorganic copper" signal upon stripping; (2) possible over-estimation of [Cu'] due to a "kinetic contribution" to the stripping current if the dissociation rate of the copper–organic complex is rapid with respect to the residence time of the copper–organic complex within the electrode diffusion layer; and (3) possible blocking of the electrode surface by adsorption of organics, thereby decreasing sensitivity or invalidating the measurements. These concerns can be minimized by using the thin mercury film rotating glassy carbon disc electrode (TMF-RGCDE) at the appropriate deposition potential. Potential problems associated with the direct electrochemical reduction of copper–organic complexes at the electrode surface can be circumvented by performing the electrodeposition step at potentials just negative enough to reduce only the labile inorganic form of copper, Cu'. Figure 2 presents a pseudovoltammogram obtained from

Fig. 2. Pseudovoltammogram showing the copper oxidation peak current (i_p) obtained by DPASV(TMF-RGCDE) at various deposition potentials for UVSW (●) and a South San Francisco Bay sample (○), both containing ≈ 47 nM Cu. The arrow indicates the deposition potential (-0.65 V) at which all DPASV analyses were performed.

[Cu$_t$] (nM)

Fig. 1. Representative CLE–CSV titration of an SFB-2 subsample using 8-hydroxyquinoline as the added competing ligand. The open circle is the x-intercept determined by extrapolation of the best-fit line through the data shown in filled circles, and represents the copper concentration (12.9 nM) bound by the strong ligand class, L_1. Linear regression results: slope = 0.519; r = 0.996.

UVSW and a South Bay sample, both containing approximately 47 nM total dissolved copper. The deposition potential of -0.65 V (indicated by the arrows) is negative enough to produce the full signal for Cu' in UVSW, yet falls within the relatively narrow window of potentials (-0.60 to -0.65 V) where electroreduction of the natural Cu–organic complexes does not occur. With deposition potentials increasingly more negative than -0.65 V, an increasing fraction of organically complexed copper is electroreducible. This is consistent with a fraction of the dissolved copper being bound in relatively labile complexes (i.e., CuL_2). Copper complexed with the class of strong ligands (i.e., CuL_1) would not be electroreducible at any of these deposition potentials. After consideration of this pseudovoltammogram, a deposition potential of -0.65 V was used for all of the South Bay DPASV studies (using both the TMF-RGCDE and the HMDE).

The theory for estimating kinetic contributions from the dissociation of labile complexes within the diffusion layer of the TMF-RGCDE is well

established [31,32]. A rotating disk electrode is well-defined hydrodynamically. At a rotation speed of 5000–6000 rpm, the TMF-RGCDE used in this study has a diffusion layer width of ca. 5×10^{-4} cm, and the residence time of a copper complex within the TMF-RGCDE diffusion layer would be ca. 10 ms. Under these conditions a metal–ligand complex with a dissociation rate constant, $k_{d'} < 1$ s^{-1} is "inert" with respect to its residence time in the diffusion layer. In contrast, inorganic complexes with $k_d > 10^5$ s^{-1} are labile, and the Cu^{2+} dissociating from these complexes is detected. Copper complexes with organic ligands such as EDTA have dissociation rate con-
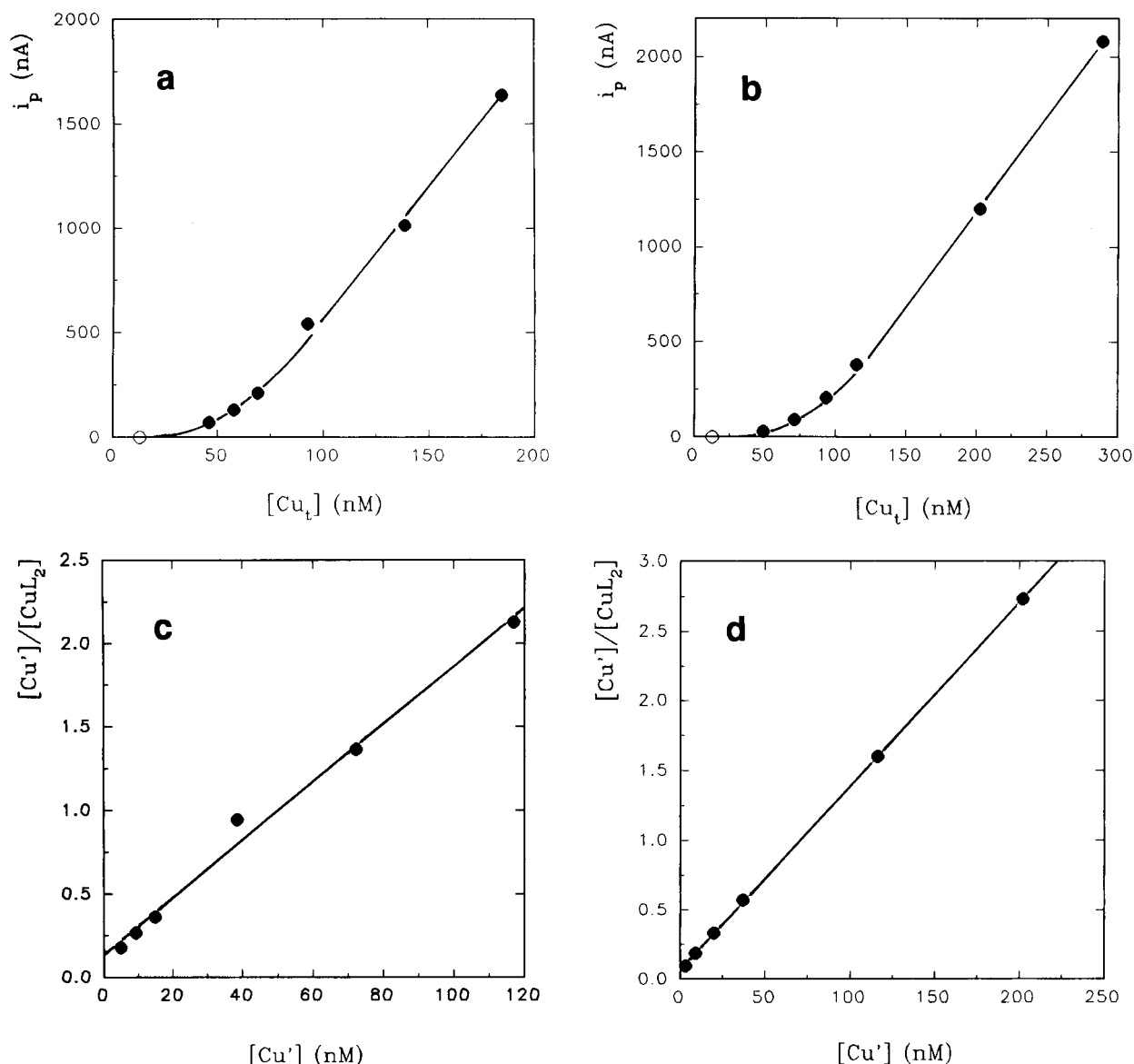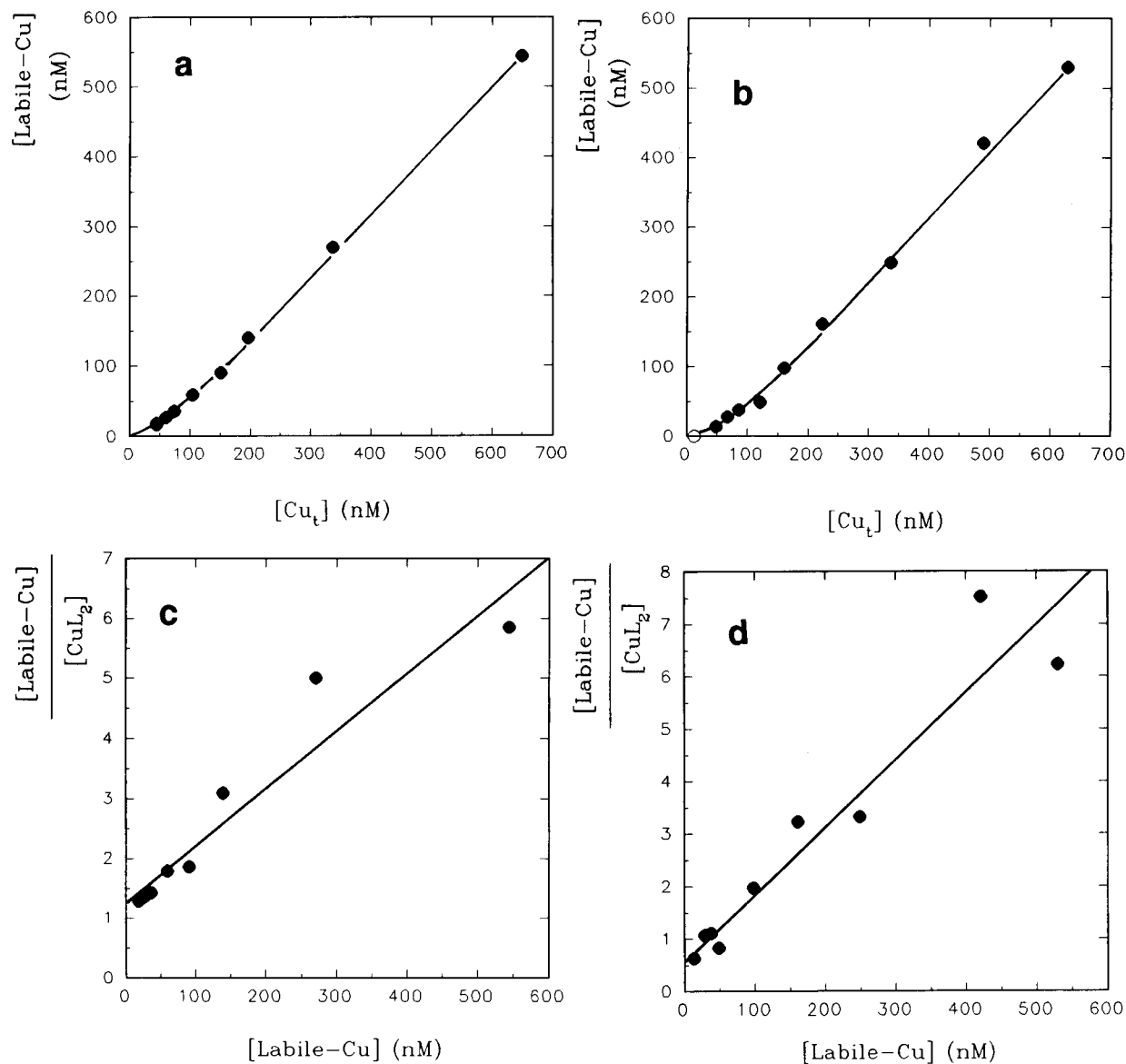

Fig. 3. Representative DPASV(TMF-RGCDE) copper titration curves for SFB-1 (a) and SFB-2 (b); linearized titration curve for SFB-1 (c) and SFB-2 (d). Open circles in (a) and (b) represent the concentration of copper complexed by the strong ligand class, L_1, determined by CLE–CSV (8-HQ). For (c): slope = 0.0173, y-intercept = 0.133, r = 0.996; for (d): slope = 0.0132, y-intercept = 0.0674, r = 0.999.

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stants in seawater of ca. 10^{-4} s^{-1} and are electrochemically inert with respect to the TMF-RGCDE. Shuman and Michael [33] found the dissociation rate constants of a relatively weaker class of copper–organic complexes in coastal waters to be ca. 2 s^{-1}. Such complexes would be relatively inert with respect to the TMF-RGCDE.

A copper titration of our UVSW, which is both free of dissolved copper and copper-complexing organic ligands, showed a linear response (r^2 = 0.998; n = 4) with an intercept of zero, indicating no apparent loss of copper due to adsorption or complexation in the absence of a complexing ligand. When 10.1 nM EDTA was added to UVSW and allowed to equilibrate with copper additions for 24 h, subsequent analyses yielded a measured concentration of 10.4 ± 1.6 nM EDTA. An average $K'_{\text{CuEDTA}} = 10^{10.0}$ M^{-1} was determined with the linearization approach, which compares favorably with that calculated from thermodynamic considerations of $K'_{\text{CuEDTA}} = 10^{10.1}$ M^{-1}. Results such as these obtained from determinations performed using well-characterized model ligands provide confidence in the accuracy of the DPASV(TMF-RGCDE) approach [12].

Representative DPASV(TMF-RGCDE) copper titration curves for the SFB-1 and SFB-2 samples are presented in Fig. 3a and b. The oxidation peak current is plotted versus the total dissolved copper. The open circle corresponds to the concentration of CuL$_1$ determined by the CLE–CSV method. The first filled data point corresponds to the peak current associated with the ambient total dissolved copper concentration for each of the sampling periods. The remaining data points correspond to the incremental copper additions in the titration. The results of triplicate analyses of these samples showed excellent precision. Linearizations of the titration curves shown in Fig. 3a and b are presented in Fig. 3c and d. In all the samples, the DPASV(TMF-RGCDE) technique determined a greater concentration of a class of weaker copper-complexing organic ligands, L$_2$, in addition to the lower concentration of strong ligand, L$_1$, existing as CuL$_1$. In each case, the second class of ligand could be modelled as a single, additional ligand class.

For SFB-1 the DPASV(TMF-RGCDE) method yielded an average total L$_2$ concentration ([L$_{2T}$]) = 63.0 ± 6.9 nM, with an average $K'_{\text{CuL}_2} = 10^{9.0 \pm 0.3}$ M^{-1}. The measured [Cu'] determined on the zero-addition aliquots yielded a value of 9.1 ± 3.8 nM. The average concentrations estimated for the different copper fractions under ambient conditions (i.e., no copper added) are: [Cu'] = 9.1 ± 3.8 nM (20% of [Cu$_T$]); [CuL$_1$] = 12.8 ± 3.7 nM (28% of [Cu$_T$] and 100% of [L$_1$]); [CuL$_2$] = 23.7 ± 5.4 nM (52% of [Cu$_T$] and 38% of [L$_{2T}$]); [L$_{2T}$] = 63.0 ± 6.9 nM; [L$_2'$] = 39.2 ± 8.8 nM (62% of total [L$_{2T}$]); [Cu$_T$] = 45.4 ± 0.6 nM; where [L$_2'$] is the concentration of L$_2$ not bound to copper in the sample.

For SFB-2 the DPASV(TMF-RGCDE) method yielded an average [L$_{2T}$] = 74.0 ± 2.0 nM, with an average $K'_{\text{CuL}_2} = 10^{9.6 \pm 0.2}$ M^{-1}. The measured [Cu'] determined on the zero-addition aliquots yielded a value of 3.9 ± 0.8 nM. The average concentrations estimated for the different copper fractions under ambient conditions (i.e., no copper added) are: [Cu'] = 3.9 ± 0.8 nM (8% of [Cu$_T$]); [CuL$_1$] = 13.2 ± 0.4 nM (27% of [Cu$_T$] and 100% of [L$_1$]); [CuL$_2$] = 31.0 ± 2.0 nM (65% of [Cu$_T$] and 42% of [L$_{2T}$]); [L$_{2T}$] = 74.0 ± 2.0 nM; [L$_2'$] = 43.0 nM ± 2.8 nM (58% of [L$_{2T}$]); [Cu$_T$] = 48.1 ± 1.8 nM.

DPASV(HMDE). The DPASV(HMDE) approach to determining copper speciation has a number of advantages and disadvantages relative to the DPASV(TMF-RGCDE) approach. The advantages are that the PAR 303A is a common, commercially available electrode system with which it is easy and quick to form reproducible HMDEs relative to the TMF-RGCDEs. The main disadvantages are: (1) lower sensitivity relative to the TMF-RGCDE due to the lower surface area of the HMDE and slower stirring speed required; and (2) the HMDE has a wider diffusion layer (ca. 2 × 10^{-3} cm) due to the slower stirring speed required which increases the potential of detecting a fraction of the weaker copper–organic complexes as labile copper, thus yielding a higher apparent [Cu']. The residence time of copper complexes within the HMDE diffusion layer is estimated to be ca. 0.2 ms; complexes whose dissociation rate constant (k_d) are less than 0.1

s^{-1} are inert (e.g. copper complexed with an organic ligand like EDTA), while those whose dissociation rate constants are greater than 10^{-4} s^{-1} are completely labile. However, the weaker copper–organic complexes studied by Shuman

and Michael [33], having $k_d \approx 2$ s^{-1}, would be partially labile with respect to the HMDE.

Representative DPASV(HMDE) titration curves for SFB-1 and SFB-2 are shown in Fig. 4a and b, and the linearizations of these titration
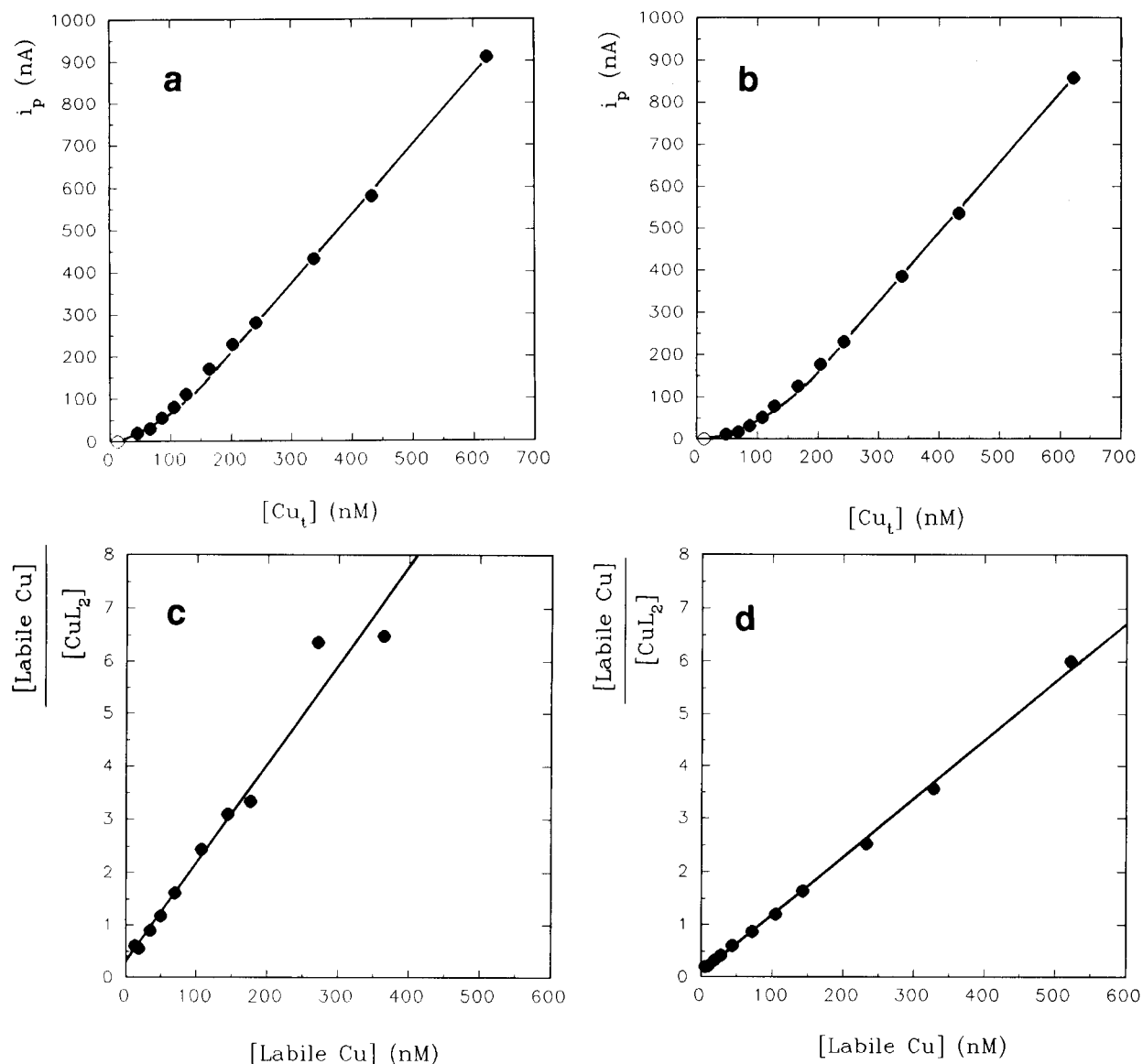
Fig. 4. Representative DPASV(HMDE) copper titration curves for SFB-1 (a) and SFB-2 (b); linearized titration curve for SFB-1 (c) and SFB-2 (d). Open circles in (a) and (b) represent the concentration of copper complexed by the strong ligand class, L_1, determined by CLE–CSV (8-HQ). Linear regression results for (c): slope = 0.0187, y-intercept = 0.333, r = 0.983; linear regression results for (d): slope = 0.0111, y = intercept = 0.0938, r = 0.999.

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data yielding ligand concentrations and conditional stability constants are presented in Fig. 4c and d. Results of the DPASV(HMDE) titrations of SFB-1 yielded a value for $[L_{2T}] = 47.0 \pm 0.6$ nM with an average conditional stability constant, $K'_{CuL_2} = 10^{9.1 \pm 0.1}$ M^{-1} (converted to $K'_{CuL_2,Cu^{2+}}$

by multiplying $K'_{CuL_2,Cu\text{-lab}}$ by $\alpha_{Cu'}$). The measured ambient concentration of Cu_{lab} on the zero addition aliquots was 12.4 nM. This value for $[Cu_{lab}]$ determined by DPASV(HMDE) is 36% higher than the value for $[Cu']$ determined by the DPASV(TMF-RGCDE) technique. This is con-
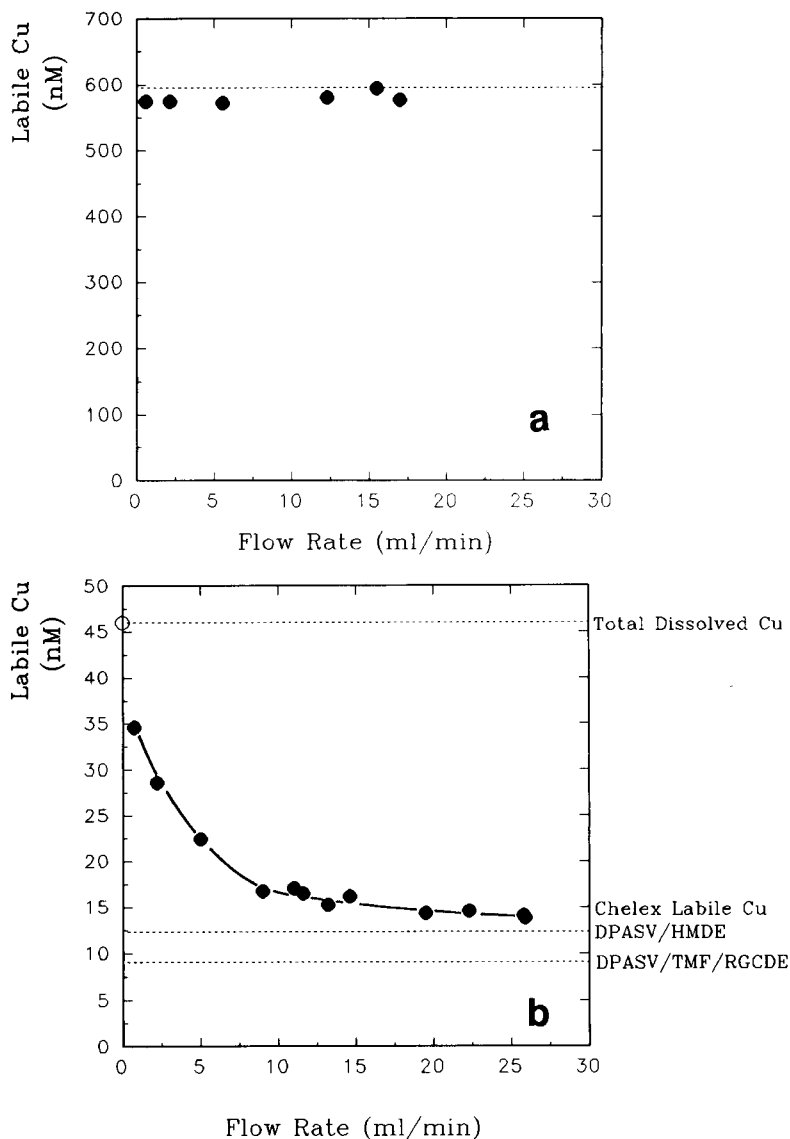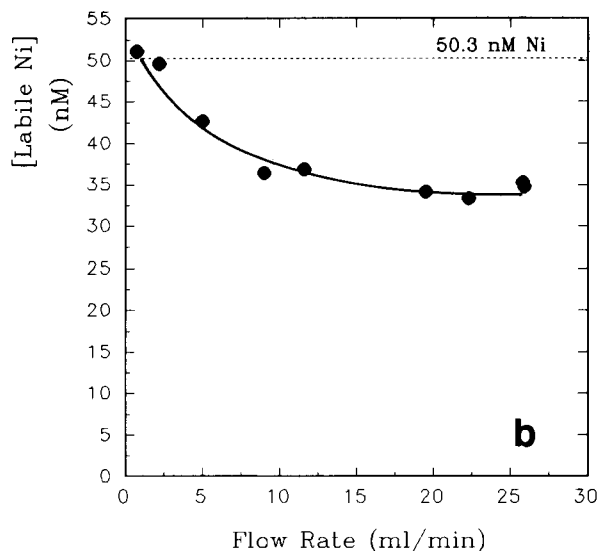

Fig. 5. Labile copper concentrations determined by CRCP–GFAAS as a function of sample flow-rate through the Chelex-100 column: (a) UVSW containing 595 nM copper; (b) SFB-1 sample.

sistent with the HMDE technique detecting a small amount of the relatively labile copper–organic ligand complexes (i.e., CuL_2).

The DPASV(HMDE) results from SFB-2 yielded a value for $[L_{2T}] = 90.5 \pm 0.6$ nM with an average conditional stability constant, $K'_{CuL_2} = 10^{9.4 \pm 0.1}$ M^{-1} (converted to $K'_{CuL_2,Cu^{2+}}$ by multiplying $K'_{CuL_2,Cu\text{-lab}}$ by $\alpha_{Cu'}$). The concentration of L_{2T} determined using the HMDE was 22% greater than that determined by the TMF-

Fig. 6. Representative CRCP–GFAAS copper titration curves for SFB-1 (a) and SFB-2 (b); linearized titration curve for SFB-1 (c) and SFB-2 (d). Open circles in (a) and (b) represent the concentration of copper complexed by the strong ligand class, L_1, determined by CLE–CSV (8-HQ). Linear regression results for (c): slope = 0.00961, y-intercept = 1.25, $r = 0.957$; linear regression results for (d): slope = 0.0129, y-intercept = 0.568, $r = 0.963$.

RGCDE, while the average conditional stability constant was slightly less ($10^{9.4 \pm 0.1}$ vs. $10^{9.6 \pm 0.2}$ M^{-1}). The ambient $[Cu_{lab}]$ measured was 6.8 ± 0.8 nM, a value 74% greater than the ambient $[Cu']$ detected by the TMF-RGCDE (3.9 ± 0.8 nM). Again, this higher value obtained with the HMDE approach is consistent with expectations due to the longer time scale of its measurement.

CRCP–GFAAS. For the Chelex-100 resin column partitioning approach, the flow-rate is the critical parameter that determines the time scale of the measurement. In order to decrease the contact time of the sample with the resin, the amount of Chelex-100 resin was decreased to approximately 2 ml volume in a 9 mm i.d. column, giving a column height of roughly 3 cm. This resin volume has adequate sites to complex all divalent trace metals in a sample, even those with the highest copper or nickel additions. Flow-rate experiments for copper are presented in Fig. 5a and b. Figure 5a demonstrates that for a UVSW sample spiked with a relatively high level of copper, the column efficiently removes the inorganic copper over a range of flow-rates up to 17 ml min^{-1}. Figure 5b presents the flow-rate dependence of the copper removal from SFB-1. The SFB-1 experiment shows that the labile copper retained by the resin column rapidly decreases with flow-rate towards an asymptotic labile copper concentration that is slightly greater than that determined by the DPASV techniques. This is consistent with the time scale of this method. For example, the contact time of the solution with the resin column at a flow rate of 15 ml min^{-1} is approximately 2.4 s. Thus, copper–organic complexes that dissociate during this contact time with the resin can be retained by the column. Copper–organic complexes inert to this technique would need a k_d less than 0.05 s^{-1}. This leads to a higher estimate of labile copper than determined by the other methods. Based upon the SFB-1 results, we selected a flow rate of 14–15 ml min^{-1} to use for the copper titrations carried out on the SFB-1, and -2 samples. A copper titration of UVSW by the CRCP–GFAAS method yielded a straight line having a slope of 0.875 and $r^2 = 0.996$ ($n = 9$). The slope of 0.875 provided an overall efficiency of the method of

87.5%, (which includes the isolation efficiency of Cu', any losses during the column rinsing steps and elution, and any slight matrix effects during GFAAS analysis). This overall efficiency was used to correct subsequent data sets. With this technique, a non-zero intercept representing an apparent 7.9 nM of copper complexation was observed in the titration of UVSW. This was not observed in the DPASV analyses of UVSW, and amounts to 10% or less of the levels of copper complexation determined in samples. We assume that this is at the detection limit of the approach and did not correct the ligand concentrations derived from the CRCP–GFAAS data for this apparent blank.

Representative titrations of SFB-1, and SFB-2 are presented in Fig. 6a and b. Linearizations of these titrations are presented in Fig. 6c and d. CRCP–GFAAS titration results for SFB-1 yielded $[L_{2T}] = 104$ nM, with $K'_{CuL_2} = 10^{8.0}$ M^{-1} (converted to $K'_{CuL_2,Cu^{2+}}$ by multiplying $K'_{CuL_2,Cu\text{-lab}}$ by $\alpha_{Cu'}$). The ambient labile copper concentration, 18.4 nM, measured by this method is twice that determined by the DPASV(TMF-RGCDE) method. Both the lower conditional stability constant and the higher labile copper concentrations are consistent with the results of the flow rate studies and expectations based upon the time scale of the measurement.

Titration results for SFB-2 yield $[L_{2T}] = 78.1$ nM and $K'_{CuL_2} = 10^{8.7}$ M^{-1} (converted to $K'_{CuL_2,Cu^{2+}}$ by multiplying $K'_{CuL_2,Cu\text{-lab}}$ by $\alpha_{Cu'}$). This weak ligand concentration is within 3% of that determined by the DPASV(TMF-RGCDE) method; however, the conditional stability constant is, once again, substantially lower than that determined by DPASV(TMF-RGCDE). The ambient labile copper concentration measured by this technique is 14.0 nM, 29% of the total dissolved copper. This labile copper concentration is, as expected, more than three times the $[Cu']$ determined by the DPASV(TMF-RGCDE) method and twice the $[Cu_{lab}]$ determined by DPASV(HMDE).

Dissolved nickel speciation

CLE–CSV. The CLE–CSV method, using dimethylglyoxime (DMG) as the added compet-
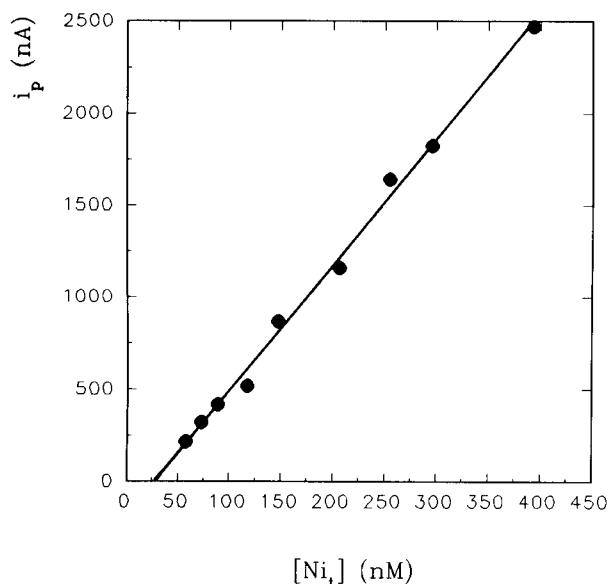
Fig. 7. Representative CLE–CSV nickel titration of a SFB-2 subsample using dimethylglyoxime as the added competing ligand. The x-intercept was determined by extrapolation of the best-fit line through the data shown in filled circles, and represents the nickel concentration (27.6 nM) bound by an extremely strong ligand class. Linear regression results: slope = 6.828; r = 0.997.

ing ligand, indicated the presence of a class of extremely strong nickel-complexing organic ligands at roughly one-third to one-half the concen-

tration of total dissolved nickel. For the SFB-1 sample, the titrations yielded a concentration of nickel complexed with this ligand ([NiL]) of 17.0 nM, with a conditional stability constant $K'_{NiL} \geq 10^{17.0}$ M^{-1}. For the SFB-2 sample (Fig. 7), the titrations yielded [NiL] = 27.6 nM, with $K'_{NiL} \geq 10^{17.9}$ M^{-1}. Due to the strong competition with the added competing ligand DMG (log $\alpha_{Ni-DMG} \approx 9.18$–9.20), this method did not detect the presence of any additional, weaker, class(es) of nickel complexing ligands. The ambient [Ni_{lab}] measured by CLE–DPCSV in the two samples was: SFB-1:[Ni_{lab}] = 33.3 nM; SFB-2:[Ni_{lab}] = 30. 1 nM. ([Ni_{lab}] includes both Ni' and any potentially labile organic nickel complexes.)
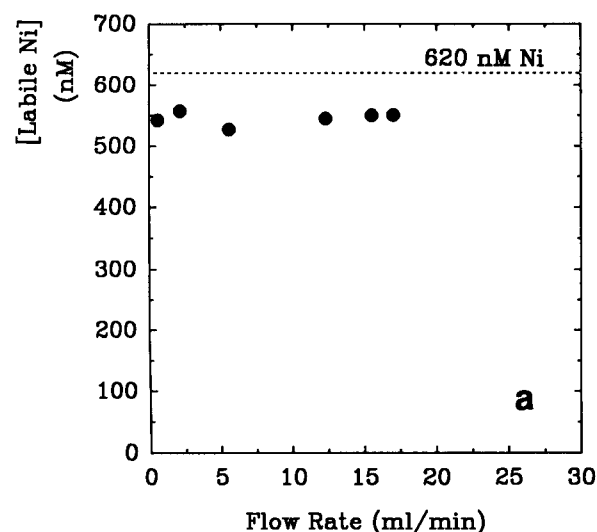
CRCP–GFAAS. Flow-rate dependence studies were carried out for nickel similar to those for copper. Figure 8a presents the results of a relatively high nickel addition to UVSW in order to examine the response to Ni'. The overall recovery efficiency of nickel in UVSW was 87.5%, a value similar to that observed for copper. The flow rate dependence of the SFB-1 sample shows the labile nickel decreasing with increased flow-rate to an asymptotic value of close to 32 nM. This is consistent with the existence of 25 nM of strongly bound NiL which is relatively inert with respect to the few seconds of contact time with the Chelex-100 resin column.

Fig. 8. Labile nickel concentrations determined by CRCP–GFAAS as a function of sample flow-rate through the Chelex-100 column: (a) UVSW containing 620 nM nickel; (b) SFB-1 sample.
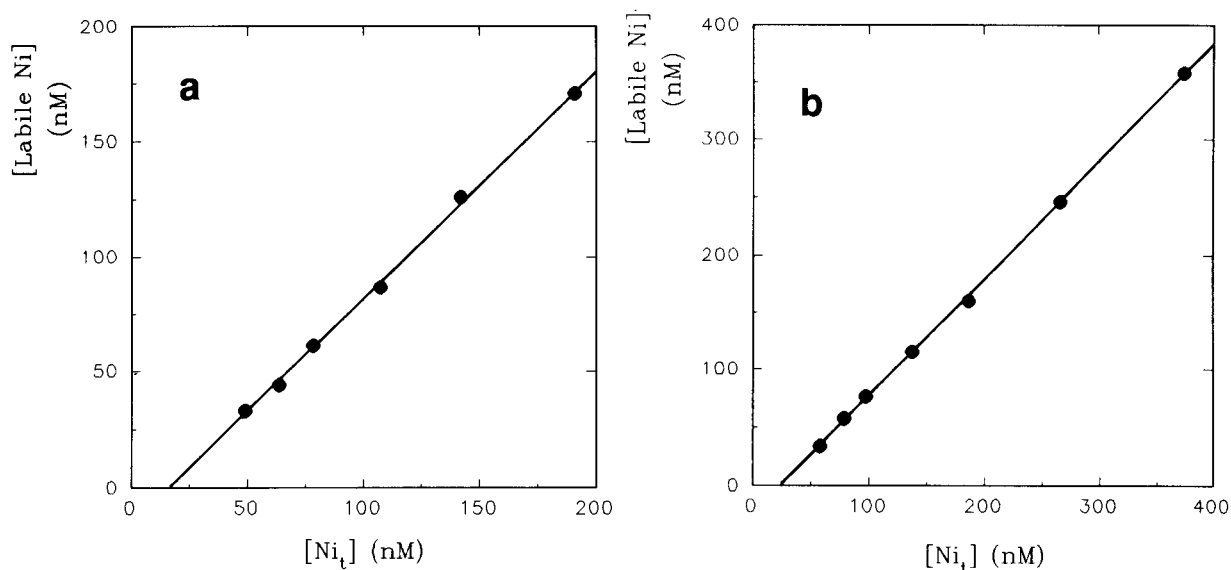
Fig. 9. Representative CRCP–GFAAS nickel titration curves for SFB-1 (a) and SFB-2 (b). Linear regression results for (a): slope = 0.979, x-intercept = 16.0 nM, r = 0.999; linear regression results for (b): slope = 1.017, x-intercept = 23.8 nM, r = 0.999. The x-intercepts represent the concentration of nickel bound by an extremely strong ligand class.

CRCP–GFAAS yielded results similar to those obtained by the CLE–CSV approach. The titrations are presented in Fig. 9a and b. Concentrations of the strong nickel-complexing organic ligand are: SFB-1, [NiL] = 16.0 nM; SFB-2 [NiL] = 23.3 nM. In each case the $K'_{NiL} \geq 10^{11}$ M^{-1}. The ligand concentrations were within 15% of those

determined by the CLE–CSV approach. This agreement indicates the presence of only one class of nickel-complexing ligands. Although this class of organic ligands forms extremely strong, inert nickel complexes, its concentrations are less than 50% of the total dissolved nickel. Thus, the ambient [Ni$_{lab}$] determined by CRCP–GFAAS in

TABLE 2

Comparison of analytical methods used to measure dissolved copper speciation in South San Francisco Bay

Analytical method	Basis for distinguishing species	Species reactivity			Comments
		Cu^{2+}, CuX [a]	CuL_1	CuL_2	
CLE–CSV(8-HQ)	Equilibrium competition with 8-HQ	Labile	CuL_1 and CuL_2 determinable if log $\alpha_{CuLi} \approx 3.5$–6.5		Provides best characterization of CuL_1; 8-HQ too strong to allow CLE–CSV to detect CuL_2
DPASV(TMF-RGCDE)	Kinetics of CuL_i dissociation	Labile	Inert	Inert for $k_d < 1$ s^{-1}	Best estimate of Cu'; differentiates Cu' from CuL_2
DPASV(HMDE)	Kinetics of CuL_i dissociation	Labile	Inert	Inert for $k_d < 0.1$ s^{-1}	Measures labile Cu; detects some CuL_2 complexes in addition to Cu'
CRCP–GFAAS	Kinetics of CuL_i dissociation and ligand exchange	Labile	Inert	Inert for $k_d < 0.05$ s^{-1}	Measures labile Cu; detects larger fraction of CuL_2 than the DPASV techniques

[a] CuX represents inorganic copper complexes.
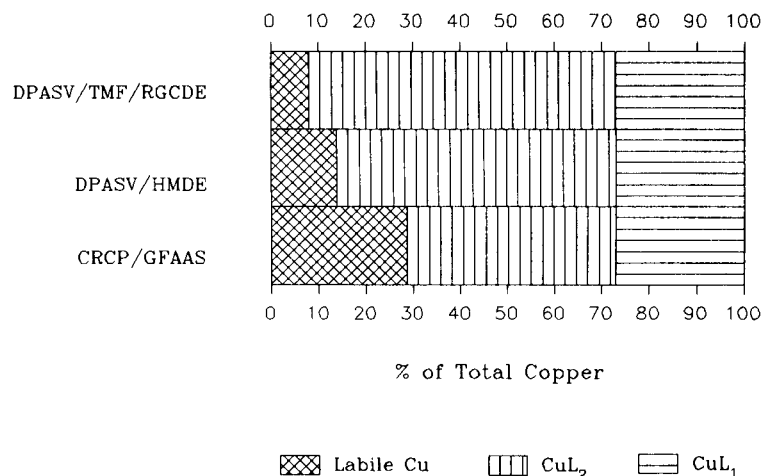
Fig. 10. The ambient dissolved copper speciation in South San Francisco Bay sample SFB-2 as measured by the various analytical methods. The [CuL$_1$] is that determined by CLE–CSV (8-HQ).

each of the two samples was: SFB-1 : [Ni$_{lab}$] = 33.0 nM; SFB-2 : [Ni$_{lab}$] = 34.2 nM.

DISCUSSION

The analytical methods utilized in this study were each useful in providing insight into the chemical speciation of copper in South San Francisco Bay. More importantly, the use of these various analytical methods in concert allowed a more comprehensive characterization of the speciation of dissolved copper and nickel in South San Francisco Bay than that provided by any single method alone. The analytical methods and the respective forms of copper that each detects as labile are presented in Table 2.

The CLE–CSV method determined the concentration of the stronger copper-complexing ligand class L$_1$. [L$_1$] could only have been roughly estimated by the other analytical methods, primarily because L$_1$ was already completely titrated by the ambient concentration of dissolved copper. CLE–CSV using 8-HQ can characterize L$_T$ and K'_{CuL} for copper–organic complexes (CuL) whose α_{CuL} value (i.e., $1 + (K'_{CuL}[L'])$, where [L'] = L$_T$ − [CuL]) lies within 1–2 decades above and below α_{Cu-8HQ} (ca. 9.3 × 10^4). Weaker organic ligands (i.e., those whose $\alpha_{ML} \ll \alpha_{Cu-8HQ}$) are out-

competed by 8-HQ, and the copper originally complexed by them would be detected as if it had been free. For organic ligands forming extremely strong copper complexes (i.e., those whose $\alpha_{CuL} \gg \alpha_{Cu-8HQ}$), estimates of L$_T$ can be made along with a minimum estimate of K'_{CuL}. CLE–CSV using 8-HQ could only provide a lower limit estimate for K'_{CuL_1} ($K'_{CuL_1} \geq 10^{13.5}$) because L$_1$ is so strong and because the concentration of L$_1$ was so low compared to the ambient copper. The value of α_{CuL_2} ($\approx 3 \times 10^2$) for the weaker class of ligands (L$_2$) detected by the other methods is too low to allow characterization of CuL$_2$ by CLE–CSV using 8-HQ, which has an α_{Cu-8HQ} value $\approx 9.3 \times 10^4$.

Although DPASV(TMF-RGCDE) could not provide a precise determination of the concentration and conditional stability constant of CuL$_1$ for the reasons given above, the DPASV(TMF-RGCDE) titration results were consistent with the existence of a strong copper-complexing ligand of the concentration and strength of L$_1$. Due to the longer time scale of its measurement, the DPASV(HMDE) technique determined a labile copper concentration that includes not only the inorganic species, [Cu'], but also a small portion of the relatively labile CuL$_2$ species. The CRCP–GFAAS approach measured the highest concentration of labile copper because the analyt-

TABLE 3

Comparison of copper- and nickel-complexing ligand concentrations and conditional stability constants reported for estuarine and coastal surface waters

Sampling location	Depth (m)	Total dissolved Cu or Ni (nM)	$[L_{1T}]$ (nM)	$[L_{2T}]$ (nM)	$\log K_1$	$\log K_2$	%Organic Cu or Ni	Method	Ref.
Copper									
South San Francisco Bay	Surface	45–48	13.0	60–80	>13.5	9.0–9.6	80–92	CLE(8-HQ)–CSV DPASV(TMF-RGCDE)	This paper
North Sea	32	3.2	16.2	ND [a]	12.4	–	≈ 100	CLE(tropolone)–CSV	[14]
North Carolina Shelf	Surface	2.8	3.3	26	13.2	10.0	> 99	CLE(EDTA)– chemiluminescence	[34]
Severn River Estuary, UK	Surface	6.3–76.4	13–196	ND	11.4–12.7	–	> 99	CLE(catechol)–CSV	[35]
Biscayne Bay	Surface	3.5	5.1	110	12.0	10.5	> 99	CLE(Acac)–solv. extr.– GFAAS	[11]
Coastal Peru	5	3.7	3.8	75	12.4	9.3	98	CLE(EDTA)–C_{18} Sep-Pak	[36]
US Northeast Coast	15–20	5.9	20	50	11.7	9.1	> 99	Fixed potential amperometry	[37]
Irish Sea	Surface	28	ND	97	–	10.2	98	MnO_2 equil.	[38]
Nickel									
South San Francisco Bay	Surface	50–58	17–28	ND	>17	–	35–50	CLE(DMG)–CSV CRCP–GFAAS	This paper
Liverpool Bay, UK	Surface	6–22	0.3–6.4	ND	17.7–18.7	–	30–40	CLE(DMG)–CSV	[18]
Menai Strait, UK	Surface	12	5.3	ND	17.8	–	40	CLE(DMG)–CSV	[16]
English Channel	40	3.7	1.8	ND	17.3	–	50	CLE(DMG)–CSV	[16]

[a] ND = Not detected.

ical time scale of its measurement is the longest, being set by the long contact time of the sample with the resin (i.e. 2–3 s). This period of time greatly increases the possibility that a larger fraction of the more labile CuL_2 complexes may dissociate and contribute to the labile copper measured by this method.

The DPASV(TMF-RGCDE) technique has the shortest analytical time scale of the three techniques [DPASV(TMF-RGCDE), DPASV-(HMDE), and CRCP–GFAAS] able to characterize L_2. Thus, of these three techniques, DPASV-(TMF-RGCDE) provides the most exact separation between dissolved inorganic copper (Cu') and organically-complexed copper (CuL_1 and CuL_2), and, therefore, DPASV(TMF-RGCDE) yields the best estimate of [Cu']. The Cu' concentrations estimated by DPASV(HMDE) and CRCP–GFAAS both include a fraction of CuL_2 and should be referred to as "labile copper" (Cu_{lab}, expressed with respect to the appropriate method).

The copper speciation determined by each of the techniques in this study for the SFB-2 sample is presented in Fig. 10. Viewed in combination, the results from these various analytical methods used in concert provide a more comprehensive characterization of the speciation of dissolved copper and nickel in South San Francisco Bay than could have been provided by any one method. Labile copper varies from 8% of the total dissolved copper measured by DPASV-(TMF-RGCDE), to 28% measured by CRCP–GFAAS. Conversely, "inert" organically complexed copper varies from 92% of the total dissolved copper as determined by the DPASV-(TMF-RGCDE) method, to 71% of total dissolved copper as determined by CRCP–GFAAS.

L_1, the strong class of copper-complexing ligands, is completely titrated at about one-third of the ambient dissolved copper. At copper concentrations near or just above ambient levels, the weaker class of copper-complexing ligands is primarily influencing the speciation of copper. At ambient conditions, Cu' comprises 8% of the total dissolved copper, with 27% existing as CuL_1 and the remaining 65% existing as CuL_2. The inorganic side reaction coefficient for copper,

$\alpha_{Cu'}$, under the ambient pH, temperature, and salinity conditions of SFB-2 was 21 (i.e., [Cu']/[Cu^{2+}] = 21). Thus, the free copper (0.2 nM) was 0.4% of the total dissolved copper. A copper speciation model based upon the copper titration results showed that as copper is added to the SFB-2 sample, excess L_2 is titrated, and additions in excess of approximately 94 nM are no longer substantially influenced by organic complexation. Any additional copper increases only [Cu']. However, at small additions near ambient levels the excess L_2' acts to "buffer" [Cu'] and thus [Cu^{2+}].

Copper-complexing ligand concentrations and conditional stability constants have been determined in different estuarine and coastal waters by different investigators using various analytical methods. The copper-complexing ligand concentrations and conditional stability constants determined in this study for South San Francisco Bay are consistent with previously reported values (Table 3). Concentrations of the stronger ligand L_1 in estuarine and coastal waters range from 3 to 200 nM with log K_1 values averaging about 12.5, and concentrations of the weaker ligand L_2 range from 26 to 110 nM with log K_2 values averaging about 9.7.

Our determinations of the relative concentrations of L_1 and L_2 differ from those reported previously. In previous studies (see Table 3), L_1 has been found at concentrations exceeding the ambient total dissolved copper concentration, causing L_1 to have the predominant impact on the speciation of total dissolved copper. In contrast, we determined concentrations of L_1 much lower (ca. 30%) than the ambient total dissolved copper concentration, causing L_1 to play a minor role in the speciation of dissolved copper in South San Francisco Bay. Instead, we found that L_2 has a greater influence on total dissolved copper speciation (see Fig. 10). Although K'_{CuL_1} is about 10^4 times greater than K'_{CuL_2}, the concentration of L_2 is about 5 times greater than that of L_1, and exceeds the total dissolved copper concentration by 10–25 nM. While L_1 complexes at most about 28% of the total dissolved copper, L_2 complexes 52–65%. However, despite the much greater fraction of total dissolved copper complexed by L_2,
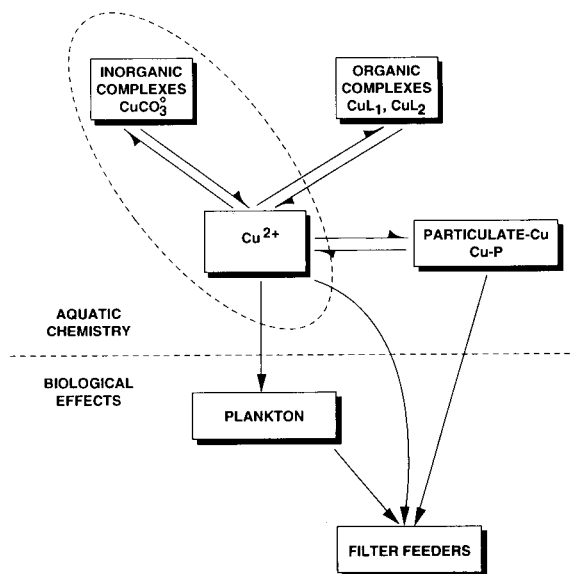
Fig. 11. Conceptual model of the relationship between the aquatic chemistry of copper and potential biological effects.

only 80–92% of the total dissolved copper was organically complexed by both ligands; previous studies of coastal and estuarine waters reported $\geq 98\%$ organically-complexed copper. Thus, the lower concentration of L_1 relative to that of total dissolved copper not only allowed L_2 to play a greater role in the speciation of total dissolved copper, but also caused organic complexation to be slightly less important to copper speciation in South San Francisco Bay than in other areas where the concentration of L_1 exceeded total dissolved copper.

A simple conceptual model of the aquatic chemistry and biological influence of copper is presented in Fig. 11. Hydrated Cu^{2+} is in equilibrium with its inorganic complexes, organic complexes, and particulate forms. $[Cu^{2+}]$ is thought to control copper assimilation and toxicity to plankton. Complexation with two classes of organic ligands, L_1 and L_2, has the greatest control on $[Cu^{2+}]$. Organically-complexed copper is generally thought to be biologically unavailable, and thus lowers the toxicity of a given concentration of total dissolved copper. Filter feeders can as-

similate copper from living plankton and non-living particles, as well as from solution.

The chemical speciation of nickel in South San Francisco Bay appears to be simpler than that for copper. The CLE–CSV method determined the presence of an extremely strong class of nickel-complexing ligands present at concentrations comprising from 35 to 50% of the total dissolved nickel. Thus, this ligand class was completely titrated by the ambient nickel concentrations. The CRCP–GFAAS method determined the same concentration of an extremely inert nickel–organic complex, and found no evidence for a weaker class of nickel-complexing ligands. The nickel complex conditional stability constant determined in this study for South San Francisco Bay is consistent with values previously reported for other estuarine and coastal surface waters (Table 3). The concentrations of the nickel-complexing ligand in South San Francisco Bay is greater than that reported for the other locations where nickel complexation has been studied, and may reflect the much higher total dissolved nickel concentration.

The extremely strong class of nickel-complexing ligands detected in South San Francisco Bay complexes essentially all of the nickel up to concentrations of 24 nM. At the ambient total dissolved nickel concentration of 57.7 nM, $[Ni_{lab}] = 34.1$ nM. Any nickel additions to the sample above the ambient concentration add to the Ni_{lab} pool, because no excess nickel-complexing ligand remains. For the pH and salinity of the SFB-2 sample, $[Ni^{2+}]$ is 65% of $[Ni']$. Assuming that Ni_{lab} is primarily Ni', the ambient $[Ni^{2+}]$ would be 22 nM. The conceptual model for copper presented in Fig. 11 is also relevant for nickel, with the exception that no evidence exists for a second class of organic nickel-complexing ligands. Thus, in contrast to copper, South San Francisco Bay appears to have no natural buffering capacity for nickel near ambient concentrations.

The nickel speciation results obtained from CLE–CSV and CRCP–GFAAS do not appear to be completely consistent. The CRCP–GFAAS flow-rate studies showed that NiL is labile at slower sample flow-rates through the Chelex-100 resin. However, the magnitude of the conditional

stability constant yielded by the CLE–CSV titration data ($K'_{NiL} > 10^{17}$ M^{-1}) suggests that NiL should be inert with respect to Chelex-100 at any flow-rate. This inconsistency deserves further study.

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Determination of copper speciation in marine waters by competitive ligand equilibration/liquid–liquid extraction: an evaluation of the technique

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Abstract

A technique for determining organic complexation of copper using a competitive ligand equilibration coupled with liquid–liquid extraction was evaluated with respect to its applicability in marine waters. Particular attention was paid to the equilibration times required, extractability of naturally occurring organic copper complexes, and consistency with results from electrochemical techniques. In general, it was found that although this approach produces results that agree well with those from other analyses of the open ocean, there are unresolved discrepancies when it is applied to copper analyses in estuarine samples. The ability to use the technique for analysis of a single sample at different competition strengths, or analytical windows, was also examined. In open ocean waters, it was possible to identify two discrete ligand classes with three different windows, although the determined extent of binding did increase with increasing competition strength.

Keywords: Atomic absorption spectrometry; Stripping voltammetry; Copper; Metal speciation; Sea water; Solvent extraction

The study of chemical speciation involves identification of the various forms of an element within a system. For a given oxidation state of a metal cation such as copper(II) in natural waters, the likely species will include the "free" hydrated ion, relatively weak and labile inorganic complexes, generally stronger and more inert organic complexes, and particulate forms associated with colloids and suspended particles, either surface adsorbed or within the lattice. Among the truly dissolved forms in sea water, thermodynamic calculations have been successful in identifying the important complexes with major inorganic anions

[1,2]. Although it is known that organic complexation of metal cations may influence steps in their biogeochemical cycles, including biological assimilation and adsorption onto particles, e.g. [3,4], we are just beginning to understand the organic speciation of metals in marine waters. Recent data suggest that for at least a few metals, very strong organic complexes with unidentified ligands existing at low concentrations can be significant or even dominant [5–11]. Although important advances have been made in the field, critical evaluation of existing techniques followed by their extensive application to the systematic study of the oceans is still necessary.

A number of analytical techniques which identify some part of the organically complexed frac-

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tion of metals in natural waters have been reported over the last decade [6,12–18]. Two fundamentally different approaches have been used to determine metal–organic ligand complexation, depending on the extent of sample manipulation. One of these approaches, utilized by several techniques, is based upon the direct detection of a metal species under ambient conditions, or with minimal sample perturbation. If the dissolved speciation of a generic metal, M^{n+}, can be represented by

$$M_T = M^{n+} + MX_i + ML_i \tag{1}$$

where naturally occurring ligands are represented by X_i (inorganic) and L_i (organic), these techniques determine M^{n+} [13], M' (where $[M'] = [M^{n+}] + [MX_i]$) [14–16], or ML_i [18]. Such direct techniques are often limited by the low concentrations at which many metals occur, particularly in sea water. Total metal concentrations can approach the detection limits of available instrumentation, and speciation analyses require the ability to determine just a fraction of that.

The second approach used to examine trace metal speciation involves a controlled and well-characterized competition for the metal between the native ligands and an added metal binding agent which also allows concentration of the sample, thus circumventing detection limitations [6,12]. In these techniques, the sample is perturbed by the addition of a competing ligand, A, and allowed to come to equilibrium before analysis of the metal in the competing complex, MA. Theoretically, any method that will quantify MA could serve as the final detection step. Both adsorptive cathodic stripping voltammetry (ACSV) [6] and liquid–liquid extraction coupled with graphite furnace atomic absorption spectrometry (GFAAS) [12] have been used as concentration/detection systems in these techniques. Titration with the metal allows determination of the natural ligand concentration(s), $[L_i]$, and the conditional stability constant(s) at the ionic strength and major ion composition of the sample, K'_{cond}, for the natural complex(es). With this information, the original unperturbed speciation can be calculated.

Recent work by van den Berg et al. [19,20] has

shown that the degree of complexation determined can be strongly influenced by the analytical technique used. Each technique can identify only a limited range (or window) of ligand binding. For the natural ligand, the extent of binding can be defined as the product of the excess ligand concentration (that fraction not bound by the metal) and the stability constant of its complex with the metal,

$$\alpha_{CuL} = [ML]/[M^{n+}] = [L']K'_{cond} \tag{2}$$

In the direct techniques, such as anodic stripping voltammetry, one limit for the determination of $[ML]/[M^{n+}]$ is set by the sensitivity and detection limit (e.g. the smallest quantity of $[M']$ observable) and the other by the precision and accuracy ($[ML_i]$ is determined from $[M_T] - [M']$). If the extent of organic complexation (Eqn. 2) is too large, $[M']$ falls below the detection limit for the technique, and if $[ML]/[M']$ is too small, it is not possible to distinguish between the presence or absence of the organic ligand. With competitive techniques, if either the natural or the competing ligand system overwhelmingly dominates, the natural ligand cannot be conclusively identified. When ML predominates, the detection limit for MA precludes analysis at low metal additions (below [L]) and only the ligand concentration can be determined, not K'_{cond}. Conversely, excessive competition by the added ligand can draw all of the metal out of the natural complexes and the titration behaves as though the native ligand is not present.

If there were only one ligand present, it would be possible to choose a technique with a suitable window and proceed. In a sample where more than one ligand class or even a continuum of binding sites exits, however, a single analytical window can give an incomplete, and often misleading, picture of the metal speciation. Therefore, in order to gain a realistic picture of the in situ speciation, either multiple techniques or those that can be modified easily to examine a number of detection windows are needed.

Although a number of techniques are available to study copper complexation, examination of copper speciation by new techniques can prove useful in evaluating their suitability to environ-

mental analyses. In this study, we have taken a closer look at a method for copper speciation reported by Moffett and Zika [12] with a view to eventually applying the approach to the analyses of other metals. The Moffett and Zika technique involves a competitive equilibration of the sample with acetylacetonate followed by extraction of the copper diacetylacetone complex and GFAAS of copper associated with the organic phase. The concentration of the natural copper-binding ligands and the stabilities of their complexes can be identified from titrations with copper. With the multi-element flexibility of GFAAS, this approach can potentially be adapted to study a wide range of metals, using a variety of competing ligands and modifying the concentration factors as necessary. Also, because this is a competitive equilibration technique, the lability of the metal complexes is less likely to influence the determined stability constants, provided sufficient time is allowed for the sample to reach equilibrium during the analysis. We have examined the applicability of the copper technique to both open ocean and estuarine samples, particularly with regards to equilibration kinetics, extractability of the naturally occurring organic complexes, and consistency with results from electrochemical analyses.

The competitive ligand equilibration/liquid–liquid (solvent) extraction (CLE–SE) approach also has a great capacity for modifying the detection window by changing both the added ligand concentration and the solvent ratio. We took advantage of this potential, examined a range of analytical windows, and attempted to distinguish between discrete ligand classes and a continuum of binding sites in both central Pacific and San Francisco Bay samples.

THEORY

The competitive ligand equilibration/solvent extraction (CLE–SE) technique for copper involves the addition of acetylacetone (acac) and toluene to a sample which is allowed to come to equilibrium before the phases are separated. Ideally, the acac is added at a concentration that establishes a competition between the added and natural ligands, rather than forcing complexation and extraction of all the Cu(II) present. The neutrally charged, dissolved copper diacetylacetonate complex, $Cu(acac)_2$, is partitioned between the water and toluene, while other copper species ideally remain in the aqueous phase. The sea water phase is discarded, and the copper in the toluene is quantitatively back extracted into dilute nitric acid. Ultimately, Cu in the acid fraction is determined by GFAAS.

The equilibrium speciation in the perturbed sample may be represented by

$$[Cu_T] = [Cu^{2+}] + [CuX_i] + [CuL_i]$$
$$+ [Cuacac^+] + [Cu(acac)_2]$$
$$+ [Cu^*] \qquad (3)$$

where the inorganic complexes are given by CuX_i, CuL_i are the naturally occurring organic complexes strong enough to compete with the acac, and $[Cu^*]$ the quantity of $Cu(acac)_2$ removed from the aqueous sample into the toluene. The values of $[Cu_T]$ and $[Cu^*]$ are independently measured and, in theory, the remaining quantities in Eqn. 3 can be calculated using the equations in Table 1.

A titration curve is generated by incrementally increasing the total metal concentration, and the speciation is calculated at each point. As shown in Fig. 1, the signal response is linear after the natural ligand is fully saturated, and extrapola-

TABLE 1

Equations for calculating speciation in a perturbed sample from one analysis

($V_x \equiv$ volume of the phase x, $K_{d(Cu)} \equiv$ distribution coefficient of $Cu(acac)_2$, $\beta_2 \equiv$ formation constant for $Cu(acac)_2$, $K_1 \equiv$ formation constant for $Cuacac^+$, $\alpha_{Cu'} \equiv$ inorganic side reaction coefficient for copper in sea water [21], $[acac^-]$ calculated from total [acac] and side reactions with major cations, $[Cu_T]$ from an independent analysis)

1	$[Cu^*] = [Cu]_{acid} V_{acid} / V_{aq}$
2	$[Cu(acac)_2] = [Cu^*] V_{aq} / (K_{d(Cu)} V_{org})$
3	$[Cu^{2+}] = [Cu(acac)_2] / (\beta_2 [acac^-]^2)$
4	$[Cuacac^+] = K_1 [Cu^{2+}][acac^-]$
5	$[CuX_i] = \alpha_{Cu'} [Cu^{2+}]$
6	$[CuL_i] = [Cu_T] - \{[Cu^{2+}] + [CuX_i] + [Cuacac^+]$
	$\quad + [Cu(acac)_2] + [Cu^*]\}$
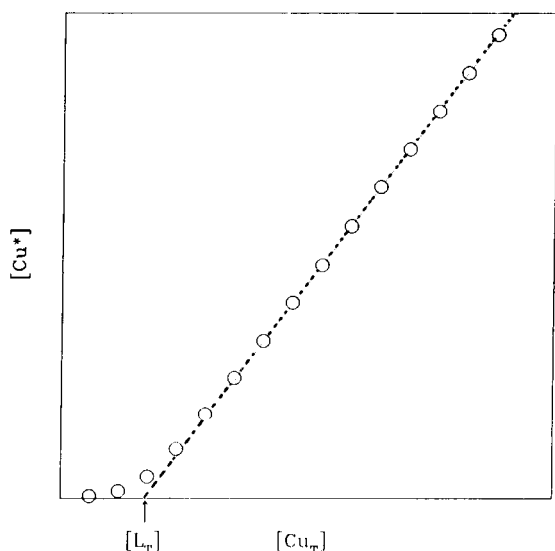
Fig. 1. Hypothetical copper titration of a sample containing an Cu-binding organic ligand. After the natural ligand is fully saturated, the copper signal, [Cu*], increases linearly with total copper (dashed line).

tion of this line to the abscissa provides an estimate of the total concentration of that ligand, [L_T]. In addition, the extent to which the data deviate from the line at low metal additions gives an idea of the strength of the natural ligand in relation to the competing ligand. If the natural ligand were much stronger than the added ligand, it would not be possible to detect a signal until the total metal concentration exceeded [L_T], whereas if the opposite were true (the added ligand outcompeting the natural one), the data would only asymptotically approach the dashed line at high copper additions [9]. More precise determinations of the concentrations of natural organic ligand classes, [L_i], and their conditional stability constants with respect to Cu^{2+}, K'_{cond}, can be determined through use of Scatchard [22] or Langmuir (applied to dissolved trace metal speciation by both Ruzic [23] and van den Berg [17] in 1982) linearizations of the data once [Cu^{2+}] and [CuL_i] have been calculated for each point.

Unfortunately, uncertainties in the conditional stability constants for the acac complexes with copper and the major cations in sea water as well as the distribution coefficients for their partitioning between sea water and toluene can induce

errors in the estimation of CuL_i at each titration point. In order to circumvent this problem, we chose to utilize an internal calibration, analogous to that used in cathodic stripping voltammetry [6,9], where the slope of the titration line at high metal addition points is used to provide a measure of the combined constants in the calculations. In the absence of a natural organic ligand, the speciation in the analysis is given by

$$[Cu_T] = [Cu^{2+}] + [CuX_i] + [Cuacac^+]$$
$$+ [Cu(acac)_2] + [Cu^*] \qquad (4)$$

or in terms of side reaction coefficients

$$[Cu_T]/[Cu^{2+}] = 1 + \alpha_{Cu'} + \alpha_{Cuacac}$$
$$+ \alpha_{Cu(acac)_2} + \alpha_{Cu^*} \qquad (5)$$

where

$$\alpha_{Cu'} = [CuX_i]/[Cu^{2+}] \qquad (6)$$

$$\alpha_{Cuacac} = [Cuacac^+]/[Cu^{2+}] \qquad (7)$$

$$\alpha_{Cu(acac)_2} = [Cu(acac)_2]/[Cu^{2+}] \qquad (8)$$

$$\alpha_{Cu^*} = [Cu^*]/[Cu^{2+}] = K_{d(Cu)}V_r\alpha_{Cu(acac)_2} \qquad (9)$$

and V_r is the ratio of toluene to sample volume. Rearranging Eqn. 5 and substituting [Cu^*]/α_{Cu^*} for [Cu^{2+}] yields

$$[Cu^*] = \alpha_{Cu^*}[Cu_T]/[1 + \alpha_{Cu'}$$
$$+ \alpha_{Cuacac} + \alpha_{Cu(acac)_2} + \alpha_{Cu^*}] \qquad (10)$$

The slope of this line, or a plot of [Cu^*] versus [Cu_T], is the same as that of the titration curve after all natural ligands present have been fully titrated (dashed line in Fig. 1) and is given by

$$S = \alpha_{Cu^*}/[1 + \alpha_{Cu'} + \alpha_{Cuacac} + \alpha_{Cu(acac)_2} + \alpha_{Cu^*}] \qquad (11)$$

In the presence of a natural organic ligand, the speciation is that in Eqn. 3 which can be solved for [CuL_i] using α-coefficients to give

$$[CuL_i] = [Cu_T] - [Cu^*][1 + \alpha_{Cu'} + \alpha_{Cuacac}$$
$$+ \alpha_{Cu(acac)_2} + \alpha_{Cu^*}]/\alpha_{Cu^*} \qquad (12)$$

or more simply, in terms of the calibration slope, S,

$$[CuL_i] = [Cu_T] - [Cu^*]/S \qquad (13)$$

Solving for $[Cu^{2+}]$, the other quantity necessary to linearize the titration data, is somewhat more complicated. Rearranging Eqn. 9, we get

$$[Cu^{2+}] = [Cu^*]/K_{d(Cu)}V_r\alpha_{Cu(acac)_2} \qquad (14)$$

Determination of this value requires the actual calculation of $\alpha_{Cu(acac)_2}$ and the direct invocation of the uncertain constants in Table 1. In order to do this, we used the experimental calibration slope and literature values for all the stability constants to estimate the distribution coefficient of the acetylacetonate complex. That value was calculated from the calibration slope using Eqn. 11 after substituting from Eqn. 9,

$$K_{d(Cu)} = S\left[1 + \alpha_{Cu'} + \alpha_{Cuacac} + \alpha_{Cu(acac)_2}\right]$$
$$/V_r\alpha_{Cu(acac)_2}(1 - S) \qquad (15)$$

The quantities α_{Cuacac} and $\alpha_{Cu(acac)_2}$ were calculated from 4 and 3 in Table 1, respectively,

$$\alpha_{Cuacac} = K_1[acac^-] \qquad (16)$$

$$\alpha_{Cu(acac)_2} = \beta_2[acac^-]^2 \qquad (17)$$

It is important to note that this calculation may not be an accurate determination of the distribution coefficient, $K_{d(Cu)}$, for $Cu(acac)_2$ between sea water and toluene. Rather, it is a calibration accounting for the errors in all of the constants. The correction was made to only $K_{d(Cu)}$ for simplicity, but K_1, β_2, or any of the other constants could also have been adjusted. Since this is an internal calibration applied to each and every titration, it can effectively account for the variations between samples (i.e. potential salinity and surfactant differences) as well as the uncertainties in the thermodynamic constants. Estimates of K_d carried out in this manner varied between 3 and 26, with an average of 7. Stary and Liljenzin's review of acetylacetone constants report a best value of 7.08 for K_d [24], and Moffett and Zika determined a distribution coefficient of 6.2 in artificial sea water [12].

The internal calibration approach used in this study is limited by the assumption that all of the ligands are fully titrated at the high metal addition points. Great care must also be taken in choosing the calibration line as the final results are strongly dependent on the slope of that line.

Moffett and Zika [12] calibrated the technique in UV-oxidized sea water using NTA and EDTA as model ligands. Because copper binding by these ligands is relatively weak in a sea water matrix, large concentrations were needed and full titrations were not practical. In that study, $\alpha_{acac^-} = [acac_T]/[acac^-]$ was determined from the distribution of copper between the model ligand and acetylacetonate. That experimentally determined value of α_{acac^-} was applied to actual titrations in natural water samples with calculations analagous to those in Table 1. Using a K_d of 6.2 for the copper–acetylacetonate complex, they found α_{acac^-} to be $(2.1 \pm 0.1) \times 10^2$. In the absence of the model organic ligands, α_{acac^-} was much more variable, underscoring the limitation of applying a single calibration to different samples. Here, we used literature values for acetylacetonate binding with major cations and protons [12,24–26], including Moffett and Zika's determination of K_{Mgacac^+}, as well as the distribution of Hacac between water and toluene [24] to calculate an α_{acac^-} value of 2.2×10^2 at pH 8.1, in excellent agreement with the previous study.

The constants used in this study were taken from the literature [12,24–26] and corrected where necessary to sea water ionic strength using the Davies approximation. The inorganic side reaction coefficients for copper at the experimental pH values were calculated using Titrator [27] and constants from Smith and Martell [28]. An $\alpha_{Cu'}$ of 33 was calulated at pH 8.10.

Both Scatchard and Langmuir linearizations were used in this study, since each has limitations when applied to scattered data. Averages of the results from each linearization technique were used whenever possible. The Langmuir linearization sometimes gave a negative intercept, thereby not allowing an estimate of the stability constant. In such cases, only the Scatchard linearizations were used.

EXPERIMENTAL

All analyses were conducted in class 100 clean areas. Separatory funnels and reagent bottles were initially cleaned by soaking for at least one

week each in 6 M HCl, 7.5 M HNO_3, and aqua regia (reagent grade) and sat for at least a month filled with dilute quartz distilled nitric acid (Q-HNO_3) before use. Reagents were made up in quartz distilled water which was also used for all rinses with the exception of extractions performed at sea which used water purified in a Millipore ion exchange system (Milli-Q water). Copper standards were made from Baker aqueous atomic spectral standards, HPLC-grade toluene (Fisher) was redistilled in a quartz sub-boiling still, and Aldrich gold-label acetylacetone and diethylenetriaminepentaacetic acid (DTPA; G. Frederick Smith) were used without further purification.

In each extraction, a known quantity of sample was added to a Teflon separatory funnel with the appropriate amount of additional copper, shaken for 1 min, and allowed to sit for at least 15 min. Acetylacetone and toluene were added and the sample was equilibrated with intermittent shaking. Sample and toluene volumes, as well as acac concentrations and maximum total copper added are shown for each titration in Table 2. The aqueous phase was drained and disposed of after

its pH was determined. The organic phase was back-extracted twice into 2 ml aliquots of 1 M HNO_3 and discarded. The combined acid fractions from each sample were evaporated to dryness in quartz beakers and redissolved in about 1.5 ml 1 M HNO_3 as an additional concentration step. Copper was determined in the final acid fraction by standard additions using GFAAS. The manufacturer's recommended program for copper analysis and graphite tubes with L'vov platforms in a Perkin-Elmer 5000 atomic absorption spectrometer interfaced with an HGA-500 graphite furnace and an AS-40 autosampler were used.

In the model ligand titrations, UV-oxidized sea water, free of trace metals and copper-complexing organic ligands (UVSW) [29] was used to provide a sea water medium. The desired quantity of DTPA was added as a model natural ligand to each UV-sea water aliquot at the same time as the copper.

The quantity of natural hydrophobic copper complexes in each sample was determined by extracting aliquots without acetylacetone, and the amount of copper determined in the organic frac-

TABLE 2

Experimental parameters for each copper speciation titration

(The center of the analytical window, $\alpha_{Cuacact} = \alpha_{Cuacac} + \alpha_{Cu(acac)_2} + \alpha_{Cu*} = [Cuacac_T]/[Cu^{2+}]$. $[Cuacac_T]$ denotes all forms of copper bound by acetylacetonate, in both the aqueous and organic phases)

Sample	Aqueous (ml)	Toluene (ml)	$[acac_T]$ (μM)	Final $[Cu_T]$ (nM)	$\alpha_{Cuacact}$
DTPA titrations					
1	200	30	300	52	2 000
2	200	30	300	51	2 000
3	150	20	300	101	2 000
San Francisco Bay					
1990	150	20	300	200	1 000
1991	200	40	1 000	230	10 000
			140	450	500
Sargasso Sea	200	20	300	200	1 000
Central Pacific					
20°S	250	25	1 000	20	9 000
			150	50	500
			20	90	20
0°	250	25	1 000	20	10 000
			150	50	400
			20	90	20
15°N	250	25	1 000	20	9 000
			150	50	400

tion was subtracted from the extracted copper signal, [Cu*], in the titration calculations. Procedural blanks were less than 1 nM for the open ocean and UVSW titrations and less than 5 nM for the San Francisco Bay titrations. These blank values were determined by subjecting Milli-Q water to the full extraction procedure and added to the known total copper in the speciation calculations.

Total copper in the San Francisco Bay samples was determined by the pyrollidine dithiocarbamate–diethylolithiocarbamate extraction method while those from the central Pacific were analyzed by Chelex-100 preconcentration [30]. The Sargasso Sea sample was analyzed for total copper by CSV after UV-oxidation [31].

Samples

Estuarine samples were collected at a depth of 1 m from the South San Francisco Bay in September of 1990 and October of 1991 in the central channel, just south of the Dumbarton bridge. As a region, South San Francisco Bay is highly contaminated with trace metals, including Cu [32]. In contrast, samples were also taken from the Sargasso Sea in April of 1989 (32°N, 64°W at 90 m) and the central Pacific in August of 1991 (135°W, 20°S at 50 m; 145°W, 0° at 40 m; 153°W, 15°N at 30 m). These were open ocean waters and include a station in the high nutrient region of the equatorial Pacific as well as three central gyre locations. All samples were collected cleanly and immediately filtered through 0.4-μm polycarbonate membrane filters (open ocean) or 0.45-μm filter cartridges (San Francisco Bay). The Sargasso Sea and 1990 San Francisco Bay samples were stored frozen prior to analysis, but those from the central Pacific were analysed at sea within three days of collection, and the 1991 San Francisco Bay sample was immediately taken back to UCSC, stored at 4°C in the dark, and analysed that same week.

RESULTS AND DISCUSSION

Model titrations

In order to evaluate the ability of CLE–SE to characterize the complexation of copper by or-
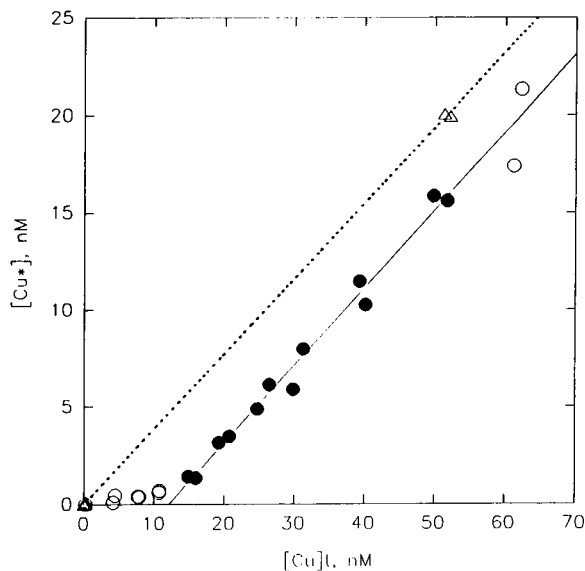
Fig. 2. Copper titration of UVSW with DTPA (○, slope = 0.399, intercept = −4.83, open data points not included in regression) and without DTPA (△, slope = 0.386, intercept = 0.00179). [DTPA]$_T$ = 14 nM. Log K'_{cond} = 12.7.

ganic ligands in sea water, model titrations were performed on DTPA in UV-oxidized sea water. DTPA was chosen as a model ligand because it forms well documented complexes with copper that do not extract appreciably into toluene and are about the same strength as the natural ligands previously reported for copper in sea water [33]. The predicted conditional formation constant for CuDTPA complexes in sea water with respect to Cu^{2+}, including side reactions with major cations, was log K'_{cond} = 12.7, calculated using Titrator [27] and constants from the literature [28,34–37], corrected to sea water ionic strength.

An example of the DTPA titrations is shown in Fig. 2, and the results from all three titrations are compiled in Table 3. The model titrations produced good estimates of the DTPA concentrations added (within 25%) and values of log K'_{cond} between 12.1 and 12.7 (within a factor of log 4 of the predicted value). In these relatively well characterized systems, the slope of the titration line at high copper concentrations does reach that of the line generated without the model ligand. When titrations were performed on actual

TABLE 3

Results from model titrations of DTPA in sea water

(Predicted log K'_{cond} = 12.7, from Titrator [26])

Titration	added [L] (nM)	[L] (nM)	log K'_{cond}	Calibration slopes	
				with DTPA	without DTPA
1	14.0	13	12.7	0.40	0.39
2	9.85	12	12.1	0.39	0.38
3	29.1	30	12.2	0.45	0.43

samples using the same conditions of acac concentration and solvent ratio as in UVSW titrations, the final slopes obtained from the natural samples were always somewhat lower (0.32 in the San Francisco Bay in 1990 and 0.21 in the Sargasso Sea compared to 0.41 ± 0.03 in UVSW). This observation indicated that either there was a large concentration of a relatively weak ligand that was not fully titrated, or some other component of the natural samples (absent from the UVSW) slightly decreases the extent to which $Cu(acac)_2$ is extracted.

Kinetics

Successful utilization of CLE–SE for the thermodynamic characterization of naturally occurring organic ligands in marine waters requires that the natural copper–organic complexes be allowed to come to equilibrium with the acac and toluene before the phases are separated. The assumption that the sample reaches equilibrium during the analysis was checked by allowing otherwise identical aliquots to sit for varying lengths of time (with intermittent shaking) after the addition of Cu(II), acac, and toluene. In the San Francisco Bay, equilibration times up to eight hours were examined (Fig. 3a). No discernable systematic change could be seen in the signal after 1 h, and the mean of all eight data points had a relative standard deviation of 12%. It was possible that the samples were reaching only a pseudo-eqilibrium, and that longer equilibrations would have illuminated a systematic variablility. If strong ligands that were kinetically inert on timescales of a day were present, they would not be discernable after only an eight hour equilibration, but could still be geochemically important [38].
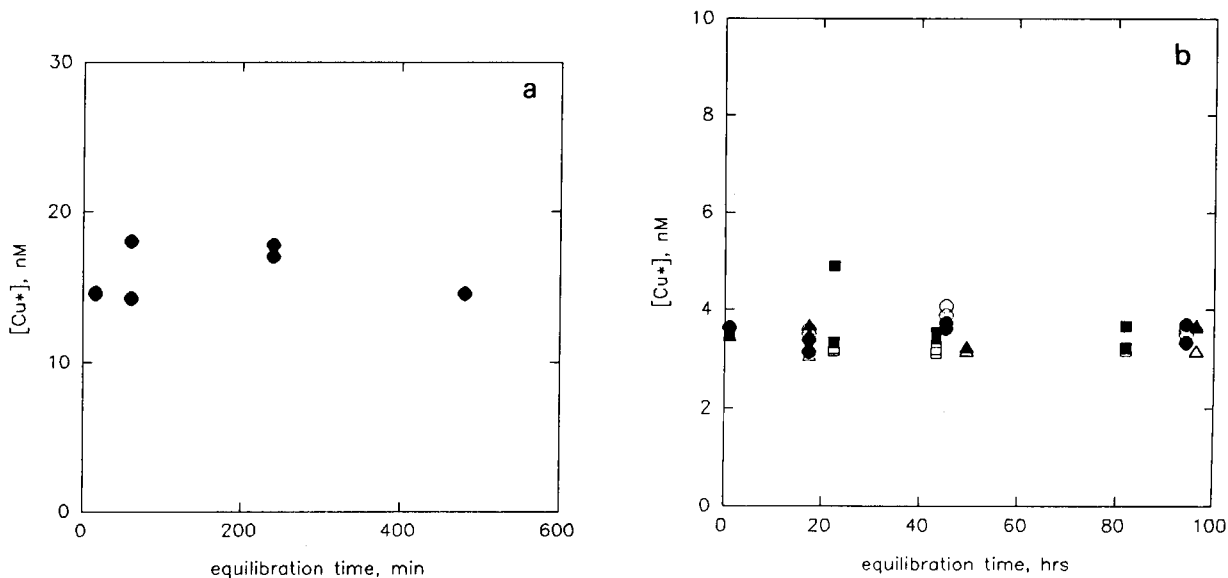
Fig. 3. Kinetic experiments on samples from (a) the San Francisco Bay ($[Cu_T]$ = 30 nM) and (b) the central Pacific ($[Cu_T]$ = 10 nM): (●) 20° S; (▲) 0°; (■) 15°N; open symbols in (b) represent hour-long control equilibrations.

Therefore, samples from the central Pacific were equilibrated for up to four days (Fig. 3b). Control extractions with hour-long equilibrations were conducted when the long-term extractions were completed. These controls indicated that significant speciation changes, independent of the analyses, were not occurring in the samples during the longer equilibrations. The values of [Cu*] determined in this experiment were identical within a relative standard deviation (R.S.D.) of 9% and demonstrate that the natural copper–organic complexes are labile on timescales of an hour and that the experimental protocol outlined above gives sufficient time for the samples to reach at least a psuedo-equilibrium that persists for several days. There still remains a possibility

that ligands may be present that would be significant at true equilibrium but are inert on timescales up to a week. However, when such long timescales come into play, it is doubtful that a true equilibrium would be representative of the important processes occurring in natural waters such as the open ocean and estuaries.

Extractability of complexes occurring in the environment

The amount of copper in natural organic complexes that extracts into toluene in the absence of acetylacetone varied between samples and was 2–7% of the total Cu. In general, the open ocean waters had a small fraction of directly extractable copper, consistent with what was seen by Donat

TABLE 4

Ligand concentrations and conditional stability constants from copper speciation titrations of marine samples

($\alpha_{CuA} \equiv$ Center of the detection window. Primary data are available from the authors. Referenced results are from analyses of the same samples by ASV and CSV)

	[Cu]t (nM)	α_{CuA}	[L] (nM)	Log K'_{cond}	α_{CuL} [a]	Ref.
Single windows						
Sargasso Sea	1.16	1 000	0.8	≥ 14.2	–	This work
		90 000	0.88 [L1]	14.0	–	CSV [40]
		–	7.4 [L2]	9.8	40	ASV [40]
San Francisco Bay						
1990	50.7	1 000	7×10 [L1]	11.6	8 000	This work
			1×10^2 [L2]	9.6	200	This work
		10^6	10 [L1]	14	–	CSV [41]
		–	20 [L1]	≥ 11	–	ASV [41]
			65 [L2]	9	10	ASV [41]
Multiple windows						
Central Pacific						
20°S	0.242	9 000	3	13.3	60 000	This work
		500	3.3	12.74	20 000	This work
		20	3	≥ 11.6	≥ 1 000	This work
0°	0.370	10 000	2.2 [L1]	13.4	50 000	This work
		400	3 [L1]	12.2	4 000	This work
			10 [L2]	10.8	600	This work
		20	15 [L1] + [L2]	10.2	200	This work
15°N	0.315	9 000	1.0	≥ 15.0	≥ 700 000	This work
		400	4	≥ 12.3	≥ 7 000	This work
San Francisco Bay						
1991	49.0	10 000	4×10	≥ 13.4	–	This work
		500	119	10.67	3 000	This work
		90 000	13.2 [L1]	≥ 13.5	–	CSV [31]
		–	74 [L2]	9.6	100	ASV [31]

[a] When [Cu_T] > [LT], α_{CuL} cannot be calculated as defined here.

et al. [39] using C_{18} solid phase extraction. The gyre stations averaged 2% extractable, while in the equatorial Pacific sample, 7% of the copper extracted into toluene without acetylacetone, indicating a possibly greater preponderance of hydrophobic ligands in the equatorial waters. In the autumn samples from the San Francisco Bay, approximately 4% of the total copper was directly extractable. This is a smaller fraction than that found by C_{18} solid phase extraction in Narragansett Bay on the east coast of North America, where values ranging from 8 to 56% of the total copper were isolated on the resin [18]. At this point, the source of this difference cannot be distinguished between effects of the type of binding material in the San Francisco and Narragansett Bays or the hydrophobicity of the different extraction media.

It is also possible that this naturally extractable fraction does not represent copper binding in hydrophobic complexes, rather it may be indicative of inefficient phase separation. At this stage, it is difficult to determine which is the correct interpretation. If the extracted copper were due to physical entrainment of water in the organic phase, more variablity within titrations and less between samples would be expected. In addition, although extractable copper was observed in UVSW, it was near the detection limit and much lower than the amount seen in the actual samples. On the other hand, if there were an actual hydrophobic class of copper complexes present, the extractable fraction would be expected to level off eventually at high total copper concentrations. Regardless, it is essential to perform extractions of each sample without acac at Cu(II) concentrations covering the range of the titrations in order to accurately correct the titration data.

Single window titrations of marine samples

Initially, two samples, one from the San Francisco Bay and the other from the Sargasso Sea, were examined by single titrations at one analytical window. Table 4 gives the results from these titrations. Because of the large sample volumes required for analysis, replicate titrations were not performed. However, it was possible to estimate

the uncertainties associated with the linearizations and, thereby, the determined ligand concentrations and stability constants. At the 95% confidence interval, the uncertainties were within 40% for [L] and 1 order of magnitude for K'_{cond}. These are maximum uncertainties which apply to all the values reported in Table 4.

For both the Sargasso Sea and the Fall 1990 San Francisco Bay samples, the analytical window was centered at $\alpha_{Cuacact} = 1000$, where $\alpha_{Cuacact}$ is defined as in Table 2. The results from the Sargasso Sea by CLE–SE are fully consistent with those by cathodic and anodic stripping voltammetry (CSV and ASV). Both CLE–SE and CSV were able to identify the same ligand class, although the former technique established a weaker competition, and we were only able to determine a minimum value for the stability constant. On the other hand, α_{CuL_2} observed by ASV is definitely below the window of the CLE–SE technique, and L_2 was outcompeted by the acac–toluene extraction system and could not be recognized by the analysis. In general, natural ligands with α_{CuL} within an order of magnitude in either direction of the center of an analytical window [19] can be identified by that window.

The San Francisco Bay data, however, are more difficult to reconcile with the results from ASV and CSV. The ligand class determined by CSV is too strong to have been resolved by CLE–SE in this analysis. Although the stability constants determined here agree well with those found by ASV, the ligand concentrations from CLE–SE are larger than those from the ASV analyses by factors of 2–3.

ASV can underestimate the ligand concentrations and stability constants if the metal–organic complexes are labile on the timescale of their residence in the diffusion layer about the electrode [33]. The ASV analyses of the San Francisco Bay sample from 1990 utilized a thin mercury film-rotating glassy carbon disc electrode at 5000 rpm, giving a residence time in the diffusion layer of about 10 ms. Copper complexes with dissociation rate constants, k_d, less than 1 s^{-1} will be inert on this timescale [31]. Copper binding material with k_d up to 2 s^{-1} has been identified in coastal waters [42], and if such complexes

were present in our samples, they could be making a small contribution to the ASV copper signal and causing a minor underestimation of the ligand concentrations and/or stability constants.

There are two other possible explanations for the lack of agreement between CLE–SE and electrochemical techniques in the San Francisco Bay. It is conceivable that the former approach cannot be applied to the study of estuarine samples. Surface active materials could influence the extraction of $Cu(acac)_2$ despite the small quantity of directly extractable CuL complexes detected. In addition, the more complex composition of the estuarine waters could lead to the formation of mixed ligand complexes with acac that may or may not extract, giving an apparently lower or
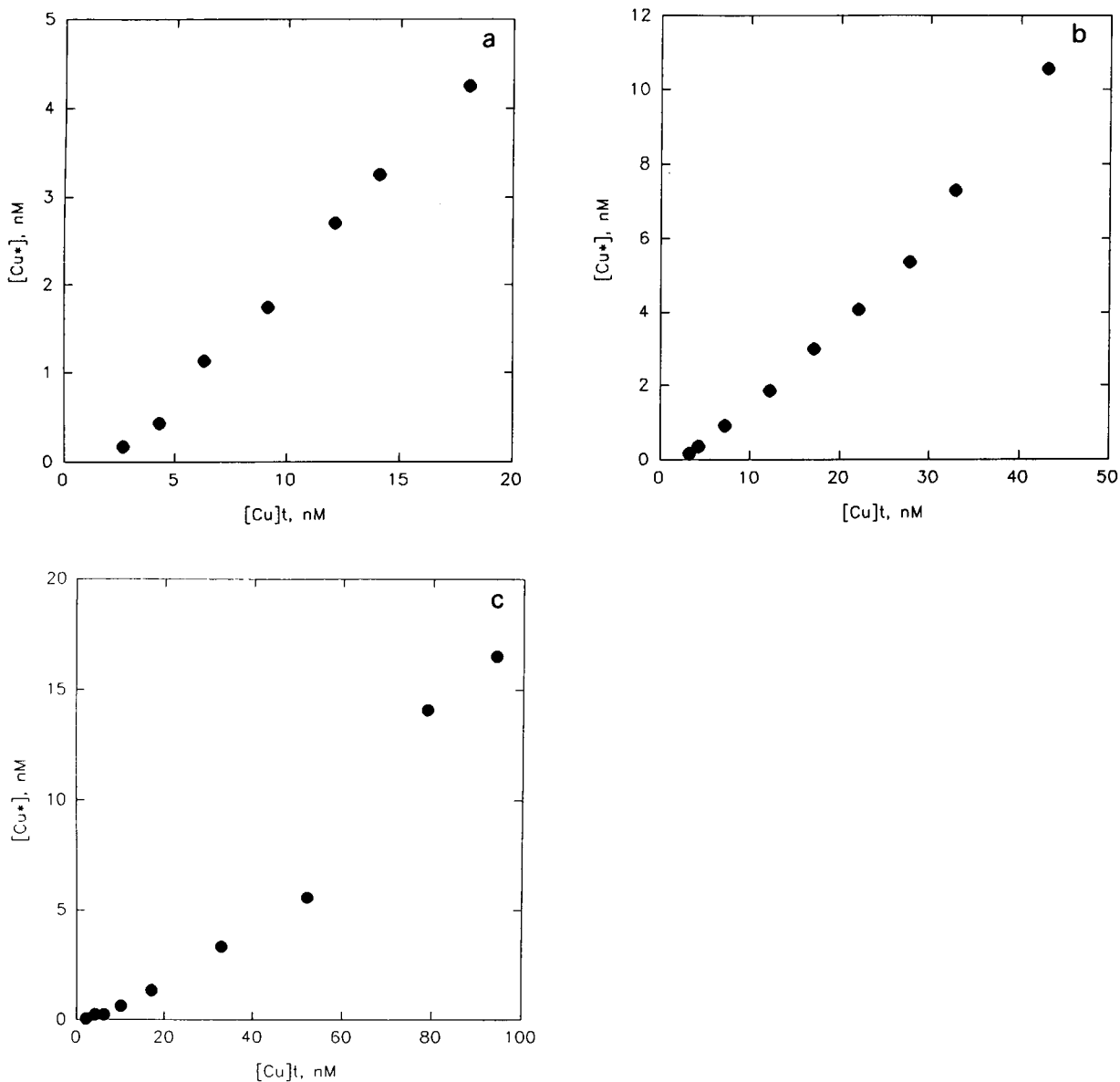
Fig. 4. Titrations of the equatorial Pacific sample at three different analytical windows. (a) $\alpha_{Cuacact} = 10\,000$. (b) $\alpha_{Cuacact} = 400$. (c) $\alpha_{Cuacact} = 20$. Note different axis scales.

higher binding capacity, respectively. Another possibility is that we are simply seeing the results of applying analytical techniques at varying detection windows to a sample with a complex mixture or even a continuum of binding sites.

Multiple window titrations

Results from the examination of both open ocean and San Francisco Bay samples with more than one detection window are shown in Table 4, along with the single window analyses. As an example, titration curves at the three analytical windows for the equatorial Pacific sample are shown in Fig. 4. Analyses of the open ocean samples were internally consistent, indicating the presence of only 1 or 2 distinct ligand classes in the range of windows examined ($20 \leq \alpha_{Cuacact} \leq 10000$). In all three samples, a strong ligand (log $K'_{cond} \geq 13$) at a concentration of about 3 nM was indicated. At the equator, an additional ligand was seen at a concentration of 10 nM with a weaker binding constant of nearly 10^{11} M^{-1}.

In view of the central Pacific data, it is apparently possible to identify a single ligand class in natural waters, even with different analytical windows. Within the uncertainties of the method,

ligands with the same characteristics are identified with windows centered at values of $\alpha_{Cuacact}$ differing by orders of magnitude. Even in samples where it was only possible to determine a minimum limit for the stability constant (because the window was too weak to fully characterize it), that minimum is consistent with the values determined using stronger windows.

On the other hand, two titrations of a San Francisco Bay sample (in fall of 1991) by CLE–SE, in which $\alpha_{Cuacact}$ differed by nearly two orders of magnitude, gave disparate results which also contradicted those from two other analytical techniques [31]. As shown in Table 4, the two CLE–SE titrations produced evidence of two different ligand classes, a relatively strong ligand at a concentration lower than [Cu$_T$] and a weaker one at a higher concentration. If taken alone, these results would indicate that there were either two different ligands present, or we were simply observing different parts of a binding continuum. However, when the results from analyses of the same samples by other techniques are also considered, it becomes clear that the interpretation cannot be so simple. A study using CSV and ASV [31] identified two distinct ligand classes, a very low
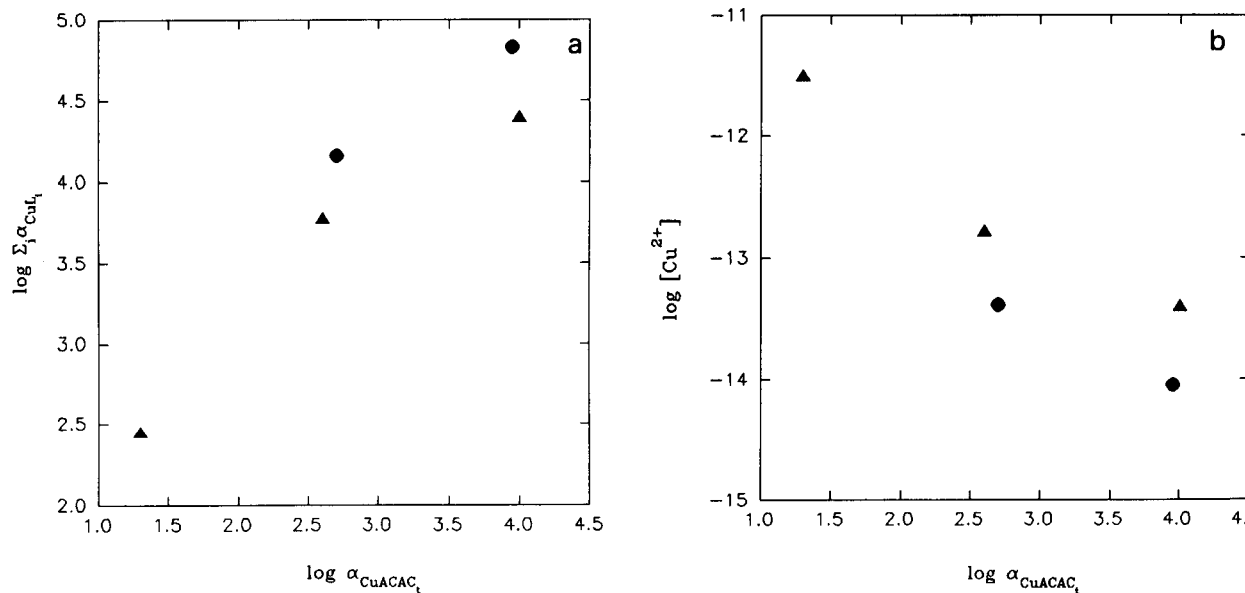
Fig. 5. Dependence of binding extent (a) and log[Cu^{2+}] (b) on analytical window in south (●) and equatorial (▲) Pacific Ocean samples as determined by CLE–SE. α_{Cuacac} gives the center of the analytical window. Log $\Sigma_i \alpha_{CuL_i}$ and free [Cu^{2+}] were calculated by modelling the original speciation from the data in Table 4 for each window.

concentration of a strong ligand and a weaker ligand at a higher concentration with $\alpha_{CuL} = 100$ (Table 4). Both of these ligand classes are at lower concentrations and, with the exception of the CSV results, form weaker copper complexes than those found by CLE–SE. The different results cannot be attributed solely to the use of varying analytical detection windows. Although CLE–SE and CSV examined very different windows in the 1990 sample, the windows used for the 1991 study were quite similar, within an order of magnitude of each other. At this point, we cannot positively identify the source of the discrepancy in the CLE–SE San Francisco Bay results, but it is clear that there remain some unresolved questions in applying this technique to the study of copper speciation in estuarine waters.

An important point to be made from the multiple window titrations is that the determined speciation does vary with the detection window, even when the same ligand class is identified. The dependence of the estimated copper speciation on the location of the detection window is shown graphically in Fig. 5. The ordinate in Fig. 5a

$$\sum_i \alpha_{CuL_i} = \sum_i [CuL_i] / [Cu^{2+}] \qquad (19)$$

and $[Cu^{2+}]$ in Fig. 5b were determined by modeling the initial copper speciation in the unperturbed sample using Titrator [27] and the characteristics for each window shown in Table 4 (those determined by CLE–SE, only). Only titrations from the southern and equatorial Pacific, those where both [L] and log K'_{cond} could be determined, were used to generate these plots. It is evident from Fig. 5 that weaker analytical windows generally result in the determination of a lower total extent of binding and higher $[Cu^{2+}]$.

In the South Pacific where only one ligand class could be identified by the two titrations, it is certainly possible to explain the decrease in calculated $[Cu^{2+}]$ as indicative of a much more complex ligand mixture, and we may be calculating average ligand concentrations and stability constants for the range detectable by the windows we used. It seems more likely, however, in view of the similarity between the ligand parameters determined with the two windows, that the ligand is

actually best described as a class with some narrow distribution of stability constants, and each window emphasizes either the lower or stronger stability side of the distribution.

A similar explanation can be applied to the results from the equatorial Pacific where two ligands were determined by three analytical windows. Here, the influence of the detection window on the calculated free copper concentration is particlularly strong because the strongest and weakest windows were each only able to identify one of the ligands. In other words, since the stronger ligand class could not be seen conclusively in the weak titration (Fig. 4c), the $[Cu^{2+}]$ determined from those results was high, and vice versa for the strong titration.

These observations emphasize the importance of conducting multiple titrations at varying windows if one wishes to realistically model the in situ speciation. Using only one titration to extrapolate the original speciation could prove misleading, particularly in complex samples.

Conclusions

In this study, we have carefully evaluated the suitability of competitive equilibration coupled with solvent extraction to determine the extent of copper–organic binding in marine waters. A fundamental requirement for the use of CLE–SE is that only a small fraction, and preferably none, of the natural organic complexes extract into the solvent used. We found that 2–7% of the natural complexes extract into toluene, regardless of where the sample originated. Although this fraction cannot be characterized by CLE–SE, it is small enough that the data can be corrected, allowing the determination of the dominant hydrophilic copper complexes. In addition, within 1 h, the analytically perturbed system reaches at least a pseudo-equilibrium that is significant on timescales of a day to a week.

Analysis of samples that were also examined by ASV and CSV gave results that were consistent with those from the electrochemical techniques in the Sargasso Sea. However, our speciation results from the San Francisco Bay were irreconcilable with those from other techniques. Although only a small fraction of the natural

organic copper extracted into toluene, there could have been some other phenomenon, such as mixed ligand complexes or surface active materials interfering with the solvent exchange.

Because CLE–SE combines a competitive equilibration with a liquid–liquid extraction, it can be used to examine analytical windows varying over at least three orders of magnitude without changing the experimental protocol or the reagents used. In samples from the central Pacific, it was possible to identify a distinct ligand class with more than one window. However, examination of stronger detection windows results in the determination of higher total complexation. This is not inconsistent with the identification of discrete ligand classes, but it does recommend caution when modelling speciation in natural waters with the results from only one titration.

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Platinum analysis and speciation in urban gullypots

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Abstract

Concentrations of platinum in the < 63-μm fraction of some Swedish road sediments have been shown to increase from 3.0 ng g^{-1} in 1984 to 8.9 ng g^{-1} in 1991. Road sediments contained 39–88% more Pt than gullypot sediments and sequential extraction showed a distinct shift from predominantly inorganic Pt on the road surface to wholly organic Pt in the gullypot. Dissolved Pt concentrations in disturbed gullypot liquor were within the range 1.7 ng l^{-1} to 3.8 ng l^{-1} and are explained by bacterial action in the gullypot sediment mobilizing organically bound dissolved Pt forms.

Keywords: Digestion techniques; Voltammetry; Catalysts; Dry ashing; Platinum

The number of automobiles equipped with catalytic converters is steadily increasing. In 1989 autocatalysts constituted 42%, 8% and 81% of total world demand for Pt, Pd and Rh, respectively [1]. Manufacturers of modern three-way (i.e. removing hydrocarbons, carbon monoxide and nitrogen oxides together) catalysts currently favour Pt and Rh as the active metals in a ratio of 5:1. Already catalytic converters are unequivocably the prime source of Pt to road surface sediments [2], albeit in low concentrations. Pt emissions from automobiles are due to the abrasion of the catalytic surface, the emitted particles having a median size of 5–10 μm with an estimated emission rate of 2–40 ng km^{-1} [3]. A comparison of Pt concentrations in size-fractionated road sediments, collected in 1984 and 1991, has shown average increases in all the fractions, but particularly in the < 63-μm fraction with an increase from 3.0 to 8.9 ng g^{-1} [2].

The low total concentrations of Pt found in road sediments require a sensitivity of analysis at the lower ng l^{-1} level and this can be achieved by using a highly sensitive voltammetric method [4,5]. Pt is quantified after the catalytic reduction of protons by either the Pt–formazone or the Pt–ethylenediamine complex preadsorbed to a mercury drop electrode [6]. The results obtained by this method (adsorptive voltammetry) are comparable to those obtained by inductively coupled plasma-mass spectrometry, at least for Pt in blood [5].

The adsorptive voltammetric method is highly sensitive for Pt but is negatively affected by even the smallest residual trace of organic material in samples, with extinction of the hydrogen wave [7]. Although aqua regia digestion [8] and ultraviolet irradiation [6] have been tested previously, it is our experience that dry ashing is the most reliable digestion procedure for the total destruction of our highly organic road sediment and gullypot (roadside catchbasin) samples. This agrees with the findings of other research groups [7].

In this article we report on the analysis of dissolved/suspended solid associated and solid phase fractionated Pt in road sediments, gullypot

liquor and gullypot sediment. Gullypots have been shown previously to act as an accumulative chamber for heavy metals from road runoff and provide a significant contribution to the end-of-pipe "first flush" effect found under wet-weather conditions [9].

EXPERIMENTAL

Sampling

Road dusts were collected by means of a wet/dry vacuum cleaner for a four-month period (June to September) at two-week intervals in 1984 and again in 1991. The samples were collected from an asphalt parking area at Chalmers University of Technology with a catchment area of 390 m^2. Two representative sub-areas on the catchment were sampled. The first was an 11.7-m^2 car parking lot and the second a 215 cm long roadside kerb of area 0.65 m^2. After collection

the road dusts were air dried, weighed and sieved prior to analysis.

The gullypots, chosen to represent different degrees of road usage by automobiles, were sampled on two occasions (A and B in Table 1). During A (29-04-1992) the gullypot was being washed by rainfall with a total of 11.4 mm for the day and 0.8, 25.2, 4.8, 8.4 and 5 mm on the previous five days. On occasion B (26-05-1992) an antecedent dry period of 14 days was recorded. On both occasions samples of undisturbed gullypot liquor were taken in a 500-ml polyethylene bottle secured to an extended stainless steel holder. On occasion A the undisturbed sample was supplemented by a disturbed sample where the gullypot contents were first vigorously stirred with a stainless steel rod. This action was meant to simulate the effect of a high-intensity summer rainfall, which typically mobilizes the gullypot contents [9].

On return to the laboratory, samples were

TABLE 1

Gullypot sample characteristics

Sampling site	Sample no.	Sampling occasion	Status [a]	Suspended solids (g l^{-1})	pH
Residential	1	A	U	0.51	7.5
			D	20.7	
	2	B	U	1.31	7.0
Parking area	3	B	U	0.04	6.5
	4	A	U	0.17	6.5
			D	62.2	
		B	U	0.4	7.0
	5	A	U	0.31	6.4
		B	U	0.19	7.0
Roundabout	6	A	U	0.09	7.0
			D	22.4	7.0
		B	U	0.63	7.0
Dual carriageway	7	A	U	1.37	7.4
			D	35.6	
		B	U	0.19	7.2
	8	A	D	57.4	7.1
	9	B	U	–	7.8
	10	A	U	0.036	7.5
			D	20.5	
		B	U	1.21	7.1
Motorway, E20	11	B	U	2.03	7.0

[a] D = disturbed; U = undisturbed.

analysed for pH and suspended solids concentration. The latter analysis was by filtration through a GF/C glass fibre filter (Whatman) and drying to constant weight. Sub-samples were filtered through a 0.45-μm cellulose acetate filter (Sartorius) and the solids dried at 105°C. All water samples were kept at 4°C before analysis.

Platinum determination

Either a dried sediment sample (25–30 mg) or a water sample (10–20 ml) was transferred to a silica crucible. A volume of 600 μl of 15 M HNO_3 was added and the crucible placed in a muffle furnace with a stepwise program to 800°C [2,5].

When cool, the ashed sample was dissolved in aqua regia (1.5 ml of 12 M HCl and 0.5 ml of 15 M HNO_3), left to stand for 4 h and taken just to dryness on a hot plate. A volume of 0.6 ml of 12 M HCl, 8.6 ml of ultrapure water, 0.4 ml of 0.4% hydrazine and 0.4 ml of 3.2% formaldehyde were added to the sample crucible. The crucible was placed directly in the voltammetric cell (Metrohm 647 VA stand). The sample was deaerated with nitrogen for 5 min and preelectrolysed at −800 mV vs. Ag/AgCl for 180 s. A glassy carbon (instead of the more common platinum) auxiliary electrode was used. The stirring was stopped for 10 s and finally cathodic stripping was recorded in the differential-pulse mode with the following instrumental settings (Metrohm 646 VA processor): modulation amplitude, 25 mV; pulse repetition rate, 200 ms; effective scan rate, 20 mV s^{-1}. Pt concentration was determined by comparison with a Pt standard curve and confirmed by standard addition analysis.

Sequential extraction of sediments

Road surface and gullypot sediments were subjected to a modified form of a sequential extraction scheme [10]. The fraction identification, extractants and extraction conditions were: (i) exchangeable, 1 M NaAc (10 ml), pH 7, 1 h, room temperature, shaking; (ii) carbonate, 1 M NaAc (10 ml), pH 5, 1 min, microwave digestion; (iii) Fe and Mn hydrous oxide, 0.04 M hydroxylammoniumchloride, pH 2, 1 min, microwave digestion; (iv) organic, 30% H_2O_2, pH 2, 5 h, 86°C in a water bath; (v) residual, 15 M HNO_3, 1 min, microwave digestion.

All extractions were carried out in a Parr 45-ml PTFE cup. For microwave extractions the cup was sealed in a Parr 4782 digestion bomb. The digestion bomb was placed in a household microwave oven at full effect. The microwave oven was calibrated [11] and full effect was found to be equivalent to 474 W. The digestion bomb and PTFE cup were opened carefully after cooling in an ice bath for at least 30 min. The H_2O_2 extraction was not carried out by microwave after initial tests showed the risk for a rapid reaction causing pressure buildup and expulsion of the heated bomb contents into the microwave oven (and the risk for explosion).

The extraction scheme was applied initially to 2 g sediment with removal of 200–300 mg for analysis after each extraction. Dried, accurately weighed sub-samples (approximately 30 mg) of the extracted sediment were analysed for the remaining total platinum concentration. In most other sequential extraction schemes the extractant is analysed for metal concentration; on the contrary, we discarded the extractants as their matrix effects on Pt analysis by adsorptive voltammetry have not been investigated. Instead the remaining Pt was analysed and subtracted from total Pt. The retained sediment after each extraction was sufficient to allow five Pt analyses, which we felt to be the minimum necessary for statistical purposes. Three samples each of the < 63-μm fraction of road sediment and gullypot sediment were analysed.

Microtox toxicity test

Microtox is an instrument which utilizes the light emitting bacterium, *Photobacterium phosphoreum*. The results are expressed as an EC_{50} value which is the toxicant concentration that causes a 50% reduction in light emission. Microtox was performed directly on road and gullypot sediments. Freeze-dried *P. phosphoreum* was reconstituted for use in the test. All tests were carried out at 15°C. Data for the solid phase test were analysed according to the manufacturers operating manual [12].

Chemical oxygen demand

Chemical oxygen demand (COD) was used as a surrogate parameter for (oxidizable) organic material and was determined by the sealed-tube method. The commercial sealed-tube method is a Dr Lange cuvette LCK 114 (COD measuring range, 150–1000 mg O_2 l^{-1}). Sediment was added to the cuvette and heated in a LASA Aqua LTK 031 thermostat. Cr^{3+} was measured in a LASA pocket photometer.

RESULTS AND DISCUSSION

Recovery and precision of Pt in road sediments

The percentage recovery for Pt added to road sediment has been tested previously [2]. For the sediments analysed in this study the absolute quantity of Pt analysed was in the range 0.015–0.3 ng which gives a recovery of between 63% and 85%. Volatile Pt compounds may explain the loss, although HNO_3 is added before dry ashing to avoid the possible loss of volatile Pt chlorides by conversion to Pt nitrate.

Because of the sensitivity of Pt analysis to incomplete digestion, some samples invariably fail

and therefore special attention has to be paid to careful replication during digestion and analysis. The analysis of Pt in road sediments, following dry ashing and adsorptive voltammetry, has a detection limit of 0.5 ng g^{-1} and a standard deviation of $\pm 2.2\%$ [2]. For the gullypot liquor samples the standard deviation between sub-samples was poorer, $\pm 29\%$, due to the heterogeneous nature of suspended solids. Repeated voltammetric analysis of the same digested sub-sample gave a better standard deviation for the dissolved, $\pm 5.1\%$, and total, $\pm 5.7\%$, measurements. No detectable peaks were found for reagent blanks, which were run with each sample batch.

Pt in sediments

A comparison of Pt concentrations and biochemical characteristics between road sediments and gullypot sediments is made in Table 2. Gullypot sediments have an oxidizable organic content which is 39–88% higher than road sediments, but a toxicity which is 260–605% lower. This suggests that road surface sediments do not wholly comprise the gullypot sediment, undoubtedly other material such as fallen leaves are significant. This

TABLE 2

Platinum concentration, COD and Microtox EC_{50} values for road and gullypot sediments

	Pt, (ng g^{-1})			COD (mg O_2 g^{-1}) < 63 μm	Microtox EC_{50} (%) < 63 μm
	< 63 μm	63–500 μm	500–1000 μm		
Road sediments					
(year)					
1984	3.0	1.5 [a]	< 0.5 [b]	119	0.1
1991	8.9	3.6 [a]	2.8 [b]	128	0.2
Gullypot sediments					
(sample No.)					
1	15.1	< 0.5	< 0.5	208	0.52
4	3.5	2.3	1.4	143	1.04
5	4.6	2.1	5.1	139	1.21
6	7.8	1.4	1.3	214	0.82
7	7.8	1.9	1.8	232	0.68
8	4.0	1.6	1.1	140	0.89
10	7.0	1.6	0.9	167	0.59

[a] 63–250 μm. [b] 250–1000 μm.
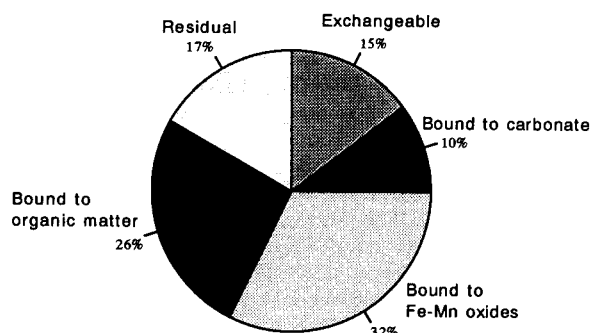
Fig. 1 Distribution of Pt between geochemical fractions extracted from road sediments (< 63 μm).

sediment dilution is reflected in the gullypot sediment which contains a 39–88% lower Pt concentration than road surface sediments in the < 63 μm fraction (with the exception of sample 1).

Pt fractionation of road sediments

Sequential extraction of Pt from road sediments shows a significant exchangeable fraction which may be released into the dissolved phase during storm events (Fig. 1). For the gullypot

sediments Pt was only found in the organic fraction (and is therefore not shown in Fig. 1). This suggests that the higher organic content of gullypot sediments results in a transformation of Pt from an inorganic to an organic bound form.

Pt in gullypot samples

In gullypot liquor samples (Table 3) the highest total Pt concentrations were found for undisturbed gullypots in a busy car park (site 5) and on a motorway (site 11) with concentrations of 13.6 ng l^{-1} and 11 ng l^{-1}, respectively. No dissolved Pt was found in the undisturbed gullypot liquor (< 1 ng l^{-1}). In the disturbed gullypot samples the high suspended solids concentration made reliable total Pt concentrations difficult to achieve and several values of dissolved and total Pt are missing from Table 3 due to difficulties of analysis at the low concentrations found. However, the dissolved concentrations were within the range 1.7 ng l^{-1} to 3.8 ng l^{-1}. These Pt concentrations might be expected in the "first flush" of a heavy summer rainfall.

Higher concentrations of Pt in a disturbed gullypot can be explained by biochemical activity

TABLE 3

Total platinum concentration and dissolved/suspended solid partitioning in gullypots

Sample no.	Sampling occasion	Status [a]	Total Pt (ng l^{-1})	Dissolved Pt [ng l^{-1} (%)]	Suspended solid Pt [ng g^{-1} (%)]
1	A	U	7.2		4.9 (34)
2	B	U	7.0		6.5 (122)
3	B	U			51
4	A	U	8.2		15 (31)
	A	D		2.02	
	B	U	7.6	1.9 (25)	17.2 (91)
5	A	U	13.6		13.8 (32)
	A	D		1.95	
	B	U	6.9		31.9 (88)
6	A	U	8.1		33 (38)
	B	U	2.4		40 (1050)
7	A	U	5.3		14 (369)
	A	D		1.7	
	B	U			
8	A	D		3.75	
9	B	U	2.5		
10	B	U	6.5	2.1 (32)	7.41 (138)
11	B	U	11.0	1.9 (17)	

[a] D = disturbed; U = undisturbed.

in gullypot sediment between storm events. This is reflected in low redox potential and dissolved oxygen concentration when gullypot sediments are mobilized [9]. Bacterial action effectively releases organically bound dissolved metal forms into the interstitial waters and eventually into the gullypot liquor. Increasing concentrations of metals have been shown, following an asymptotic curve, between storm events in the gullypot liquor [9]. A similar situation appears to exist for Pt as dissolved Pt is found after the dry period (B). The gullypots in the busy parking area (site 4), dual carriageway (site 10) and motorway (site 11) had dissolved concentrations amounting to 25%, 32% and 17%, respectively, of total Pt concentration in the gullypot liquor. We have tried, unsuccessfully, to physically separate fractions of Pt in these samples by dialysis. There is certainly a need both to be able to separate dissolved inorganic/organic Pt species and to be able, in the light of possible bioaccumulation, to separate biomethylated Pt.

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Extraction and spectrophotometric determination of Nd(III), Th(IV) and U(VI) in synthetic brine using Chlorophosphonazo III

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Abstract

A simple method has been developed to determine micromolar concentrations of Nd(III), Th(IV) and U(VI) in brine. The method involves the extractive separation of these cations from brine matrix species by using Chlorophosphonazo III as extractant with 1-butanol as solvent, and the spectrophotometric determination of the concentration of extracted species in the butanol phase. The lowest concentrations that could be determined are estimated to be 5×10^{-7} M for Nd(III) and 2×10^{-7} M for Th(IV) and U(VI).

Keywords: Spectrophotometry; Brines; Chlorophosphonazo III; Extraction; Neodymium; Thorium; Uranium

Several national plans for disposal of high level nuclear wastes consider the feasibility of deep geological storage in salt deposits [1]. Any subsequent dissolution and migration of the radionuclides would, at least initially, involve brines of the salt deposits. To evaluate the effect of the brine, solubility measurements of the elements of concern are underway. In this laboratory, research has been directed to the measurement of the solubility of Nd(III), Th(IV) and U(VI) in a brine representative of those formed in the Waste Isolation Pilot Plant (WIPP) project of the United States Department of Energy (USDOE). Our solubility studies have been done with brine solutions which contain only one of the ions [Nd(III), Th(IV) and U(VI)] (i.e., UO_2^{2+}) of interest.

Such solubility studies require determination of the concentration of the f-element ions in the

presence of a large amount of matrix elements. Some analytical methods, such as inductively coupled plasma mass spectrometry (ICP-MS) [2,3], neutron activation analysis (NAA), isotope dilution mass spectrometry (ID-MS) [5] and UV–visible absorption spectrophotometry [6–10], have been used for the determination of these f-element cations in sea water and mineral ore samples. In these methods, it is common practice to remove matrix elements by a series of procedures [e.g., coprecipitation with Fe(III)] coupled with further separation on ion exchange resin columns. However such an approach suffers from limitations of residual matrix contamination and poor sensitivity.

Chlorophosphonazo III has previously been used as a chromogenic reagent in the spectrophotometric determination of a variety of metal ions [6–9]. Such determinations were usually performed directly in aqueous solutions and did not involve extraction of the Chlorophosphonazo III complexes into an organic phase. Determination

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of lanthanides are typically done in pH 3 solutions whereas Mg(II) and Ca(II) analyses are performed at pH 7–8 [11]. Interference from Mg(II) and Ca(II) in the brine matrix can be eliminated by performing an extraction of the f-elements, at high hydrogen ion concentration (pH < 3) prior to the spectrophotometric measurement.

In the present procedure, extraction of each f-element ion from the acidified (ca. 1 M HCl) brine matrix with butanol is combined with spectrophotometric measurement using Chlorophosphonazo III as both extractant and dye.

EXPERIMENTAL

Reagents

Chlorophosphonazo III, hereafter designated as H_4L, (Fluka) was dissolved in deionized water to prepare a 0.05% (w/w) solution. The synthetic brine solution was prepared by dissolving 100.1 g of NaCl, 292.1 g of $MgCl_2 \cdot 6H_2O$, 1.66 g $CaCl_2$, 6.20 g Na_2SO_4, 1.95 g $Na_2B_4O_7 \cdot 10H_2O$, 0.96 g $NaHCO_3$, 0.52 g NaBr, 57.2 g KCl in deionized water to make 1.0 l of solution (the Brine A recipe of the WIPP program). Aliquots of this solution were centrifuged and filtered, immediately prior to use, with a Nylon Acrodisc syringe filter (Gelman Sciences) having 0.2 μm pore size.

Standard neodymium solution was prepared by dissolving Nd_2O_3 (99.9%) with 6 M HCl and diluting the solution to 9.0×10^{-4} M. Standard uranium(VI) solution was prepared similarly by dissolving U_3O_8 (99.9%) with a mixture of HNO_3 and HCl, followed by dilution to 3.4×10^{-5} M. Thorium nitrate was used directly to prepare the standard solution of Th(IV) (4.4×10^{-4} M) whose pH was adjusted to 2 with dilute HNO_3. Solutions of Nd(III) and Th(IV) were standardized by titration against EDTA using xylenol orange as indicator [12,13] while U(VI) solution was standardized gravimetrically [14]. Aliquots of the standard solutions were diluted with brine to give concentration ranges of 1×10^{-7} to 1×10^{-5} M.

ACS grade 1-butanol was used without further purification. All other reagents were of analytical grade and were used without further purification.

Procedure

To evaluate the method, samples of brine solution with known concentrations of one of the metal ions of interest were adjusted to the desired H^+ concentration with 6 M HCl and to the desired concentration of Chlorophosphonazo III. The total volume of this solution was made 3.0 ml with deionized water. At this point, the aqueous solution had a purple color and some precipitate was observed. Exactly 3.0 ml of butanol was added and the two phases were mixed by mechanical shaking for 10–15 min. The aqueous phase became colorless within 1 min of shaking and the precipitate dissolved, following the addition of butanol, within the shaking time. The phases were separated by centrifugation and after standing for at least 1 h, samples of the butanol phase were removed for absorbance measurements.

Measurements

The absorption spectra were recorded on a Cary-14 spectrophotometer (rebuilt by On-Line Instrument Systems) interfaced to a Zenith 248 computer, in 1 cm quartz cells. The baseline was corrected by setting the mean absorbance at 740–745 nm range to be zero. In the 740–745 nm region, neither the Chlorophosphonazo III nor its complexes show observable absorption. The absorbance reading error is estimated to be ± 0.001 by this treatment.

RESULTS AND DISCUSSION

Chlorophosphonazo III and its complexes with Nd(III), Th(IV) and U(VI) can be extracted from the aqueous solution into the butanol phase in an acidity range from 0.05 to 3.5 M. In neutral aqueous solution, they are not extracted and in high acidity ($H^+ \geq 2$ M) the butanol dissolves in the aqueous phase. As a result measurements were done in solutions with 1 M acidity where there is quantitative extraction with little dissolution of butanol in the aqueous phase.

Figure 1 shows the spectra of Chlorophosphonazo III and of the Nd complex in the butanol solution (the spectra of the uranium and thorium complexes are very similar to that of the

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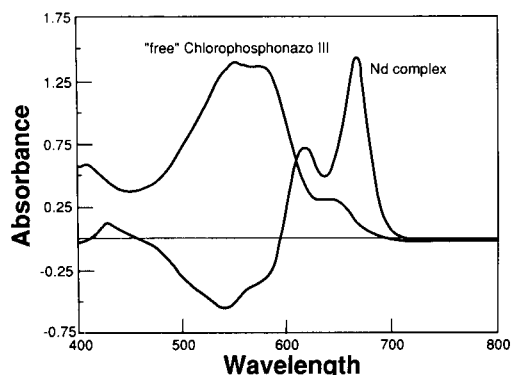
Fig. 1. Spectra of "free" Chlorophosphonazo III and its complex with Nd(III) in butanol. [H$_4$L]: 3.3×10^{-5} M, [HCl]$_{aq}$: 1.0 M. Blank solution for Nd(III) complex spectrum was Chlorophosphonazo III in butanol.

Fig. 2. Dependence of the apparent molar absorptivities of the Chlorophosphonazo III complexes of Nd(III), Th(IV) and U(VI) near 670 nm on acidity. [H$_4$L]: 3.3×10^{-5} M.

neodymium). There is no observable difference between these spectra in butanol and those of the complexes in aqueous solution. In the wavelengths of interest, the Chlorophosphonazo III spectrum has absorption peaks at 543, 574 and 640 nm (shoulder), while the spectra of the complexes have peaks at 640 and 667 nm for Nd(III), 640 and 669 nm for U(VI) and 640 and 675 nm for Th(IV), respectively. The peaks near 670 nm for all three cations show much stronger absorption than that at 640 nm and are less affected by the absorption from uncomplexed Chlorophosphonazo III. Hence this peak was chosen for use in the spectrophotometric measurements.

The apparent molar absorptivities of the 667–675 nm peaks of the Chlorophosphonazo III complexes with Nd(III), Th(IV) and U(VI) in butanol are shown in Fig. 2 as a function of hydrochloric acid concentration of the brine solution. Figure 3 shows the dependence of the apparent molar absorptivities in butanol solution as a function of the total Chlorophosphonazo III concentration. Complete extraction from the aqueous phase was confirmed by the absence of spectral evidence for Chlorophosphonazo III in all aqueous samples. As in the previous figure, the extraction of Nd varies with concentration more than that of the other two cations. The extraction of Nd(III) by the Chlorophosphonazo III corresponded to a second power dependency on the Chlorophos-

phonazo III concentration and a third power dependency on [H$^+$].

For all three cations, the degree of extraction is very high and the complexes in butanol have large molar absorptivities. A series of experiments showed that the extraction efficiency of these cations are $96 \pm 4\%$ ([H$_4$L] = 5.0×10^{-6} M; [H$^+$] = 0.25 M), $97 \pm 3\%$ ([H$_4$L] = 3.3×10^{-6} M; [H$^+$] = 1.0 M) and $92 \pm 5\%$ ([H$_4$L] = 6.3×10^{-6} M; [H$^+$] = 1.0 M) for Nd(III), Th(IV) and U(VI), respectively. The lowest concentrations of these f-elements in brine that could be determined by this technique are 5.0×10^{-7} M for Nd(III) and 2.0×10^{-7} M for Th(IV) and U(VI). These represent the concentration of cation cor-
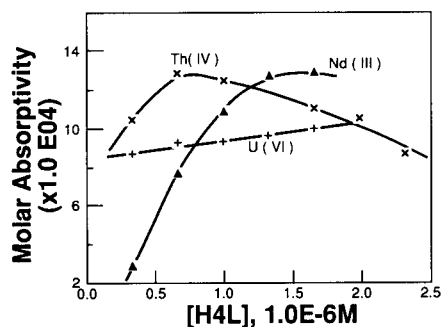
Fig. 3. Dependence of the apparent molar absorptivities of the Chlorophosphonazo III complexes of Nd(III), Th(IV) and U(VI) near 670 nm on the concentration of Chlorophosphonazo III. [HCL]$_{aq}$: 1.0 M.
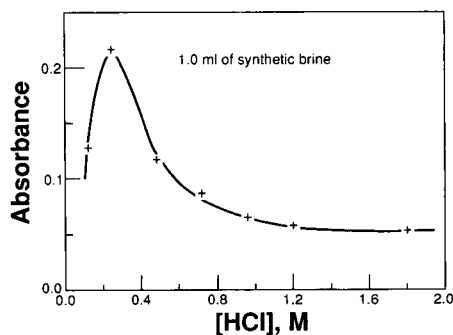
Fig. 4. Dependence of the absorbance of 1.0 ml of brine matrix at 667 nm on acidity. [H_4L]: 3.3×10^{-5} M.

responding to the lowest absorbance (0.015) that gives a satisfactory signal to noise ratio ($S/N \geq 5$).

The concentration of the cations Na(I), K(I), Mg(II) and Ca(II) is sufficiently large in the brine solution that their possible interference with the determination of Nd(III), Th(IV) and U(VI) had to be investigated. In weakly acidic and neutral solution, precipitation occurred immediately upon addition of the Chlorophosphonazo III to the brine. In 1.0 M HCl solution, a small amount of precipitate formed which disappeared (and the solution became colorless) when contacted with butanol. The absorption spectrum of the organic phase showed peaks at 520 and 640 nm, presumably due to complexes with some of the metal ions in the brine which were extracted into the butanol phase.

The interference of brine constituents on the absorbance of Nd(III), Th(IV) and U(VI) was determined. The absorbance of brine extract at 667 nm (see Fig. 4) was found to be lower than that of the Nd(III), Th(IV) or U(VI) complexes (1.0 M [H^+]) in the concentration range used for the determination of the cations. Only Mg(II) and Ca(II) have measurable interference at the brine concentration used (e.g., the absorbance of Mg extracted with 3.3×10^{-5} M Chlorophosphonazo III is 0.08 and that of Ca is 0.1).

Calibration graphs were prepared for each cation by plotting absorbance versus concentrations of Nd(III), Th(IV) and U(VI) extracted from brine with butanol. Linear graphs were obtained up to 8.1×10^{-6} M Nd(III), 9.0×10^{-6} M U(VI) and 4.0×10^{-6} M Th(IV). The equations ob-

tained by least-squares fitting ($n \geq 6$) of the data were:

Nd(III); $A = 30460C + 0.0058$ ($r = 0.9992$)

Th(IV); $A = 84880C - 0.0093$ ($r = 0.9987$)

U(VI); $A = 77654C - 0.0025$ ($r = 0.9983$)

where A is the absorbance, C the concentration in mol l^{-1} (M) and r the correlation coefficient.

A standard procedure was developed for determination of each of the three cations. A sample of 1.5 ml of the brine solution with the metal ion was mixed with 0.5 ml of 6.0 M HCl and the total volume was adjusted to 3.0 ml. After addition of 3.0 ml of butanol, the procedure followed that described previously in the Experimental section. This acidity does not result in maximum extraction of Nd(III) but does eliminate spectral interference from the brine constituents. The extraction equilibrium is reached within a few minutes of shaking the mixture as judged by the complete disappearance of color from the aqueous phase. The small amounts of precipitate formed in the aqueous phase dissolve after shaking the aqueous butanol mixture for 10–15 min. The mixture is centrifuged to separate the phases and allowed to stand for at least 1 h so that the small amount of aqueous solution dispersed into the organic phase can coalesce and separate from the butanol phase. Also allowing the mixture to stand for 1 h permits complete color development. The absorbance for the three complexes were found to be stable for at least 72 h after extraction.

A least-squares spectral deconvolution method was developed to remove the effect of the interference of Mg and Ca. An observed spectrum is the sum of the spectra of the sample and that of the interfering ions. Three reference spectra were recorded separately and used to fit the observed spectrum of the extracted sample by a least squares fitting method, the three spectra are: the spectrum of 3.3×10^{-5} M "free" Chlorophosphonazo III; that of 1.0 ml of synthetic brine solution with no Nd, Th or U present; that of 2.7×10^{-6} M of the Nd(III), Th(IV) or U(VI) complex (not extracted from brine). Figure 5 shows the results of the least squares fitting routine for Nd(III). With this technique, it was possi-

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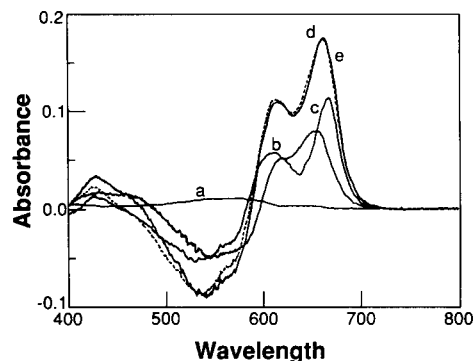
Fig. 5. The least squares fitting result of three reference spectra [Chlorophosphonazo III, synthetic brine with no metal and Nd(III), Th(IV) or U(VI)], recorded in butanol, to the observed sample spectrum. a = Free Chlorophosphonazo III; b = synthetic brine; c = neodymium; d = measured spectrum (solid line); e = fitted spectrum (dashed line).

ble to determine the concentrations of Nd(III), Th(IV) or U(VI) in brine samples containing interfering ions. Given the similarity in their spectra, this procedure cannot be used as described herein for simultaneous analysis of the three cations directly.

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Aluminum–pyrocatechol violet reactivity with various complexing agents

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Abstract

A spectrophotometric method for determining the speciation of aluminum using pyrocatechol violet (PCV) is tested on a series of known ligands over a range of concentrations and at two fixed pH values. The procedure assumes that the colorimetric signal is defined by the competition of the Al–ligand complex with Al–PCV. The stability constants determined are in agreement with literature values. Time to steady state is more than 10 min. A procedure for determining the conditional stability constant, the total ligand concentration and the reactive Al at a fixed pH is proposed.

Keywords: Spectrophotometry; Aluminium; Complexometry; Pyrocatechol violet

Aluminum chemistry and its speciation in natural waters has been an area of intensive study. Aluminum is ubiquitous in nature, being the third most abundant element in the crust of the earth. Aluminum becomes increasingly labile in waters with pH < 6. In laboratory experiments, Al has been shown to be toxic to many forms of life. Aluminum speciation is important to the toxic effect [1].

Many speciation techniques have been developed to assess Al. All can be criticized as being "operationally defined" [2]. The techniques have been categorized into (a) reaction rate, (b) cation-exchange resin techniques, (c) exclusion by filtration, dialysis etc., (d) ion chromatographic separations, and (e) indirect estimations using a secondary probe (e.g. F$^-$ electrode) [3]. Clarke et al. [2] give a good summary of the various methods.

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One analytical technique that has been used quite extensively is the color development of Al–pyrocatechol at a fixed pH of about 6 [4]. Pyrocatechol violet (PCV) has been used for the analysis of "reactive" Al in a number of studies [5–7]. It has been automated for continuous analysis [8] and adapted to flow-injection analysis schemes [2,9,10]. Here we assess the reaction competition of Al–PVC with other Al–ligand complexes to determine conditional stability constants for these ligands and Al. The method does require analysis within a small pH range (about 5.3–6.2) to optimize the colorimetric signal. It is free of many interferences with the exception of Fe(III) [5]. Speciation determination obtained by the competition of PCV with natural ligands for Al has the potential of eliminating many of the debatable procedures common to separation or column methods.

In this paper, we explore the feasibility of using PCV as a means of defining free and bound Al and establishing stability constants for natural systems. We test a number of reasonably well

defined ligands. We assess the ability to define the aquo Al species by measuring the PCV signal colorimetrically. Since Al chemistry is largely irreversible in such systems, we evaluate differences in sequences of procedures in the measurements. This calibration of the method with known ligands gives more confidence to the procedure for natural waters.

MATERIALS AND METHODS

The analytical system was configured on a Technicon auto-analyzer assemblage or in a stirred reactor. The system was then fed through a peristaltic pump to a flow-through 5 cm light cell where absorbance was measured at 590 ± 5 nm. Chemicals were generally BDH AnalaR or Assured with the exception of PCV which was Alfa (99% assay). Stock concentrations and system diluted concentrations in parentheses were: hydroxylammonium chloride, 0.10937 M (187.6 μM); 1,10-phenanthroline, 0.00145 M (38.1 μM); PCV, 97.15 μM (40.88 μM); and Tris buffer 0.693 M (0.148 M). This results in an ionic strength of about 0.145 M. The Tris buffer was adjusted to a pH of 5.40 with 4 M HCl and was used to obtain a final mixed solution pH of 5.337 $\pm$ 0.018 for all runs. We also carried out analyses near the optimum signal pH of 6.00. Under these conditions, the calibration graph is linear to about 9 μM with a detection limit of 0.1 μM.

The ligands tested were malate, salicylate, citrate, tartrate and fluoride. These were added in various amounts (1–450 μM) to different Al concentrations (1.85 and 3.75 μM, i.e. 50 and 100 ng ml^{-1}); a dilution factor of 0.690 resulted for Al and ligands.

1,10-Phenanthroline/hydroxylammonium chloride are added to suppress the reaction of Fe(III) with PCV. Runs with and without the phenanthroline solution were made to test possible interferences.

The temperature was not controlled, but was monitored and varied from 22–24°C for all experiments.

The normal procedure was to add acid (pH $\approx$ 2.2) and phenanthroline solution to an Al–ligand

mixture for about 2 min, to add and mix PCV for 5 min, and to stabilize with the pH buffer for 5 min. The system was calibrated for Al–PCV without ligand. The suppression of the Al–PCV signal with ligand was then measured.

The hypothesis for the procedure depends upon the attainment of equilibrium or steady state for the following reaction:
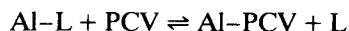
$$Al-L + PCV \rightleftharpoons Al-PCV + L$$

Then

$$K' = \frac{[Al-PCV][L]}{[Al-L][PCV]} \tag{1}$$

where K' is the conditional stability constant and square brackets are concentrations. The test is to ascertain if the Al–pyrocatechol violet absorbance, proportional to the total [Al–PCV], is dependent solely on the colorimetric signal. For a known addition of PCV, we see from Eqn. 1 that the bound and free ligand, [Al–L] and [L], then need to be determined. This can be resolved by addition (titration) of either Al, PCV, or a known ligand. Mass balance equations used to obtain [Al–L], [Al–PCV], [PCV] and [L] are written for total ligand, [L]$_t$, total soluble Al, [Al]$_t$, total pyrocatechol violet, [PCV]$_t$, and for the Al–PCV species, [Al–PCV]$_t$ for the defined systems:

$$[L]_t = [H_3L] + [H_2L] + [HL] + [Al-L]$$
$$+ 2[Al-L_2] + 3[Al-L_3] \tag{2}$$

$$[Al]_t - [Al-PCV]_t$$
$$= [Al-L] + [Al-L_2] + [Al-L_3] \tag{3}$$

$$[PCV]_t = [H_2-PCV] + [H-PCV] + [PCV]$$
$$+ [Al-PCV] + 2[Al-PCV_2]$$
$$+ 3[Al-PCV_3] \tag{4}$$

and

$$[Al-PCV]_t = [Al-PCV] + [Al-PCV_2]$$
$$+ [Al-PCV_3] \tag{5}$$

In this experiment, the total values, adjusted for dilution by mixing, are known. Table 1 gives the stability constants used in the calculations. Since the colorimetric response is calibrated against the solution without ligand at constant pH (e.g. Eqn.

TABLE 1

Acid dissociation and Al complex stability constants used in the calculations

Substance	pK_{a_1}	pK_{a_2}	pK_{a_3}	$\log \beta_1$	$\log \beta_2$	$\log \beta_3$	Ref.
D-Tartaric	2.73	3.78		5.32	9.77		11
Salicylic	2.92	13.1		12.9	23.2	29.8	12
Citric	3.13	4.76	6.40	7.37	13.9		13
Malic	3.46	5.10		5.14	8.52		13
Fluoride				6.13	11.15	15.00	14
PCV	9.23	13.00		16.3	29.3	37.6	12

3), the hydrolysis species and the aquo ion would cancel in mass balance equations. For different pH values, any Al–OH–L species change would have to be considered, however. The total Al concentration was low enough that mononuclear species would be predominant.

The calculations were not corrected for ionic strength, but they should be consistent since the ionic strength stayed constant for the small additions of ligand and Al.

Eqns. 2–5 can be simplified in all cases, since not all of the terms are significant for the constant pH (6.0) and the concentrations used. Thus for the concentrations and pH range $[L]_t$ and $[Al]_t$ are simplified depending upon the specific ligand and:

$$[PCV]_t \approx [H_2\text{-}PCV] + [Al\text{-}PCV]_t \qquad (6)$$

and

$$[Al\text{-}PCV]_t \approx [Al\text{-}PCV] \qquad (7)$$

Various preliminary tests were carried out on the system. First, the precision of the absorbance measurement was determined for blanks and different Al solutions. Then the potential interference to the blank reading of different levels of ligands was determined. The time required to reach a stable reading was determined for different ligands and concentrations. Then the change in the Al–PCV signal with and without the phenanthroline solution were tested. Finally, differences in absorbances for different sequences of adding acid, ligand and Al were determined.

After completion of the stability and interference tests, conditional stability constants were calculated.

RESULTS

The first assessment was the stability of repeated measurements of different concentrations of Al. For reference, the analytical background noise was about 0.6% of the full signal. Four replicates on four dates over one month for four different reaction configurations and two Al concentrations (100 and 150 ng ml^{-1}) gave an average precision of 0.72% with a range from 0.3 to 1.4%. This is very similar, though slightly elevated from the background noise. We concluded that the precision was excellent for the different reaction times and mixtures although the absolute signal varied for different setups.

The effect of the ligands on the PCV absorbance signal was assessed by comparison to the blank. Ligands were added individually and in combinations up to a total concentration of 0.01 M. At 0.01 M, malic and tartaric acid blanks differed from the PCV blank and gave an apparent Al concentration of 20 and 17 ng ml^{-1} respectively. None of the ligands showed an interference within the experimental working range of 0 to 450 μM, however.

The time to reach steady state or equilibrium is important. This was assessed by the time required to reach maximum and stable color development. This time was assessed by mixing the PCV with Al and ligand for different times and then adding the pH buffer at different intervals. Stable well-formed signals were obtained after a pH stabilization of 10 min and a reaction time ranging from less than 1 min for malic acid to 10 min for salicylic acid. The Al standard alone stabilized with PCV after 9–15 min depending upon the concentration of Al. Therefore a reaction time of Al–PCV–ligand of 5 min followed by a stabilization time of 10 min after pH buffer is considered minimal to achieve system uniformity. These times are similar to those suggested by Seip et al. [6]. Furthermore they fall within the 1 to 8 min estimates that can be made from kinetic data for selected for Al organic ligands [15].

Memory contamination is possible since a continuous flow automated analysis system was used. We checked this possibility by running decreasing

and increasing standard solutions of Al in the range 0–5.5 μM. No bias was measured.

The sequence of adding acid to pH of 2.5, Al and ligand were altered using Al, tartaric acid and HNO_3. There was a deviation of less than 1% for the Al–PCV signal, using all possible combinations. It was concluded that there was no bias due to sequence of adding ligand, Al and acid.

Another test ascertained the difference in Al–PCV signal with and without the Fe(III) sequestering solution of phenanthroline. We experimented with all of the ligands at 15 and 450 μM. We found no significant difference for the low concentration ligands, but we did find interestingly a small suppression of the Al–PCV signal at elevated concentrations of citrate, malate tartrate and fluoride in the presence of phenanthroline.

TABLE 2

Estimation of Al–L stability constants
(Values are at 25°C and 0.1 M ionic strength. Errors on log K are 1σ and reflect deviation in the Al–PCV measurement only. Runs were for 3.71 μM total Al except as noted. Values of Al and ligand concentrations are before dilution. Number of runs are given in parentheses. Al–PCV is given in percent of Al total signal without ligand.)

Ligand (μM)	Al–PCV measured			Calculated log K'	
	pH 6.00	pH 5.34		I	II
Malic acid					
15	–	87.9 ± 2.3 (5)		–	11.62 ± 0.02
30	81	78.2 ± 1.7 (5)		10.53	11.62 ± 0.01
150	43	33.0 ± 2.9 (5)		11.23	11.45 ± 0.04
450	22	17.4 ± 3.1 (5)		10.51	11.56 ± 0.07
Summary, log K'	Lit. value: 11.76 [14]		Average	10.8 ± 0.4	11.6 ± 0.1
Salicylic acid					
30	94	100 (5)		4.07	–
150	86	83.7 ± 2.7 (5)		4.41	4.91 ± 0.01
450	75	52.5 ± 2.5 (5)		4.67	4.77 ± 0.02
Summary, log K'	Lit. value: 4.0 [14]		Average	4.4 ± 0.4	4.8 ± 0.1
Citric acid					
1.5	–	83.7 ± 2.0 (7)		–	9.31 ± 0.01
3	–	60.2 ± 3.0 (6)		–	9.02 ± 0.01
15	–	18.0 ± 2.2 (7)		–	9.50 ± 0.05
30	–	13.1 ± 0.4 (7)		–	9.60 ± 0.01
Summary, log K'	Lit. value: 9.53 [14]		Average		9.4 ± 0.3
Tartaric acid					
150	70.4 ± 2.2 (6)	–		11.62 ± 0.01	
450	27.8 ± 1.7 (5)	–		11.70 ± 0.03	
Summary, log K'	Lit. value: 11.58 [14]		Average	11.7 ± 0.1	
Fluoride					
5	93	–		10.42	–
10	82	73.1 ± 5.9 (3)		10.32	10.41 ± 0.03
50	30	24.5 ± 0.5 (3)		10.49	10.77 ± 0.01
100	17	12.9 ± 1.4 (3)		10.81	11.04 ± 0.04
Summary, log K' (1.86 μM Al$_t$)	Lit. value: 10.77 [14]		Average	10.5 ± 0.2	10.7 ± 0.3
5	90	–		10.24	–
10	70	–		10.05	–
100	21	–		10.95	–

Finally, we determined the effect of various mixing times in the procedure. With manual mixing, we experimented with development of signal after addition of the buffer. We found that a stable colorimetric signal was developed 10 min after addition of the pH buffer to a solution containing Al, ligand, PCV and with and without phenanthroline. Different time delays in adding the PCV or phenanthroline reagents over 1–2 min did not affect the final signal. Using all levels of ligands and Al concentrations of 50, 100 and 150 ng ml^{-1}, we concluded that a delay of 13 min or longer was sufficient to obtain a stable colorimetric signal.

Determination of conditional stability constants

The stability constants for complexes with various ligands were obtained without phenanthroline and for a constant total Al of 3.71 μM (2.56 μM in diluted final solution). We also ran a few experiments with F$^-$ at a total Al concentration of 1.86 μM (1.28 μM after system dilution). The pH was held constant at 5.34 and at 6.00, and the absorbance was measured after 15 min. Table 2 summarizes the experimental results and shows the log K' (Eqn. 1) determined and expected using information in Table 1. The standard deviation for multiple runs of the same concentration depends only upon the deviation in the Al–PCV absorbance. This deviation, shown in Table 2, is greater than but similar to the analytical precision. Averages for estimated K' are given to ± 0.1, which reflects deviation of runs of different total ligand concentration. Experiments were run for L$_t$ from 1 to 450 μM. When data are not shown for low L$_t$ concentrations, there was no effect on the Al–PCV signal by the ligand.

A nearly constant value for K' was found over a wide ligand concentration and at constant pH for the species tested. The fluoride values are constant for changes in pH and Al concentration. They also bracket the expected value. Tartaric and citric acid are also constant, but they were only run at one pH. They also bracket the literature value. The values for salicylic acid and malic acid do not correspond to the literature values well, and perhaps more importantly, they change with the pH of the experiment. Therefore a pH

dependent species, Al(OH)$_b$L$_c$, may be predominant instead of simply AlL$_c$. A detailed study of Al–salicylates [16] shows that in the pH range of 5–6, an Al(OH)L$_2^{2-}$ may be an important species. Citrate acid shows an even more complicated speciation in this range [17]. Therefore if the method gives a different apparent constant for Eqn. 1, H$^+$ may be invoked as a variable, and the equation would become:

$$H^+ + Al-OH-L + PCV = Al-PCV + L + H_2O$$
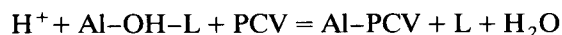
$$K' = \frac{[Al-PCV][L]}{[H^+][Al-OH-L][PCV]}$$

These species can explain the difference of reported literature values which are typically of the form, AlL$_n$. This modification becomes more complex for an unknown system because the predominant species, which may or may not involve proton, may change with pH. In any event, the change in the estimated pK' values in Table 2 are in the right direction for change in pH.

DISCUSSION

Overall the Al–PCV seems to recover the Al–L values quite well for some of the ligands. The complications of the speciation, specifically involving pH, make comparison at different pH values more of an challenge. When apparent constants change with pH, a Al–OH–L needs to be considered. The change in the magnitude of the apparent pK' and the pH will indicate change in predominance of species or merely the effect of pH.

The results suggest that this analytical technique can be used for Al speciation in the pH range of 5.3 to 6 or greater than 6. For unknowns, the stability constant(s) of the Al–L, the total ligand concentration and the total reactive Al concentration need to be determined. The system can be defined by titrating Al or PCV$_t$ at one or more pH values. Since there may be one or more ligands and complexes, we define a composite term as:

$$K'_L = \frac{[Al'][L']}{[Al-L']} \tag{8}$$

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where $[Al-L']$ and $[L']$ are the total concentrations of Al–L and L ligands and $[Al']$ is the reactive Al, defined by Al–PCV, at the pH. Given this conditional constant, we can write a mass balance for Al and L as:

$$[Al_o] + [Al_a] = [Al-PCV] + [Al-L'] + [Al'] \tag{9}$$

where Al_o is the initial total reactive Al, Al_a is the added Al, and $[Al-PCV]$ is equal to the PCV absorbance. All values are corrected for dilution due to the addition of Al_a and are for a fixed pH. For negligible $[Al_o]$ and $[Al']$, Eqn. 9 becomes:

$$[Al-L'] = [Al_a] - [Al-PCV] \tag{10}$$

The total ligand concentration, L_t, is:

$$L_t = [L'] + [Al-L'] \tag{11}$$

In addition, the labile Al, as defined by PCV, can also be calculated from β for Al–PCV:

$$[Al'] = \{[Al-PCV_t]\}\{\beta_{Al-PCV}([PCV_t]$$
$$- [Al-PCV_t])10^{(K_1+K_2)}[H^2]\}^{-1} \tag{12}$$

Substituting Eqns. 10–12 into 9 and rearranging gives:

$$Y = -K'_L + L_t\frac{Y}{X} \tag{13}$$

where

$$Y = [Al'], \quad X = [Al_t] - [Al-PCV_t]$$

Eqn. 13 is linear and can be solved for K'_L and L_t for a series of Al additions and Al–PCV readings.

The various assumptions regarding $[Al_o]$ and $[Al']$ being negligible in Eqn. 9 must be checked for a specific data set. In addition, there is a basic assumption that the PCV reacts only with Al. The test can be run at the two limiting pH values for the method. If different K'_L and L_t values are obtained, then one may assume and estimate additional Al–L species. Alternately there may be a measurable change in the ligands, due to a change in protonation.

This test would appear to give a comprehensive and independent assessment for Al speciation. The definition of $[Al']$ is subjective, however, in that it is characterized by the Al–PCV part of the system. Other rapid kinetic methods [2] for obtaining $[Al']$ can be compared to the results obtained here. In general, one would anticipate that this method would overestimate $[Al']$, compared to "fast" methods.

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Detection of a strong ligand for copper in sea water and determination of its stability constant

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Abstract

A method for the detection and characterization of an organic ligand with a high conditional stability constant but a low concentration in natural waters is proposed. The method is based on a combination of a procedure that involves the ligand-exchange reaction between natural ligands and chelating reagents and the analysis of the experimental results on metal speciation by a simple equilibrium model. Application of this method to samples of sea water from different marine environments suggested the presence of a strong ligand for copper in most samples of sea water examined. The lower limits of the concentration of the strong ligand in oceanic water were evaluated to be < 0.01–0.20 nM. The conditional stability constant for the copper complex with this ligand was evaluated to be higher than $10^{13.8}$–$10^{14.3}$ l mol^{-1} at an ionic strength lower than 10^{-5} M at pH 5.71 and 4°C. In coastal water, the concentration of the strong ligand was higher than in oceanic water, but the conditional stability constant was the same. The conditional stability constant for the strong ligand, evaluated by the present method, is equal to or one order of magnitude greater than that of ethylenediaminetetraacetic acid under the same conditions.

Keywords: Copper; Demetallization; Sea water; Speciation; Stability constants

Metal–organic interactions in natural waters have recently attracted increasing interest and it appears that they exert a significant influence on the patterns of chemical speciation of several trace metals in natural waters. The understanding of copper speciation in sea water has been revised, and copper is now believed to exist mainly as undefined, highly stable organic complexes in sea water [1–8].

Sea water contains high levels of major inorganic salts and low levels of various minor inorganic and organic constituents. The concentrations of heavy metal ions and organic ligands are extremely low. The contamination by metal ions of sea-water samples during collection, storage

and processing is a serious obstacle to accurate studies of metal speciation (e.g., [7]). High levels of major inorganic salts also complicate the complexation equilibria in sea water via side-reactions with both metal ions and organic ligands [9].

The earlier literature in this field indicated the occurrence of relatively high concentrations (50–1000 nM) of natural ligands (L) with relatively low conditional stability constants, e.g., $K'_{\text{CuL}} = 10^7$–10^{10} l mol^{-1} [10–12]. Application of more highly sensitive electrochemical techniques and "clean" techniques has revealed that there exist natural ligands with higher conditional stability constants and that copper dissolved at extremely low levels binds quantitatively to these ligands in oceanic surface water [7].

Determinations of conditional stability constants of organic ligands for copper have mostly been based on the titrimetric measurement of the

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low levels of free or electrochemically labile copper. Such results are clearly restricted by the sensitivity of the technique. In measurements by the metal titration method, the side-reactions of natural ligands with ambient metal ions present in sea water may make it difficult to elucidate the actual details of the complexation of the metal in sea water. In particular, the occurrence of low levels of strong ligands may not have been detected because such ligands have been complexed quantitatively in situ with ambient metal ions. It is not clear whether or not all ligands that are actually responsible for the speciation of dissolved metals in sea water have been detected.

Midorikawa et al. [13] developed a method for the determination of complexing abilities of organic ligands with molecular weights > 1000 in sea water. In this procedure, natural ligands in sea water were concentrated and desalted by lyophilization and dialysis. The procedures also involved the removal of metals that were bound to natural ligands (demetallization process) by the ligand-exchange reaction in the presence of a large excess of ethylenediaminetetraacetic acid (EDTA) using a dialysis membrane. Two types of ligand with different conditional stability constants were characterized by the copper titration method using an ion-selective electrode (ISE). During the course of the investigation, it was suggested that a third ligand, which was stronger than the two ligands characterized by the copper titration method, was present in the coastal water [14]. In this study, a methodology based on the combination of the demetallization procedure and the simple equilibrium model was developed and applied to samples of oceanic water to confirm the occurrence of the strong ligand and to estimate its complexing ability. The ability of this method to characterize the low levels of natural organic ligands that form highly stable complexes with copper is discussed.

EXPERIMENTAL

Materials and reagents

Analytical-reagent grade reagents were used unless indicated otherwise. All solutions were prepared with deionized water (Milli R/Q system; Millipore, Bedford, MA). A solution of Cu(II) was prepared from the nitrate and standardized against a 0.01 M solution of Titriplex III disodium EDTA (Merck, Darmstadt). HNO_3, $HClO_4$ and KOH (30% solution) were of Suprapur grade (Merck).

All glassware was washed with HCl and HNO_3. Siliconized glassware was used for the preconcentration and demetallization to prevent any possible adsorption of organic ligands on the walls of the glassware.

Spectrapor 6 dialysis tubing with a molecular weight cut-off (MWCO) of 1000 (Spectrum Medicine Industries, Los Angeles, CA) was used for dialysis. The method for pretreatment of the membranes to remove contaminants was as described previously [13].

Samples

Sea-water samples were obtained from the western North Pacific (site P: 41°32′N, 147°00′E) and the Japan Sea (site J: 44°15′N, 130°58′E) in August 1987 (M.S. Kofu-Maru, Hakodate Marine Observatory); from the coastal area off the Muroto Cape (site A: 33°17′N, 134°14′E) in February 1988 (M.S. Tosa-Kaiyo-Maru, Kochi Prefectural Fisheries Station); and from Suruga Bay (site S-1: 34°56′N, 138°41′E; site S-2: 35°01′N, 138°30′E) in April 1988 (M.S. Ryofu-Maru, Japan Meteorological Agency). Sea water was collected with a 30-l non-metallic Niskin sampler and a 3-l bucket and filtered through a membrane filter (Millipore HA, 0.45-μm pore size) immediately after sampling. The samples were frozen (ca. −20°C) and stored until use. Hydrographic data for the sampling stations are listed in a previous paper [13].

Demetallization procedure

The procedural sequence is shown schematically in Fig. 1. Natural ligands with molecular weights > 1000 were isolated from sea water. The details of the procedure can be found elsewhere [13]. Natural ligands in sea water were concentrated and desalted by twice-repeated rounds of both lyophilization and dialysis, and also subsequent electrodialysis. By this proce-
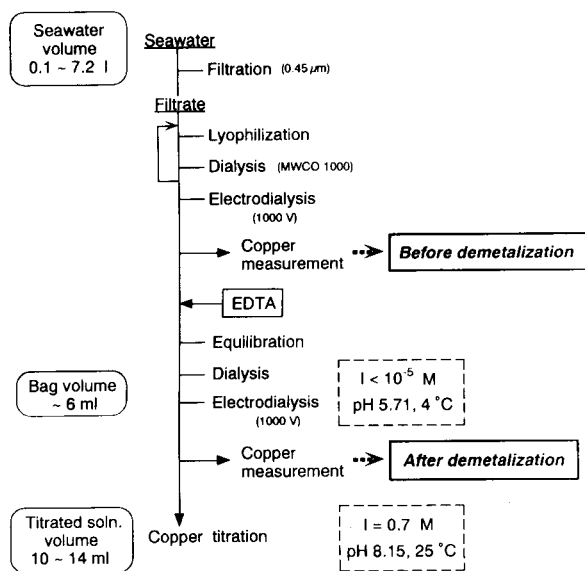
Fig. 1. Schematic representation of the procedure.

dure, ca. 10 l of the sea-water sample were concentrated to a final volume of less than 50 ml, and then one aliquot of the concentrated solution was subjected to copper measurement and another was subjected to the demetallization procedure.

Removal of metals bound to natural ligands in the concentrated solution was performed by the ligand-exchange reaction using excess of EDTA (demetallization process). An aliquot of the concentrated solution was subjected to this procedure as follows. After addition of 2 ml of 0.1 M EDTA (adjusted to pH 8 by addition of KOH), a sample solution was lyophilized to a few millilitres, then 10 μl of diethyl pyrocarbonate (Aldrich, Milwaukee, WI) were added to the solution as a disinfectant and the solution was equilibrated by stirring for 24 h at 4°C. The equilibrated solution, the volume of which was diluted to about 6 ml, was dialysed for 2 days at 4°C against 5 l of deionized water, with two changes, and then subjected to electrodialysis at a constant voltage of 1000 V (d.c.) for 2–4 days at 4°C against 1 l of deionized water with three to five changes. Electrodialysis was performed in an electrophoresis chamber (SJ-1060DCII; Atto, Tokyo). After demetallization, the sample solu-

tion inside the bag was at a pH of 5.71 and concentrations of major inorganic salts from the sea-water sample were below 10^{-5} M [13].

To estimate the permeability of the dialysis membrane to EDTA and copper–EDTA, the concentrations of copper and EDTA in the solution inside the dialysis bag (referred to as the bag hereafter), during the course of dialysis and electrodialysis, were monitored as a function of time in control experiments. A 6-ml volume of a solution containing 0.35 mM Cu(II) and 33 mM EDTA in dialysis tubing with a flat width of 12 mm were dialysed with stirring at 4°C against 5 l of deionized water. For electrodialysis, the same bag containing 4×10^{-8}–2×10^{-5} M Cu(II) and 1.3 mM EDTA was electrodialysed at 1000 V at 4°C against 1 l of deionized water. Electrodialysis was performed for 1–100 h. The entire solution inside the bag was subjected to analysis for copper or EDTA.

In this study, samples were always treated in parallel with a control during the course of the demetallization procedure and corrections were made for every measurement using the control data, as mentioned below, to compensate for the effect of possible adsorption of copper ions on the dialysis membrane and variations in the conditions of electrodialysis.

Analysis

Copper was determined as follows. The entire solution inside the bag was digested with several millilitres of concentrated HNO_3 and $HClO_4$, diluted to 0.2 ml in the case of samples from sites S and A and to 3 ml in the case of samples from sites P and J, and analysed by atomic absorption spectrometry (AAS) with a Model 170-50A spectrometer (Hitachi, Tokyo) and inductively coupled plasma atomic emission spectrometry (ICP-AES) with a model JY-48 spectrometer (Seiko, Tokyo). Precautions were taken to avoid contamination by trace metals, especially during the analysis of metals after demetallization.

The concentration of EDTA was determined as its methyl ester by gas chromatography and combined gas chromatography–mass spectrometry. After the addition of cyclohexane-1,2-diaminetetraacetic acid (CyDTA) and nonade-

canoic acid as internal standards, the sample solution inside the bag was evaporated to dryness under a stream of nitrogen. The EDTA in the dried sample was methylated with 14% BF_3 in absolute methanol in a sealed tube for 5 h at 110°C. The reaction mixture was washed twice with distilled water and the resulting methyl ester derivatives were extracted three times with distilled chloroform. The combined extract was evaporated to dryness under a stream of nitrogen gas and the residue was dissolved in chloroform. The methyl ester of EDTA in chloroform was determined using a Model 9APF gas chromatograph (Shimadzu, Kyoto) under the following conditions: DB-5 glass capillary column (25 m × 0.25 mm i.d.); oven temperature, 150–300°C, programmed to increase at 10°C min^{-1}; and injection port and detector temperatures, 280°C. The methyl ester of EDTA was identified by use of an INCOS 50 combined gas chromatograph–mass spectrometer (Finnigan MAT, San Jose, CA) with electron impact ionization (70 eV).

The symbols used and their definitions are listed in Table 1.

RESULTS

The concentrations of copper in the concentrated solutions of the sea-water samples from sites S, A, J and P before and after demetallization (Fig. 1) were converted into those in the original sea water and are given in Table 2. The concentrations of copper before demetallization for the samples from oceanic water (sites P and J) were determined to be in the range 0.33–2.7 nM. These values are comparable to those reported for the total concentration of dissolved copper in the surface water in the oceanic regimes (e.g., 0.4–1.2 nM in the North Pacific [15]), indicating that the samples are not significantly contaminated. The values for coastal water (sites S-1 and 2) are higher than those for oceanic water. The concentrations of copper in the sample bag after demetallization (Table 2, column A) are lower than the values before demetallization (Table 2, column B).

TABLE 1

Symbols and their definitions

Symbol	Definition
M	Metal ion
X	Inorganic ligand
Y	EDTA
L_i	Organic ligand of type i
L_N	Undemetallizable ligand
C_M	Total concentration of metal M
[M]	Concentration of free metal ion
[M']	Concentration of all inorganic forms of metal
pM	$-Log[M]$
C_Y	Total concentration of EDTA
[Y']	Concentration of EDTA not bound to copper
C_L	Total concentration of ligand L
[L']	Concentration of ligand L not bound to copper
K'_{ML}	Conditional stability constant for complex ML
α_M	Inorganic side-reaction coefficient for M
C_{Cu}^{min}	Sensitivity limit of ion-selective electrode for total copper

TABLE 2

Concentrations of copper in the original samples of sea water (B) before and (A) after demetallization

Site [a]	Depth (m)	C_{Cu} [b] (nM) B	A
S-1 [c]	0	1.20 ± 0.02	0.79 ± 0.04
	0	1.49 ± 0.03	0.77 ± 0.04
	0	1.44 ± 0.04	0.80 ± 0.06
	0	1.33 ± 0.10	0.74 ± 0.15
S-2 [c]	0	4.14 ± 0.14	1.08 ± 0.20
	0	4.09 ± 0.12	1.04 ± 0.18
A	0	0.49 ± 0.02	0.31 ± 0.03
P	0	2.70 ± 0.03	0.200 ± 0.004
	191	0.55 ± 0.03	0.071 ± 0.004
J	0	0.33 ± 0.03	< 0.01
	523	0.68 ± 0.03	0.050 ± 0.002
	1071	0.87 ± 0.03	0.170 ± 0.004

[a] Samples of sea water can be classified into three groups: sea water from sites P and J is oceanic; site A is in the region where oceanic and coastal sea water mix; and sites S-1 and 2 are coastal. [b] The concentration of copper is given as that in the original sea water: the amount of copper detected in the sample bag before demetallization was converted into the concentration in sea water after subtraction of the blank value for copper measurement; the concentration of copper in the sample bag after demetallization was corrected by subtracting that in the corresponding control bag. Each concentration was calculated on the basis of the volume of sea water used and the volume of the bag, shown in Table 3. The uncertainty represents the standard deviation (1σ). [c] Multiple measurements made on subsamples of surface sea water from Suruga Bay.

Concentrations of copper and EDTA in the control bag

The changes in the concentrations of copper and EDTA in the bag during the demetallization process were examined. As a control experiment, a bag that contained a solution of Cu(II) and EDTA without a sample of sea water was treated in the same way as one that contained a sample. After two successive rounds of dialysis for 16 h, the concentration of copper, C_{Cu}, decreased from 0.35 to 0.03 mM (Fig. 2), and the concentration of EDTA, C_Y, decreased from 33 to 1.3 mM in an identical experiment [13]; 91.7% of the copper and 96.1% of the EDTA in the initial solution inside the bag were removed by dialysis. Changes in the levels of EDTA and copper in the bag during the electrodialysis are shown in Figs. 3 and 4. C_Y in the bag decreased rapidly during the first 20 h before reaching an approximately constant value of 61 ± 17 nM. The amount of copper in the bag decreased logarithmically during the course of the electrodialysis, independently of any differences in the initial concentration of copper.

The differences between the value of $-\log C_{Cu}$ provided by the least-squares line (Fig. 4) and the actual values in each experiment are in the range 0.01–0.17 (as a logarithmic value) with

Fig. 3. Decrease in the concentration of EDTA, C_Y, inside the bag during electrodialysis. The initial concentration of EDTA was 1.3 mM (○) and the initial concentration of copper was 4×10^{-8} M.

a standard deviation (σ) of 0.091. This value of σ is not very high but each difference is not negligible, indicating that the control value varies in each electrodialysis. As a procedural matter, however, the influence of the variation in the values of C_{Cu} in the sample bag can be compen-

Fig. 2. Time course of changes in the concentration of copper, C_{Cu}, inside the bag during the dialysis. The values (●) were calculated from C_{Cu} in the external water. The direct determinations of C_{Cu} in the bag (○) agree well with the calculated values. The initial concentration of EDTA was 33 mM.
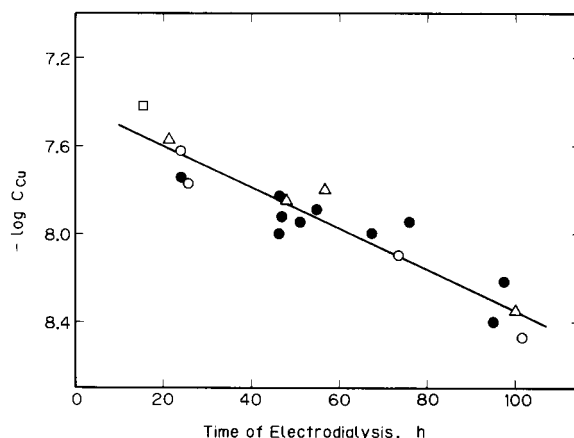
Fig. 4. Decrease in the concentration of copper, C_{Cu}, inside the bag during electrodialysis. Four experimental results are combined for initial concentrations of copper as follows: □ = 2×10^{-5} M; △ = 4×10^{-6} M; ○ = 4×10^{-7} M; ● = 4×10^{-8} M. The initial concentration of EDTA was fixed at 1.3 mM as in the experiment for which results are shown in Fig. 3. Regression equation: $-\log[C_{Cu} \text{ (M)}] = (9.35 \pm 0.79) \times 10^{-3}T + (7.41 \pm 0.05)$, $R = 0.947$, where T (h) represents the duration of electrodialysis.

sated for by correction using the value of the control that was processed in the same cell in parallel with exactly the same treatment of the sample throughout the demetallization process. Table 2 (column A) shows that the values of C_{Cu} are in good agreement with each other among four sets of subsamples for S-1 and between two sets of subsamples for S-2.

Concentrations of copper in concentrated solutions inside the sample bags

The concentrations of copper in concentrated solutions inside the sample bags before and after demetallization and in the corresponding control bags are summarized in Table 3. Amounts of copper in the sample bag after demetallization (Table 3, column A) were usually low compared with the values before demetallization (Table 3, column B). The values in column A include the concentration of copper bound to EDTA, which was added during the demetallization process, whereas those in column B do not include this concentration. An amount of copper corresponding at least to the differences in concentration between those in column B and those in column A is removed from the sample bag during the

demetallization process, apart from the amount of copper in the copper–EDTA complex. It is clear that significant amounts of copper were detected in almost all the demetallized samples as compared with those amounts in the corresponding control bags.

Modelling

A model was generated of the chemical speciation of the copper detected in the sample bag after demetallization (Table 3, column A) on the assumption that equilibria existed among the species of copper present in the bag under the conditions for electrodialysis, i.e., at an ionic strength lower than 10^{-5} M at pH 5.71 and 4°C. There are three possible sources of the copper.

(1) The EDTA complex (CuY): formation of a copper–EDTA complex, CuY, as an artifact during the procedure, occurred because EDTA introduced exogenously during the demetallization process still remains (61 ± 17 nM) in the bag after demetallization (Figs. 2–4).

(2) Inorganic complexes (CuX_n): formation of copper–inorganic complexes, CuX_n, with inorganic anions (X) derived from sea water may have occurred.

TABLE 3

Concentrations of copper in concentrated samples of sea water (B) before and (A) after demetallization and in the dialysis bag of the corresponding control (C) experiments

Site	Depth (m)	Sea-water sample				Control		Ref.
		Sea-water volume (l)	Bag volume (ml)	C_{Cu} [a] (nM)		Bag volume (ml)	C_{Cu} [a] (nM)	
				B	A		C	
S-1 [b]	0	0.49	5.9	100	79	6.0	13	13
	0	0.49	6.4	115	71	6.0	11	14
	0	0.25	5.1	69	47	5.0	8.4	14
	0	0.15	7.4	27	25	6.5	10	This work
S-2 [b]	0	0.10	6.9	60	21	6.8	5.4	14
	0	0.10	6.1	67	36	6.0	19	14
A	0	1.05	8.9	58	62	8.4	26	This work
P	0	4.33	6.2	1890 *	145	5.6	5.3	This work
	191	4.32	6.4	371 *	53	5.6	5.3	This work
J	0	6.16	7.0	290 *	22	6.2	13	This work
	523	7.23	5.9	833 *	68	5.6	6.5	This work
	1071	4.75	5.6	738 *	151	5.6	6.5	This work

[a] The concentration of copper is given as that in the dialysis bag. The precision in the determination of copper is generally ± 2 nM (1σ). However, it is ± 22 nM (1σ) for samples marked with an asterisk. [b] Multiple measurements made on subsamples of surface sea water from Suruga Bay.

(3) Natural organic complexes (CuL_i): this type of complex can be further divided into two categories, namely copper–organic complexes that are originally present in sea water and copper–organic complexes that are formed as artifacts during the concentration of the sea water.

Chemical forms of copper in the control

Before describing the analysis of copper speciation in the sample solution, let us first consider the behaviour of copper in the control solution. The equilibrium between EDTA and copper in the control solution is given as follows:

$$Cu + Y' \xrightleftharpoons{K'_{CuY}} CuY \tag{1}$$

$$K'_{CuY} = \frac{[CuY]}{[Cu][Y']} \tag{2}$$

where the concentration of EDTA that is not bound to copper, [Y'], is

$$[Y'] = C_Y - [CuY] \tag{3}$$

The concentrations of the copper–EDTA complex [CuY] and of free copper ions [Cu] are related to the total concentration of copper, $C_{Cu,C}$, in the control bag, by the equation

$$C_{Cu,C} = [Cu]\alpha_{Cu} + [CuY] \tag{4}$$

where α_{Cu} represents the inorganic side-reaction coefficient [16] for copper and is calculated to be 1.01 at pH 5.71 from the values of the conditional stability constants for copper–inorganic complexes, such as $CuOH^+$, $CuCO_3$ and $Cu(OH)_2$, which were based on the values given by Turner and Whitfield [17], corrected for the present conditions of electrodialysis. The fraction $[Cu]\alpha_{Cu}$ corresponds to the total concentration of inorganic copper species [Cu']. [Cu'] is approximately equal to [Cu] because $\alpha_{Cu} = 1.01$ under the present conditions.

[Cu'] in the control bag can be calculated from Eqns. 2–4 using the results of the control experiments. The conditional stability constant for the copper–EDTA complex, K'_{CuY}, was taken from the literature and corrected for the conditions of the present experiment, i.e., the value given by Turner and Whitfield [17] at infinite dilution and at 25°C was corrected for the temperature of 4°C,

using the value of the enthalpy of reaction 1, ΔH, given by Martell and Smith [18]. This calculation yields log $K'_{CuY} = 13.78$ at pH 5.71, taking into account the protonation reaction involving free EDTA (Y), under the present conditions of equilibrium systems. The total concentrations of EDTA and copper in the control bag were obtained from the results of the control experiments, i.e., $C_Y = 61 \pm 17$ nM (Fig. 3); for $C_{Cu,C}$, each value of the total concentration of copper in Table 3 (column C) is used. From Eqns. 2–4, the total concentrations of inorganic copper, [Cu'], in all the control solutions were found to be lower than $10^{-13.9}$ M. This result indicates that copper in the bag after demetallization binds quantitatively to EDTA. Thus,

$$C_{Cu,C} = [CuY] \tag{5}$$

in the solution used in the control experiment.

Chemical forms of copper in the sample

The chemical forms of copper in the sample bag after demetallization were determined from the experimental results and from calculations using an equilibrium model. The evaluations of the concentrations of the three types of copper, namely copper complexed with EDTA, copper complexed with inorganic ligands and copper complexed with natural organic ligands, were carried out under the conditions of electrodialysis (at an ionic strength lower than 10^{-5} M at pH 5.71 and 4°C; Fig. 1). A significant difference in the measurements of levels of copper was observed between the results for the control and those for the sample (Table 3). Each value for copper in the sample after demetallization (Table 3, column A) was corrected by subtracting the control value (Table 3, column C). The corrected value was called the excess copper, $[Cu]_{ex}$, and is given by

$$[Cu]_{ex} = C_{Cu,A} - C_{Cu,C} \tag{6}$$

The excess copper in the sample bag was characterized as follows.

EDTA complex (CuY). For demetallization, a large excess of EDTA was added to the sample solution and, accordingly, the concentration of each species in the sample solution was con-

trolled predominantly by [Y'], which was further regulated by the rate of dialysis that decreased depending on its own concentration ([Y']) as in the case of the control bag. On the assumption of a rapid response [19] of all copper species to a decrease in [Y'], [CuY] in the sample solution was effectively equal to that obtained from the control experiment (Table 3) [14,20]. As copper in the control bag is quantitatively complexed with EDTA (Eqn. 5), Eqn. 6 can be rewritten as follows:

$$[Cu]_{ex} = C_{Cu,A} - [CuY] \qquad (7)$$

The excess copper in the sample over the value for the control is given in Table 4. The excess copper in the sample solution is distinguishable from and separate from the Cu–EDTA complex.

Inorganic complex (CuX$_n$). The excess copper is not in an inorganic form for the following reason. Almost all major inorganic salts derived from sea water were already removed from the sample solution during the dialysis and electrodialysis steps [13] and, therefore, inorganic anions and, consequently, their copper complexes are present at low levels. The inorganic side-reaction coefficient for copper, α_{Cu}, is calculated to be 1.01 in both the sample solution and the control. [Y'] and [CuY] are also the same in both the

sample and the control solutions, as mentioned above. [Cu] and [Cu'] in the sample solution are, therefore, buffered to be at low levels by reaction 1 in the presence of excess Y' and they can be calculated in a similar manner to that shown above for the control. Thus [Cu'] is equal to the value calculated from the results of control experiments based on Eqn. 2. From the control values of [CuY] and [Y'] (Table 4), values of [Cu'] in the sample solutions after demetallization are all lower than $10^{-13.9}$ M (Table 4). This value is negligible in comparison with [Cu]$_{ex}$.

Natural organic complex (CuL$_i$). Copper in the solution inside the bag is not in the inorganic form but binds quantitatively to EDTA or to natural ligands after the addition of EDTA. As shown in Eqn. 7, the excess copper in the sample solution is free from EDTA; thus, the excess copper is in the form of natural organic complexes (CuL$_i$), that is,

$$[Cu]_{ex} = \sum_i [CuL_i] \qquad (8)$$

It was shown previously, by the copper titration method, that there are at least two kinds of natural ligand, L_1 and L_2, in the sample solution [13,14]. Let us now examine whether the excess copper can be explained as being CuL$_1$ (and

TABLE 4

Concentrations of excess copper detected and each copper species evaluated in the sample solution inside the dialysis bag at an ionic strength below 10^{-5} M at pH 5.71 and 4°C

Site	Depth (m)	Concentration (nM)						pCu
		[Cu]$_{ex}$ [a]	[CuY] [b]	[Y'] [c]	[Cu'] [d]	[CuL$_1$]	[CuL$_2$]	
S-1	0	66	13	48	4.5×10^{-6}	0.001	0.0001	14.3
	0	60	11	50	3.7×10^{-6}	0.001	9×10^{-5}	14.4
	0	39	8.4	53	2.6×10^{-6}	0.0004	4×10^{-5}	14.6
	0	15	10	51	3.3×10^{-6}	0.0002	2×10^{-5}	14.5
S-2	0	16	5.4	56	1.6×10^{-6}	0.0003	1×10^{-5}	14.8
	0	17	19	42	7.5×10^{-6}	0.002	7×10^{-5}	14.1
A	0	36	26	35	1.2×10^{-5}	0.003	0.0002	13.9
P	0	140	5.3	56	1.6×10^{-6}	0.002	0.0001	14.8
	191	48	5.3	56	1.6×10^{-6}	0.001	0.0003	14.8
J	0	9	13	48	4.5×10^{-6}	0.02	0.001	14.3
	523	61	6.5	54	2.0×10^{-6}	0.007	0.0009	14.7
	1071	144	6.5	54	2.0×10^{-6}	0.007	0.001	14.7

[a]–[c] The precision (1σ) is (a) 3, (b) 2 and (c) 17 nM. [d] The value of [Cu'] is approximately equal to that of [Cu] (see text).

CuL_2). The ligand L_1 is in the equilibrium of the following ligand-exchange reaction with EDTA:

$$CuL_1 + Y' \xrightleftharpoons{K_1} L_1' + CuY \qquad (9)$$
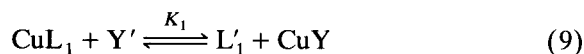
where the equilibrium constant is defined as

$$K_1 = \frac{K'_{CuY}}{K'_{CuL_1}} = \frac{[L_1'][CuY]}{[CuL_1][Y']} \qquad (10)$$

For the concentration of copper bound to ligand L_1 rewriting Eqn. 10 yields

$$[CuL_1] = \frac{K'_{CuL_1}[L_1'][CuY]}{K'_{CuY}[Y']} \qquad (11)$$

For $[L_1']$ and K'_{CuL_1}, the values of C_{L_1} and K'_{CuL_1} in Table 5 are available from the copper titration. The values of $[CuY]$ and $[Y']$ are obtained from the control experiment (Table 4). For K'_{CuY}, the same value as corrected above is available (log $K'_{CuY} = 13.78$). The calculated concentrations of CuL_1, i.e., $[CuL_1]$, are given in Table 4. The concentrations of CuL_1 are negligible compared with the level of the excess copper. The level of CuL_2 is also negligible because CuL_2 is less stable than CuL_1 (Table 5). Therefore, the

excess copper is not in the forms of natural organic complexes CuL_1 and CuL_2.

In conclusion, it follows form the above considerations that the excess copper found in the demetallized sample cannot be derived from an EDTA complex, from an inorganic complex, or from natural organic complexes with L_1 and L_2. Hence the concept begins to emerge that the excess copper may be in the form of a more stable complex with an alternative and uncharacterized natural ligand, an undemetallizable ligand, that is stronger than natural ligands L_1 and L_2 which were characterized by the copper titration in our procedure.

Evaluation of the conditional stability constant of the undemetallizable ligand for copper

If it is assumed that the natural ligand bound to copper after demetallization is a single unique species of undemetallizable ligand, designated L_N, the conditional stability constant, K'_{CuL_N}, can be calculated from the concentration of each species of copper in the sample after demetallization. The equilibrium with respect to the ligand L_N in the solution inside the bag after demetal-

TABLE 5

Concentrations of ligands and sensitivity limits for samples in the dialysis bag after demetallization and conditional stability constants deduced from results of copper titration experiments [a]

Site	Depth (m)	Ligand concentration (nM) [b]		Conditional stability constant		Sensitivity limit [b]: $C_{Cu}^{min'}$ (nM)	Ref.
		C_{L_1}	C_{L_2}	Log K'_{CuL_1}	Log K'_{CuL_2}		
S-1	0	720	1590	8.61	7.25	173	13
	0	664	1470			159	
	0	413	913			99	
	0	174	385			42	
S-2	0	136	710	9.12	7.05	64	14
	0	154	803			72	
A	0	130	578	9.26	7.40	45	This work
P	0	1540	5100	8.89	7.09	473	13
	191	2630	3710	8.41	7.75	305	13
J	0	1060	5720	9.60	7.57	592	13
	523	1350	4900	9.44	7.94	407	13
	1071	2970	9330	9.05	7.77	675	13

[a] Data were obtained at an ionic strength of 0.7 M at pH 8.15 and 25°C. [b] Concentrations of ligand and sensitivity limits are those for solutions in the dialysis bag. Each concentration was calculated from the volume of sea water used and the volume of the bag, shown in Table 3.

lization is expressed by the following equation:

$$CuL_N + Y' \xrightleftharpoons{K_N} L_N' + CuY \qquad (12)$$
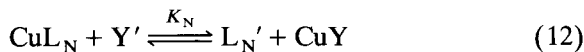
Therefore,

$$K_N = \frac{K'_{CuY}}{K'_{CuL_N}} = \frac{[L_N'][CuY]}{[CuL_N][Y']} \qquad (13)$$

For K'_{CuL_N}, Eqn. 13 can be rewritten as

$$K'_{CuL_N} = \frac{K'_{CuY}[CuL_N][Y']}{[CuY][L_N']} \qquad (14)$$

On the basis of the discrete-ligand model, the principal species responsible for the equilibrium with the ligand L_N in the sample solution can be considered to be the four species shown in Eqn. 12.

In Eqn. 14, the concentrations of the copper–EDTA complex [CuY] and of free EDTA [Y'] are obtained from the control experiment (Table 4). Natural ligands other than L_N in the solution are quantitatively demetallized by the ligand-exchange reaction (as are L_1 in Eqn. 9), which is attributed to the large excess of EDTA during the demetallization process. Therefore, copper in the solution inside the bag binds quantitatively to L_N or to EDTA. The total concentration of copper after demetallization is given as follows:

$$C_{Cu,A} = [CuL_N] + [CuY] \qquad (15)$$

From Eqns. 7 and 15,

$$[Cu]_{ex} = [CuL_N] \qquad (16)$$

The concentration of the CuL_N complex $[CuL_N]$, in the sample solution, is given as $[Cu]_{ex}$ in Table 4. The conditional stability constant of copper–EDTA, namely K'_{CuY}, has the same value as that used in the evaluation of $[CuL_1]$ (log $K'_{CuY} = 13.78$). Only the concentration of free L_N (concentration of ligand not bound to copper), $[L_N']$, in Eqn. 14 could not be measured experimentally.

For the calculation of K'_{CuL_N}, $[L_N']$ needs to be evaluated. $[L_N']$ falls below the sensitivity of the Cu(II) ISE measurement because no ligand of the L_N type was detected by the copper titration method [13]. However, an approximate estimation of $[L_N']$ may be possible if we introduce the sensitivity limit, C_{Cu}^{min}, as was discussed by Buffle [21]. This limit is defined as the minimum value of the total concentration of copper, C_{Cu}, for which a correct measurement of the concentration of free copper ions, [Cu], can be obtained. In the copper titration experiments, C_{Cu}^{min} was 200–400 nM (Table 6). C_{Cu}^{min} in the sample solution when the copper titration was performed was converted into the concentration, $C_{Cu}^{min'}$, (Table 5) in the solution inside the bag on the basis of the difference between the volumes of the solutions (Tables 3 and 6). The converted concentration, $C_{Cu}^{min'}$, can be assumed to be the upper limit of

TABLE 6

Concentrations of ligands and sensitivity limits in the copper-titrated solutions

Site	Depth (m)	Sea-water volume (l)	Titrated solution volume (ml)	Ligand concentration [a] (nM)		Sensitivity limit [a]: C_{Cu}^{min} (nM)	Ref.
				C_{L_1}	C_{L_2}		
S-1	0	1.66	14.0	1020	2250	245	13
S-2	0	0.80	14.0	537	2800	251	14
A	0	7.51	12.0	688	3070	238	This work
P	0	4.33	10.9	874	2900	269	13
	191	4.32	10.0	1680	2380	195	13
J	0	6.16	10.9	678	3670	380	13
	523	7.23	10.0	795	2890	240	13
	1071	4.75	10.9	1530	4790	347	13

[a] Concentrations of ligand and sensitivity limits are those for the titrated solutions. Each concentration was calculated on the basis of the volume of sea water used and the volume of the titrated solution, from the results of the copper titration.

TABLE 7

Estimated lower limits for the concentration of the undemetallizable ligand, L_N, and the conditional stability constant for copper in various samples of sea water at an ionic strength below 10^{-5} M at pH 5.71 and 4°C

Site	Depth (m)	Lower limit	
		C_{L_N} [a] (nM)	Log K'_{CuL_N}
S-1 [b]	0	0.79 ± 0.04	13.9 ± 0.2
	0	0.77 ± 0.04	14.0 ± 0.2
	0	0.80 ± 0.06	14.2 ± 0.2
	0	0.74 ± 0.15	14.0 ± 0.3
	Av.	0.78 ± 0.04	14.1 ± 0.1
S-2 [b]	0	1.08 ± 0.20	14.2 ± 0.3
	0	1.04 ± 0.18	13.5 ± 0.3
	Av.	1.06 ± 0.13	14.0 ± 0.2
A	0	0.31 ± 0.03	13.8 ± 0.3
P	0	0.200 ± 0.004	14.3 ± 0.3
	191	0.071 ± 0.004	14.0 ± 0.3
J	0	< 0.01	– [c]
	523	0.050 ± 0.002	13.9 ± 0.3
	1071	0.170 ± 0.004	14.0 ± 0.3

[a] Concentration of ligand is that in sea water. [b] Values estimated from multiple subsamples of sea water from Suruga Bay are averaged. [c] Not determined.

$[L_N']$ in the bag after demetallization, i.e.,

$$[L_N'] < C_{Cu}^{min'} \qquad (17)$$

The concentration of free L_N is restricted by the results of the copper titration (Table 5). Now, K'_{CuL_N} can be calculated from Eqn. 14. The conditional stability constant for the ligand L_N was evaluated to be higher than the range $10^{13.8}$–$10^{14.3}$ l mol^{-1} for the samples originating from sea water under the present conditions, i.e., at an ionic strength lower than 10^{-5} M at pH 5.71 and 4°C (Table 7).

Evaluation of the total concentration of the ligand L_N

The total concentration of the ligand L_N, C_{L_N}, is defined as the sum of the concentrations of the copper–L_N complex and of the ligand L_N not bound to copper by the following equation:

$$C_{L_N} = [CuL_N] + [L_N'] \qquad (18)$$

In this study, the value of $[Cu]_{ex}$ is taken as the lower limit for C_{L_N} and is converted into that in sea water. On the basis of considerations of copper speciation, the ligand L_N was detected in the

form of a copper complex, CuL_N, and its concentration was estimated to be equal to $[Cu]_{ex}$ from Eqn. 16; it appears that the total concentration of the ligand L_N is at least equal to, and may be greater than, $[Cu]_{ex}$. This estimate is conservative for C_{L_N} because it does not include the fraction of free L_N, the concentration of which, $[L_N']$, could not be determined. The lower limits for C_{L_N} in oceanic water (sites P and J) were determined to be in the range < 0.01–0.20 nM (Table 7).

Table 7 shows that there are no significant vertical variations either in the concentrations or in the conditional stability constants for the ligand L_N at the two oceanic sites. The concentrations of L_N are one to two orders of magnitude lower than those of L_1 or L_2 [13]. The conditional stability constants for L_N are four to six orders of magnitude greater than those of L_1 and L_2 (Table 5). The values for the concentration of the ligand for coastal water (sites S-1 and 2) are higher and those for the conditional stability constants are about the same as those for oceanic water.

DISCUSSION

Influence of contamination by metals on the procedure

The same levels of copper as those reported for open-ocean water were detected in the sample bag before demetallization (Table 2). These results give two insights regarding the magnitude of contamination in the procedure, that is, the samples were not significantly contaminated during the sampling and/or processing of samples by exogenous copper, and ambient copper in sea water (endogenous copper) may have been bound to organic ligands with high molecular weights during the concentration of the sample and retained in the bag during the dialysis and subsequent electrodialysis.

Consider the effects of two types of contamination, that is, by exogenous and endogenous copper, on the methodological performance. When samples are contaminated by exogenous metals, although the magnitude of this type of

contamination was insignificant in the present samples, the following two pieces of evidence indicated that exogenously introduced copper did not influence the copper contents of samples after demetallization. After the addition of copper to the sample solution and subsequent equilibration of reactions with natural ligands, the exogenously introduced copper ions were removed completely by the demetallization procedure and the same amount of copper as that detected without the addition of copper was detected in the sample after demetallization [14]. There were no detectable differences among the results of the three sequential experiments by repeated rounds of both demetallization and copper titration using the identical natural ligands from sea water, indicating that copper ions titrated in the sample solution were quantitatively removed after each titration [13].

Copper–organic complexes may have been endogenously formed between ambient copper and natural ligands as artifacts during the course of the concentration of the sample. The copper bound to organic ligands with high molecular weights would not be removed during the course of dialysis and subsequent electrodialysis, although copper–inorganic complexes with inorganic anions derived from sea water would be removed completely, as mentioned above. The resultant copper contents of samples would be influenced by different treatments, such as degree of concentration and desalting. The following three pieces of evidence verified that the demetallization was efficient in removing artifactual effects by endogenous contamination. First, when the first concentration procedure by lyophilization (Fig. 1) was omitted, the copper contents of samples after the second dialysis and electrodialysis were lower than those after the first concentration procedure, depending on the degree of concentration, whereas the values after demetallization closely resembled each other and were not influenced by the modification of the procedure [13]. Second, in the present work, concentrations of copper detected after demetallization converted into those in the original sea water were not dependent on the volumes of sea water used, i.e., the concentration ratio. Third, in the

copper titration experiment, the ligand L_1 in the deep water was not detected before demetallization because it was quantitatively masked by the relatively high level of ambient copper ions in the concentrated solution, but it was detected after demetallization [13].

The above observations indicate that the demetallization procedure allows interferences by both exogenous and endogenous contaminations by metals to be disregarded in the present discussion.

Relationship between the copper titration and demetallization experiments in the evaluation of the complexing ability of ligand L_N

No strong ligand with K'_{CuL_N} above 10^{14} l mol^{-1} was detected in the copper titration of an identical demetallized sample [13]. The high values of lower limits for K'_{CuL_N} (Table 7) estimated in this study suggest that the ligand L_N formed a complex with copper quantitatively in the initial sample solution after demetallization but before addition of copper. The concentration of free L_N was too low to be detected by using the Cu(II) ISE. From a tentative analysis based on the results of copper titration, however, it was demonstrated that the concentration of the CuL_N complex in the titrated solution was constant during the copper titration and was in good agreement with that obtained in the demetallization experiment [22]. The existence of the ligand L_N, which is characterized by the model, does not conflict with the results of the copper titration. It is recognized that the concentration of free L_N for each sample is appreciably lower than the value of the corresponding sensitivity limit (Table 6) used in the above-mentioned model, and consequently the estimation of the conditional stability constants of CuL_N, K'_{CuL_N}, shown in Table 7 may be fairly conservative. The tentative estimation of $[L_N']$ by using the results of both experiments further leads to values for K'_{CuL_N} one to two orders of magnitude higher than those shown in Table 7 [22].

Influence of other metals on the determination of $[L_N']$ and K'_{CuL_N}

In the above model, the influence of metal ions other than copper was ignored in the deter-

mination of $[L_N']$ and K'_{CuL_N}. As alkaline earth metal ions were removed during the demetallization process [13], these metals should have neither kinetic nor thermodynamic effects on the present calculations. However, the low levels of heavy metals may possibly interact with EDTA and/or with the ligand L_N, which are not bound to copper in the equilibrium reaction in Eqn. 12. When trace metals other than copper are present, these metal competition effects involving the side-reactions of EDTA finally lead to an overestimation of log K'_{CuL_N} by a magnitude comparable to the errors in its estimation (Table 7) [20].

When significant competition exists between copper and other metals in complexing with the ligand L_N, such reactions may increase the total concentration of the ligand L_N not bound to copper, but cannot increase the concentrations of free and protonated forms of the ligand L_N. Such effects result in a conservative estimate of the lower limit for C_{L_N} and would have no serious influence on the estimation of conditional value for K'_{CuL_N} which is controlled by the protonation reactions alone but is independent of the existence of other metals [20].

Other methodological considerations

In the model, the concentrations of CuL_1 and CuL_2 were calculated by use of K'_{CuL_1} and K'_{CuL_2}, which were both determined under conditions different from those of electrodialysis, that is, at an ionic strength of 0.7 M at pH 8.15 and 25°C (conditions of titration; Fig. 1). Use of these values leads to an overestimation of $[CuL_1]$ and $[CuL_2]$ at pH 5.71 primarily as a result of the influence of the difference in pH because K'_{CuL_1} and K'_{CuL_2} at pH 8.15 are relatively higher than those at pH 5.71, given the general properties of organic ligands (e.g. [21,23,24]). The contributions of $[CuL_1]$ and $[CuL_2]$ to $[Cu]_{ex}$ are, therefore, assessed to be even smaller and can be ignored.

For the evaluation of K'_{CuL_N}, the value of [CuY] in the sample bag was postulated a priori to be the same as that in the corresponding control bag, on the assumption of the rapid response of all copper species to the decrease in $[Y']$ with increasing duration of dialysis and sub-

sequent electrodialysis. The concentrations of all copper species including CuY and CuL_N are controlled by $[Y']$, which is the highest among the concentrations of the components permeable to the dialysis membrane. Although the response of the equilibrium in Eqn. 1 to the decrease in $[Y']$ in the sample bag is rapid [19], that of the equilibrium in Eqn. 12 for CuL_N may be slow. In such a case, [CuY] in the sample bag may be lower than that in the control bag and the value of $[CuL_N]$ measured would be conservative [20]. Consequently, the use of both the observed data for $[CuL_N]$ and the control value of [CuY] in Eqn. 14 leads to a conservative estimate of K'_{CuL_N}.

A significant difference in the concentrations of copper before (C_{Cu}, column B in Table 3) and after demetallization ($[Cu]_{ex}$ in Table 4) was clearly observed. The actual difference corresponds to the "demetallizable" ligands in the present procedure, namely ligands that cannot be demetallized by electrodialysis alone but are demetallizable by the demetallization procedure (in the presence of EDTA). In the present study, this fraction of the demetallizable ligands cannot be distinguished from the ligand L_1 or from the ligand L_N. However, the observed difference before and after demetallization suggests that some ligands with conditional stability constants between those of L_N and L_1 (log K'_{CuL} = 10–14) may possibly be present at low levels in sea water.

Occurrence of a strong ligand for copper in sea water

The conditional stability constant for the ligand L_N, evaluated in this study, represents a marked difference from results of observations of the complexing abilities of humic-type substances in sea water, and also of the humic substances isolated from fresh water, soil and sediment (e.g., [10,25]). The concentration of humic ligands in sea water estimated by Mantoura et al. [10] is four orders of magnitude higher but the conditional stability constant is almost four orders of magnitude lower than those for L_N evaluated in this study. Although detailed information about the nature of this ligand cannot be provided, the high value of K'_{CuL_N} in this study suggests that the ligand L_N may not be associated with so-

called humic-type materials. The occurrence of a strong ligand with $K'_{CuL} = 10^{13}-10^{14}$ l mol^{-1} has been reported and this ligand dominates the complexation of copper in surface sea water [7,26]. These values are similar to those evaluated for the ligand L_N in this study. It may be possible to relate the ligand L_N to the previously reported ligands or to some fraction of those ligands. However, the difference in the conditions of measurements between the previous and present studies makes it difficult to compare the present results directly with those of other workers until further investigations have been performed to evaluate the dependence of copper–L_N complexation on pH, temperature, ionic strength and side-reactions with Ca(II) and Mg(II) [24]. As the present estimate of K'_{CuL_N} is conservative, it is suggested that the ligand L_N detected in this study may be stronger than those reported previously.

Coale and Bruland [7,8] reported that the concentrations of a strong ligand with $K'_{CuL} = 10^{13}$ l mol^{-1} were 1.3–3.2 nM in surface waters, that the values decreased with increasing depth below the mixed layer and that the ligand was not detected below depths of 100–300 m. In this study, there was no apparent vertical change in the concentration of the ligand L_N and it was still detectable in the mid-depth water. The concentration (Table 7) of the ligand L_N at such depths was lower than that found by Coale and Bruland in surface waters. The strong ligand may not have been detected in the mid-depth water in the studies of Coale and Bruland [7,8], by using their method, for the following reason. The concentrations of the strong ligand at mid-depths were lower than those at the surface, whereas the total concentration of copper increased with increasing depth. Almost all the strong ligand and some fraction of the weaker ligands were titrated naturally by the increasing concentrations of ambient copper ions in the deep water. Consequently, low levels of the strong ligand that complexed quantitatively with copper became difficult to detect by their method because the concentration of the complexed weak ligand increased relative to that of the strong ligand at mid-depths. In the present experiment, the demetallization procedure allowed the detection of the strong ligand.

The determination of the complexing ability of the L_N-type ligand based on the simple equilibrium model suggests that such a copper complex is remarkably stable. The ligand L_N may play an important role in the geochemical cycle of copper in the ocean because of its high affinity for Cu(II) ions. It is of great interest to determine the nature of this strong organic ligand and to determine the relationship between the apparent lack of vertical structure in the profile of its concentration and the sources and sinks of the ligand of the L_N type. More detailed studies are required for the characterization of the undemetallizable ligand of the L_N type.

Conclusions

The occurrence of natural ligands (molecular weights > 1000) with high complexing abilities but low concentrations in sea water was established by a method based on a combination of a procedure that involves the ligand-exchange reaction between natural ligands and EDTA and the analysis of the experimental results on metal speciation by a simple equilibrium model.

The conditional stability constant for a copper complex with the strong ligand isolated from sea water was evaluated to be higher than the range $10^{13.8}-10^{14.3}$ l mol^{-1} for conservative estimate at an ionic strength lower than 10^{-5} M at pH 5.71 and 4°C. This type of strong ligand was detected from the entire water column of both oceanic and coastal waters whereas it would not be detected in deep water by the widely adopted titrimetric method because such ligands have been already titrated in situ with ambient metals.

The methodology involves the processes of concentration and desalting of natural ligands in sea water and of the removal of metals bound to natural ligands. As a result, the enormous complexity of the equilibrium systems in sea water becomes reduced and a simple model system of complexation equilibrium is applicable to the analysis of the experimental results.

The simplification provided by the present methodology yields some useful merits, namely versatility and that natural ligands from different sources (oceanic water, heavily polluted coastal water, river water, etc.) can be compared with

each other in terms of complexing ability and also with synthetic complexing reagents on the same basis.

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Conditional stability constants of metal complexes of organic ligands in sea water: past and present, and a simple coordination chemistry model

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Abstract

The conditional stability constants of complexes of organic ligands with trace metals (Cu and Zn) that have been determined by solution chemistry techniques since 1980 are summarized. The present common understanding on the chemical speciation of trace metals is that > 99% of copper dissolved in sea water is complexed with natural organic ligands; more than one strongly binding organic ligand exists in sea water. A simple coordination model, including two non-specific discrete strong organic ligands such as EDTA, is introduced to understand the complex features of recent metal speciation. The model, consistent with the result of the ligand titration, permits the wide range of conditional stability constants determined by metal titrations and the present concentration levels of several trace metals in sea water to be explained. These findings suggest that organic metal complexes play a significant role in the geochemical cycle of reactive trace metals.

The chemical speciation of trace metals is the most important concern in the field of marine chemistry because the bioavailability and scavenging of metals are closely related to the chemical forms of the metals. The speciation techniques generally suffer from difficult problems because sea water is a multi-component system and the concentrations of the trace metals of interest are extremely low (usually less than 10 nmol 1^{-1}). Hence highly sensitive analytical methods, including cleaning techniques, and a knowledge of thermodynamics and coordination chemistry are required [1–3]. Further, the results obtained by speciation techniques must be refined by chemi-

cal modelling, based on the concepts of thermodynamic calculations and coordination chemistry as applicable under the conditions of sea water, as Sillen presented in his "sea-water model" [1] in 1961.

A final goal in marine chemistry is to give a reasonable model able to explain the concentration levels of metal in sea water, containing horizontal and vertical distributions, using the chemical thermodynamics involved in non-equilibrium processes such as scavenging and dissolution, and which also satisfies biological requirements in the marine system. The least known constituent of sea water is organic matter, especially that which interacts with metals. In this connection, Mantoura [4] pointed out about a decade ago that despite the great number of independent experimental studies that show, directly or indirectly,

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the existence of organic–metal interactions in natural water, it has not been possible actually to isolate and identify the resulting complexes, mainly because of the lack of information on the molecular nature of 90% of the dissolved organic matter [5,6].

The current purpose of speciation studies is, in general, not only to study metal speciation but also the characterization of organic ligands in sea water. To achieve this, various steps are necessary; the first step is to measure the concentration of ligands present, the second to clarify their chemical properties, e.g., monodentate or polydentate, unique for a metal or not, and molecular weight, and the final step is to clarify the molecular structure in solution.

The purpose of this paper is to review the knowledge of the conditional stability constants of metal complexes of organic ligands with trace metals in sea water and to understand the properties of the organic ligands dissolved in sea water. A typical feature is that the conditional stability constants of trace metal complexes determined by metal titration cover a wide range. To clarify the implications of metal titrations involving complex processes, a simple coordination chemistry model is introduced that includes two discrete strongly binding organic ligands, which is consistent with the results of the ligand titration. Finally, an attempt is made to predict the concentration levels of several trace metals based on this model.

RESULTS AND DISCUSSION

Weak organic complexes in sea water

Weakly binding organic ligands are present in sea water but weakly binding organic complexes of metals cannot exist as a major species in sea water [2,3]. The usual organic materials in sea water such as amino acids, lipids and carbohydrates have a complexation ability with metals. However, the known organic materials bind too weakly to form metal complexes as major species because the concentrations of organic materials are too low in sea water [3].

The following preconditions should be accepted as a common understanding to determine whether the stability constants of metal complexes are large for a complex to be formed as one of major species under the conditions in sea water, i.e., low organic ligand concentrations, and in the presence of competitive reactions.

The estimation of the upper limit of organic ligand concentration is a difficult problem without solution chemistry techniques. The open ocean waters contain only small amounts of organic matter, the maximum concentration of total organic carbon observed in surface waters being less than about 200 μmol l^{-1} according to current data [7]. Dissolved organic carbon (DOC), however, provides no direct information on the ligand concentration. Taking account of the observation that simple organic molecules such as monocarboxylic acids and amino acids are minor constituents in oceanic organic matter, the concentration of a ligand that shows the same affinity to a metal is less than 1 μmol l^{-1} [3]. Therefore, 1 μmol l^{-1} is used as the upper limit of the concentration of an organic ligand to reject the possibility of the presence of weak complexes under the conditions in sea water. This value is a overestimate about one order of magnitude larger than that determined by metal speciation techniques.

The determination of an organic ligand in sea water gives lower limits of the conditional stability constants of trace metals, which are 10^5 l mol^{-1} for a 1 : 1 complex under the conditions of 10% complexation for trace metals. This estimate rejects the possibility of the presence of weakly bound complexes as a major species because the stability constants of organic monodentate ligands are less than 10^5 l mol^{-1} for most metal ions; the same applies for complexation between dicarboxylic acids, which react as a bidentate ligand, and mono- or bivalent metals [8].

Sea water is a typical multi-component system that contains many metals and inorganic and organic ligands. For the chemical speciation of metals in sea water, competitive reactions generally cannot be ignored. In sea-water systems, there are two types of competitive reactions. The first type is described as follows

$$MI + L = ML + I \qquad (1)$$

where M is a metal ion and I and L are inorganic and organic ligands, respectively. Anionic species in sea water, including chloride, sulphate, carbonate and hydroxide, can also act as ligands.

Taking the competitive reactions into account, a lower limit of the conditional stability constant is estimated from the following equation:

$$K_{ML}^c = R_{MT}(1 - R_{MT})^{-1}\alpha_{M(I)}[L] \qquad (2)$$

where R_{MT} is the concentration ratio of the organic metal complex to the total metal ($R_{MT} = [ML]/C_M$) and [L] is the free organic ligand concentration, whose maximum value is estimated to be 1 μmol l^{-1}. The side-reaction coefficient, $\alpha_{M(I)}$, which contains the effect of competitive reactions, is represented as follows:

$$\alpha_{M(I)} = 1 + K_{MCl}[Cl^-] + K_{MSO_4}[SO_4^{2-}]$$
$$+ K_{MOH}[OH^-] + K_{M(OH)_2}[OH^-]^2 + \dots \qquad (3)$$

where K_{MX} is the stability constant of the inorganic complex MX.

The lower limit of the conditional stability constant of an organic complex in sea water is calculated from Eqns. 2 and 3, using the data for inorganic speciation [9–16]. The results are summarized in Table 1, together with the corresponding side-reaction coefficients.

The second type of competitive reactions [17–19] is represented as

$$M_1L + M_2 = M_2L + M_1 \qquad (4)$$

where M_1 and M_2 are metal ions and L the organic ligand. These reactions are significant in

understanding the implications of metal titration if the organic ligand is non-selective for metal ions. This competition, described in detail later, is important to know whether most of the organic ligands are present in a free form or not.

Strongly bound organic complexes in sea water

The existence of strongly bound organic metal complexes in sea water had been recognized qualitatively until 1980 [20–23]. The development of bioinorganic chemistry revealed the presence of strongly bound organic metal complexes in biological systems; some metals (Fe, Cu, Zn, etc.) occupying the reactive centre in enzymes, and highly stable complexes, such as siderophores, were also discovered. In this connection, these metals are trace elements essential to both plants and animals. At high concentrations these metals are toxic, but more generally they are considered to be micronutrients [24–26]. If one does not inquire whether strongly bound organic metal complexes are major species or not, biological knowledge requires the presence of strongly bound organic metal complexes in sea water because the organic matter in sea water is essentially of biogenic origin.

Since 1980, the complexing characteristics of sea water have been widely studied for several transition metals (e.g., Cu, Zn) using two distinct concepts, viz., metal titration and ligand titration, although most studies were focused on copper because of its strong complexation and the analytical tool available. Metal titration methods have been extensively employed for the chemical speciation of trace metals in natural waters, with MnO$_2$ adsorption [27,28], electrochemical [29–40], chromatographic [41,42], bioassay [43], liquid–liquid extraction [44,45] and chemiluminescence techniques [46] being used. On the other hand, ligand titrations, which involve the competitive reactions between natural and artificial organic ligands, have hardly been developed although initial work on the use of EDTA was carried out in 1982 [47].

The recent common understanding based on experimental studies is that strongly complexing ligands exist in sea water; more than 99% of copper dissolved in sea water is present as complexes with organic ligands, whose conditional

TABLE 1

Side-reaction coefficients and lower limit values of conditional stability constants for metals under the conditions in sea water

Metal ion	Log $\alpha_{M(I)}$	Log K_{ML}^c
Th^{4+}	14.8	19.8
Fe^{3+}	11.3	16.3
Hg^{2+}	14.9	19.9
Cu^{2+}	1.77	6.8
Zn^{2+}	0.3	5.3
Ca^{2+}	0.08	5.1
Mg^{2+}	0.03	5.0

TABLE 2

Conditional stability constants of organic Cu complexes in sea water

Sampling location	pH	pCu	Log K_1^c	Log K_2^c	Log K_3^c	L_1 (nmol l^{-1})	L_2 (nmol l^{-1})	L_3 (nmol l^{-1})	Ref.
Western North Pacific	8.1	11.1	13.8	11.8	–	7	21	–	47
Irish Sea	8.2	–	–	–	10–10.4	–	–	60–150	28
Southeastern Gulf of Mexico	8.2	–	–	> 12	9.8	–	5	15	43
Cape San Blas, FL	8.2	11.5	–	11.2	9.0	–	13	80	43
Mississippi River plume	8.1	11.3	–	11.1	8.9	–	20	130	43
South Atlantic	7.7	–	–	12.2	10.2	–	11	33	29
Atlantic	–	–	–	–	9.9, 9.0	–	–	31, 87	31
Atlantic	–	–	–	–	9.7, 8.6	–	–	60, 120	31
North Sea	7.9–8.2	–	–	–	8.9–9.1	–	–	80–103	30
Tamor Estuary	–	–	–	–	8.6–9.1	–	–	390	32
North Atlantic	8.0–8.3	–	–	–	7.8–8.2	–	–	50–82	33
North Atlantic	–	12.2–12.7	–	9.8–12	7.4–8.9	–	4–144	2–440	34
Biscayne Bay	–	–	–	12	10.5	–	5.1	110	44
Naragansett Bay	8.0	12.5	–	12.4	10	–	50	100	41
Naragansett Bay	8.0	12.1	–	12	10	–	20	100	41
Coastal Peru	8.2	11.4	–	12.3	9.2	–	4.5	70	41
Chistiansen Basin	8.0	11.8	–	11.7	9.1	–	50	68	35
Montauk Point	8.2	12.2	–	11.7	9.1	–	20	50	35
Northeast Pacific:									
Surface	–	13.9	–	11.9	9.5–10.6	–	1.8	7.6	36
Deep	–	11	–	–	8.1–9.1	–	–	5–10	36
North Pacific	–	–	13.0	–	10.0	1–3	–	5	37
Southwestern Sargasso Sea	–	–	13.2	–	9.7	2.0	–	80	43
Shelf water off North Carolina	8.1	12.5	13.2	–	10.0	3.3	–	26	46
Severn Estuary	–	11.1–12.8	–	11.4–12.8	–	–	13–196	–	38
Indian Ocean	–	–	–	12.6	–	–	4.13	–	40
North Sea	–	–	–	12.4	–	–	16.2	–	40

stability constants are in the range $10^9–10^{14}$ l mol^{-1}. The conditional stability constants of organic copper complexes in sea water, which have mainly been determined since 1980, are summarized in Table 2.

Based on the conditional stability constants of organic copper complexes, organic ligands in sea water are conveniently divided into the three classes: the strongest, L_1 (log $K_{CuL}^c > 13$), strong, L_2 (log $K_{CuL}^c \approx 12$) and weak ligands, L_3 (log

TABLE 3

Conditional stability constants of organic Zn complexes in sea water

Sampling location	pH	pZn	Log K_1^c	Log K_2^c	Log K_3^c	L_1 (nmol l^{-1})	L_2 (nmol l^{-1})	L_3 (nmol l^{-1})	Ref.
Western North Pacific	8.1	8.7	10.7	9.3	–	5	8	–	47
South Atlantic	–	–	–	–	7.4	–	–	30	49
Irish Sea	–	–	–	8.4	7.5	–	26	64	48
Central North Pacific Gyre	–	–	11.0	–	–	1.2	–	–	50
Northeast Pacific	8.2	–	10.2–11.3	–	–	1.6–2.3	–	–	51
Atlantic, etc.	–	–	–	6.3–8.1	5.8–6.6	–	–	–	16
Narrangansett Bay	–	–	–	8.3–9.4	7.4–7.7	–	4–46	32–104	52

$K^c_{CuL} < 10$). It must be noted that in no case have the three kinds of organic ligands simultaneously been detected in the same experiment. As a general trend, however, the concentrations of the organic ligands complexed with Cu increase in the order $L_1 < L_2 \ll L_3$. Taking account of the total concentration of Cu dissolved in sea water, which is usually less than that of the organic ligands, L_2, L_1 or $L_1 + L_2$, it is clear that most of the organic ligand, L_3, is not bound with Cu under the conditions in sea water; in other words, most of the dissolved Cu is present as complexes with the organic ligands, L_1 and/or L_2. This situation is consistent with the fact that the organic ligand, L_3, cannot be detected by the ligand titration method [47].

The speciation of zinc in sea water has recently been studied by several workers [48–52], although inorganic speciation of Zn in sea water has been carried out from initial stage [9,11,12]. The conditional stability constants of organic Zn complexes, which were determined by the ligand and metal titration methods, are summarized in Table 3. The conditional stability constants are divided into three classes similarly to Cu. The stability of organic Zn complexes is systematically lower than those of Cu. This tendency is reasonable according to the Irving–Williams series for transition metals [2], although there has been no discussion about whether the organic ligands associated with Zn are the same as those of Cu or not.

All studies suggest that it is significant to characterize the chemical properties of the organic ligands, L_1 and/or L_2. This includes resolving the problem of whether the organic ligands determined by titration methods can be considered as discrete or not. An additional problem is the role that the major organic ligand, L_3, plays from oceanographic and chemical points of view, including analytical artifacts. The main reason why the features of the strongly binding organic ligands in sea water could not be clarified lies in the organic geochemistry as pointed out be Mantoura [4]. Another reason is that most studies regarding speciation in sea water have been limited to Cu and partly Zn.

Determination of conditional stability constants of trace metals

A knowledge of the chemical thermodynamics of metal complexation in solution, especially linear free-energy relationships (LFER), will become a tool for understanding the chemical forms of metals and ligands in sea water. LFER techniques have recently been developed for the determination of missing values of metal complex formation constants in several areas of chemical oceanography [16,53,54].

There is still little information on the properties of organic ligands, except that organic ligands may be macromolecules such as humic compounds originating from biogenic matter [4]. Assuming that natural organic ligands in sea water are non-specific and non-selective regarding the complexation of metal ions and aminopolycarboxylic acids, then it is expected that there is an "artificial" ligand which is in an LFER with a natural organic ligand. In this case, the following equation for an "artificial" ligand as a reference is established:

$$\log K_{ML} = \log K_{MY} + A \tag{5}$$

where K_{MY} is the stability constant of the metal complex with an artificial ligand and A is a constant.

The conditional stability constants of natural organic metal complexes have been extensively measured by various techniques. However, the data set applied to the LFER technique must satisfy the following conditions: conditional stability constants for more than one metal ion being determined with the same method and/or the same quality, and including no effect of competitive reactions between metals. The data obtained by metal titration contain the possibilities of conditional stability constants reflecting metal exchange reactions. The available data sets, therefore, are limited to the results for Cu and Zn obtained by the ligand titration [47]; two kinds of organic ligands, L_1 and L_2, co-exist in sea water, in which the stability constants are of the same orders as those determined in recent studies (Tables 2 and 3).

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TABLE 4

Predicted stability constants of metal complexes with DTPA- and EDTA-type organic ligands in sea water

Metal ion	DTPA-type ligand		EDTA-type ligand	
	Log K_{MY_1} [a]	Log K_{ML_1}	Log K_{MY_2} [a]	Log K_{ML_2}
Th^{4+}	28.8	21.1	23.2	16.1
Fe^{3+}	28.0	20.1	25.1	18.0
Hg^{2+}	26.7	19.0	21.7	14.6
Cu^{2+}	21.6	13.8	18.8	11.7
Zn^{2+}	18.4	10.7	16.5	9.4
Ca^{2+}	10.8	3.1	11.0	3.9
Mg^{2+}	9.3	1.6	9.1	2.0

[a] K_{MY_1} and K_{MY_2} are the stability constants of metal complexes with DTPA and EDTA, respectively.

Some artificial ligands, the stability constant of whose complexes were selected to determine under the same conditions (the same ionic strength, etc.), were examined with respect to satisfying the LFER relationship for the organic ligands L_1 and L_2. It was found that LFER relationships with a slope of unity are established between the organic ligand L_1 and diethylenetriaminepentaacetic acid (DTPA) and between the organic ligand L_2 and ethylenediaminetetraacetic acid (EDTA), the constant A in Eqn. 5 being 7.7 and 7.1 for the organic ligands L_1 and L_2, respectively. To avoid confusion between ligand terms in the ligand and metal titration methods, henceforth the terms DTPA- and EDTA-type organic ligands are used for the strongly binding organic ligands L_1 and L_2, respectively, determined by ligand titration. The LFER technique permits the stability constants of natural organic complexes in sea water to be determined for metals with known stability constants of EDTA and DTPA complexes. The calculated results are summarized in Table 4, together with the corresponding stability constants of EDTA and DTPA complexes [55,56].

The predicted stability constants for alkaline earth metals are relatively low (log $K_{CaL} = 3.9$ and log $K_{MgL} = 2.0$ for the EDTA-type ligand), which is consistent with the fact that the major species of Ca and Mg are the free ions. As specific interactions between alkali and alkaline earth metal ions and organic ligands in natural waters have, in general, escaped the attention of aquatic chemists, there has been only a limited study to determine the conditional stability constants of Ca and Mg complexes with marine organic matter. Mantoura et al. [57] measured the conditional stability constants of Ca and Mg with marine humic compounds: log $K^c_{CaL} = 3.87$ and log $K^c_{MgL} = 3.4$. It is surprising that the conditional stability constant of the organic Ca complex in sea water is in fair agreement with that of the EDTA-type organic ligand predicted by the LFER technique. This finding does not simply imply that one of the natural organic ligands is completely characterized by the LFER technique because the conditional stability constants for several transition metals with humic compounds determined by Mantoura et al. [57] were much lower than the current data. However, these low conditional stability constants can also be explained by the competitive reactions between metals, as discussed later. Therefore, the result permits the hypothesis that the chemical properties of the EDTA-type organic ligand are similar to those of humic compounds specified by Mantoura et al. [57].

For reactive metals in sea water, e.g., Th, Fe and Hg, the LFER technique can lead to some significant ideas regarding complexation with organic matter in sea water. Concerning reactive metals, the role of iron in marine ecology is receiving increasing attention in view of recent data showing that it may limit primary production in parts of the ocean [58]. This limitation is due to low Fe concentrations (0.05–1 nmol l^{-1}) in oxic sea water [59–61] or the unavailability of dominant Fe species. To solve the ecological problems that govern the acquisition of Fe in the marine environment, therefore, it is essential to clarify the chemical form of Fe in sea water because the rate of uptake of Fe by phytoplankton depends on the reactivity of dissolved Fe species. In this connection, there is no evidence of direct uptake of colloidal Fe by algae [62].

The stability constants predicted for organic Fe^{3+} complexes by the LFER technique, both of which are higher than the lower limits in Table 1, suggest the possibility that Fe^{3+} in sea water forms organic complexes. Recently, Hudson et al. [63] suggested that organic complexation of

Fe(III) is possible in sea water from the results with model and natural systems. Hudson and Morel [64] studied Fe transport in marine phytoplankton, in which the half-saturation constant for the Fe cell surface complex of phytoplankton was measured kinetically. The stability constant of the Fe^{3+} cell surface complex can be evaluated to be $\log K_{FeL}^{c} = 20.2$ from the results of Hudson and Morel [64], which is in fair agreement with the value ($\log K_{FeL} = 20.1$) predicted for the DTPA-type organic ligand. This led to the idea that the DTPA-type organic ligand dissolved in sea water is characterized as fresh organic matter originating from the phytoplankton cell surface and then directly related to the primary production.

Thorium in sea water has been extensively studied in the field of chemical oceanography with respect to scavenging processes [65]. A typical feature for Th in sea water is that even recent concentration levels ($0.02–2$ pmol l^{-1}) [66–71] are supersaturated with regard to the dissolution of inorganic salts (the solubility of thorianite is about 0.01 pmol l^{-1}) [72]. To understand the mechanism of scavenging and dissolution phenomena, it is also significant to clarify the chemical form of particulate and dissolved Th. A recent leaching study [73] suggests that Th in the particulate matter is present as organic complexes. Dissolved Th is also considered to be present as complexes with humic substances [74,75]. The stability constants predicted by the LFER technique suggest that only the DTPA-type organic ligand, relatively fresh biogenic matter, can form a complex with Th. The EDTA-type organic ligand, which is the major one of the humic compound type, cannot be complexed with Th under the conditions in sea water. These findings are consistent with the scavenging model that new production is the main controlling factor for the vertical transport of particle-reactive metals [76,77].

Mercury is the most toxic metal in the environment. The XAD-2 adsorption method [78] suggests the presence of organically bounded Hg in sea water. However, the stability constants predicted by the LFER model, both of which are lower than the lower limit value in Table 1, suggest that Hg cannot exist as complexes with the organic ligands discussed.

Chemical forms of the organic ligands

There is little information on the chemical properties and molecular structures of the organic ligands. However, it can be discussed whether the major form of the organic ligands is free or not, although this problem has received little attention so far.

The ratio of the complexed ligand concentration to the free ligand concentration for individual metal ions, R_L, is easily deduced from the definition of the stability constant:

$$R_L = [ML]/[L] = K_{ML}[M^{n+}] \qquad (6)$$

where $[M^{n+}]$ is the free ion concentration of a metal.

The free ion concentrations of metals are the most significant factor for understanding the biological uptake processes and the chemical speciation of metals. For trace metals, a current problem is what the levels of free ions are in sea water and what factors control these free ion levels. However, the free ion concentration levels of major metals in sea water, e.g., Ca and Mg, can be estimated from an inorganic equilibrium system although there may be some discussion on the precise concentrations of these free metal ions. On the other hand, for some trace metals, which are supersaturated regarding the dissolution of inorganic salts, the free ion concentrations are controlled by the dissolution equilibrium of inorganic salts, which are calculated from the solubility products [8,79]. For metals that are undersaturated regarding the dissolution of inorganic salts in sea water, e.g., Cu and Zn, it is difficult to estimate the free ion concentrations thermodynamically. The free metal concentrations of Cu and Zn in sea water are thus tentative values, taking the direct measurements of free metal ion concentrations into account (Table 2). It must be noted that these free ion concentrations of Cu and Zn are optimum levels with regard to phytoplankton growth [80–85]. The free metal concentration levels of some metals in sea water are summarized in Table 5.

TABLE 5

Free metal concentrations and ratios of the complexed ligand concentration to the free ligand concentration under the conditions in sea water

Metal ion	Free ion: $\log [M^{n+}]$	DTPA-type ligand: $\log R_{L_1}$	EDTA-type ligand: $\log R_{L_2}$
Th^{4+}	-23.0	-1.9	-6.9
Fe^{3+}	-19.3	0.8	-1.3
Cu^{2+}	-12.0	1.8	-0.2
Zn^{2+}	-11.0	-0.3	-1.6
Ca^{2+}	-2.07	1.0	1.8
Mg^{2+}	-1.49	0.1	0.5

The ratios, R_L, for some metals were calculated from Eqn. 6 using the corresponding stability constants predicted by the LFER technique. The results are given in Table 5. A typical feature concerning the chemical form of organic ligands is that most of the organic ligands are present as complexes with metals. Fig. 1 shows the percentage of the metal-bound ligand with respect to the total for the DTPA- and EDTA-type organic ligands. The values for the free ligands are less than 1% and 2% for DTPA- and EDTA-type organic ligands, respectively. Most of the binding site for the major EDTA-type ligand is occupied by calcium ion, whose possibility was discussed by

Reuter and Perdue [17]. There is no change in this situation as calculated from the data of Mantoura et al. [57] even if one cannot recognize the LFER technique. On the other hand, one of the major forms of the DTPA-type organic ligand is considered to be Cu complex.

Determination of concentration levels of trace metals

During the past decade, major developments in analytical and sampling techniques have provided new concentration levels and distributions for many trace metals in sea water. The present concentration levels for trace metals [86–100], as shown in Table 6, are about one to two orders of magnitude lower than those determined before 1975. Nevertheless, even the very low concentrations of some metals, e.g., Th, are supersaturated with regard to the dissolution of corresponding inorganic salts. However, there is no model that can explain the present concentration levels of trace metals in sea water. On the other hand, Fe(III) in sea water may be near supersaturation of inorganic salts because the solubility of iron(II) hydroxide is about 0.5 nmol l^{-1} [101].

The concentration levels of organic complexes of Th, Fe, Cu and Zn, which are the most interesting issue for marine chemists because of their
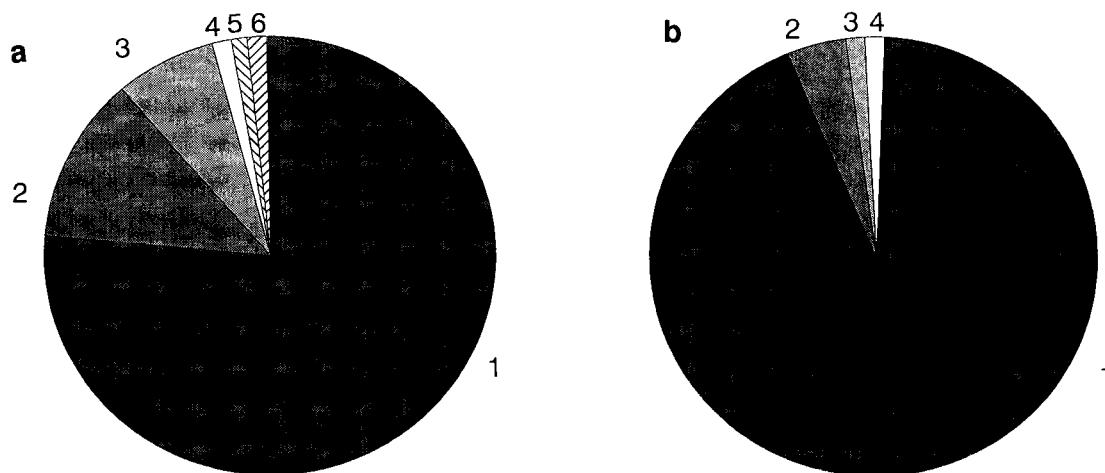
Fig. 1. Percentages of metal-bound ligand to the total for the organic ligands L_1 and L_2. (a) Organic ligand L_1: $1 = CuL_1$; $2 = CaL_1$; $3 = FeL_1$; $4 = MgL_1$; $5 = L_1$; $6 = ZnL_1$. (b) Organic ligand L_2: $1 = CaL_2$; $2 = MgL_2$; $3 = L_2$; $4 = CuL_2$.

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important role in the biogeochemical cycle [58], can be estimated if the free ligand concentrations of the DTPA- and EDTA-type organic ligands are known. Taking account of the total ligand concentrations as shown in Table 2 and estimates of the free ligand concentrations for ligand titration [47] (0.01–0.05 nmol l^{-1} for the DTPA-type organic ligand and about 4 nmol l^{-1} for the EDTA-type organic ligand), 0.05 and 2 nmol l^{-1} were adopted for the DTPA- and EDTA-type organic ligands, respectively, as tentative values of the free ligand concentrations. The calculated results for several metals are given in Table 6.

The calculated concentrations of Th, Fe, Cu and Zn dissolved in sea water are 0.6 pmol l^{-1} and 0.9, 4.4 and 0.1 nmol l^{-1}, respectively, which agree with the present concentration levels in open ocean surface waters, although colloidal Fe and Th cannot be evaluated. The model suggests that the major chemical forms of Th, Fe and Cu dissolved in sea water are the complexes with the DTPA-type organic ligand, 100, 75 and 75% of the total organic complex for Th, Fe and Cu, respectively, being associated with the DTPA-type organic ligand, whereas Zn associated with the DTPA-type organic ligand is only 38% of the total organic complex. In other words, reactive trace metals react preferentially with the DTPA-type organic ligand, which may originate directly from the phytoplankton cell surface and play a significant role concerning the scavenging and the bioavailability of trace metals. In fact, about 75% of the DTPA-type organic ligand is associated with Cu. Ca is the second most important metal bound with the DTPA-type organic ligand. An-

other typical feature is that the amount of the DTPA type organic ligand is only a few percent of the EDTA-type organic ligand.

Most of the EDTA-type organic ligand in sea water is associated with Ca, as discussed previously, although the organic Ca complex is less than 0.002% of the total. For trace metals, only Cu can form a meaningful amount of complex with the EDTA-type organic ligand. The total concentration of the EDTA-type organic ligand is calculated to be about 130 nmol l^{-1}, which may correspond to that of the weakly binding organic ligand, L_3, determined by metal titration because this method gives correct information on the total organic ligand concentration.

There is the possibility that the concentration of the DTPA-type organic ligand varies widely in sea water because it is due to the fresh biogenic matter, in contrast to humic substances such as the EDTA-type ligand, which may be preserved in sea water. When the free ligand concentration of the DTPA-type organic ligand in sea water is lowered to 0.005 nmol l^{-1} in the model, the concentrations of Th, Fe, Cu and Zn in sea water are 0.06 pmol l^{-1} and 0.6, 2 and 0.07 nmol l^{-1}, respectively, which are also in the range of the present concentration levels.

There is an idea that relatively high concentrations of trace metals (Cu, Fe, etc.) dissolved in sea water (several tens of nmol l^{-1}) are due to unknown organic complexes, which may be adsorbed on some adsorber such as XAD-2 [23]. The LFER technique will remove this possibility because the DTPA- and EDTA-type organic ligands show the strongest affinity with "hard" acids

TABLE 6

Concentrations of the organic complexes (nmol l^{-1}) and the total predicted by the coordination chemistry model (nmol l^{-1}) under the conditions in sea water

Metal ion	Organic metal complex [a]		Inorganic	Total		Ref.
	Ligand L_1	Ligand L_2		Predicted	Measured	
Th^{4+}	6×10^{-4}	2×10^{-7}	–	6×10^{-4}	$(0.02–2) \times 10^{-3}$	65–71
Fe^{3+}	0.3	0.1	0.5 [b]	0.9	0.01–1	58–61
Cu^{2+}	3.1	1.3	0.05	4.5	0.5–10	86–96
Zn^{2+}	0.03	0.05	0.02	0.1	0.01–6	97–100

[a] The concentrations of the organic metal complexes were calculated using free organic ligand concentrations [L_1] = 0.05 nmol l^{-1} and [L_2] = 2 nmol l^{-1}. [b] From Ref. 101.

such as Th^{4+} and Fe^{3+} in oxic sea water, except in the case of siderophores, taking into account that the properties of the natural organic ligands discussed are similar to those of aminopolycarboxylic acids. Further, it is difficult to believe that more than nmol l^{-1} levels of special ligands such as siderophores are generally present in open ocean waters.

Implications of the metal titration

To determine the conditional stability constants of organic complexes with trace metals, metal titration methods have been widely applied [27–46]. A typical feature is that the given conditional stability constants of Cu and Zn complexes increase with increase in the analytical sensitivity regarding pCu and pZn measurements but less than that of the DTPA-type organic ligand determined by ligand titration [47], as shown in Tables 2 and 3. This tendency has been confirmed from a theoretical comparison of the metal and ligand titration methods [102].

The coordination chemistry model suggests that Ca and Mg complexes in sea water are present as major forms of the EDTA- and DTPA-type organic ligands. As metal titrations for the chemical speciation of trace metals have usually been carried out under the conditions of sea water, the total concentrations of the DTPA- and EDTA-type organic ligands are written as follows:

$$C_{Li} = [L_i] + [CaL_i] + [MgL_i] + [ML_i]$$
$$= [L_i]\alpha_{L_i(Ca,Mg)} + [ML_i] \qquad (7)$$

where $i = 1$ and 2 and $\alpha_{Li(Ca,Mg)}$ is the side-reaction coefficient for the organic ligand, L_i, including the effect of metal exchange reactions; $\alpha_{L_i(Ca,Mg)} = 1 + K_{CaL_i}[Ca^{2+}] + K_{MgL_i}[Mg^{2+}]$. The side-reaction coefficients are kept constant because there is no change in free ion concentrations of Ca and Mg in the process of metal titration.

When metal exchange equilibria are established during the titration process, the total concentration of an added metal, C_M, is represented as follows:

$$C_M = [M^{n+}]' + [ML_1] + [ML_2]$$

$$= [M^{n+}]\alpha_{M(I)} + K_{ML_1}C_{L_1}[M^{n+}]$$

$$\times \left\{\alpha_{L_1(Ca,Mg)} + K_{ML_1}[M^{n+}]\right\}^{-1}$$

$$+ K_{ML_2}C_{L_2}[M^{n+}]$$

$$\times \left\{\alpha_{L_2(Ca,Mg)} + K_{ML_2}[M^{n+}]\right\}^{-1} \qquad (8)$$

We now introduce conditional stability constants of organic metal complexes including the effect of metal competitive reactions, which are defined as follows:

$$K^c_{ML_i} = K_{ML_i}\alpha_{L_i(Ca,Mg)}{}^{-1} \qquad (9)$$

where $i = 1$ and 2. By using the conditional stability constants, the following equation is obtained:

$$C_M = [M^{n+}]\alpha_{M(I)}$$

$$+ K^c_{ML_1}C_{L_1}[M^{n+}]\left(1 + K^c_{ML_1}[M^{n+}]\right)^{-1}$$

$$+ K^c_{ML_2}C_{L_2}[M^{n+}]\left(1 + K^c_{ML_2}[M^{n+}]\right)^{-1}$$
$$\qquad (10)$$

This equation corresponds to a typical relationship for a metal titration system containing two different ligands. The result reveals that the presence of competitive reactions between metals affects only stability constants. Hence the metal titration method gives true ligand concentrations and the number of ligands.

The logarithmic conditional stability constants for the complexes of Cu and Zn with the major EDTA-type organic ligand are calculated to be 10.0 and 7.5, respectively, from Eqn. 9, which are in good agreement with recent values for the organic complexes with the weakly binding organic ligand, L_3, determined by the metal titration. These findings suggest that the weakly binding complexes with the organic ligand, L_3, reflect metal exchange reactions between Ca and added metals for the EDTA-type organic ligand. The logarithmic conditional stability constants for the complexes of Cu and Zn with the DTPA-type organic ligand are calculated to be 12.7 and 9.6, respectively, from Eqn. 9, which correspond to recent values [37,40,46,52] for the organic complexes with the strongly binding organic ligand, L_2, determined by metal titration. These findings

suggest that the two organic ligands detected by ligand titration are consistent with those given by the metal titration. The existence of the two organic ligands, therefore, is essential to establish the chemical form of trace metals in sea water.

Bruland [50] and Donat and Bruland [51] determined a higher conditional stability constant of an organic zinc complex by metal titration (log $K_{ZnL}^c = 10$–11). It is difficult to explain the presence of this complex by the equilibrium model. This may suggest the presence of a new organic ligand. It must be pointed out, however, that Bruland's conditional stability constant apparently coincides with the stability constant for the DTPA-type organic ligand. If the kinetic effect, due to relatively slow dissociation of the Ca complex, is present in extremely low-level titrations of Zn, there is a possibility that the stability constant of the complex for the free DTPA-type ligand was measured.

Bruland [50] pointed out that strongly binding organic ligands obtained by metal titration are specific for the corresponding metals from the experiment involving doubling the concentration of possible interfering metals. It is never decided from such an experiment, however, whether strongly binding organic ligands are specific for the corresponding metals or not. The model suggests that the major part of the DTPA- and EDTA-type ligands is complexed with Ca^{2+} ion. Then the effect of the additional Cu (0.6 nmol l^{-1}) for the Zn titration is absorbed by the Ca complex pool, which is a typical ligand buffer. Therefore, the lack of change observed in the titration curve for small additions of Cu or Cd does not provide evidence that the strongly binding organic ligand is specific to Zn, but indicates that the strongly binding ligand determined by the Zn titration is in a ligand buffer system.

The analysis of the metal titration curves must consider the effect of the competitive reactions between metals. There are several theoretical studies on metal titration that include the effect of competitive reactions. However, recent theoretical developments are inadequate to introduce the correct parameters from all of the metal titration curves because there are excessive simplifications in the processes used to deduce the theoretical equations [18] and these equations cannot contain two strong ligands [19]. However, although the metal titration includes many reactions, e.g., complex formation for the free organic ligands and competitive reactions between Ca (and partly Mg and other metals) and an added metal, the overall titration curves can be expressed by a simple equation (Eqn. 10) for the two discrete ligand model usually used.

Robinson and Brown [103] found that there is a general relationship between log K_{ML}^c and log C_L by comparison with other data, in which the conditional stability constant decreases as the total ligand concentration increases. However, there is no clear relationship between log K_{ML}^c and log C_L for a strongly binding complex. Although they mentioned that this relationship is a real effect, this tendency can be explained by an artifact accompanying the metal titration: for high organic ligand concentrations, which means high concentrations of the organic ligand, L_2, the major reaction in the process of the metal titration is metal competition between Ca and an added metal for the organic ligand, L_2, whereas complex formation of the free organic ligand and competitive reactions for the organic ligand, L_1, are important for low organic ligand concentrations.

Conclusion

The current understanding of the chemical speciation of trace metals in sea water is as follows.

Chemical modelling including chemical thermodynamics and coordination chemistry is essential for the speciation of trace metals in sea water, in which concentrations of free metal ions and unbound organic ligands are the most important factors in controlling the chemical forms of the trace metals.

The model requires that only two types of non-specific strongly binding organic ligands coexist in sea water: the DTPA type, the concentration of which is relatively low and may be freshly produced by phytoplankton, and the EDTA type, which is a major ligand.

The model is helpful in understanding the present wide range of the conditional stability

constants of natural organic complexes determined by metal titration.

A coordination chemistry model based on the LFER technique permits an explanation for the present concentration levels of several trace metals (Th, Fe, Cu and Zn) in sea water.

The model predicts that there is the possibility that a major part of reactive trace metals is associated with the DTPA-type organic ligand included in the model.

To characterize the chemical form of metals in sea water, it is necessary to examine the chemical form of the organic ligands. In this case, the effect of alkaline earth metals on the complexation of trace metals in sea water must be taken into account; in fact, the major part of the EDTA-type organic ligand is associated with Ca.

The ligand titration method provides directly no information about the total ligand concentration, although it can provide correct conditional stability constants for the organic complexes of trace metals, whereas the metal titration method, which includes more complex processes, gives information on the total ligand concentration.

The most serious problem for metal speciation is that the DTPA- and EDTA-type strongly binding organic ligands cannot be isolated and chemically identified. Therefore, the present purpose of chemical speciation studies is not metal speciation but the characterization of the organic ligands. There is no direct information on the molecular properties of the organic ligands. When the organic ligands have not been isolated, metals may become a good tool for characterizing the chemical properties of natural organic ligands.

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Comments on trace metal speciation in seawater or do "onions" grow in the sea?

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Abstract

Recent research has suggested that trace metals in seawater are almost completely complexed by organic compounds. The organic compounds seem to be very selective for a given metal, and form complexes having very high conditional stability constants (log K_{cond} = 10–18). Measurements of the strong copper complexing capacity of seawater (using a technique based on competition with Chelex-100 resin), in the presence of weak ligands and reducing agents, are incompatible with this model. We propose that trace metals in seawater are mainly in the colloidal state with coordination bonds holding the colloid particles together. The speciation of trace metals in seawater is determined by kinetics and not by thermodynamics.

Keywords: Atomic absorption spectrometry; Complexation in natural waters; Organic compounds; Sea water; Trace metals; Waters

Over the last thirty years, a great deal of effort has been dedicated to the study of trace elements and trace metals in seawater. While the primary intent was probably to obtain accurate concentrations of these elements in ocean waters, it has become clear that the measurement of concentration is inextricably related to the measurement of speciation, i.e. the physical and chemical forms that elements assume in seawater. It was further realized that the biological and geological properties of trace metals were also closely related to chemical form rather than the total metal concentration. Thus, more efforts were, and are presently being, directed to the study of speciation.

The first indications of differences due to physical and chemical form were probably obtained when it was realized that different analytical methods, applied equally conscientiously to replicates of the same sample, could produce different concentrations. A second indication of speciation in seawater was the observation that changing the pH of a seawater sample could produce different values for the trace element concentration. Finally, it was observed that techniques which concentrated trace metals from seawater nearly always produced a quantitative recovery of metals added to the sample, while recovery of the natural metals was often much lower.

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Attributing speciation to a particular source or phenomenon, has not been a simple matter, however, since virtually all evidence for speciation is indirect. Traditionally, the basic speciation scheme has been to pass seawater through a 0.45-μm filter. This separates the particulate from the "dissolved" fraction. "Dissolved" is used in quotes since colloidal particles, very small procaryotes, and protein fragments all pass through the filter. It is this definition of "dissolved" matter that presents a major uncertainty when we try to determine the speciation of a trace metal. An element, in its natural state in the ocean may either be bound to dissolved or colloidal matter, which may be organic, inorganic, or both. In any case, its physico-chemical state is very different from the aqueous ion that is used almost universally as the analytical standard.

The purpose of this paper is to review the present and past literature which explicitly or implicitly indicate differences due to trace element speciation, for the purpose of seeing whether the experimental evidence suggests some physical or physico-chemical model that is generally applicable to most trace elements in seawater. The implications of this model and its oceanographic and analytical consequences are discussed. Because much of the experimental work has been focused on copper, this element will assume a central point in our discussion. However, the overall model should have wider application.

EXPERIMENTAL

Copper complexing capacity (CuCC)

All containers and laboratory materials ware were made from polyethylene, PTFE or silica which had been washed with detergent, acid-soaked, and rinsed with Milli-Q water. Sample manipulations, additions of reagents, photooxidations, and analyses were performed in a Class-100 Clean Laboratory.

Surface seawater was collected from the South Pacific at 30°S 167°E and stored in 250-ml polyethylene bottles. Blank samples were prepared from deep, aged seawater which was spiked with hydrogen peroxide, and UV photooxidized (1000 W high-pressure mercury lamp) in silica tubes for 5 h. The seawater was then filtered (0.45 μm) and stored in a polyethylene container. The CuCC was determined as follows: the sample was spiked to 800 nM with copper sulfate (and additional compounds, see below), stored for a month, and then passed through a column of Chelex-100: the eluate was collected in a silica tube, photooxidized (1000 W high-pressure mercury lamp), and the copper measured by graphite furnace atomic absorption spectrometry (GF-AAS) after preconcentration by a mixed dithiocarbamate/Freon procedure. Full details are given elsewhere [1–6].

The CuCC was determined on the surface sample, the blank sample, and after the addition to each of (i) Tris (0.5 mM) + Tris–HCl (0.5 mM) buffer (pH 8.2), (ii) Tris buffer–ascorbic acid (10 μM), (iii) Tris buffer–hydroxylamine (10 μM), (iv) Tris buffer–ethylenediamine (10 μM), (v) Tris buffer–cetyltrimethylammonium bromide (10 μM), and (vi) Tris buffer–sodium dodecylsulfate (10 μM). Some samples were also ultrasonicated for an hour.

PAST WORK

Non-electrochemical methods

There is overwhelming evidence of strong interactions between trace elements and "dissolved" organic matter in seawater. Slowey et al. [7] reported that up to 50% of total dissolved copper (as well as fractions of other metals) in seawater can be extracted into chloroform. Slowey and Hood [8] found that copper present in seawater is non-dialysable and confirmed the observation of Corcoran and Alexander [9] that higher copper concentrations were obtained when seawater was treated with a strong oxidizing acid.

Here, we need to point out that this work was carried out before the possibility of contaminating the sample during sampling was fully realized, and that highly purified reagents were not available. Thus, virtually any chemical treatment of the sample tended to increase the concentrations of the metals in question. Be that as it may, the

observations above have withstood the test of time. The development of a photooxidation procedure for seawater by Armstrong et al. [10] provided an elegant, non-invasive means of destroying organic matter, and Williams [11] was able to give robust "additional indirect information for organically associated copper in seawater". While the problems of contamination were not fully appreciated at that time, UV photooxidation was probably less likely to contaminate samples than many other speciation schemes derived from a range of chemical and physical manipulations. Williams [11] was careful to point out that both dissolved and colloidal organic matter were susceptible to photolysis. Thus, the values reported by him could represent either molecular copper–organo complexes or copper occluded by organic matter.

The evidence for trace metal association with chemically inert, possibly organic, matter has been summarized by Mackey [12] and includes the following: (i) there is incomplete extraction of metals by complexation and solvent extraction [13,14], (ii) chelating resins such as Chelex-100 or Chitosan only partially extract "naturally occurring" metals from seawater [15–18], (iii) there is an increase in the amount of metal that can be determined after acidification [19–21], chemical oxidation [22–24], and UV photooxidation [11, 13,20,25–28], (iv) a substantial fraction of copper, lead and zinc can be retained by ultrafiltration and dialysis [7,14,29–33], (v) equilibrium is reached very slowly between naturally occurring metals and added radioactive tracers of ^{55}Fe, ^{64}Cu, ^{109}Cd, ^{210}Pb, ^{54}Mn, ^{60}Co and ^{65}Zn [20,32,34–37], (vi) the marine cyanobacteria, *Oscillatoria theibautii*, is inhibited at a pCu of 12, which is a much lower activity of free copper ion than can be accounted for on the basis of inorganic complexation [38], (vii) copper in natural waters can be directly extracted into organic solvents [7,39], (viii) trace metals can be adsorbed onto hydrophobic resins and eluted from these resins by organic solvents [12,40–48].

While the above observations may indicate metal-organo association, they give no specific information as to the physical and chemical nature of the metal-organo matrix, nor do they give any direct measurements of the fraction of the total metal that is present as a dissolved metalorganic complex. The cited methods can only give an estimate of the fraction of metal that is "labile", i.e. responsive to the method and, by difference, the fraction that is "non-labile". They are in fact, operational definitions, dependent on the technique employed. Additionally, by drastically altering the system during analysis, they give no information about the state of the natural system.

Electrochemical methods

Electrochemical techniques have a great advantage over most techniques since they can cause minimal perturbation of the aquatic medium during measurement. Closest to ideal are methods which use ion-selective electrodes (ISEs). These do not appreciably consume or alter the sample, but detect an equilibrium potential produced by the interaction of the electrode surface and the active components of the solution. Unfortunately no such devices are currently available for the detection of trace metal ions at the very low activities that occur in seawater.

Voltammetric techniques such as anodic stripping voltammetry (ASV) also do not appreciably disturb the bulk sample and thus are very useful for obtaining information about in situ speciation. Early experiments with ASV also showed that copper, lead and zinc peak currents increased when the pH of filtered seawater was lowered [37,49–51]. This occurred for both added and natural metals, in artificial seawater and in photooxidized seawater, and it was observed that a peak potential shift was associated with the change in current [50,51]. Added cadmium did not show pH dependence. The pH dependence of the zinc, lead and copper peak current and peak potential was explained in terms of an inorganic model which predicted that zinc and copper in seawater were associated with hydroxides, lead with carbonate and cadmium with chloride [52]. More recently, copper has been shown to be more associated with carbonate rather than with hydroxide, but the pH dependence is identical. While there was a direct connection between peak potentials and the model, the peak current

dependency was only implicitly explained by the model.

Because the computed ion activity products of the proposed species were substantially below the solubility products, an increase in peak current at lower pH could be produced if free ions, with large diffusion constants, were displaced by H^+ from a Lewis base having a smaller diffusion constant or if some other kinetic hindrance was removed by the acid. Of course, the Lewis base could equally be a dissolved organic complex or any colloidal surface known to adsorb these metals. If the matrix, for instance, were hydrated colloidal iron oxide, then the matrix itself would be destroyed by the added acid, releasing the free metal ions and producing an increase in current. Similarly, if the metal oxide or carbonate predicted by the model were incorporated in an iron or manganese oxide colloid, the effect would be the same but there would be no adsorption. The identical weak-base effect would also be observed if the metal-binding matrix was composed of colloidal material of both organic and inorganic composition.

In voltammetry, by judiciously controlling the applied potential and cell conditions (ASV), or the concentration and nature of the added ligand [cathodic stripping voltammetry (CSV)], it is possible to restrict those species undergoing chemical reaction, and to limit analytical detection to the most labile (reactive) species. Pseudo-amperometric titrations of seawater, in which the ASV signal is used rather than the direct measurement of the reduction current, can be conducted at low overpotentials. When Cu^{2+} is added to the sample under these conditions, the titration plots of peak current, vs. Cu^{2+} added, show an obvious transition from a low slope zone to a region of much greater slope. The equivalents of Cu^{2+} added to the break in the curve are taken as a measure of the "complexation capacity" of the sample, i.e. as a measure of the Lewis bases that have bound to the Cu^{2+}. After transformation of the data, it is possible to compute a conditional stability constant for the assumed copper-organic complex [53–56]. Implicit in this work, of course, is the assumption that the added Cu^{2+} is in equilibrium with the natural sample.

Recently the copper complexation in seawater has been determined by a ligand competition technique using ethylenediamine with voltammetric measurement of the labile metal fraction [57]. An advantage of this method is that, when applied to raw seawater, it allows the investigation of natural copper complexes, while in spiked seawater it allows "newly formed" complexes to be determined. Additionally, they used a technique known as stripping or pseudopolarography to produce characteristic current–potential plots for the same seawater samples.

For all the samples analyzed in this manner, the data showed that even very large overpotentials (> 1200 mV) were only half as successful in rendering copper reducible as 2×10^{-3} M ethylenediamine, even though for reversible systems, the change in Gibbs free energy equivalent to the overpotential was overwhelmingly greater than that for complexation. For a divalent metal, such as copper, each increase of 29.5 mV in the overpotential should enable the electrodeposition of metal-organic complexes having effective stability constants one order of magnitude higher. At an overvoltage of 1.5 V, metal-organic complexes having effective stability constants greater than 10^{50} should be dissociated. Such large stability constants are beyond the realm of thermodynamic possibility. Nevertheless, all the copper in seawater is not electrochemically labile at overvoltages of 1.5 V. This suggests that (i) the system is not reversible and (ii) the direct reduction of the natural copper is strongly hindered by kinetic or steric factors.

LACK OF EQUILIBRIUM

There is good reason to believe that trace metal ions, when added to seawater in the quantities commonly used in routine analysis, are far out of equilibrium with the natural system. Estimates of free ion concentrations in seawater from various determinations range from about 10^{-11} M to 10^{-18} M and when 10^{-8} to 10^{-9} M metal additions are made in the course of an analysis, the added metal must come to equilibrium with the sample during the time allowed for analysis.

It is most likely that the trace metal spike added to seawater does not come to rapid equilibrium with either the dissolved organic or particulate fractions. Disequilibrium between a radiotracer added to seawater and naturally occurring isotopes of the same element can remain even after one year in the case of ^{65}Zn [32,37]. Thus, the interpretation of analytical results on the assumption of chemical equilibrium is fraught with uncertainty. Of course, many analyses call for the displacement of the natural conditions to another condition in which the added standard exchanges freely with the original material. Acidification of the sample is an example of this type of treatment. Such methods can only produce concentration values and virtually no data about natural speciation.

Another argument for disequilibrium can be made on the basis of the Irving–Williams series [58] which states that copper is expected to form stronger complexes in seawater than other metals present at the same concentrations. If a large excess of copper is added, other metals should be displaced by the copper from metal-organic complexes and only copper complexes should remain. In fact, the ligands that appear to complex trace metals at picomolar concentrations also appear to be highly selective. The formation of zinc [59] or lead [61] complexes is little affected by the presence of an excess of copper ions. On the basis of the Irving–Williams series, this is unexpected and casts into doubt many experiments which determine the complexing capacity of seawater by converting all ligands and pre-existing metal complexes to copper complexes. It also leads away from the view that natural, metal-binding, dissolved, organic materials in seawater can be modelled with simple dissolved ligands.

One possible explanation for the apparent selectivity of organic ligands in seawater is that the stereochemical requirements of heavy metals differ. Copper and nickel prefer square planar coordination, zinc and cadmium prefer tetrahedral coordination and iron and manganese prefer octahedral coordination. Since there is a large range of organic molecules in seawater that could bind to trace metals, it is conceivable that those molecules that could achieve tetrahedral coordination would preferentially form complexes with zinc rather than copper. However, such a zinc-organic complex must contain many other polar functional groups to remain in solution. These functional groups would also be able to coordinate to heavy metals. Hence it is likely that some combinations of functional groups on the molecule can occupy square planar coordination sites around a copper ion or octahedral sites around an iron ion. Moreover, the Irving–Williams series is generally valid for multidentate ligands, suggesting that the stereochemical requirements of the central metal are not of paramount importance. If heavy metals and organic ligands were truly in chemical equilibrium in the dissolved state, then other metals should be displaced by an excess of copper regardless of stereochemistry.

ADSORPTION OF METALS BY HYDROPHOBIC RESINS

Colloidal particles should not be retained by a hydrophobic resin such as C_{18}-bonded silica or XAD-4, and inorganic metal ions that are adsorbed by impurities in the resin, or by free silanol groups, are not eluted by organic solvents [12,62–64]. It is therefore assumed that the fraction that is adsorbed by the resin, and is subsequently eluted by an organic solvent, must represent metal-organic compounds present in the original seawater. However, this fraction of trace metal is typically only 10–30% for copper, 5–10% for zinc, and less than 5% for iron and nickel, even though there is good evidence that these metals are almost totally "complexed" in seawater [5,65].

Compounds that can be adsorbed from seawater on to hydrophobic resins can be analyzed by liquid chromatography (LC). By coupling the liquid chromatograph to a multichannel atomic fluorescence (AF) detector, it is possible to simultaneously monitor a range of metals in the eluent. Using this equipment, it was shown [4,41–43] that heavy metals were associated with organic compounds having a wide range of polarities and molecular weights. The chromatograms of cop-

per, nickel, iron and zinc were very similar and there was no evidence of sharp peaks that could be attributed to individual metals forming complexes with metal-specific ligands such as tetrapyrroles from chlorophylls. When excess copper is added to seawater, there is only a small change in the amount of zinc that can be adsorbed from seawater on to C_{18}-bonded silica and eluted by organic solvents and there is little change in the resulting LC-AF chromatograms of copper and zinc [5].

The fraction of trace metals that can be adsorbed onto hydrophobic resins would be in qualitative agreement with predictions based on the Irving–Williams series if there were insufficient organic ligands present to complex all the heavy metals. However, ASV and CSV titrations of seawater have been interpreted on the basis of essentially complete complexation of copper [66], zinc [59,60], nickel [67], cobalt [68] and lead [61]. If these metals are completely complexed by organic ligands, the fact that the fraction of trace metal adsorbed by hydrophobic resins is in the order Cu > Zn > Ni ≈ Fe [5,65] implies that, for example, zinc complexes are more polar than copper complexes. This is in disagreement with

the LC-AF analysis of those compounds that are adsorbed by hydrophobic resins [5,43] and does not make a great deal of sense since the polarity of a metal-organic complex, particularly a large one, should depend on the nature and position of the functional groups not attached to the metal.

These observations are in general agreement with the electrochemistry results described earlier. First, copper does not seem to displace zinc from zinc complexes and secondly, for a particular metal, there are sufficient organic ligands in seawater to complex the metal completely but there is little excess complexing ability which is available to added metals.

CONCENTRATION OF NATURALLY OCCURRING LIGANDS

As electrochemical (and other) techniques improved in sensitivity, the concentrations of trace metal reported for seawater decreased. At the same time, trace metals were found to be essentially non-labile and this was explained by the presence of low concentrations of ligands that formed very stable metal-organic complexes with

TABLE 1

Speciation of metals in seawater as determined by metal titration

Trace metal	Depth (m)	Method	Trace metal concentration (nM)	Ligand concentration (nM)	Log K_{cond}	Organic ligand bound to trace metal (%)	Reference
Pb	0	ASV	0.017	0.22	10.0 [a]	50	61
						70	
Co	0	CSV	0.35	0.40	15.6 [a]	100	68
Ni	0	CSV	7.5	3.7	18.7 [a]	60	67
Zn	22	ASV	0.30	0.98	10.7 [b]	> 98	59
	400	ASV	1.62	1.3	10.4 [b]	> 98	
	60	CSV	0.12	1.60	10.3 [a]	> 95	60
		ASV	0.12	1.76	11.2 [a]	> 95	
	150	CSV		2.14			
		ASV		2.22			
Cu	0	ASV	0.58	> 1.8	> 11.5 [b]	> 99.7	66
	400	ASV	1.5	7.4	8.4 [b]	50	
				7.6	8.4 [b]	70	

[a] Conditional log $K'_{cond,F}$ values calculated with respect to free metal. [b] Conditional log $K'_{cond,I}$ values calculated with respect to inorganic metal. Log $K'_{cond,F}$ = log $K'_{cond,I}$ × α_M, where α_M is the inorganic side reaction coefficient for the metal.

high effective stability constants. Many models based on titration of seawater with excess metal also found evidence for the presence of higher concentrations of weaker ligands, but these ligands played no part in the complexation of metals in unspiked seawater.

A selection of such data is given in Table 1. The concentrations of trace metals and organic ligands can be extremely variable in estuarine and coastal waters, particularly those that are subject to high levels of humic substances or to industrial or urban pollution. For open ocean seawater, it is noteworthy that, in many cases, the concentration of "strong ligand" is almost the same as the concentration of trace metal. This is either a remarkable coincidence or it suggests that the concentrations of trace metal and strong ligand are dependent on each other. Since the authors of these papers often report that the ligands are strongly selective for a given metal, it seems unlikely that a trace metal such as zinc should form complexes with a particular fraction of the total organic ligands available, and that all similar ligands, in excess of this amount, should be removed from the water column. In general, all strong ligands, in excess of those required to selectively bind trace metals, must also be removed from the water column even though much higher concentrations of weaker ligands are often reported in the same experiments. It is difficult to imagine how this could occur.

It may be argued that metal-specific ligands are secreted by organisms to actively control the concentration of trace metals in seawater. While such a mechanism could be postulated for essential metals such as copper or iron, it is difficult to imagine why organisms would produce ligands that have very high specificity for the non-essential metal lead [61] which is present in seawater at total concentrations far below levels that are toxic to most marine organisms. The nutrient-like behaviour of many trace elements, essential, non-essential, and toxic, argues against the active production of ligands having high specificity for biologically important elements. Also, as mentioned previously, the LC-AF analyses of naturally occurring metal-organic compounds provide no evidence of the existence of such ligands.

If ligands are controlling the concentration of trace metal, it is not obvious why this requires the presence of an equal concentration of a selective ligand that forms complexes with an effective stability constant that is sufficient to complex most of the total amount of trace metal. The concentration of organic carbon in seawater is sufficiently high that the concentration of potential donor groups (carboxylic acids, phenols, N- and O-hetererocycles) greatly exceeds the concentration of all trace metals [69]. The sequestering of trace metals could just as readily be achieved by the presence of larger amounts of weaker ligands. Such ligands have not been observed in the absence of "strong ligands".

Summary of existing data

Table 2 summarizes the existing experimental observations and data and indicates the conclusions that may be drawn. For argument's sake let us assume that the binding matrix for metals is in true solution and not colloidal (item 1). Item 2 requires that there must be a substantial binding capacity. Either small quantities of compounds with large formation constants or large quantities of compounds with small formation constants, or both.

Then item 3 requires that there be a spectrum of ligands, all in solution, with variable metal-bond strengths, and forming metal complexes of varying labilities. The ligands would also need to be of largely differing sizes and molecular weights (item 4). Item 5 requires that much of the binding matrix be organic in nature, although it need not be exclusively organic. Item 6 requires that the ligands release metals upon titration with H^+. This condition is only partially met, since we know that oxidations with strong acids are required to completely release all the metal in a sample.

Item 6 puts strong restrictions on our assumption of complete solubility. It is not immediately clear why a titration plateau is reached for zinc, for instance, that is much lower than one predicted from the total zinc content [37,70]. Strong, dissolved, metal-binding ligands such as EDTA and nitrilotriacetic acid (NTA) are easily titratable. Thus, our hypothesized compounds must be

TABLE 2

Observations of the behaviour of trace metals in seawater

No.	Observation	Indication
1	Metals pass through 0.45-μm filter	Metals either in true solution or colloidal
2	Marine organisms are sensitive to very low (10^{-12} M) levels of free metals. This level is lower than predicted by inorganic speciation models	Free ion activity controlled by excess Lewis bases in natural seawater
3	"Stronger" chemical treatments yield increasingly greater concentrations of trace metals	Availability of metals varies according to bonding and physical environment
4	"Metals non-dialysable" and separable by ultrafiltration	Metal attached to large molecules or colloidal particles
5	Labile metal increases with exposure to UV light	Metals bound to or adsorbed on organic molecules/matter
6	Labile metal increases with decreasing pH	Metal attached to Lewis base: dissolved organic ligands, hydrated metal oxides or both
7	Exchange equilibrium is reached very slowly	Ligands are inert. There are physical barriers to exchange
8	Overpotential not effective in releasing metal XAD-4 or C_{18}-bonded silica remove organically bound trace metal in the order Cu > Zn > Fe $\approx$ Ni	Kinetic or diffusional barriers to direct reduction. The polarity of metal-organic complexes increases in the order Cu < Zn < Fe $\approx$ Ni
9	The concentration of strong ligands approximately equals concentration of trace metal	Excess strong ligands are removed from the water column
10	Little competition between trace metals for strong ligands	Strong ligands are selective for a given trace metal

different from them. Similarly, it is also not obvious how the organic matrix can bind zinc with a large formation constant and the zinc not be

displaced by copper or mercury ions which generally have much larger formation constants for identical ligands (items 7 and 11).

Item 8: once again, if we employ the model of a mixture of dissolved ligands with varying complexation strengths, it fails to explain why extremely large over-potentials on copper fail to reduce it quantitatively and why ethylenediamine is more successful at releasing copper from the binding matrix. The failure of the overpotential implies that there is resistance to counter the applied voltage, i.e. an insulating matrix which shields much of the copper from coming in direct contact with the electrode or a very large compound (one with a very small diffusion constant). Ethylenediamine appears to be successful in dissolving this matrix, at least partially.

Item 9 requires that the polarity of a metal-organic complex depends on the nature of the coordinated metal ion rather than on the excess functional groups that determine the hydrophilicity of the organic compound. Items 10 and 11 infer that metal-specific ligands bind to particular trace metals and excess ligands somehow disappear from seawater.

In summary, items 6 to 11 seriously challenge our hypothetical dissolved ligand model. As mentioned earlier, this may be due to the fact that we define the "dissolved" phase as that fraction that passes through a 0.45-μm filter and do not consider the possible contribution of colloidal matter having a chemistry very different from that of a hypothetical dissolved metal-organic complex.

THE ONION MODEL

A more satisfactory explanation of the interaction between metals and organic compounds in seawater can be found in the "onion" model. This model, as the name implies, is based on the existence of colloids consisting of layers (or concentric spheres). The layers are built up from organic molecules of varying sizes which are held together by hydrogen bonds and coordination bonds between trace metals and ligand donor atoms. Individual layers will also be held together by similar bonds. The reactivities of the layers
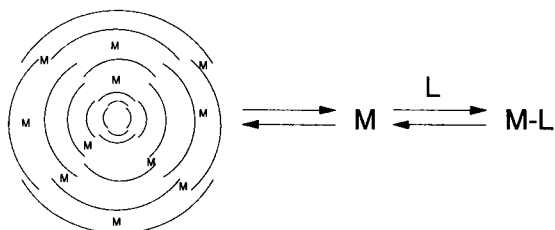
Fig. 1. Schematic diagram of the "onion" model. Trace metals (M) act as "glue" holding together individual organic molecules via coordination bonds. Organic ligands (L) capable of forming strong complexes with metals may also be present in solution. For copper, ca. 10–30% is present as M–L complexes while for metals such as zinc, cobalt, nickel and lead, the equilibrium lies strongly to the left.

become progressively lower towards the core of the "onion" because of the steric hindrance provided by the outer layers. We need not attribute any particular structure to the individual organic molecules apart from the fact that sufficient polar functional groups must be present to make the molecule hydrophilic. These functional groups will be of the same type as can bond to trace metals or form intermolecular hydrogen bonds. Such molecules are known to be present in seawater and freshwater; they are called humic and fulvic acids. As far as the "onion" model is concerned, the composition of the organic component of the matrix could be described by any of the proposed structures for these compounds [69,71–73].

In Fig. 1, we have given a schematic diagram to illustrate the overall speciation of trace metals in seawater. Metals, in the outer, hydrated, spheres are labile, and therefore easily measurable; the inner spheres become progressively more inert. Metals in the inner spheres can be measured by "peeling the onion", i.e. dissociating the outer spheres.

The major fraction of the matrix which forms the onion is undeniably organic. However, there may also be inorganic components such as silica, calcium carbonate and iron and manganese oxides. Thus, the onion might be termed an irreversible pseudocolloid [74]. The in situ formation of carbonates and of oxides/hydroxides of Mn(III), Mn(IV) and Fe(III) would provide a substrate with high affinity for the adsorption of organic compounds with polar functional groups.

Trace metals could then be adsorbed either on to the oxide/hydroxide phases or could form complexes with organic ligands in the matrix. In either case the amount of metal associated with the "onion" would be pH dependent.

Over time, by continually adsorbing materials from seawater, the onion would grow layers at the surface while compacting at the centre. By continually growing and by colliding and fusing with other colloidal particles, the onion would ultimately reach a size where its density was greater than that of seawater and would then begin to sink to the sediments.

Evidence for the onion model—copper complexing capacity (CuCC)

The method for determining CuCC detects those ligands that can form complexes with copper that are kinetically or thermodynamically stable to dissociation in the presence of a large excess of Chelex-100. On the assumption of thermodynamic equilibrium, simple calculations show that the method measures organic ligands at a concentration of ≈ 30 nM if they form copper complexes having K_{cond} greater than about $10^{11.3}$ [5]. Under the conditions of this experiment, NTA in seawater is not determined even at 10^{-5} M (Cu–NTA, $K_{cond} = 10^{8.8}$) while EDTA would not be determined in seawater at nM concentrations (Cu–EDTA, $K_{cond} = 10^{10.2}$).

If we add other ligands to seawater and then measure the copper complexing capacity, the value of CuCC will increase if the ligands are capable of forming strong complexes with the added copper or there will be no change if the ligands form weak complexes that will be dissociated in the presence of Chelex-100. If the speciation of copper is controlled by thermodynamics, the addition of other complexing agents to seawater cannot decrease the measured value of CuCC.

However, if the original (unspiked) copper is incorporated into colloidal matter, the copper need not be held by strong chemical bonds and the inertness and non-bioavailability [75–77] of the copper could be due to kinetic rather than thermodynamic effects. The particles may be small enough to enter the pores of Chelex-100 resin but would not be retained because the

TABLE 3

Copper complexing capacity of seawater under various perturbations
(Tris = 1 mM tris(hydroxymethyl)aminomethane, ascorbic = 10 μM ascorbic acid, NH_2OH = 10 μM hydroxylamine, en = 10 μM ethylenediamine, $CetMe_3NBr$ = 10 μM cetyltrimethylammonium bromide, SDS = 10 μM sodium dodecylsulfate.)

Description	CuCC (nM)
Sample	2.4 ± 0.4 (n = 6)
Sample (sonicated)	2.0 ± 0.1 (n = 2)
Sample + Tris	0.9 ± 0.1 (n = 2)
Sample + Tris + ascorbic acid	1.4 ± 0.4 (n = 3)
Sample + Tris + NH_2OH	0.9 ± 0.1 (n = 2)
Sample + Tris + en	1.0 ± 0.1 (n = 2)
Sample + Tris + $CetMe_3NBr$	1.3 ± 0.3 (n = 3)
Sample + Tris + $CetMe_3NBr$ (sonicate)	1.3 ± 0.0 (n = 2)
Sample + Tris + SDS	1.3 ± 0.3 (n = 3)
Sample + Tris + SDS (sonicate)	1.3 ± 0.2 (n = 2)
Blank	≤ 0.5
Blank (sonicate)	< 0.5
Blank + Tris	0.9
Blank + Tris + ascorbic acid	< 0.5
Blank + Tris + NH_2OH	< 0.5
Blank + Tris + en	< 0.5
Blank + Tris + $CetMe_3NBr$	0.7 ± 0.1 (n = 2)
Blank + Tris + $CetMe_3NBr$ (sonicate)	0.5
Blank + Tris + SDS	< 0.5
Blank + Tris + SDS (sonicate)	0.5

particles would be negatively charged. These colloids would then be measured as CuCC. If such colloids could be broken up in the presence of compounds that could either form weak complexes with copper, react with the ligand groups, or could hinder the formation of colloids, then this could lead to a decrease in the measured value of CuCC. In order to ensure that there were no significant changes in pH when additional reagents were added, the CuCC was measured in the presence of a large (1 mM) excess of Tris buffer. This buffer has a pH that is essentially the same (8.2) as that of seawater.

From Table 3, it is clear that in all cases where we have added other reagents or complexing agents, the measured value of CuCC decreased. Even though the reagents were present at much higher concentrations (1 mM Tris and 10 μM for the other reagents) than the naturally occurring strong ligands, the decrease(s) in CuCC cannot be explained on the basis of a mass action effect

with copper being complexed by added organic ligands. However, the results can be explained if trace metals act as a "glue" holding together individual layers of organic compounds. This "glue" consists of trace metals such as copper or zinc which can react with added ligands (such as ethylenediamine) or of trace metals such as iron(III) or manganese(III/IV) which can be reduced (by ascorbic acid or hydroxylamine) to the divalent state, thus forming much weaker bonds to the organic ligands. While Tris does not form very strong complexes with trace metals, the high relative concentration of Tris ensures that it is as effective as any of the other reagents in breaking up the colloids.

There is some indication that ultrasonication may break up the colloids since the CuCC decreases from 2.4 ± 0.4 nM to 2.0 ± 0.1 nM, but any effect is small. The addition of cationic (cetyltrimethylammonium bromide) or anionic (sodium dodecylsulfate) surfactants produce values of CuCC (1.3 ± 0.3 nM) that are intermediate between the natural sample and the natural sample plus Tris. This could be explained if the surfactants hindered the breakup of colloidal particles.

It is seen from Table 3 that no procedure produces values of CuCC as low as the blank values in UV photooxidized seawater. This implies that not all the copper is released from colloidal matter, or that some other form of copper is present in solution. We prefer the latter hypothesis since there are experimental procedures that can be used to quantitatively preconcentrate metals such as zinc but fail to determine all the copper. Our results in Table 3 can be explained if approximately 20–30% of the copper exists as dissolved copper complexed by organic ligands. This is consistent with the earlier work showing that in seawater 20–30% of copper is not dissociated by Chelex-100 [18] and 20–30% of copper is adsorbed by XAD resin or by C_{18}-bonded silica [5,65].

While the "onion" can be dissociated by compounds that react with the metal ions that are present as a "glue" which holds individual organic compounds to make up the colloidal particle, the addition of metal ions should increase the

number of such bonds. This would hinder the exchange of trace metals between the "onion" and the solution, and explain the slow equilibration between naturally occurring trace metal and added trace metal [20,32,34–37,78] as well as the observation that copper does not readily displace other trace metals in seawater [5,59,61].

Other work

Studies of sub-micrometre particles in the ocean [79] have shown that > 95% of particles in the range 0.38–1 μm are non-living and occur in the upper 50 m of the water column. These particles are fragile, readily dissociated by ultrasonication and many of them can pass through a GF/F filter. The concentration of these particles decreases rapidly with depth. Smaller particles, < 120 nm, have been studied by Wells and Goldberg [80], who found that these smaller particles (i) are more than three orders of magnitude more abundant than the larger particles studied by Isao et al. [79], (ii) have a distribution with depth that varies with season but there can be maxima in the concentrations at the surface, near the thermocline and near the bottom (900 m), (iii) are rounded in shape, mainly organic but containing iron, silica and other trace metals, (iv) seem to be aggregates of smaller granules 2–5 nm in size, and (v) bear a striking resemblance to soil-derived fulvic acids.

The particles observed by Isao et al. [79] may be precursors to those observed by Wells and Goldberg [80]. As more trace metals bond to individual organic compounds, water will be excluded and a smaller, more compact, colloidal particle will be produced. These small particles will then sink and aggregation will lead to a size distribution that increases with depth as observed. This is in agreement with observations [69] that the high-molecular-weight (> 100 000 daltons) fraction of refractory organic matter in seawater seems to increase with depth. The variations in size distribution and concentration of 5–120 nm diameter particles imply that these particles have short residence times and are largely controlled by biological processes. Wells and Goldberg [80] conclude that "the apparent close association of metals with these colloids

suggests that they may play an important part in the transport and fate of trace elements in seawater". We would go even further and suggest that these small particles dominate the speciation of trace metals in seawater.

This does not necessarily imply that organic matter is predominantly colloidal in nature since the concentration of organic matter in seawater is far greater than the total concentration of the metals considered in this paper. If coordination bonds between trace metals and organic compounds are the primary cause of the formation of colloids, it is quite possible that the bulk of the organic matter consists of comparatively small discrete molecules.

Conclusions

There is evidence that 10–20% of copper in seawater is present in solution as Cu-organic complexes having large (log $K_{cond} \approx 11$) stability constants. The speciation in seawater of the remaining copper, and other trace metals such as zinc, cobalt nickel and lead, can be explained if they are incorporated into particles in the size range 10–100 nm. These metals cannot readily exchange with other metals added to seawater.

These particles are probably negatively charged and too large to enter the pores of XAD-4 (4 nm) or C_{18}-bonded silica (12.5 nm) and hence only a small fraction of trace metals are retained by adsorption on to hydrophobic resins. In natural seawater (no acidification or adjustment of pH), the behaviour of these particles towards Chelex-100 seems to be more complex. While some fraction of zinc may be retained by reaction of the particles with the ligand groups of Chelex-100, copper is poorly retained. In the presence of a large excess of copper, other metals should be displaced from the surface of the particle, and the particle should pass through the column of Chelex-100 and so contribute to the measured CuCC.

We have chosen to describe the postulated colloidal matter in terms of an "onion" model as opposed to a "plum-pudding" model. While many of the observations described in this manuscript could equally well be explained in terms of a gel-like or heterogeneous assemblage of metals

and organic compounds, the expression "onion" does highlight the important concept that different techniques will determine different fractions of the total trace metal.

The apparent presence in seawater of organic ligands capable of forming strong complexes with trace metals with high specificity for a given trace metal is probably an artifact. The speciation of trace metals is determined by a combination of kinetic and thermodynamic effects.

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Capabilities of supported liquid membranes for metal speciation in natural waters: application to copper speciation

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Abstract

The transport of Cu^{2+} through a supported liquid membrane (SLM) consisting of 1,10-didecyldiaza-18-crown-6, a fatty acid and a mixture of toluene and phenylhexane (1 + 1) under natural water conditions was studied. Long-chain fatty acids having various numbers of carbon atoms C_n ($n = 10$–18) were tested to obtain a reasonably high Cu^{2+} transport rate. The rate of transport of Cu(II) was found to be fast when lauric acid ($n = 12$) was included in the membrane phase. The transport of Cu^{2+} by the SLM in the presence of Cu(II) complexants such as Tiron and oxalate was investigated. The results showed that the flux of Cu(II) through the membrane depends specifically on the free metal ions. These results were verified by means of a Cu(II) ion-selective electrode. The SLM was applied to the determination of Cu^{2+} in fresh waters and the results were compared with those obtained by ultrafiltration.

Keywords: Atomic absorption spectrometry; Copper; Metal ions; Speciation; Supported liquid membrane; Waters

In natural aquatic systems, trace metals exist in different chemical forms such as the free hydrated ions, inorganic and organic complexes and metals associated with colloidal particles. These species play various roles in the geochemical and biological cycling of elements and the determination of each of the species, in particular the free ion concentration, is essential [1–4]. Several techniques are available for making these measurements, including electroanalytical techniques [direct potentiometry, anodic stripping voltammetry (ASV), adsorptive cathodic stripping voltammetry (ACSV)], ion exchange, dialysis, liquid–liquid extraction, ultrafiltration and computer modelling

[1,5–7]. All these methods have certain advantages and disadvantages. For example, ion-selective electrode potentiometry has the advantage that it is at present the only method that can measure free metal ions in solution, but it suffers from poor sensitivity. ASV is one of the most sensitive techniques but its use is limited to electro-active species and in addition adsorption problems may hamper the interpretation of the results. ACSV is a very sensitive method applicable to a wider range of elements than ASV but it suffers from interference problems from competitive adsorption by surface-active agents. A separation method such as ultrafiltration (UF) has the advantage that it is applicable to a wide range of elements and it does not perturb the equilibrium of the test system provided that the volume of the filtered sample is small compared with the initial

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volume of the sample [1]. However, UF is limited to the determination of metal complexation with macromolecular ligands, the lowest molecular weight usable being dependent on the porosity of the membrane employed. In this paper, we propose an alternative method for trace metal speciation by combining a simultaneous separation and concentration method such as facilitated transport through a supported liquid membrane, with a highly sensitive analytical technique such as ASV or atomic absorption spectrometry with electrothermal atomization (ETAAS).

In a previous paper [8], the transport of heavy metal ions through a supported liquid membrane containing a neutral macrocyclic carrier was described. A supported liquid membrane consists of a microporous support impregnated with a hydrophobic organic solvent containing a cation carrier [9,10]. This membrane is placed between the sample (source phase) and receiver (strip phase) solutions (Fig. 1). At the sample/membrane interface the metal ion reacts with the cation carrier, R, dissolved in the membrane, to form a neutral complex and partitions into the membrane. The neutral metal complex diffuses across the membrane and the metal is released at the membrane/strip interface by complexing with the ligand L. If the strip phase volume is less than the sample volume, and/or its complexing strength is greater than the source phase, then the metal ions of interest can be concentrated [11]. The driving force for this transport is the free metal ion activity gradient facilitated by a pH gradient (Fig. 1b) if an acidic extractant is used as the metal carrier; a metal accompanying anion, A^-, counter gradient (Fig. 1a) if a neutral carrier is used; or another metal ion, M'^+, counter gradient (Fig. 1c). Transport of ions in the SLM is diffusion limited and the mechanism of transport of metals through the SLM in SLM systems containing non-cyclic and macrocyclic extractants together with transport models built on diffusion processes have been reviewed [9,12–18].

Non-cyclic compounds have been used for separation and/or preconcentration of copper ions [9,19,20] (see also citations in [8]). In particular, Cox and Bhatnagar [11] studied the use of an SLM for the analytical separation and preconcen-
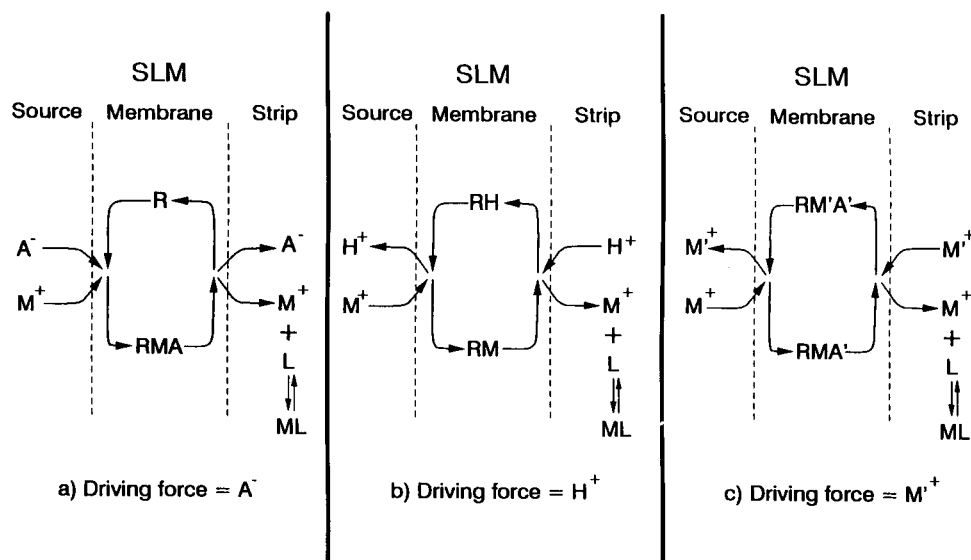
Fig. 1. Schematic representation of the supported liquid membrane system using various driving forces (a–c) to produce a flux of metal ions from the source to the stripping solution. R = metal ion carrier in the membrane phase (R may be alone, protonated or complexed with M); L = complexant of M^+ in the stripping solution; A'^- = lipophilic anion in the membrane phase; M'^+ = metal ion other than the test metal; M^+ = test metal ion in the source solution; A^- = counter anion present in the source solution. Ions bearing one single charge are shown for the sake of simplicity.

tration of zinc using a non-cyclic acidic extrac-
tant. Very few studies have been reported regard-
ing the separation, extraction or transport of
transition metals using macrocyclic ligands
[8,10,21–27], and to our knowledge the applica-
tion of SLM to trace metal speciation studies has
not been reported. Proton-ionizable macrocyclic
compounds have been used for the separation
and preconcentration of metals [28,29], but in
such cases the transport of metal is proton driven.
These, however, are inapplicable to speciation
studies in natural waters, as they lead to alter-
ation of the test medium.

In an earlier paper [8], it was shown that by
using a neutral carrier such as 1,10-didecyl-1,10-
diaza-18-crown-6 (22DD), this problem may be
obviated and Cu^{2+} ions can be transported across
the liquid membrane under pH conditions close
to those of natural waters. A lipophilic counter
ion, bis(2-ethylhexyl)phosphoric acid (HDEP,
equivalent to A'^- in Fig. 1c), was also incorpo-
rated in the organic phase. This removes the
need for a simple counter ion to cross the
source/organic interface and also retards the
leaching of carrier from the membrane. Although
at neutral pH values copper ions could be trans-
ported through a 22DD–HDEP–toluene SLM
system, the transport rate was not sufficiently fast
for applying this method to the separation of
metal ions from natural waters. Therefore, ways
were sought to improve the metal ion transport
rate before attempting to use SLM for speciation
studies. A means of enhancing the separation
step is to replace the carrier counter ion HDEP
by another hydrophobic counter ion, in particular
by one of the readily deprotonated fatty acids.
Lindoy and Baldwin [24] proposed hexadecanoic
acid (palmitic acid) for improved transport of
copper(II) ions through macrocyclic ligands. In
this work, SLM systems containing 22DD as the
metal ion carrier and long-chain fatty acids hav-
ing various chain lengths in toluene (or a mixture
of toluene and phenylhexane) were tested to se-
lect the fatty acid most suitable for obtaining
enhanced copper transport. This was then used
for Cu^{2+} speciation measurements and finally
applied to the determination of Cu^{2+} ions in
fresh waters.

EXPERIMENTAL

Apparatus, membranes and reagents

Transport measurements were made in a spe-
cially designed diffusion cell similar to that de-
scribed by Danesi [9] for metal ion transport.
Metal ions were determined by means of flame
atomic absorption spectrometry (FAAS) using a
Pye Unicam SP9 atomic absorption spectrometer
or by ETAAS using a Perkin-Elmer HGA 500
spectrometer.

All the reagents used were of analytical-re-
agent grade (Merck). All solutions were prepared
using freshly prepared doubly distilled water.

Supported liquid membrane

Celgard 2500 (Celanese Plastic, Charlotte, NC)
polypropylene hydrophobic membrane (surface
area = 8.19 cm^2, porosity = 45%, thickness = 2.5
$\times 10^{-3}$ cm and effective pore size = 0.04 μm)
was impregnated with ca. 100 μl of 0.1 M 22DD
+ 0.1 M fatty acid in toluene [or toluene–phenyl-
hexane (1 + 1, v/v)] carrier solution. Long-chain
carboxylic acids having 11–18 carbon atoms were
examined. After impregnation, the membrane was
rinsed with water by dipping it into five small
troughs containing water to remove excess of
carrier.

Source and stripping solutions

The membrane was then placed between the
two compartments of the diffusion cell. The feed
solution, i.e., the solution containing the metal
ion (called the source), was placed in one half cell
and the strip solution was placed in the other;
10^{-6}–10^{-4} M Cu(II) was used in the source
solution. The pH of the solution was kept con-
stant at 6 by means of 10^{-2} M N-morpholino-
ethanesulphonic acid (MES)–LiOH buffer. MES
does not complex with transition metal ions, par-
ticularly Cu^{2+}, and is not soluble in the organic
phase. Lithium hydroxide was used for neutraliz-
ing MES because Li^+ ions are not transported by
22DD, and therefore they do not act as interfer-
ents in copper ion transport experiments. The
stripping solution consisted of 5 $\times 10^{-4}$ M cyclo-
hexanediaminetetraacetic acid (CDTA) (pH 6).
As this concentration was found to be optimum

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for Cu^{2+} transport through the 22DD–HDEP–toluene SLM reported earlier [8], the same stripping solution concentration was used in this study. For comparison purposes, 1×10^{-3} M sodium pyrophosphate was also used as the stripping solution. Solutions were stirred at 480 rpm and aliquots of the source and stripping solutions were withdrawn at various intervals and analysed by FAAS or ETAAS.

RESULTS AND DISCUSSION

As mentioned earlier, the flux of copper ions with the type of SLM system described in the previous study [8] is not fast enough to allow the separation of metal ions present at trace levels such as those found in natural waters. Moreover, by varying the ligand concentration of the stripping solution, no substantial improvement in the transport rate was observed using this type of SLM system. Therefore, attempts were made to improve the experimental conditions for metal transport through the SLM before its application to speciation studies in known solutions and in natural waters.

Choice of organic solvent

The low metal ion flux observed previously might have been due to the high solubility of toluene (and therefore the macrocyclic carrier R) in the aqueous phase. A very low solubility of R in the source phase is a prerequisite for the metal species in the test medium to remain unaltered. However, although the solubility of the chosen organic solvent in water should be low, its dielectric constant should not be too low, as this would lower the distribution coefficient of the metal in that solvent. The solubilities of various solvents in water are given in Table 1. The water solubility of phenylhexane has been reported to be low but no value for its solubility could be found. An attempt was made to determine it by gas chromatography. Water saturated with phenylhexane was injected into the gas chromatograph (Hewlett-Packard Model 5890). A very small peak corresponding to the retention time of phenylhexane was observed

TABLE 1

Solubilities of liquid membrane solvents in water

Solvent	Water solubility (mol dm^{-3})	Ref.
Chloroform	8×10^{-2}	30
Toluene	6×10^{-3}	30
Phenylhexane	$\leqslant 5 \times 10^{-6}$	This work
Kerosene	Not reported	

and from its surface area the solubility of phenylhexane was estimated to be $\leqslant 5 \times 10^{-6}$ M.

Initial experiments were performed by using palmitate as counter ion, A'^{-}, in the membrane as proposed by Lindoy and Baldwin [24]. Typical plots of copper ion concentration C vs. time, using toluene and phenylhexane–toluene as the carrier solvents, are shown in Fig. 2. The transport rate is faster than that observed with HDEP as the counter ion in the membrane [8]. The rate was also found to be faster using pure phenylhexane as the solvent. However, a blue precipitate was formed on the membrane very rapidly with pure phenylhexane, and only after 30 min with toluene–phenylhexane. This is probably due to the low solubility of the neutral copper complex formed with 22DD and palmitate in the mem-
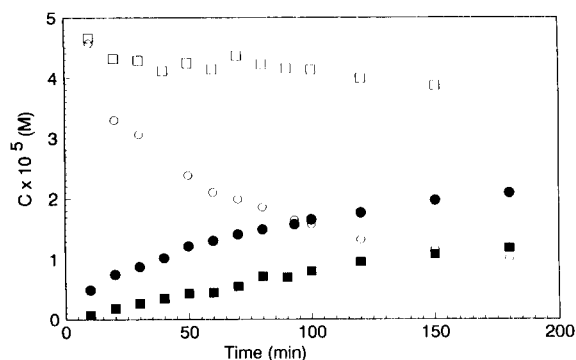
Fig. 2. Cu(II) transport through 22DD–palmitate (PA)–organic solvent SLM: typical plots of C vs. time. Conditions: source solution, 5×10^{-5} M Cu^{2+} in 1×10^{-2} M MES–LiOH buffer (pH 6); stripping solution, 5×10^{-4} M CDTA (pH 6.4); carrier, (■, □) 0.1 M 22DD and 0.1 M PA in toluene and (●, ○) 0.1 M 22DD and 0.1 M PA in toluene–phenylhexane $(1 + 1, v/v)$; volumes of stripping and source solutions, both 80 ml. Open symbols = source solution and solid symbols = stripping solution.

brane. This precipitation explains why the source phase Cu^{2+} concentration decreases much more rapidly than the corresponding increase in the stripping phase concentration in Fig. 2.

The palmitic acid (PA) concentration in the carrier was varied to see whether an analogous transport rate could be obtained without the formation of a precipitate. At concentrations $\leqslant 10^{-2}$ M, no precipitate appeared but the transport rate was about ten times slower than with 0.1 M PA in toluene–phenylhexane and comparable to that found with 0.1 M PA in toluene. Initial tests with a higher concentration of PA (5×10^{-2} M) and various ratios of phenylhexane to toluene revealed that the blue precipitate is formed on the membrane even at a phenylhexane to toluene ratio of 1:3, indicating the very low solubility of the neutral complex formed in phenylhexane and in addition that the transport rate at this ratio is lower than with a lower proportion of toluene in the mixture; in addition, the transport rate is optimum at a phenylhexane to toluene ratio of 1:1.

The blue precipitate on the membrane mentioned above appeared when pure phenylhexane or a mixture of phenylhexane and toluene was used as the membrane phase solvent but not when toluene alone was used as the solvent, indicating that the precipitate is not copper hydroxide. Under the experimental conditions used, the solubility calculation also supports the fact that copper hydroxide is not formed. Hence the blue precipitate is probably Cu–22DD–palmitate, which is sparingly soluble in phenylhexane. However, chemical analysis of the precipitate was not carried out to identify this product.

Choice of fatty acid

The results obtained with the 22DD–palmitic acid–[toluene–phenyl hexane (1 + 1)] SLM suggests that enhanced metal transport can be achieved. However, the low solubility of the neutral complex in the organic solvent is a shortcoming of the SLM system used. This problem can be overcome by selecting another long-chain fatty acid. Fatty acids having 11–18 carbon atoms were tested. Experiments were performed with 0.1 M 22DD and 0.1 M fatty acid in toluene–phenylhexane (1 + 1). Among the fatty acids tested (Fig. 3a and b), lauric acid seems to be the most suitable for copper ion transport. Although tridecanoic and myristic acids gave comparable transport, a blue precipitate appeared on the membrane. With capric acid no precipitate was observed but the copper ion transport rate was markedly lower, possibly owing to the higher water solubility of capric acid (Table 2) and the subsequent formation of a Cu–caprate complex. Hence laurate (LA) was chosen as the counter ion (in the organic phase) in subsequent studies.

The existence of copper transport via the mechanism depicted in Fig. 1c (where $M' = Na$)
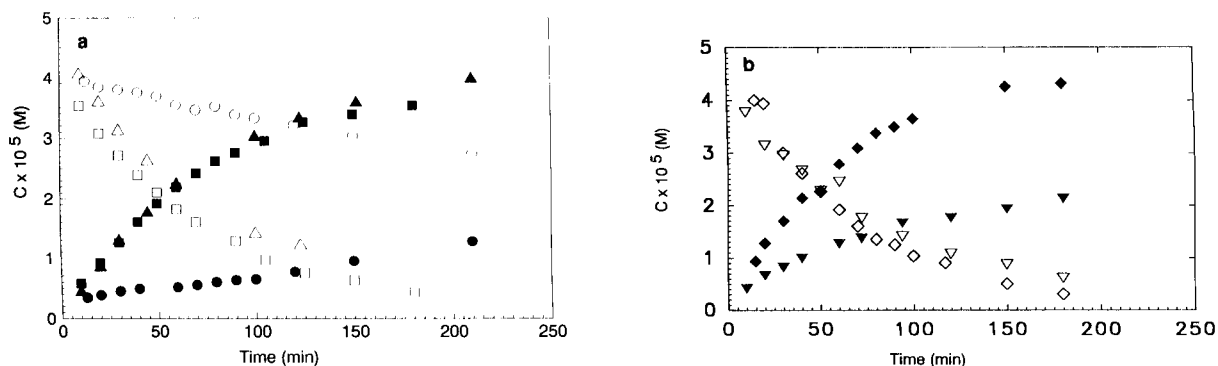
Fig. 3. (a) Effect of the nature of the fatty acid used in the carrier phase on Cu^{2+} ion transport: plots of C vs. time. Conditions and open/closed symbols in Fig. 2 except that PA was replaced with 0.1 M of various fatty acids having different chain lengths. ●, ○ capric acid (C_{10}); ▲, △ = lauric acid (C_{12}); ■, □ = myristic acid (C_{14}). (b) Same as (a) except with different fatty acids in the carrier phase: ◆, ◇ = tridecanoic acid (C_{13}); ▼, ▽ = palmitic acid (C_{16}).

TABLE 2

Solubility of long-chain carboxylic acids ($C_{n-1}H_{2n-1}COOH$) in water [31]

Fatty acid	n	Solubility (mol dm^{-3})
Capric acid	10	1.15×10^{-3}
Lauric acid	12	3.4×10^{-4}
Tridecanoic acid	13	1.3×10^{-5}
Myristic acid	14	2.5×10^{-5}–4.4×10^{-5}
Palmitic acid	16	2.7×10^{-5}
Stearic acid	18	2.1×10^{-5}

was checked by means of control experiments which showed that copper is not transported by LA alone or by the solvent alone. Fig. 4, for instance, shows that copper(II) ion transport in the absence of LA is much slower than in its presence. Moreover, in the presence of LA, the copper ion transport rate approaches that found for metal transport in a proton-driven SLM system [11] and is about ten times larger than that of the copper ion transport through the 22DD-HDEP SLM under similar conditions.

Choice of complexant in the stripping solution

In the investigations on the effect of the nature of the stripping solution on copper ion transport reported previously [8], 1×10^{-3} M sodium pyrophosphate was found to be a good stripping agent for copper ions. In this work, the results
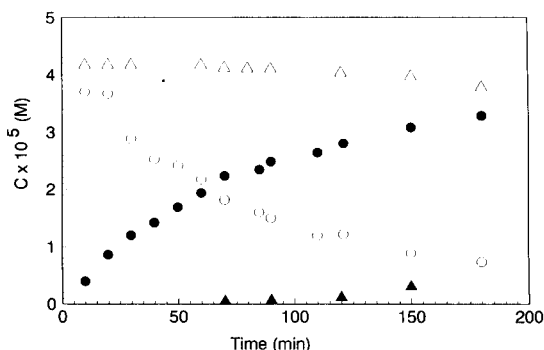
Fig. 4. Transport of Cu(II) through the SLM in the presence and absence of laurate (LA) in the carrier. Conditions as in Fig. 3. ▲, △ = No laurate in the carrier; ●, ○ = 0.1 M laurate present in the carrier.

obtained with sodium pyrophosphate and CDTA were compared and were found to be comparable, CDTA being slightly better. Therefore, in subsequent experiments CDTA was used as the stripping solution. It must be mentioned, however, that pyrophosphate might be preferable when the detection technique used is ASV. In fact, for Cu(II), either CDTA or pyrophosphate can be used but for the other transition metal ions such as Pb^{2+} the latter has to be used as the strong Pb–CDTA complex cannot be analysed by ASV.

Preconcentration of copper ions

The advantage of using SLM is that metal separation and concentration can be done in a single step by simply using a stripping volume smaller than the sample volume. This is of interest when determining metal ions present in traces such as those found in most natural waters. In order to attain large concentration factors, $F = C_{st}/C_{so}$, the volume, V_{st}, of the stripping solution (subscript st) should be much less than the volume, V_{so}, of the source solution (subscript so). C_{so} is the initial concentration of metal ion in the sample solution and C_{st} its concentration in the stripping solution. Note that C_{st} is a function of time, as described by Cox and Bhatnagar [11].

Experiments were carried out using 100 ml of 5×10^{-6} M Cu^{2+} in 1×10^{-2} M MES buffer (pH 6) as the source and 5 ml of 5×10^{-4} M CDTA as the stripping solution. A specially designed diffusion cell with one half cell having a 100-ml cell capacity and the other half cell with an 8-ml cell capacity was used. The surface area of the membrane was 8.19 cm^2. The cells were stirred and aliquots of sample and stripping solutions were withdrawn at various intervals of time and analysed by FAAS. A typical plot of F vs. time is shown in Fig. 5. Analogous results have been obtained for zinc preconcentration by a proton-driven SLM by Cox and Bhatnagar [11]. The reproducibility of the experiments was determined by performing four replicate measurements with 5×10^{-6} M Cu^{2+} solution, the other conditions being the same as described above. The average relative standard deviation was 5% over the entire time range 10–180 min. These
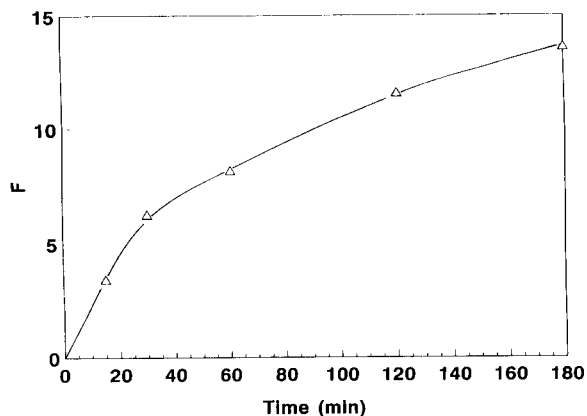
Fig. 5. Preconcentration of Cu^{2+} using the 22DD–LA–toluene–phenylhexane SLM: plot of F vs. time. Conditions: source solution, 5×10^{-6} M Cu^{2+} in 1×10^{-2} M MES–LiOH buffer (pH 6); stripping solution, 5×10^{-4} M CDTA (pH 6.4); source solution volume, 100 ml; stripping solution volume, 5 ml; carrier, 0.1 M 22DD and 0.1 M LA in toluene–phenylhexane $(1 + 1, v/v)$.

results demonstrate that the SLM can be used for the analytical preconcentration of metal ions under neutral pH conditions.

Pb^{2+}, Cd^{2+} and Zn^{2+} ions are also transported through the 22DD–LA–toluene–phenylhexane SLM. The flux of Pb^{2+} was equal to that of Cu^{2+}, but the fluxes of Cd^{2+} and Zn^{2+} were less than that of Cu^{2+} when their concentrations were equal in single cation transport experiments. The flux of a given metal ion (e.g., Cu^{2+}) in a mixture of these four ions present in equal concentrations is the same as that found in pure solutions containing the individual ion. In particular, the concentration factor of copper(II) ions was unaffected by the presence of other ions. This feature is very important for the application of SLM to trace metal separation and speciation studies in natural waters.

SLM for metal ion speciation studies

In order to check whether the SLM can be applied to metal speciation studies, the interaction of Cu^{2+} with Tiron (1,2-dihydroxybenzene-3,5-disulphonic acid) and with oxalate was studied. Both are good complexing ligands for Cu(II).

Complexation of Cu(II) by Tiron. Tiron (symbolized below by T) forms strong complexes with Cu^{2+}, i.e., CuT, CuT_2 and probably CuTH [32–34]. To determine whether the SLM can be used for the measurement of free metal ion concentrations, metal ion transport experiments through the 22DD–toluene–phenylhexane SLM were performed in parallel with Cu^{2+} ion-selective electrode measurements, using samples containing a total copper concentration $[Cu]_t = 5 \times 10^{-5}$ M in 0.1 M $LiNO_3$ and 1×10^{-2} M MES buffer (pH 6.2). The total Tiron concentration was varied between 5×10^{-4} and 1×10^{-2} M. Source and stripping volumes of 100 and 5 ml, respectively, were used. The source and stripping solutions were stirred as described earlier and aliquots of stripping solutions were withdrawn at various intervals of time and analysed by ETAAS. The free copper ion concentrations in the source solutions measured by SLM ($[Cu^{2+}]_{SLM}$), were calculated from the measurements of copper ion concentration in the stripping solution after 180 min and the F values in Fig. 5, by considering that the flux of Cu(II) through the membrane is proportional to the free copper ion concentration in the source solution. The free copper ion concentration in the source solution was also measured ($[Cu^{2+}]_{ISE}$) as a function of time using a Tacussel copper(II) ion-selective electrode and an Ag/AgCl/3 M KCl/0.2 M $NaNO_3$// reference electrode. Stable potential readings were measured when the potential drift was < 0.05 mV per 15 min. The free copper ion concentrations were then calculated from a preconstructed calibration graph. A plot (Fig. 6) of $[Cu^{2+}]_{SLM}$ vs. $[Cu^{2+}]_{ISE}$ for various concentrations of Tiron (pH 6.2) shows that the SLM and ISE give the same results, at least for values of the degree of complexation, $\alpha = [Cu]_t/[Cu^{2+}]$, up to 2000. This confirms that the SLM can be effectively used for the measurement of free metal ion concentrations in the source solution.

Complexation of Cu(II) by oxalate. The use of the SLM for the determination of free metal ion concentration was also tested with oxalate (OX). Like Tiron, OX is predominantly present as multi-negatively charged ions at pH > 5, and previous studies have shown that such compounds do

not pass through the membrane [8]. Cu^{2+} forms the following moderately strong complexes with oxalate [32–34]:

$$Cu^{2+} + OX^{2-} \overset{\beta_1}{\rightleftharpoons} Cu(OX) \qquad (1)$$

$$Cu^{2+} + 2\,OX^{2-} \overset{\beta_2}{\rightleftharpoons} Cu(OX)_2^{2-} \qquad (2)$$
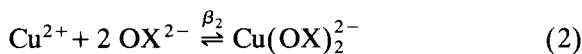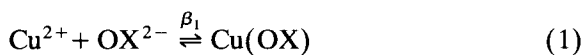
where β_1 and β_2 are the formation constants of $Cu(OX)$ and $Cu(OX)_2^{2-}$ complexes, respectively:

$$\beta_1 = \frac{[Cu(OX)]}{[Cu^{2+}][OX^{2-}]} \qquad (3)$$

$$\beta_2 = \frac{\left[Cu(OX)_2^{2-}\right]}{[Cu^{2+}][OX^{2-}]^2} \qquad (4)$$

The concentration of each Cu(II) species, either free or complex, can be calculated by combining the corresponding equilibrium constants [32] with mass balance equations for Cu(II) and oxalate (OX):

$$[Cu^{2+}]_t = [Cu^{2+}] + [CuOX] + \left[Cu(OX)_2^{2-}\right] \qquad (5)$$

$$[OX]_t = [OX^{2-}] + [CuOX] + 2\left[Cu(OX)_2^{2-}\right]$$
$$+ [HOX^-] + [H_2OX] \qquad (6)$$

where HOX^- and H_2OX are the protonated species of oxalate.
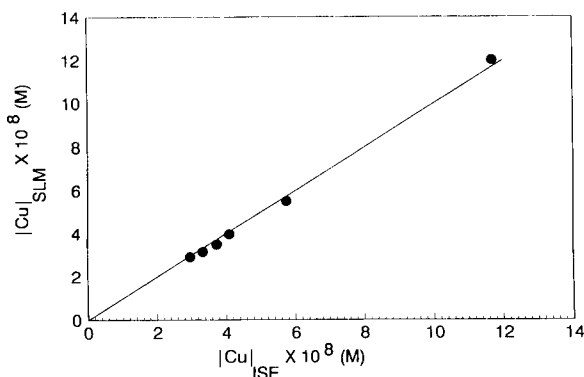
Fig. 6. Free Cu^{2+} concentration measured by means of Cu^{2+} ISE and with the SLM. Conditions: source solution, 5×10^{-5} M Cu^{2+} + various concentrations of Tiron in 0.1 M $LiNO_3$ – 1×10^{-2} M MES buffer (pH 6.2). The total Tiron concentration was varied between 5×10^{-4} and 1×10^{-2} M.
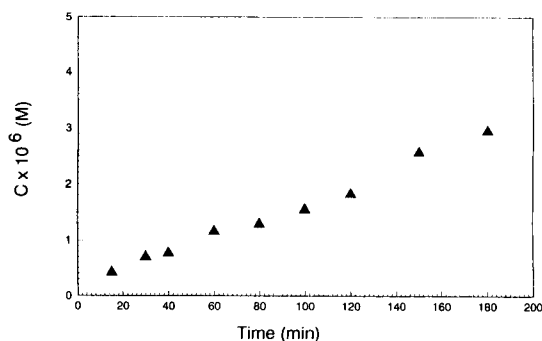
Fig. 7. Influence of oxalic acid on Cu^{2+} transport through the SLM. Conditions: source solution, 5×10^{-5} M Cu^{2+} and 1×10^{-3} M oxalic acid in MES buffer (pH 6); stripping solution, 5×10^{-4} M CDTA; source solution volume, 100 ml; stripping solution volume, 5 ml.

Metal ion transport experiments in the presence of oxalate were performed under the same conditions as those described for Tiron, except that oxalate was used instead of Tiron and the pH was 6.0, i.e., $[Cu]_t = 5 \times 10^{-5}$ M in 0.1 M $LiNO_3$ and 0.01 M MES buffer; $[OX]_t$ was varied between 5×10^{-4} and 1×10^{-2} M. Source and stripping solution volumes of 100 and 5 ml, respectively, were used. The procedure used was the same as that described earlier. Aliquots of stripping solutions were withdrawn at various intervals of time and analysed by ETAAS. A typical plot of copper concentration in the stripping solution, C, vs. time is shown in Fig. 7. It can be seen that the flux of Cu^{2+} is about 20 times smaller in the presence of oxalate than in its absence (compare with Fig. 4, curves with laurate in the time range 0–30 min), although the total Cu(II) concentrations are the same in Figs. 4 and 7. This results from the fact that the flux of Cu(II) through the membrane depends on the free (and not the total) ion activity gradient between the source and the stripping solutions.

The free Cu^{2+} ion concentrations for various concentrations of oxalate were calculated from the measurements made after 180 min, as indicated for the Tiron experiment. The degree of complexation, α, defined as

$$\alpha = [Cu]_t / [Cu^{2+}] \qquad (7)$$

was calculated for each ligand concentration, and β_1 and β_2 were then determined from the linear
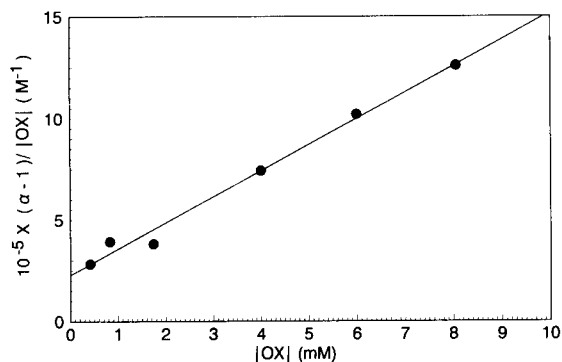
Fig. 8. Plot of $(\alpha\text{-}1)/[OX]$ vs. $[OX]$ for the complexation of Cu^{2+} by oxalate. Conditions: source solution, 5×10^{-5} M Cu^{2+} and various concentrations of oxalic acid (pH 6); stripping solution, 5×10^{-4} M CDTA (pH 6.4); source solution volume, 100 ml; stripping solution volume, 5 ml.

plot corresponding to Eqn. 8, obtained by combining Eqns. 3, 4, 5 and 7:

$$\frac{\alpha - 1}{[OX]} = \beta_1 + \beta_2[OX] \qquad (8)$$

where $OX \equiv OX^{2-}$. The correct values of $[OX]$ were obtained by combining Eqn. 8 with Eqn. 6 starting with $[OX] = [OX]_t$ and using four successive iterations, with $[HOX^-] = [H_2OX] = 0$, since these two species are negligible at pH 6.0.

The result of such a plot is shown in Fig. 8 and values of $\log \beta_1$ and $\log \beta_2$ were found to be 5.2 ± 0.3 and 8.1 ± 0.5, respectively under the conditions used ($T = 25°C$ and 0.1 M $LiNO_3$). Reported $\log \beta_1$ and $\log \beta_2$ values at an ionic strength of 0.1 M are in the range 4.5–5.75 and 8.4–10.6, respectively [32–35]. The β values obtained in this work fall in these ranges, confirming the applicability of the SLM for free metal ion concentration measurements. An interesting observation, however, is that the value of $\log \beta_2$ is at the lower end of the range of values reported in the literature. This is discussed under Conclusion.

Application of the SLM to Cu^{2+} determination in natural waters

Application of the SLM to the speciation of Cu(II) was tested with two types of natural waters, from Lake Bret (Vaud, Switzerland) and

River Arve (Geneva, Switzerland). Lake Bret is a eutrophic lake. Samples were collected at a depth of 8 m in the middle of the lake, i.e., at a location where most allochthonous material is eliminated by sedimentation. As a result, the major complexing agents are natural dissolved organic compounds. The River Arve originates directly from the Alps and clay particles and colloids form a significant fraction of its complexing material.

Bret water samples after collection were filtered through a 0.2-μm Schleicher and Schüll membrane. A 100-ml volume of this sample (pH 8.1) was placed in one compartment of the diffusion cell and 5 ml of 5×10^{-4} M CDTA (pH 6.4) were placed in the other compartment. The solutions were stirred and the concentration of Cu(II) in the source and in the stripping solutions were measured at time zero and after 180 min by ETAAS. $[Cu]_t$ and $[Cu^{2+}]$ were found to be 30.3 $\pm$ 0.5 and 8.4 $\pm$ 0.2 nM, respectively. For comparison purposes, free $[Cu^{2+}]$ in a lake Bret sample was determined by ultrafiltration, as described [36]. A 40-ml volume of the lake water prefiltered through a 0.2-μm membrane was ultrafiltered through an Amicon UM05 membrane with a pore diameter of 10 Å. The Cu(II) concentration in the filtrate (which corresponds to the free $[Cu^{2+}]$ [36]) was measured by ETAAS and was found to be 9.0 $\pm$ 0.4 nM, i.e., in good agreement with that found using the SLM.

A similar result was obtained for the speciation of Cu(II) in river Arve water filtered through a 0.45-μm filter. [Cu(II)] was determined in the source and stripping solutions both by ETAAS and by differential-pulse anodic stripping voltammetry (DPASV). The free copper ion concentrations in river Arve water, measured by combining the SLM with these two techniques, were found to be 12.0 $\pm$ 0.3 and 12.3 $\pm$ 0.1 nM, respectively. "Total" dissolved copper concentrations measured by DPASV and ETAAS directly on the filtered sample were found to be 33.0 $\pm$ 0.3 and 55.0 $\pm$ 0.5 nM, respectively. This difference is not surprising as ETAAS measures the true total copper concentration, whereas DPASV applied directly to the filtered water without pretreatment (as was used here) measures only the free copper ions plus the labile complexes small

enough to diffuse significantly towards the electrode surface. In particular, copper bound to most clay particles are not measured by DPASV. Therefore, SLM, DPASV and ETAAS measurements directly applied to the water sample give complementary information, viz., the concentrations of free Cu^{2+}, the small labile complexes and the ensemble of copper species, respectively.

Conclusion

Although the present results are preliminary, they demonstrate the analytical utility of the 22DD–LA–toluene–phenylhexane system for the speciation of trace elements in natural waters using an SLM. The advantage of this technique is that no sample treatment apart from separation using the membrane is required and that simultaneous separation and preconcentration of metal ions can be achieved. By judiciously choosing the sample to stripping volume ratio, high concentration factors can be attained, and by combining the SLM with highly sensitive detection techniques, such as voltammetric stripping techniques, very low detection limits may be achieved.

The small value of β_2 obtained for the $Cu(OX)_2$ complex might suggest a possible limitation of the SLM. As noted earlier, the value obtained here is in the range of reported values and might be correct. However, it might also be underestimated as the neutral species CuOX might partly pass through the membrane, owing to its non-ionic character. When α is very large ($[Cu^{2+}] \ll [Cu]_t$) the passage of even a small fraction of CuOX might then result in a significantly too large value of Cu(II) in the stripping solution (compared with that of the flux of Cu^{2+} alone), and therefore in turn in an underestimation of the degree of complexation. This effect might occur in natural waters containing neutral, liposoluble complexes and the larger the α value, the more important this "interfering" effect will be. This effect should be studied in more detail, not only because it may play a limiting role in the application of SLMs for speciation studies, but also because small neutral complexes are likely to play an important role in the uptake of metals by organisms. More generally, the passage of small neutral Cu(II) complexes through the SLM, ad-

sorption of uncharged macromolecules on the membrane or leaching of membrane material might be limitations of SLM. However, the results obtained with natural waters seem to suggest that these problems are not so important. Further, it must be realized that most natural complexing agents are either macromolecular or/and highly negatively charged [1]. The experiments with Tiron and with Arve and Bret waters clearly suggest that in such instances an SLM combined with sensitive detection techniques may be a very powerful tool for measuring free metal ions in complex natural systems.

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Ion chromatographic separation and on-line cold vapour atomic absorption spectrometric determination of methylmercury, ethylmercury and inorganic mercury

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Abstract

The ion chromatographic separation of methylmercury, ethylmercury and inorganic mercury as cysteine complexes was investigated. The chromatographic apparatus was interfaced with cold vapour atomic absorption spectrometry for detection using an on-line continuous flow cell coupled with a reduction system (sodium tetrahydroborate). The influence of the composition of the eluent (pH, ionic strength, cysteine concentration) and stripping gas flow-rate was investigated. The method was optimized by coupling an on-line preconcentration step. The detection limits, evaluated on 100.0-ml samples, were 2, 10 and 4 ng for Hg, CH_3Hg and C_2H_5Hg, respectively. The method was applied to synthetic mixtures and natural samples (tap water) and furnished satisfactory results.

Keywords: Atomic absorption spectrometry; Ion chromatography; Ethylmercury; Mercury; Methylmercury; Preconcentration; Speciation; Waters

Speciation analysis techniques for trace metal ions in natural waters are becoming increasingly important, as the toxicity of each element is dependent not only on its total concentration, but also on its distribution among different forms. Efficient liquid chromatographic (LC) separation techniques have proved useful for the speciation analysis of trace organometals. Inorganic and organomercury compounds are usually eluted on reversed-phase columns in situ incorporating chelating agents or after extraction of organometallic species from samples by charged or neutral ligands [1–5].

The ability of cysteine to give complexes with inorganic mercury and organomercury compounds [6,7] is well known and is usually used in

extraction procedures with biological samples [8,9]. The purpose of this work was to evaluate the efficiency of in situ cysteine complexation for the ion-exchange separation of methylmercury, ethylmercury and inorganic mercury accomplished with on-line reduction and determination by cold vapour atomic absorption spectrometry (CVAAS). Experiments were also carried out with a preconcentration procedure in order to achieve lower detection limits.

EXPERIMENTAL

Apparatus and materials

The ion chromatographic system consisted of a Dionex (Sunnyvale, CA) Model QIC or a gradient pump equipped with a variable-wavelength UV–visible detector and a cold vapour atomic absorption spectrometric detector (Milton Roy,

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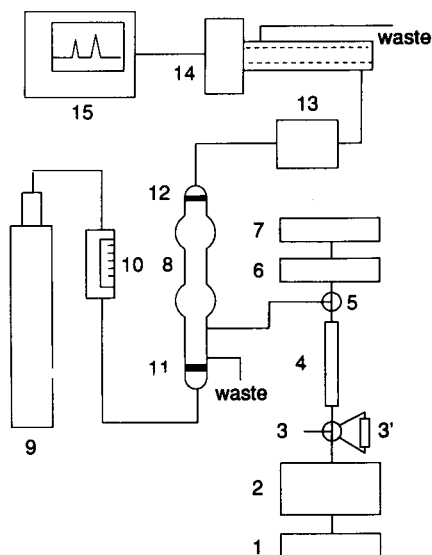
Fig. 1. Schematic diagram of the instrumentation. 1 = Eluent reservoir; 2 = chromatographic system; 3 = injector; 3' = concentration column; 4 = chromatographic column; 5 = T-mixer; 6 = pump manometric module; 7 = $NaBH_4$ reservoir; 8 = flow cell; 9 = carrier (N_2); 10 = flow meter; 11, 12 = glass frits; 13 = $MgClO_4$ traps; 14 = CVAAS detector; 15 = recorder. Flow cell: body, 11 mm i.d., height 170 mm; expansions, 27 mm i.d.

Stone, UK). Figure 1 shows a schematic diagram of the system.

An HPIC-CS-5 cation-exchange separation column (Dionex) was used. Samples were introduced into the injection loops (25–100 μl) or passed either through a LiChroCART RP-18 (5 μm) microcolumn (25 × 4 mm i.d.) (Merck, Darmstadt) or HPIC-CG-5 cation-exchange guard column (Dionex) with the aid of a Dionex (Model DQP-1) pump for the preconcentration procedure. The loading flow-rate was 4.0 ml min⁻¹. A pump and manometric module (Gilson Model 302 and 802, respectively) ensured, through a T-mixer, the addition of the reductive solution to the eluted chromatographic fractions before the CVAAS detection. The mixed solutions containing the dissolved elemental mercury were introduced into a laboratory-made Pyrex flow cell (FC) which also acted as gas–liquid separator. A peristaltic pump (Gilson Minipuls 2) was used for draining the solution from the gas–liquid separator and for ensuring a constant volume into the

cell. The CVAAS signal due to mercury was collected on a IBM PS/2 (Model 40) computer system equipped with an Axion 7272 acquisition/integration data system. For pH measurements a digital Orion pH meter equipped with a combined glass–calomel electrode was used.

High-purity water (HPW) from a Milli-Q system (Millipore, Bedford, MA), supplied with deionized water, was used for preparing all solutions. Sodium hydroxide, magnesium perchlorate, mercury nitrate and methylmercury chloride were obtained from Merck and ethylmercury chloride from Alfa (Karlsruhe). Acetic acid (Merck) was purified with a sub-boiling distillation apparatus (Kurner, Rosenheim). All other reagents were of analytical-reagent grade. Cysteine (Cys) (Merck) was used as received. The reducing solution, containing 1.0 g l⁻¹ sodium tetrahydroborate (Fluka) adjusted to pH 11.5 with 0.1 M NaOH, was purified by bubbling with nitrogen overnight and filtered just before use. All solutions were degassed and filtered through membrane filters (Millipore Type HA, 0.45 μm) before use.

Preparation and measurement of solutions

Working methylmercury, ethylmercury and inorganic mercury standard solutions were prepared daily by dilution of 100.0, 100.0 and 1000 mg l⁻¹ stock standard solutions, respectively, with HPW. Standards (solvent, methanol for methyl- and ethylmercury and water for inorganic mercury) and samples were stored in the dark and refrigerated.

Chromatographic analyses were performed, after optimization (see below), with a mobile phase consisting of 1.0 mM acetic acid–1.0 mM $NaClO_4$–5.0 mM Cys and NaOH up to pH 4.4. The flow-rates were 1.5–2.0 and 0.6 ml min⁻¹ for the eluent and reductant, respectively.

RESULTS AND DISCUSSION

Optimization of procedure

The method is based on the formation and separation of metal–cysteine complexes followed by UV (210 nm) or CVAAS (253.7 nm) detection. The eluent composition was optimized with re-

$$^{+}H_3N \diagdown CH - CH_2 - S - Hg - S - CH_2 - CH \diagdown ^{NH_3^+}$$
$$HOOC \diagup \qquad\qquad\qquad\qquad\qquad COOH$$

Hg(Cys)₂

$$^{+}H_3N \diagdown CH - CH_2 - S - Hg - CH_3$$
$$HOOC \diagup$$

CH₃HgCys

$$^{+}H_3N \diagdown CH - CH_2 - S - Hg - C_2H_5$$
$$HOOC \diagup$$

C₂H₅HgCys

Fig. 2. Structure of cysteine complexes.

spect to the separation procedure and to the reductive reaction which permits the detection of mercury. The cysteine complexes are positively charged (Fig. 2) at the selected pH values and the main mechanism expected for the chromatographic separation is cation exchange. The effects of ionic strength, pH and ligand concentration were evaluated in order to achieve the maximum selectivity, sensitivity, reproducibility and resolution. Figures 3 and 4 show the behaviour of the capacity factors (k') for the considered species as a function of ionic strength (I) (eluent composition: 1.0 mM acetic acid–0.10 mM Cys, pH 4.1) and pH ($I = 1.0$ mM NaClO₄), respectively. The great influence of both parameters on k' confirms that ion exchange is the acting mechanism. On the other hand, the elution order of compounds is inverted with respect to the classical reversed-phase procedure [5].

Different concentrations (0–40 mM) of cysteine in the eluent were also investigated. The results (Fig. 5a) show significant variations especially for k' of inorganic mercury. This behaviour can be attributed to the greater polarity of inorganic mercury than organomercury compounds. It is believed that this behaviour arises from interaction of the charged uncomplexed species at equilibrium with the cysteine complex. The decrease in the retention time of Hg²⁺ on addition of higher cysteine concentrations means that the ligand concentration affects the complexation of

Fig. 3. Dependence of the capacity factor (k') of cysteine complexes on the ionic strength of the eluent. Eluent, 1.0 mM acetic acid–0.10 mM Cys, NaOH up to pH 4.1, NaClO₄ as shown; flow-rate, 1.5 ml min⁻¹; sample, 25 μl; detection, UV (210 nm). ● = CH₃Hg⁺; ▲ = C₂H₅Hg⁺; ■ = Hg²⁺, 125 ng as Hg; ○ = Cys, 3 μg.

mercury species and in addition it avoids adsorption phenomena on the column. In particular, as the concentration of the ligand in the mobile phase increased, the retention time of the analyte decreased and the peak area increased (Fig. 5b).

Figure 5c and d show the effect of cysteine concentration on the peak width at half-height ($W/2$) and asymmetry factor (AF10, 10 means the height at 10% of total peak height), which confirm that 5.0 mM cysteine is the most suitable to optimize these parameters.

Fig. 4. Dependence of the capacity factor (k') of cysteine complexes on the pH of the eluent. Conditions as in Fig. 3, except pH adjusted with NaOH. Symbols as in Fig. 3.

The UV–visible detection of mercury complexes was hindered by the increased background absorbance when a higher concentration of reagent was used in the mobile phase; CVAAS was employed for cysteine concentrations > 0.1 mM to optimize the method. The effect of cysteine concentration was evaluated with respect to the reductive reaction permitting the detection of Hg(0) by CVAAS. Experiments were performed on CH_3Hg^+, $C_2H_5Hg^+$ and Hg^{2+} samples (100 ng as Hg) injected (100-μl loop) into the system without or with the separation column present. The results (Figs. 6 and 7) show that the cysteine concentration positively affects the mercury reduction up to a maximum value in both instances. The optimum concentration was higher for chromatographic separation and resulted in a lower signal. It should be noted that the phenomenon is greater for inorganic mercury, and this seems to confirm the interaction between mercury and the column indicated above. Taking into account the chromatographic and detection performances with respect to cysteine, a 5.0 mM concentration was selected.

In order to perform CVAAS determinations, the detector was interfaced with the chromatographic system through a flow cell (FC) which allowed Hg(0) recovery after the reduction of the eluted species. The chromatographic eluate, connected with reducing reagent with a T-joint, was passed to the FC through a 0.3 mm i.d. reaction coil (Tefzel type; Dionex). Experiments showed
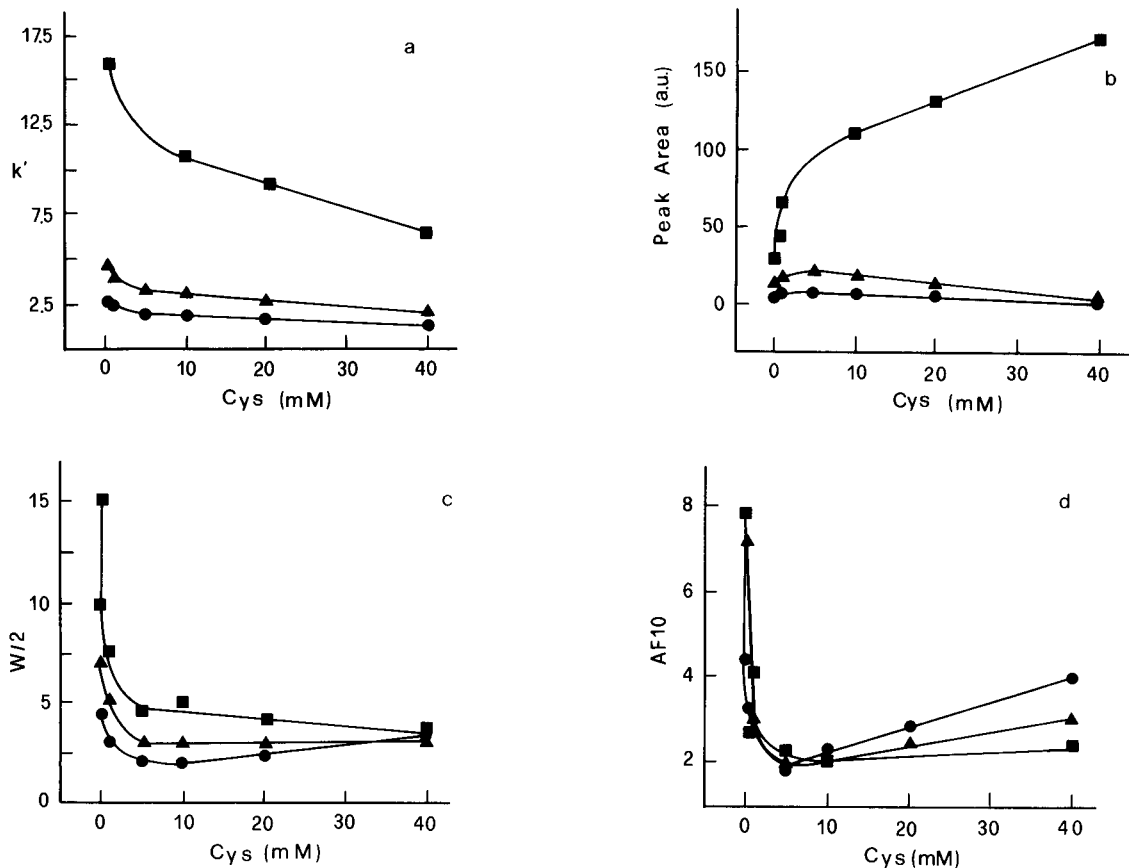
Fig. 5. Effect of cysteine concentration on (a) capacity factor (k'), (b) peak area, (c) $W/2$ and (d) asymmetry factor (AF10). Eluent, 1.0 mM acetic acid–1.0 mM $NaClO_4$ (pH 4.4)–Cys; flow-rate, 2.0 ml min^{-1}; sample, 100 μl; detection, CVAAS. $\bullet$ = CH_3Hg^+; $\blacktriangle$ = $C_2H_5Hg^+$, $\blacksquare$ = Hg^{2+}, 100 ng as Hg.
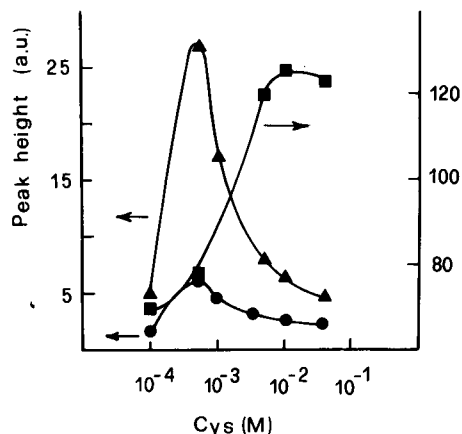
Fig. 6. Effect of cysteine concentration on peak height without separation column. Eluent 1.0 mM acetic acid–1.0 mM $NaClO_4$ (pH 4.4)–Cys; flow-rate, 2.0 ml min^{-1}; sample, 100 μl; detection, CVAAS. $\bullet$ = CH_3Hg^+; $\blacktriangle$ = $C_2H_5Hg^+$; $\blacksquare$ = Hg^{2+}, 100 ng as Hg.

that the length of the tube from 50 to 500 mm did not affect the reduction of mercury species before the sample is introduced into the FC. The design of the cell was such as to ensure a residence time of chromatographic fractions sufficient to permit complete removal of mercury without overlap of the previously separated species. The FC (Fig. 1) was optimized to reduce the residence time of the eluate, to minimize the vapour dispersion and to permit the maximum flow-rate of carrier according to the maximum
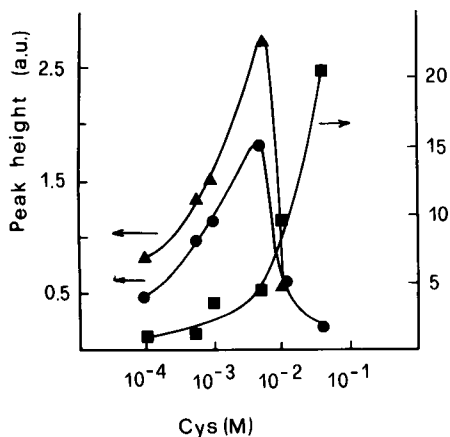
Fig. 7. Effect of cysteine concentration on peak height with separation column. Conditions and symbols as in Fig. 6.

detection sensitivity. A countercurrent flow of nitrogen into the FC swept out the reduced mercury from an outlet located on top of the separator and then introduced it into the CVAAS system, after removal of water through a trap filled with $Mg(ClO_4)_2$ (replaced daily).

The effect of the flow-rate of the reducing solution on detection was also investigated. The maximum peak height was obtained at 0.6 ml min^{-1}; higher flow-rates affected the peak width and symmetry, probably owing to dilution phenomena, without improving the sensitivity. In order to optimize the sensitivity, the effect of nitrogen flow-rate was investigated. Figure 8 shows the variation of peak height, $W/2$ and $H/W10$ as function of flow-rate (the ratio $H/W10$ is the peak height/peak width at 10% of height, and is helpful in defining the flow cell efficiency). As expected, with too low a flow-rate the sensitivity is decreased and the resulting peaks are broadened. However, the flow corresponding to the maximum peak height cannot be selected as in this range the peak broadening is still too large, with poor resolution. In order to avoid these phenomena a flow-rate of 360 ml min^{-1} was selected, at which the sensitivity is lower but good symmetry and reproducibility are achieved.

The retention times obtained using the optimized procedure were 2.6, 3.6 and 6.6 min for methylmercury, ethylmercury and inorganic mercury with relative standard deviations ($n = 6$) of 0.5, 0.4 and 0.4%, respectively.

Preconcentration procedure

Studies were performed on the ability of a microcolumn to preconcentrate samples with an on-line complexation procedure. The 100-μl loop was replaced with a C_{18} (5 μm) (25 × 4 mm i.d.) or HPIC CG-5 microcolumn. Samples were loaded without addition of cysteine. Flow-rates up to 4.0 ml min^{-1} gave good reproducibility in the preconcentration step but an increase in pressure and reduced recoveries resulted for higher flow-rates. Samples of 10–100 ml were passed at 4.0 ml min^{-1} through the microcolumn, in a counter-flow elution step, followed with two 10.0-ml HPW washings to remove the matrix. A counter-flow was preferred for the preconcentra-

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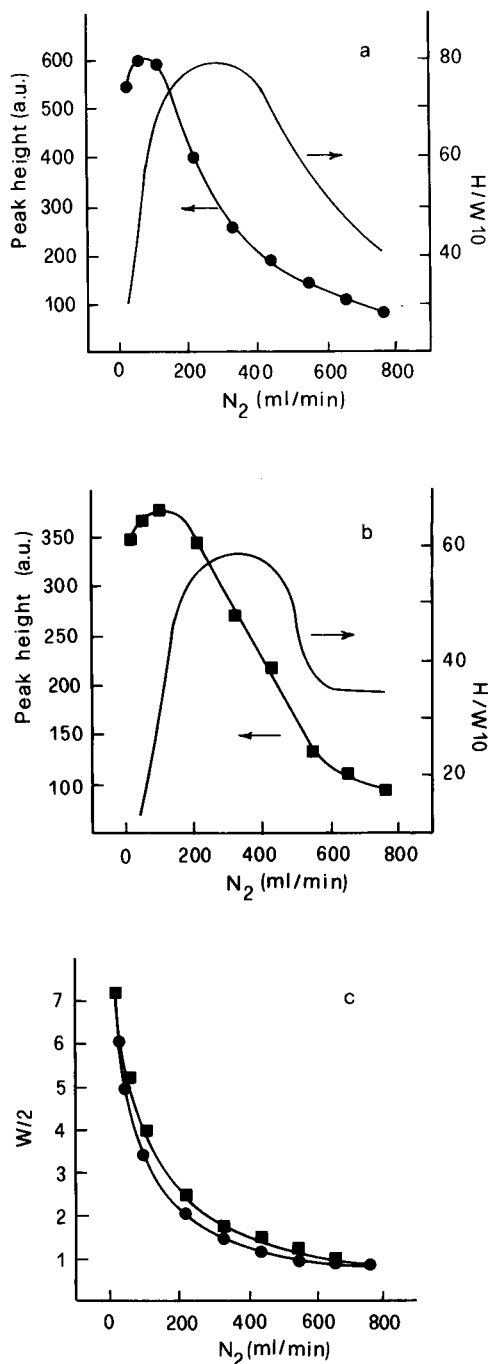
Fig. 8. Effect of nitrogen flow-rate on (a,b) peak height and $H/W10$ and (c) $W/2$ (for definitions, see text) for (●) CH_3Hg^+ and (■) Hg^{2+}, 5 and 1 ng as Hg, respectively. Eluent, 1.0 mM acetic acid–1.0 mM $NaClO_4$ (pH 4.4)–5.0 mM Cys; flow-rate, 2.0 ml min^{-1}; sample, 100 μl; detection, CVAAS.

TABLE 1

Recoveries obtained using the preconcentration procedures

Column	Volume loaded (ml)	Recovery (%)		
		CH_3Hg^+	$C_2H_5Hg^+$	Hg^{2+}
CG-5	10	51	67	55
	100	54	65	51
C_{18}	10	12	19	70
	100	11	18	74
C_{18} [a]	10	20	37	72
	100	21	36	78

[a] 0.5 M NaCl.

tion procedure to avoid interferences in the chromatographic behaviour. Each sample solution, containing 10 to 100 ng (as Hg) for inorganic and organic species, respectively, was analysed in triplicate. The analyte recoveries were evaluated by comparing the heights of the peaks obtained for samples analysed by this procedure with those obtained for 100-μl samples containing the same absolute amounts of mercury species analysed without preconcentration. Table 1 gives the results obtained with CG-5 and C_{18} microcolumns. The recovery was unaffected by the volumes loaded and there was a 500-fold concentration factor for all species (CG-5) with 100-ml samples. Taking into account the lipophilic nature of organomercury compounds and the tendency of Hg(II) to form neutral complexes with chloride or species with reduced polarity, preconcentration from synthetic sea water (0.5 M NaCl) was evaluated using a C_{18} column. Table 1 gives the recoveries, which resulted in preconcentration factors of around 200–300 for CH_3Hg and C_2H_5Hg and 700 for inorganic mercury. This shows the greater efficiency of the ion exchanger for organic species and the suitability of C_{18} for inorganic mercury. Good reproducibility (peak height ⩽ 6%) was obtained over the range of concentrations and sample volumes studied.

Detection limits

As shown by the previous results (optimization of the parameters), there is a great difference between the sensitivity of inorganic mercury and organic species. The detection limits (DL) were

TABLE 2

Detection limits

System [a]	Sample loaded (ml)	Detection limit ($\mu g\,l^{-1}$)		
		CH_3Hg^+	$C_2H_5Hg^+$	Hg^{2+}
Loop (100 μl)	0.1	50.0	20.0	10.0
CG-5 [b]	10	1.0	0.40	0.20
CG-5[b]	100	0.10	0.04	0.02
C_{18} [b,c]	10	2.0	0.60	0.10
C_{18} [b,c]	100	0.20	0.06	0.01

[a] Eluent: 1.0 mM acetic acid–1.0 mM Cys–1.0 mM NaClO$_4$ (pH 4.45). [b] Preconcentration procedure. [c] 0.5 M NaCl.
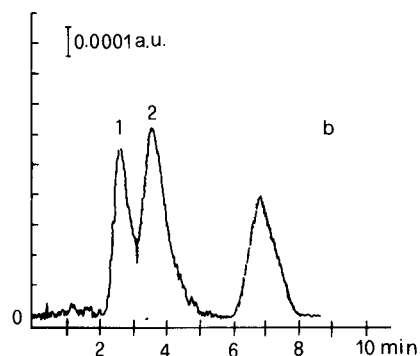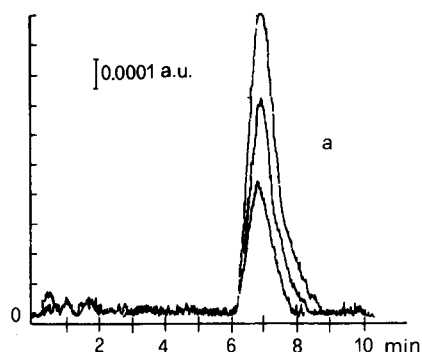
Fig. 9. Results of analysis using the on-line preconcentration procedure: (a) tap water with 0, 50 and 100 ng l^{-1} Hg; (b) tap water spiked with (1) CH$_3$Hg$^+$ and (2) C$_2$H$_5$Hg$^+$, 2.0 and 0.5 μg l^{-1} as Hg, respectively. Eluent, 1.0 mM acetic acid–1.0 mM NaClO$_4$ (pH 4.4)–5.0 mM Cys; flow-rate, 2.0 ml min^{-1}; sample, 50 ml; detection, CVAAS.

determined for each analyte in ultrapure water and synthetic sea water with a 100-μl loop and for 10–100 ml on-line preconcentrated sample procedures. Table 2 compares the DLs calculated (three times the blank value) for the tested procedures.

Real sample analysis

The performance of the proposed system was evaluated on a real sample of tap water. The sample was filtered (0.45 μm) and analysed as such or as spiked with 50–100 ng l^{-1} of inorganic mercury. Figure 9 compares (a) the raw and spiked tap water chromatograms with (b) the sample spiked with organic species at ng ml^{-1} levels. The sample concentration, evaluated by the standard addition procedure, was 77.8 ng l^{-1} for inorganic mercury with a linear regression coefficient $R = 0.994$ ($n = 3$). Hence the developed method appears to be suitable for speciation analysis studies on surface and sea waters.

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AUTHOR INDEX

Analytical Voltammetry

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Series editor: **G. Svehla**, University College, Cork, Ireland

The aim of this volume is to review the state-of-the-art in analytical voltammetry with regard to theory and instrumentation, and show how these relate to the analysis of inorganic, organometallic, organic and biological molecules. Modern voltammetric techniques have practical applications in biological, pharmaceutical and environmental chemistry. The growing importance of voltammetry in the development of modified electrodes and biological electrodes and chemical and biological sensors is also highlighted.

Contents:
1. Theory of Analytical Voltammetry (J.F. Cassidy). Introduction to analytical voltammetry. Classical techniques. Modern techniques. Electroanalysis in flowing streams. Microelectrodes, microelectrode arrays and hydrodynamically modulated rotating disc electrochemistry. Mathematical, coulostatic and reflectance methods applied to voltammetry. Conclusions. **2. Instrumentation** (J.N. Barisci, P.J. Riley, G.G. Wallace). Introduction. The electrochemical cell. The working electrode. The reference electrode. The auxiliary electrode. Electronics. **3. Analytical Voltammetry of Biological Molecules** (J.M. Séquaris). In vivo voltammetry in neurochemistry. Electrochemical immunoassay. Voltammetric analysis of biological macromolecules: proteins and nucleic acids. **4. Analytical Voltammetry In Pharmacy** (P.M. Bersier, J. Bercier). Introduction. Polarographic and voltammetric techniques applied to drug analysis. Practical applications of polarography, voltammetry and hybrid techniques in drug analysis. **5. Analytical Voltammetry In Environmental Science. I. Inorganic Species** (J.M. Fernandez, P.J. Hayes, M.R. Smyth). Introduction. General aspects of trace analysis. Polarography and voltammetric methods. Trace metals. Application of polarographic and voltammetric methods to trace metal analysis. **6. Analytical Voltammetry In Environmental Science. II. Organic and Organometallic Species** (P.M. Bersier, J. Bersier). Introduction. Use of advanced polarographic, voltammetric and hybrid techniques for the determination of organometallic and organic pollutants. Polarographic and voltammetric determination of organometallic and organic pollutants in air. Polarographic and voltammetric determination of organometallic and organic pollutants in aqueous environment. Determination of organic pollutants in soils. Determination of organometallic and organic pollutants in foodstuffs- and biological matrices. **7. Theory and Analytical Applications of Modified Electrodes** (R.J. Forster, J.G. Vos). Introduction. Preparation of modified electrode surfaces. Characterisation of modified electrodes. Theoretical aspects of mediation processes at modified electrodes. Analytical applications of modified electrodes. Conclusion. **8. Amperometric Biosensors** (J. Rodriguez Flores, E. Lorenzo). Introduction. Principles of biosensors. Fundamentals of amperometric biosensors. Classification. Applications. **Subject Index.**

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Chimica	281/2	282/2	283/2	284/1	285/1–2	286/2	287/3	288/3	290/1			
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