Determination of Metal Speciation by Reverse Titrations

ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 2005

Impact Factor: 5.64 · DOI: 10.1021/ac049078e · Source: PubMed

2 AUTHORS:

Jochen Nuester
California State University, Chico
16 PUBLICATIONS **306** CITATIONS
SEE PROFILE

Constant M. G. van den Berg
University of Liverpool
231 PUBLICATIONS **8,242** CITATIONS
SEE PROFILE

Anal. Chem. **2005,** *77,* 11−19

Articles

Determination of Metal Speciation by Reverse Titrations

Jochen Nuester† and Constant M. G. van den Berg*

Department of Earth and Ocean Sciences, Liverpool University, Liverpool L69 3PG, U.K., and MPI-Bremen, Celsiusstr. 1, 28359 Bremen, Germany

A new method is proposed to determine metal speciation by varying the concentration of a competing ligand at a constant metal concentration, with detection by cathodic stripping voltammetry. The free metal ion concentration is gradually lowered from its natural level while the method probes progressively deeper into the already complexed metal fraction: it is therefore a *reverse titration* rather than the *forward titration* which is used for conventional complexing ligand titrations. The sensitivity is greatest at the lowest free metal ion concentration, where it matters most, and the method can be carried out in a single sample aliquot in the voltammetric cell. The method is applied here to copper speciation, but in principle, it can also be used for other metals. Modeling shows that this method has good sensitivity at ligand concentrations near the metal concentration (lower as well as greater). Comparative measurements of copper speciation using reverse and forward titrations of representative water samples of oceanic and coastal origin show good agreement. The data showed that these samples did not contain low levels of strong ligands in addition to the ligands detected by the forward titrations.

The chemical speciation of copper, iron, cobalt, zinc, and several other metals in natural waters is controlled by organic metal binding ligands.[1−7] For some metals, the speciation is complicated by redox reactions and the pH causing the formation of unstable, transient species. Copper has been extensively studied, and it has been suggested that relatively low Cu^{2+} concentrations in their hydrated form are toxic to many microorganisms, including bacteria,[8] phytoplankton,[9] and crab larvae.[10] In this work, we concentrate on copper, of which the speciation is known to be dominated by stable organic complexes in waters from oceanic, coastal, estuarine, and freshwater origin.[4,11−13]

The organic ligands participating in complexation of copper (II) fall in at least two major groups (L1 and L2). Several studies indicate that strong organic ligands (L1) dominate the copper complexation in open ocean surface waters, whereas the weaker ligands (L2) are involved in the complexation in coastal waters.[14−18] A third group has been determined in some cases.[18]

Little is known about the composition of the ligands. A recent study has shown that thiols are likely ligands for copper complexation in estuarine waters,[4] but humic and fulvic acids are also thought to act as strong copper binding ligands.[19] In addition to organic ligands, sulfide clusters could also account for metal-binding ligands in freshwater,[20] and sulfides are present in seawater at nanomolar concentration.[21] Sulfhydryl-containing ligands, e.g., metallothionein-like proteins, could be present in colloids, which could also act as complexing agents.[22]

The usual method to determine the concentration of natural complexing ligands (L) and their complex stability is by means of a titration with the metal while following the free (not complexed by L) concentration by cathodic stripping voltammetry (CSV) with competitive ligand equilibration (CLE),[23] by graphite furnace atomic absorption spectroscopy of the extracted complex,[24,25] by anodic stripping voltammetry,[26] or by adsorption on

* To whom correspondence should be addressed. E-mail: vandenberg@liv.ac.uk.

† MPI-Bremen.

(1) Ellwood, M. J.; van den Berg, C. M. G. *Mar. Chem.* **2001**, *75*, 33−47.
(2) Gledhill, M.; Van den Berg, C. M. G. *Mar. Chem.* **1994**, *47*, 41−54.
(3) Gordon, L. I.; Codispoti, L. A.; Jennings, J. C.; Millero, F. J.; Morrison, J. M.; Sweeney, C. *Deep-Sea Res., Part II* **2000**, *47*, 3095−3117.
(4) Laglera, L. M.; van den Berg, C. M. G. *Mar. Chem.* **2003**, *82*, 71−89.
(5) Moffett, J. W. *Deep-Sea Res., Part I* **1995**, *42*, 1273−1295.
(6) Saito, M. A.; Moffett, J. W. *Mar. Chem.* **2001**, *75*, 49−68.
(7) Van den Berg, C. M. G. *Anal. Chim. Acta* **1984**, *164*, 195−207.
(8) Hering, J. G.; Sunda, W. G.; Ferguson, R. L.; Morel, F. M. M. *Mar. Chem.* **1987**, *20*, 299−312.
(9) Brand, L. E.; Sunda, W. G.; Guillard, R. R. L. *J. Exp. Mar. Biol. Ecol.* **1986**, *96*, 225−250.
(10) Sanders, B. M.; Jenkins, K. D.; Sunda, W. G.; Costlow, J. D. *Science* **1983**, *222*, 53−55.
(11) Moffett, J. W. *Deep-Sea Res., Part I* **1995**, *42*, 1273−1295.
(12) Xue, H.; Sigg, L. *Limnol. Oceanogr.* **1993**, *38*, 1200−1213.
(13) van den Berg, C. M. G. *Mar. Chem.* **1984**, *14*, 201−212.
(14) Coale, K. H.; Bruland, K. W. *Limnol. Oceanogr.* **1988**, *33*, 1084−1101.
(15) Donat, J. R.; Van den Berg, C. M. G. *Mar. Chem.* **1992**, *38*, 69−90.
(16) Coale, K. H.; Bruland, K. W. *Deep-Sea Res., Part I* **1990**, *37*, 317−336.
(17) Moffett, J. W.; Zika, R. G.; Brand, L. E. *Deep-Sea Res., Part I* **1990**, *37*, 27−36.
(18) Kozelka, P. B.; Bruland, K. W. *Mar. Chem.* **1998**, *60*, 267−282.
(19) Kogut, M. B.; Voelker, B. M. *Environ. Sci. Technol.* **2001**, *35*, 1149−1156.
(20) Rozan, T. F.; Lassman, M. E.; Ridge, D. P.; Luther, G. W. *Nature* **2000**, *406*, 879−882.
(21) Luther, G. W.; Tsamakis, E. *Mar. Chem.* **1989**, *27*, 165−177.
(22) Tang, D. G.; Warnken, K. W.; Santschi, P. H. *Limnol. Oceanogr.* **2001**, *46*, 321−330.
(23) Van den Berg, C. M. G. *Mar. Chem.* **1984**, *15*, 1−18.
(24) Moffett, J. W.; Zika, R. G. *Environ. Sci. Technol.* **1987b**, *21*, 804−810.
(25) Moffett, J. W.; Zika, R. G. *Mar. Chem.* **1987a**, *21*, 301−313.

10.1021/ac049078e CCC: $30.25
Published on Web 12/03/2004

MnO_2.[27] Often more than one ligand is detected in natural waters. This tends to complicate the data interpretation[28,29] when the detection method does not have the resolution to discriminate among more than 2 or 3 ligands.[30]

The existing speciation methods titrate ligands one after the other by adding metal. Ligands with similar complex stability are titrated to some extent in parallel, which is what is causing problems with the interpretation of the data. For instance, it is well-known that starting a titration at a different detection window (which includes either using a different range of metal concentrations or a different concentration of the added competing ligand) tends to give different detected concentrations of natural ligands, with lower complexing stabilities for the ligands of higher concentrations detected at higher metal concentrations. Using CLE techniques, the titration starts below the original, free, metal ion concentration as the metal is distributed over the added and original ligands. During the titration with metal, the strong species are formed first, followed by the weaker species, which are less important with respect to the metal speciation in the original sample. For this reason, titrations should be carried out at a high detection window and at a realistic range of metal concentrations, unless it is the intention to detect the weaker ligands present at higher concentrations.

In this study, we propose a new procedure aimed specifically at the detection of the strong complexing ligands that may be present at concentrations similar to that of the metal (lower as well as higher) to get information on those ligands that are most important to the metal speciation in natural waters. This procedure is based on varying the detection window in CLE from low to high with detection by CSV (CLE/CSV) while keeping the metal concentration constant. The detection window is varied by gradually increasing the concentration of the added competing ligand. By lowering the free metal ion concentration, the method probes progressively deeper into the metal species originally present in the water, as if by a reverse metal titration. The ligand concentration is estimated either by curve-fitting or data linearization. Here, we use copper for the first application and testing of this method. The copper speciation was determined by following the peak height of the copper salicylaldoxime ($CuSA_2$) complex during titrations with SA. In this study, we present the theory, testing against EDTA, and titrations on bulk oceanic samples and coastal waters; the titrations are compared against the usual forward complexing ligand titrations.

MATERIALS AND METHODS

Equipment. The voltammetric system consisted of a Metrohm VA-Stand (663 VA-Stand, Metrohm, Switzerland) connected via an IME-663 module to a computer-controlled voltammeter (PGSTAT 10, Eco Chemie, Netherlands). The working electrode was a hanging mercury drop electrode (HDME, drop surface area 0.38 mm^2). The reference electrode was a double-junction Ag/AgCl (3 M KCl) electrode, and the counter electrode was a platinum wire. The solutions were stirred by a rotating PTFE rod during the deposition step of the measurements.

Sample bottles (LDPE, Nalgene) were cleaned by soaking 1 week in 50% HCl, followed by at least 1 week in 10% HCl, and were subsequently stored partially filled with 0.01 M HCl. The voltammetric measurements were performed in a glass cell cleaned with 2 M HCl and 0.01 M HCl.

Reagents. Water was purified by reverse osmosis (Milli-RO, Millipore) followed by ion-exchange (Milli-Q). Ammonia and hydrochloric acid (Analar grade, Merck) were further purified by subboiling quartz-distillation. Solutions of copper were prepared by dilution of an atomic adsorption spectrometry standard solution (BDH, SpectrosoL grade) in 0.01 M HCl. A pH buffer containing 1 M boric acid (Analar grade) and 0.35 M ammonia was UV-digested for 1 h to remove organic matter. An addition of 150 μL of this buffer to 10 mL of seawater resulted in a pH of 8.35. A 0.1 M solution of salicylaldoxime (SA) (BDH) stock solution was prepared in 0.1 M HCl.

Sample Collection and Storage. Seawater from the North Atlantic Ocean was collected from various depths, filtered (0.1-μm filtration cartridge), and mixed during cruises with the RV Pelagia (MERLIM cruise 1999, and COMET cruise, 2001) and stored as bulk seawater in 50-L HDPE containers. These samples are called here MERLIM and COMET, respectively. This water was stored at room temperature in the dark. For some experiments this water was digested with UV-light (1 h, 125 W high-pressure mercury vapor lamp) to remove any organic ligands and was then indicated as UV-seawater (UV-SW). The water of Venice Lagoon was collected November 2002 using a peristaltic pump from a depth of 0.5 m; it was filtered on-line through a 0.1-μm filtration cartridge and stored frozen.

Procedure to Determine Total Dissolved Copper Using CSV. Total copper was determined by CSV after 45 min of UV digestion of samples in silica tubes at the original pH (pH 8). The silica tubes were conditioned with each sample before use to minimize the loss of copper due to adsorption onto the tube walls. A 15-mL portion of the samples was pipetted into the voltammetric cell; mixed with 150 μL of borate buffer and 37.5 μl of SA; and then deaerated for 5 min using oxygen-free, water-saturated, nitrogen gas; then the solution was left to equilibrate for 10 min.

The measurement parameters were deposition potential of −0.15 V; deposition time 60 s while stirring; a quiescence period of 15 s; CSV scan in the square-wave mode from −0.15 to −0.6 V with a modulation frequency of 10 Hz; step height 2.5 mV, pulse height 25 mV. The measurements were repeated after addition of copper standard to calibrate the sensitivity. Between measurements, the deaeration time was 15 s.

Reverse Titration Procedure to Determine Copper Complexation. A 15-mL portion of seawater was transferred to a voltammetric cell, and 150 μL of borate buffer was added. The concentration of SA was increased stepwise from 0.01 to 70 μM. Thus, the entire titration was performed in a single, small, sample aliquot in a voltammetric cell. The start and final SA concentrations can be varied from those used here, depending somewhat on the original copper and natural ligand concentration (for instance, lower SA concentrations could be used for higher copper

(26) Chau, Y. K.; Gachter, R.; Chan, K. L. S. *J. Fish. Res. Board Can.* **1974**, *31*, 1515.
(27) Van den Berg, C. M. G.; Kramer, J. R. *Anal. Chim. Acta* **1979**, *106*, 113−120.
(28) Filella, M.; Buffle, J.; Vanleeuwen, H. P. *Anal. Chim. Acta* **1990**, *232*, 209−223.
(29) Buffle, J.; Altmann, R. S.; Filella, M.; Tessier, A. *Geochim. Cosmochim. Acta* **1990**, *54*, 1535−1553.
(30) Van den Berg, C. M. G.; Donat, J. R. *Anal. Chim. Acta* **1992**, *257*, 281−291.

concentrations). The titration was halted when the increase in the peak height for the copper/SA complex leveled off. Each SA addition was equilibrated for 30 min, and a purging time of 10 s was allowed after each addition; the solution was blanketed with nitrogen at all times. The concentration of the $Cu(SA)_2$ complex was determined by CSV using the square-wave modulation with a frequency of 10 Hz and a step potential of 2.5 mV; the deposition potential was −0.15 V for 60 s, the quiescence period was 15 s, and the potential scan was from −0.15 to −0.6 V. A titration of UV-digested seawater was used as the control, and a UV-digested aliquot with added EDTA was used for comparative purposes.

Forward Copper Complexing Ligand Titrations. Comparative copper-complexing ligand titrations were carried out using the conventional metal-titration technique in which the ligands are titrated with metal additions to 28 mL capped, polystyrene, tubes (Sterilin).[31] Approximately 150 mL of sample water was transferred to a PTFE bottle, borate buffer was added to a concentration of 0.01 M, and 5−10 μM SA. The copper concentration was varied between 0 and 400 nM in 10 Sterilin tubes, and 10 mL of the seawater was added to each tube. The samples were equilibrated at least 8 h or overnight at room temperature (~20 °C). The Sterilin tubes were conditioned three times as described above. The samples were transferred to the voltammetric cell, and the concentration of the copper/SA complex ($Cu(SA)_2$) was determined using CSV as before.

Data Analysis. Data-fitting was carried out using the EXCEL plug-in Solver. The measured data (ratio of maximum current to the actual current) were subtracted from data modeled using estimated values for K'_{CuL} and C_L. The differences were squared and summed; the sum was minimized by Solver by adjusting the values for K'_{CuL} and C_L by least-squares regression. Where necessary, values for a second ligand and for the maximum current (i_{pmax}) were fitted simultaneously.

THEORY

Modeling of the CSV Response During a Reverse Titration of Seawater with and without Natural Ligands. In a reverse titration, the dissolved metal is bound progressively by additions of the ligand used to give the CSV response. During the titration, the CSV response increases until all metal is bound as the electroactive species; at this point, the peak height is maximal (i_{pmax}). Because the response is directly related to the concentration of the electroactive, adsorptive, species, the CSV response can be modeled by calculation of the abundance of this species during the titration. There are two models possible because there are two species, CuSA and $CuSA_2$,[31] which can adsorb. The previous work suggested that the $CuSA_2$ species adsorbs, and this was confirmed by the modeling here, so the relevant model will be discussed in detail, whereas the 1:1 CuSA model will be treated more briefly.

Rather than modeling absolute peak heights, ratios of peak heights were used; this way, there is no need to know the sensitivity (response/metal concentration), as is required for the usual forward titrations. The plot of relative peak height, X, as a function of the SA concentration was modeled using

$$X = [CuSA_2]/C_{Cu} \quad (1)$$

where C_{Cu} is the dissolved copper concentration. X is the ratio of the actual over the maximum current: $X = i_p/i_{pmax}$ and $0 < X < 1$ at concentrations of SA from low to high. The CSV response (i_p) is maximal ($i_p = i_{pmax}$) when all copper occurs as $CuSA_2$. The relationship between the CSV response and $[CuSA_2]$ depends on the sensitivity, $S = i_p/[CuSA_2]$. The sensitivity changes with the concentration of SA, and this is varied continuously during the titrations. Fortunately, there is no need to know the sensitivity for these titrations because it cancels out of the equation for X. Instead, it is necessary to have a good estimate for i_{pmax}, which is optimized through fitting of the titrations to the model.

Substitution of the mass balance for copper,

$$C_{Cu} = [Cu'] + [CuSA] + [CuSA_2] + [CuL_n] \quad (2)$$

into eq 1, the following more detailed ratio is obtained, more useful for data fitting

$$X = [CuSA_2]/([Cu'] + [CuSA] + [CuSA_2] + [CuL_n]) \quad (3)$$

where CuL_n is one of the complexes with natural organic ligands of type L, and [Cu′] is the concentration of inorganic copper (not complexed by any organic ligand). The concentration of each species is related to that of Cu^{2+} through an α-coefficient, e.g.

$$[Cu'] = \alpha_{Cu}[Cu^{2+}]$$

$$[CuSA] = \alpha_{CuSA}[Cu^{2+}]$$

and

$$[CuSA_2] = \alpha_{CuSA_2}[Cu^{2+}].$$

A value for α_{Cu} of 35 was calculated from an ion-pairing model as before.[31] Values for the α-coefficients of Cu^{2+} and SA were calculated using

$$\alpha_{CuSA} = K'_{CuSA}C_{SA}$$

and

$$\alpha_{CuSA_2} = B'_{CuSA}C_{SA}$$

where K' and B' are the conditional stability constants valid for seawater of the same salinity. The total concentration of SA (C_{SA}) was used here because it was much greater than the concentration of copper. This was not done for the unknown ligands, L, which were present at much lower concentrations, so a correction had to be made for the amount of ligand tied up by copper. For species of the type CuL (CuL_1 and CuL_2), the following relationship was used.

$$[CuL] = K'_{CuL}[Cu^{2+}]C_L/(1 + K'_{CuL}[Cu^{2+}]) \quad (4)$$

(31) Campos, M.; Van den Berg, C. M. G. *Anal. Chim. Acta* **1994**, *284*, 481−496.

By substitution of the concentration terms in eq 3, the following relationship was obtained for the case of adsorption of $CuSA_2$.

$$X = \alpha_{CuSA_2}/(\alpha_{Cu}' + \alpha_{CuSA} + \alpha_{CuSA_2} + K'_{CuL}C_L/(1 + K'_{CuL}C_L[Cu^{2+}])) \quad (5)$$

Values for K'_{CuL} and C_L were calculated by nonlinear least-squares regression of *experimental* values of X (from i_p/i_{pmax}) to the *modeled* values of X using eq 5 with optimization of initial estimates for the ligand concentration and conditional stability constant.

If two ligands of type L (L_1 and L_2) are present, this equation changes to

$$X = \frac{\alpha_{CuSA_2}}{\left(\alpha_{Cu'} + \alpha_{CuSA} + \alpha_{CuSA_2} + \frac{K'_{CuL_1}C_{L_1}}{(1 + K'_{CuL_1}[Cu^{2+}])} + \frac{K'_{CuL_2}C_{L_2}}{(1 + K'_{CuL_2}[Cu^{2+}])}\right)} \quad (6)$$

More terms for additional ligands can be added to the denominator as required, but in practicality, it may be difficult to fit more than two ligands as the number of parameters increases. The complexing stability constants (K'_{CuL1} and K'_{CuL2}) and ligand concentrations (C_{L1} and C_{L2}) of the natural organic ligands were calculated by least-squares regression of experimental X values to those calculated using eq 6.

Free concentrations of Cu^{2+} for eqs 5 and 6 were calculated (see below) using the fitted values for C_{Ln} and K'_{CuLn} and were part of the optimization process.

Calibration of the Conditional Stability Constants for CuSA and $CuSA_2$. Values for B'_{CuSA_2} and K'_{CuSA} (the conditional stability constants for $CuSA_2$ and CuSA, respectively) were calculated by curve-fitting of experimental values of $X = i_p/i_{pmax}$ (obtained in UV-SW in the absence of natural ligands) to the modeled values. The following equation was used for X, which is derived by setting the concentration C_L in eq 5 to 0.

$$X = \alpha_{CuSA_2}/(\alpha_{Cu}' + \alpha_{CuSA} + \alpha_{CuSA_2}) \quad (7)$$

After substitution of the α-coefficients, this is changed to

$$X = B'_{CuSA_2}[SA]^2/(\alpha_{Cu}' + K'_{CuSA}[SA] + B'_{CuSA_2}[SA]^2) \quad (7')$$

This equation is valid for adsorption of $CuSA_2$. In the case of adsorption of CuSA but in the presence of $CuSA_2$ at high concentrations of SA, the following equation is valid:

$$X = \alpha_{CuSA}/(\alpha_{Cu}' + \alpha_{CuSA} + \alpha_{CuSA_2}) \quad (8)$$

And in the case of CuSA adsorption without the presence of $CuSA_2$,

$$X = \alpha_{CuSA}/\alpha_{Cu}' + \alpha_{CuSA}) \quad (9)$$

The three relationships were compared to see which best described reverse titrations of UV-SW and were used to calibrate the complex stability of Cu with SA and confirm the identity of the adsorbing species.

We attempted to fit the stability constants for the two expected species (CuSA and $CuSA_2$) using the relative peak height as a function of the concentration of SA in eq 7. Preliminary calculation showed that it was not possible to fit both constants using eq 7 because both constants occur in the denominator and one in the numerator. A number of good fits could be obtained, all having a constant ratio for the complex stabilities of CuSA and $CuSA_2$. Therefore, a second relationship was obtained from the sum of the α-coefficients for copper complexation by SA determined by competition against EDTA at 25 μM SA (Campos and van den Berg, 1994): $(\alpha_{CuSA} + \alpha_{CuSA_2}) = 10^{5.83}$ for seawater. One of the two constants was then eliminated in eq 7 (and similarly in eqs 8 and 9) using

$$K'_{CuSA}[SA] = (\alpha_{CuSA} + \alpha_{CuSA2}) - B'_{CuSA_2}[SA]^2$$

This meant that only one parameter (e.g., B'_{CuSA_2}) remained to be fitted by the least squares regression and could be used to calculate a value for K'_{CuSA}.

$[Cu^{2+}]$ Calculation. In UV-SW (used for calibration), values for the concentration of Cu^{2+} were calculated from

$$[Cu^{2+}] = C_{Cu}/(\alpha_{Cu} + \alpha_{CuSA} + \alpha_{CuSA_2})$$

In the presence of a single natural ligand of type L, the following quadratic equation was obtained by substitution of the concentration terms of the individual species in the mass balance of the metal concentration (eq 2).

$$[Cu^{2+}]^2K'_{CuL}(\alpha_{Cu} + \alpha_{CuSA} + \alpha_{CuSA_2}) + [Cu^{2+}](\alpha_{Cu} + \alpha_{CuSA} + \alpha_{CuSA_2} - K'_{CuL}C_{Cu}) + K'_{CuL}C_L = 0 \quad (10)$$

Concentrations of Cu^{2+} were calculated with the usual solution for this quadratic equation.

Values for K'_{CuL} and C_L were initially estimated and then optimized during the iterative least-squares fitting process; these values were used to calculate values for $[Cu^{2+}]$ at every SA concentration during the reverse titration. The calculated concentrations of Cu^{2+} were then used to model the X ratio at each SA concentration in eq 5 for one ligand of type L.

In the presence of two ligands of type L, the following equation was obtained by substitution of eq 2.

$$[Cu^{2+}]^2K'_{CuL}(\alpha_{Cu}' + \alpha_{CuSA} + \alpha_{CuSA_2} + \alpha_{CuL_2}) + [Cu^{2+}](\alpha_{Cu}' + \alpha_{CuSA} + \alpha_{CuSA_2} - K'_{CuL1}C_{cu} + K'_{CuL1}C_{L1}) - C_{Cu} = 0 \quad (11)$$

This equation was not fully resolved because α_{CuL2} occurred within the bracketed part. Values for this were calculated using eq 12 and using estimated values for $[Cu^{2+}]$.

$$\alpha_{CuL2} = (C_{L2} - C_{Cu} + C_{L1})K'_{CuL2} \quad (12)$$

Linearization Method to Fit Natural Ligands. Although the curve-fitting procedure was effective to fit the reverse titrations, we evaluated whether it is possible to use a linear procedure, as commonly used in the forward titrations. For this reason, the van den Berg−Ruzic linearization method[27,32−34] was used to evaluate the complexing ligand concentration (C_L) and the conditional stability constant K'_{CuL}.

$$[Cu^{2+}]/[CuL] = [Cu^{2+}]/C_L + 1/(C_L K'_{CuL}) \quad (13)$$

A plot of $[Cu^{2+}]/[CuL]$ as a function of $[Cu^{2+}]$ is linear if only one ligand of type L is present and curved if there are two or more (e.g., van den Berg, 1982); this is convenient for checking whether the data can be fitted to a one-ligand model because this is not immediately apparent from the nonlinear methods.

Experimental values for the concentration of Cu^{2+} were obtained using the following relationship, which assumes that only $CuSA_2$ adsorbed during the voltammetric measurement,

$$[Cu^{2+}] = XC_{Cu}/\alpha_{CuSA_2} \quad (14)$$

and the concentration of CuL was obtained from the mass balance of copper.

$$[CuL] = C_{Cu} - [CuSA] - [CuSA_2] - [Cu'] = C_{Cu} - [Cu^{2+}](\alpha_{Cu} + \alpha_{CuSA} + \alpha_{CuSA_2}) \quad (15)$$

The experimental X values in eq 14 were used to obtain values for $[Cu^{2+}]$ and the ratio $[Cu^{2+}]/[CuL]$. Values for K'_{CuL} and C_L were calculated from the slope and the Y axis intercept of the plot of $[Cu^{2+}]/[CuL]$ as a function of $[Cu^{2+}]$ by linear least-squares regression according to eq 13. These values for C_L and K'_{CuL} were then used to model values for X using eq 5 to allow comparison of modeled and experimental X values.

RESULTS AND DISCUSSION

Model Calculations Showing the Response Expected for Reverse Titrations of Copper Complexes with SA. Free ionic copper concentration and the relative CSV response (X values) were calculated as a function of the SA concentration, using eqs 10 and 5, for given values of B'_{CuSA_2}, K'_{CuSA}, K'_{CuL}, C_{Cu}, and C_L. Values for these parameters were taken from Campos and van den Berg.[31] Previously,[31] it was demonstrated that either of two copper (II)/SA complexes can adsorb at the electrode, the CuSA or the $CuSA_2$ species, but it was considered more likely that the $CuSA_2$ species adsorbed. It will be demonstrated below that, indeed, the $CuSA_2$ species best fits the adsorption model, so in this model calculation, it is assumed for now that only the $CuSA_2$ is electroactive.

The modeled X values (representing the relative CSV response) are summarized in Figure 1, showing the expected S-shaped CSV response as a function of log[SA]. This means that the response first increases and subsequently levels off in a symmetric and predictable fashion.

Modeling of Effects of Variations in Analytical Parameters: Natural Ligand Concentration, Salinity, and the Copper Concentration, on the Shape of the Reverse Titration. At a given complex stability, K'_{CuL}, the modeled CSV response is very sensitive to variations in the concentration of the natural ligand (C_L) also when this is less than the copper concentration C_{Cu} (Figure 1A). This is important, because this means that it should be possible to detect low ligand concentrations using reverse titrations. With the usual complexing ligand titrations, it is difficult to determine ligand concentrations less than, or only slightly greater than, the metal concentration. The natural ligand is then nearly metal-saturated, causing a titration with little or no curvature and making it difficult to establish the titration endpoint. Also in the usual complexing ligand titrations, a ligand (L1) at a concentration less than metal would be missed in the presence of another ligand (L2) at higher concentration, even if ligand L1 binds the metal more strongly. The modeling suggests that the reverse titration method would be more suitable to detect low ligand concentrations because the method works by removing previously complexed copper. It therefore detects the ligand(s) actually binding the metal in the original sample condition, rather than detecting the excess ligand, as is done in the forward titrations.

The modeling showed that the CSV response is diminished more at low SA than at higher SA concentrations when the concentration of ligand L is increased from less than to greater than the copper concentration, causing the S-shape to become steeper (Figure 1A).

Furthermore, the modeling showed (Figure 1A) that titrations with SA have to be in the range of 10^{-7}−$10^{-3.5}$ M SA to cover expected complexes for copper.

The influence of variations in the salinity was modeled by varying the values for the stability constants for the CuSA and $CuSA_2$ species using the known salinity relationship for these constants (Campos and van den Berg, 1994),

$$\log B'_{CuSA_2} = 15.78 - 0.53 \log(\text{salinity})$$

and

$$\log K'_{CuSA} = 10.12 - 0.37 \log(\text{salinity})$$

The salinity effect on the reverse titration response (Figure 1B) is most pronounced at low salinities: a change in the salinity from 35 to 15 (i.e., from normal seawater to brackish water) caused only a minor change in the CSV response, but a salinity change to freshwater conditions affected the modeled CSV response more dramatically. This is in line with expectation because the change in ionic concentrations is orders of magnitude in the low salinity range and varies by only a factor of 2 in the higher range. It shows that repeat calibrations of the Cu/SA complex stability on UV-SW are not necessary at salinities encountered in normal seawater, but it may be necessary to refine the published data for low salinities.

The effect of variations in the background copper concentration is shown in Figure 1C. Because the data are plotted as a ratio of

(32) Van den Berg, C. M. G. *Mar. Chem.* **1982**, *11*, 307−322.
(33) Van den Berg, C. M. G. *Mar. Chem.* **1982**, *11*, 323−342.
(34) Ruzic, I. *Anal. Chim. Acta* **1982**, *140*, 99−113.

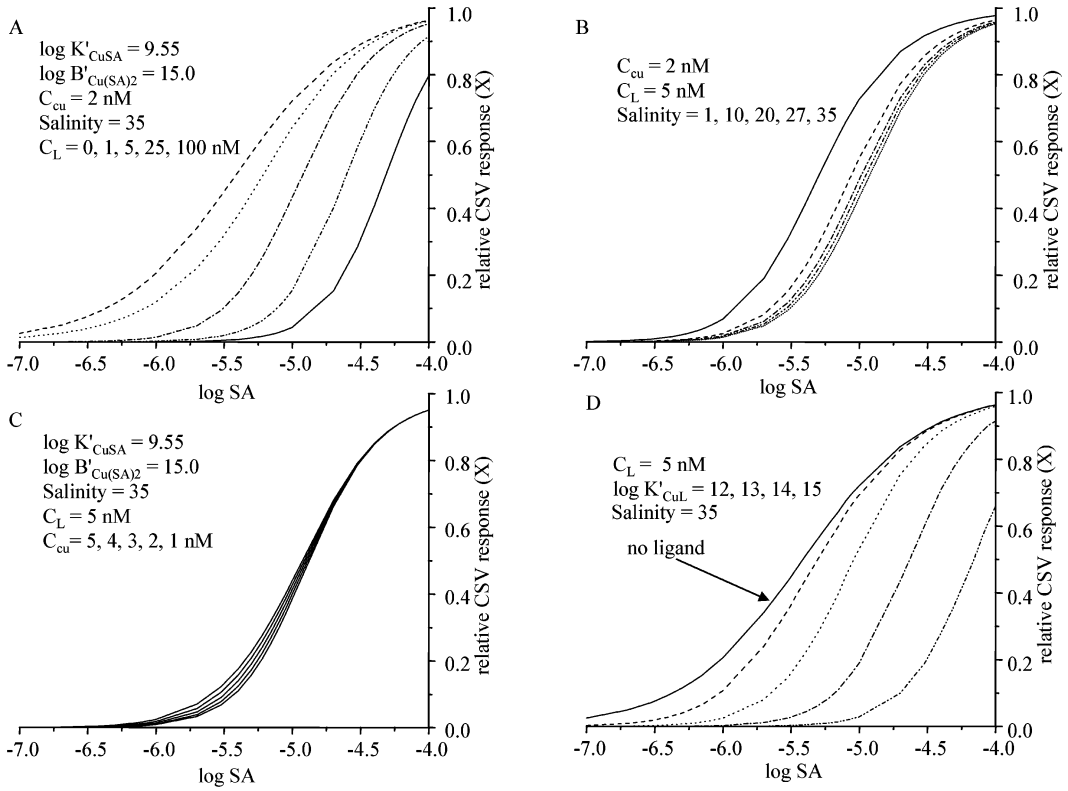

Figure 1. Modeling of the effect of variations in analytical parameters on the CSV response (the *X* ratio) during the reverse titrations: (A) as a function of C_{Cu}, (B) as a function of salinity, (C) as a function of changes in C_{Cu} at $C_{Cu} < C_L$, and (D) as a function of changes in C_L at $C_{Cu} < C_L$.

i_p/i_{pmax}, the effect of the variations in the copper concentration is small, although the effect on i_p and i_{pmax} individually would be directly related to the copper concentration and could be large. The ratio of the ligand over the copper concentration is affected by the changes in the copper concentration, and it can be seen that the titration curve flattens somewhat when the copper concentration is similar to or greater than the ligand concentration. The reason for this is that then, some of the copper is "free" (not bound by L), and this fraction, therefore, gives a peak at lower SA concentrations than does the complexed copper. The effect is not apparent at higher SA concentrations because then, the change in the *X* ratio is controlled by CuL.

Increasing the stability of the CuL complexes at constant concentration of L causes a shift of the S-shaped titrations to higher SA concentration, because more SA has to be added to dissociate stronger complexes (Figure 1D). To a first approximation, the complex stability of the Cu/SA complexes is exactly balanced by that of the CuL species when $X = 0.5$. This means that the shift in the titrations must exactly balance any changes in the stability of CuL species. The shift is approximately one decade in SA for each 2 decades change in α_{CuL} because the stability of the Cu/SA species is dominated by that of $CuSA_2$.

Kinetic and Sensitivity Aspects. Kinetic aspects were tested in preliminary experiments by following the CSV peak height as a function of time at various concentration points throughout a titration with SA. The data showed that the solutions reached equilibrium after 20−30 min; longer equilibration times of up to 8 h did not change the analytical signal in a systematic manner. For this reason, a 30-min equilibration time was used after each SA addition. This way, a titration in a single sample aliquot took ~8 h. This is a longer measuring time than would be required (3−4 h) if the sample had been subdivided into 10−15 aliquots, allowing these to equilibrate, and then these had been measured in quick succession. However, advantages of the single voltammetric cell titration are that a much (10−15 times) smaller sample size (10−15 mL for the titration) can be used and that the entire titration can, in principle, be automated by using an autoburet for the additions of the competing ligand (SA).

***I*$_{pmax}$ Correction.** There is a practical problem related to establishing the maximum peak current (i_{pmax}) of the titration. The peak current continues to increase to high SA concentration because of the presence of α_{CuSA} as well as α_{CuSA_2} in the denominator of eqs 5 and 6. For this reason,the modeled value for *X* is 0.96 (i.e., 4% less than unity) for 100 μM SA in the absence of competing natural ligands. In the presence of a competing ligand, *X* is slightly less again: for instance, *X* is 0.95 in the presence of 5 nM type L ligand with a log $K'_{CuL} = 13.3$. It is, therefore, not correct to simply equate the current obtained at the highest SA concentration with i_{pmax}, because X then would be overestimated by ~5%.

Modeling showed that the nonlinear method of data-fitting is sensitive to the value of i_{pmax}. Model fitting with hypothetical data showed a large variation in calculated values for K'_{CuL} and C_L at values for i_{pmax} varying by between 5 and 10% of the correct value, as shown in Table 1. For instance, at an actual ligand concentration of 5 nM, the calculated ligand concentration would increase to 43 nM if the value for i_{pmax} was underestimated by 5%, but it would drop to 2 nM if it was overestimated by 5%. This shows that the ligand concentration calculated by this curve-fitting method is very sensitive to establishing the correct value for i_{pmax}. However, an

Table 1. Model Calculations to Evaluate the Sensitivity of Data Fits to Selection of an Incorrect Value for i_{pmax} on the Calculated Values for C_L and K'_{CuL}[a]

i_{pmax} used in model (nA)	found C_L (nM)	found logK'_{CuL}	log α_{CuL}
90	915	10.8	4.76
95	43	12.2	4.83
100	**5**	**13.3**	**5.00**
105	2.4	13.8	5.18
110	1.9	14.1	5.38

[a] Modeled seawater composition: C_L = 5 nM, log K'_{CuL} = 13.3, i_{pmax} = 100 nA. Correct values are shown in bold.

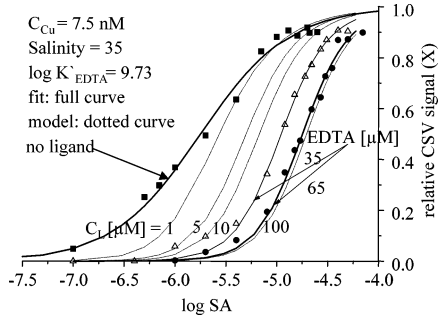

Figure 2. Reverse titrations of UV-SW with and without EDTA (30 and 65 μM). The plot shows the titrations as data points, with modeled data fits as solid curves going through the points; modeled titrations for additional EDTA concentrations (1, 5, 10, 100 μM EDTA) are shown as dotted curves.

error of 5% is readily spotted visually by plotting modeled values for X (calculated using the fitted values for C_L and K'_{CuL}) on top of the actual X values. Furthermore, i_{pmax} can be fitted to the data along with C_L and K'_{CuL}. Modeling showed that this worked well in the case of a single ligand, but poor fits (errors in i_{pmax}) were obtained for a 2-ligand model because the number of parameters including i_{pmax} increased to 5.

In our data fits (below), the value of i_{pmax} was initially based on the highest peak height obtained during the titration, and this was subsequently corrected by modeling of the titration curves using the fitted parameters.

Reverse Titrations of Copper in UV-Digested Seawater with and without EDTA. Additions of SA to seawater caused the peak height for copper to increase in accordance with the model calculations and with previous work.[31] In UV-digested seawater, the reduction signal could be recorded from SA concentrations starting from 0.1 μM. The peak height increase with increasing SA concentrations was accompanied by a shift of the copper peak potential in line with expectation because of the increased complex stability.

The titration curve was shifted to higher SA concentrations in the presence of 30 and 65 μM EDTA (Figure 2). The data points of titrations with and without added EDTA are shown in this diagram along with modeled titrations for various EDTA concentrations, showing that the modeled response agrees with the data. It can be seen that the shift in the curve was in accordance with the complex stability, with a greater shift for higher EDTA concentration. Data fits gave values of 9.5 and 9.6 for log K'_{CuEDTA}, slightly below that estimated for CuEDTA in seawater (log K'_{CuEDTA} = 10.1). The stability constants for the Cu/SA complexes have

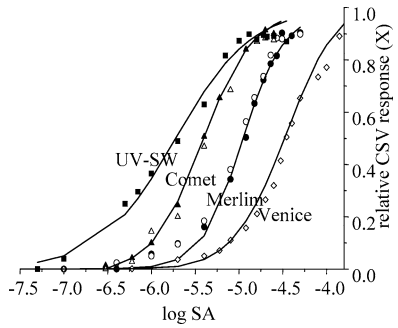

Figure 3. Reverse titrations of copper complexes in UV-SW and three seawater samples from differing environments. The curves represent model fits to the experimental data, which are shown as data points.

themselves been calibrated against EDTA,[31] suggesting that it may be necessary to recalibrate the stability of the Cu/SA complexes.

Reverse Titrations of Seawater Samples. Two samples of the Atlantic (bulked filtered seawater, collected during different cruises), and one from near-shore waters (Venice lagoon) were selected to test the reverse titration method because they represented both open oceanic and near-shore waters, with the expectation that the lagoon waters might present a special challenge due to probably containing higher levels of organic matter. Two of the samples were also analyzed by the conventional method to determine the complexing ligand concentration by titration with copper.[31] The reverse copper titrations of the samples are shown next to that for UV-SW in Figure 3. The sample titrations were shifted to much higher SA concentrations than in the UV-digested seawater, and large differences were apparent between the samples.

Determination of the copper concentration by copper additions at the end of the reverse titrations, at SA concentrations where the CSV signal response had approximately leveled off, resulted in calculated copper concentrations ~10% lower than obtained with a SA concentration of 25 μM after UV digestion. This suggests that at the end of these titrations, for instance, at a SA concentration of 30 μM with a value for α_{CuSA} of 10^6, 10% of the copper was still complexed by the natural ligands. It is likely that this was an inert fraction that was not resolved by these titrations; possibly this could be resolved by using overnight equilibration of the entire titration, but this was not further investigated.

Determination of K'_{CuL} and C_L and Comparison of Different Fitting Methods. Curve-fitting was used to fit values for K'_{CuL} and C_L to the reverse titrations. This was done by matching the experimental X values to model values (eq 5) by varying K'_{CuL} and C_L with optimization by least-squares regression. The results are shown in Table 2, where they can be compared to values obtained by fitting to two ligands of type L (eq 6) instead of one, to values obtained by linearization of the data according to eq 12, and to values obtained by the conventional complexing ligand titrations. All samples could be modeled by a single ligand and to two ligands. The two-ligand model described the data better, but the improvement was small. The concentration of the second ligand was smaller, and the complex stability was weaker than the first ligand and, therefore, not relevant to the copper speciation. We will therefore discuss the single ligand fit in more detail.

The detected ligand concentrations varied between 3 nM for the Merlim Atlantic sample and 35 nM for the lagoon sample.

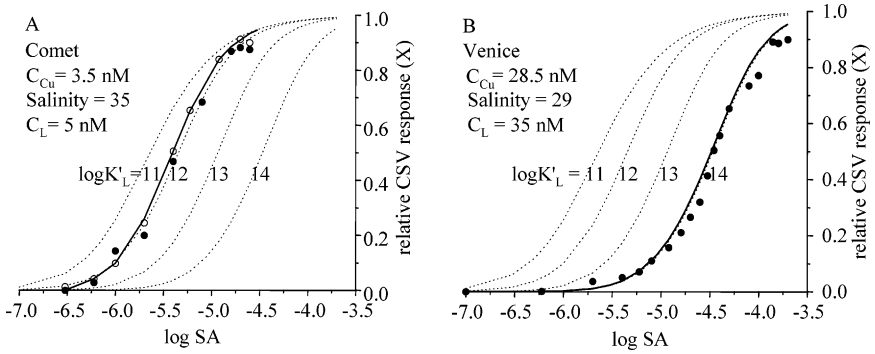

Figure 4. Effect of varying the values for the complex stability in the model fits to the data for a sample from the North Atlantic (Comet 2001) (A) and one of the Venice lagoon (B). The fits to the experimental data points are shown as solid curves, whereas the dotted curves represent modeled data for different complex stabilities.

Table 2. Fitting of Ligand Concentrations and Conditional Stability Constants to the Sample Titrations Shown in Figure 3, and Comparison to Results from Forward Complexing Ligand Titrations

	direct fit one ligand, nM	direct fit two ligands, nM	linearization one ligand, nM	linearization one ligand, nM
		Reverse Titration		Forward Titration
		Venice Lagoon		
C_{L1}	34.5	34.2	34.8 ± 5.0	35.4 ± 2.8
C_{L2}		0.7		
log K'_{CL1}	13.8	13.5	13.7 ± 0.1	13.3 ± 0.8
log K'_{CL2}		11.9		
		North Atlantic, Comet Sample		
C_{L1}	4.1	4.4	4.4 ± 0.3	6.1 ± 0.2
C_{L2}		0.4		
log K'_{CL1}	13.1	12.5	12.55 ± 0.05	13.1 ± 0.3
log K'_{CL2}		11.3		
		North Atlantic, Merlim Sample		
C_{L1}	2.7	2.5	2.7 ± 0.5	not analyzed
C_{L2}		0.8		
log K'_{CL1}	13.9	13.6	13.6 ± 0.1	not analyzed
log K'_{CL2}		11.8		

The complex stabilities varied between 12.5 and 13.6 for log K'_{CuL}. The complex stability was greater in the Merlim Atlantic sample (log K'_{CuL1} = 13.71) than in the other Atlantic sample (log K'_{CuL1} = 12.82), but no conclusions should be drawn from this, because these were stored and bulked samples, of use only to test the method.

Comparison of the reverse titrations to the usual forward complexing ligand titrations showed that the same ligand concentration was found by both methods for the lagoon sample (both 35 nM), but a lower ligand concentration (4 instead of 6 nM) was found in the Comet Atlantic sample. The detected complex stability was the same by the two methods. On the whole, the agreement between the reverse and forward titrations was good. A detailed study of copper speciation using forward complexing ligand titrations in the presence of either SA (as used here) or tropolone[35] found two ligands of significantly differing complex stability: L1 at concentrations of 3.5 ± 1.0 nM with log K'_{CuL1} = 13.2 ± 0.3 and a weaker ligand at a lower detection window. The copper species found here using the reverse titrations (and confirmed by the forward titrations) are in the same range as the L1 ligands detected in that study, suggesting that there is general agreement between the values obtained by the reverse method and the conventional forward titrations technique.

(35) Achterberg, E. P.; van den Berg, C. M. G. *Deep-Sea Res.*, submitted.

GENERAL DISCUSSION

The modeling suggests that the method of reverse titrations proposed and tested here may be especially useful to detect low ligand concentrations. Experimentally, similar ligand concentrations were found in comparative titrations by the reverse and the forward CC titrations, indicating that the reverse method can be used to successfully determine copper speciation. The data also showed that in these samples, there was no evidence of an additional ligand-binding copper at lower ligand concentration than that found with the conventional, forward, titrations. Although this finding is very preliminary (for only a few stored samples), this could be good news for the forward complexing ligand titrations because it suggests that no ligands of importance to copper speciation are being missed by that method.

The curve-fitting method, based on a least-squares regression and using a readily accessible spreadsheet, was used to fit speciation parameters (ligand concentrations and stability constants) to the experimental X values (peak heights/maximum peak height) to model parameters: this approach appeared to work well and could also be used to optimize the value estimated for i_{pmax}. Comparison to the linearization method showed that similar results were obtained using the two methods. The linearization procedure has the added advantage of making it easy to spot whether a second ligand is present; furthermore, the more simple statistics make it possible to get values for the standard deviation of the ligand concentration and the complex stability from internal consistency within a single titration, which was not possible using the curve-fitting method. Standard deviations in this case, therefore, have to be estimated from repeated titrations, although the residual error of the least squares regression could be reported and could be compared between titrations.

In the reverse titration method, the shift of the experimental CSV response compared to an UV-SW titration is a direct measure of the complex stability (α_{CuL}); in the forward titration method, on the other hand, the shift in the response is a measure of the ligand concentration. An advantage of the reverse titrations method is that low ligand concentrations at levels equal to or less than that of the metal can be detected. An advantage of the forward

titrations is that the position of the curvature in the titration data is a direct measure of the ligand concentration and gives a visual estimate of the concentration of the first and strongest ligand. So each method has advantages: for samples with ligand concentrations > metal concentration, the more intuitive output of the forward ligand titration may be more convenient, whereas at lower ligand concentrations, the reverse procedure will give more accurate results and shows better the complex stability of CuL.

During the reverse metal titration, the free metal concentration is progressively reduced with increasing concentration of the added ligand (SA in this study). At the highest concentration of the added ligand (SA), the sensitivity for the metal is greatest (see Figure 1) when also the free metal concentration is at its lowest. So rather unexpectedly, greatest sensitivity is obtained at the lowest free metal ion concentration. During the titration, the free metal ion concentration is lowered by 5 decades in UV-SW (from an α value of 35 for inorganic complexation to 10^6 at 30 μM SA), and by 1−2 decades when the copper is already complexed by natural ligands, as occurring in seawater.

For this method to work, it is essential to be able to accurately model the CSV response as a function of the added ligand concentration. This worked well for copper and SA, in which the response appeared to be exactly related to the formation of the $CuSA_2$ species. Preliminary experiments using iron and zinc have indicated that the CSV response with their adsorptive ligands may be more complicated and less easy to model.

The usual forward ligand titrations require conditioning of the titration vessels with the titrated samples to eliminate the problem of variable results that commonly occur for the first two or three titrations. In this work, each entire titration was carried out in the voltammetric cell. The vessel could, therefore, not be conditioned for each concentration of SA, though it could be conditioned for the copper concentration. Relatively stable peaks indicated that there were no major adsorption problems during these titrations; however, it was found that the titrations improved after the first one, indicating that a general conditioning of the voltammetric cell took place during the titrations.

ACKNOWLEDGMENT

The stay of J. Nuester in the laboratory of the Marine Electrochemistry research group at Liverpool University was funded by a Marie Curie Host Fellowship (Contract No. HPMT-CT-2001-00218). The samples from the Atlantic were collected during cruises with support of the PIs (de Baar and Timmermans) and officers and crew of the RV Pelagia (NIOZ, Netherlands).

Received for review June 24, 2004. Accepted September 9, 2004.

AC049078E