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Comparison of [Cu^{2+}] Measurements in Lake Water Determined by Ligand Exchange and Cathodic Stripping Voltammetry and by Ion-Selective Electrode

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While there has been a proliferation of techniques for measuring free metal ion concentrations and complexation in natural waters, there has been little direct intercomparison among methods. Here we report the results of an intercomparison among different methods used to determine free cupric ion concentration ([Cu^{2+}]) and organic complexation in lake water samples. The techniques used were potentiometric measurement with a cupric ion-selective electrode (Cu-ISE) and ligand exchange methods involving the addition of three well-characterized ligands with different binding affinities for copper (catechol, 8-hydroxyquinoline, and tropolone) followed by measurement of the Cu chelates of these ligands by adsorptive differential pulse cathodic stripping voltammetry (DPCSV). Good agreement was found among relationships between [Cu^{2+}] and the total concentration of natural copper species ([Cu_{nat}]) determined via titration with the three ligand exchange/DPCSV methods. Relationships between [Cu^{2+}] and [Cu_{nat}] measured by these methods also agreed with those determined with a cupric ion electrode within the overlapping range of detection windows for each method ([Cu_{nat}] = 40−100 nM). At lower Cu concentrations, the Cu electrode gave erroneously high [Cu^{2+}] readings, apparently due to a failure of the electrode membrane to equilibrate with the sample. The agreement among the different methods provides support for the validity of each individual method of measuring [Cu^{2+}] and natural Cu complexation. By combining Cu titration data measured by DPCSV and Cu-ISE, we obtained a more complete description of the Cu-complexing characteristics of water samples from two Swiss lakes than could be obtained with any single method. Equilibrium modeling of this composite data indicated the presence of at least three ligand classes. These ranged in concentration from 38 ± 19 nM for the strongest binding ligand class, which had conditional binding constants in the range of 10^{15} at pH 7.8−8.0, to 8 ± 2 μM for the weakest class of ligands, which had a conditional constant of $10^{8.6}$ M^{-1}.

Introduction

Copper is an essential element that is also toxic at elevated concentrations. Its reactivity and biological uptake are strongly influenced by its free ion concentration ([Cu^{2+}]), which is controlled by the extent of Cu complexation to ligands (*1*, *2*). Copper is highly complexed by organic ligands in natural waters, and the level of this complexation controls the biogeochemical cycling, nutritional availability, and toxicity of copper in these systems (*2*−*7*).

Numerous techniques have been used to measure [Cu^{2+}] in marine and freshwater samples, each with its own advantages and limitations. Potentiometric measurement with a cupric ion-selective electrode (Cu-ISE), for example, allows one to directly determine [Cu^{2+}], but this method has insufficient sensitivity for reliable measurement at the low ambient Cu concentrations in most natural waters (*7*). In addition, it cannot be used in seawater because of a chloride interference (*8*). Another electrochemical method, differential pulse anodic stripping voltammetry (DPASV), also is often too insensitive for measurement at ambient concentrations (*6*). Furthermore, this method measures concentrations of labile copper species (including inorganic and labile organic complexes) rather than free cupric ions and, therefore, suffers from uncertain data interpretation (*9*). To determine [Cu^{2+}] with this method, one must know the ratio of free cupric ions to both labile inorganic complexes and, more problematically, labile organic complexes.

One set of indirect measurement techniques, involving exchange equilibria with added ligands, has received considerable recent attention. These methods overcome the sensitivity and specificity problems of Cu electrodes and DPASV and have been widely used to measure [Cu^{2+}] and Cu complexation in both seawater and freshwater samples. In this set of techniques, a ligand whose complexation equilibria are well characterized is equilibrated with the sample, and then the concentration of copper complexes that formed with the added ligand is quantified by a variety of methods: liquid/liquid extraction (*10*, *11*), chemiluminescence (*12*), and differential pulse cathodic stripping voltammetry (DPCSV) (*3*, *13*−*17*). The free cupric ion concentration is then computed from equilibrium relationships among the concentrations of free cupric ions, free added ligand, and Cu complexes with the added ligand.

Accurate results with ligand exchange methods requires that several criteria be met: (1) ligand exchange equilibrium must be established between the added ligand and the natural ligands in the sample, (2) the concentration of specific Cu complexes with the added ligand must be accurately measured, (3) equilibrium relationships between the concentrations of these complexes and [Cu^{2+}] must be reliably known and accurately computed, and (4) the relationship between [Cu^{2+}] and the concentration of copper bound to natural ligands must not be altered by the addition of the competing ligand. Fulfillment of the last two of these criteria can be problematic since, in practice, they are based on several assumptions whose validity may be difficult to establish in natural water samples: (1) that chemical reactions such as adsorption or oxidation do not significantly decrease the concentration of added ligand in solution, (2) that mixed ligand complexes are not formed between the added ligand and natural ligands in the sample, and (3) that competitive interactions between copper and other trace metals for complexing with either the added or natural ligands are insignificant and do not affect the overall Cu^{2+} equilibria.

Despite the increasing use of ligand exchange methods in determining free ion concentrations and complexation of Cu in lacustrine and marine waters, there has been little intercomparison among different ligand exchange methods and among ligand exchange and other methodologies. Such intercomparison among techniques would provide an im-

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portant step in testing the validity of the results obtained. The present paper describes the results of an intercomparison among $[Cu^{2+}]$ measured in lake water samples with ligand exchange/DPCSV methods utilizing three different added ligands and values measured by Cu-ISE. The three ligand exchange/DPASV methods were based on equilibration of samples with added catechol (*3*, *13*), tropolone (*14*), and oxine (*16*). The catechol method has been used previously to determine free cupric ion concentrations in both seawater and lake water samples, while the other two methods have been used before only in marine samples.

Experimental Section

Sampling. The lake water samples used in the present study were taken from Lakes Greifen, Sempach, and Lucerne in Switzerland and from Lake Orta in northern Italy. The morphometric characteristics of all four lakes have been described elsewhere (*18*). Lakes Greifen and Sempach are both eutrophic and receive high levels of nutrients and other pollutants from sewage discharge and agriculture. Lake Lucerne, on the other hand, is a large oligotrophic lake. Lake Greifen has a maximum depth of 31 m and a seasonally anoxic hypolimnion. Lake Sempach has a maximum depth of 87 m and formerly possessed an anoxic hypolimnion. Its hypolimnion has been transformed to an oxic condition since 1984 by large-scale artificial oxygenation. Samples from Lake Greifen were collected on October 13, 1993, and October 25, 1994, while those from Lake Sempach were taken on October 11, 1994. The samples were collected from a depth of 2.5 m near the deepest point in the lakes. The Lake Lucerne sample was collected on July 22, 1991, from a depth of 20 m. The total depth at the sampling station was 112 m.

The final lake sampled, Lake Orta, is industrially polluted and contains high levels of copper and acid contamination (*19*). Liming has been used intermittently in this lake to help neutralize its acidity. Surface water samples were collected in the central and southern basins of the lake on June 19, 1994. Go-Flo sampling bottles (General Oceanics, 5 L) were used to collect the subsurface samples from the three Swiss lakes. The Lake Orta surface samples were collected with a 2-L polyethylene ladle. The samples were transferred to polyethylene bottles and were passed through 0.45-μm pore Millipore filters in a laminar flow clean hood within 2−3 h of collection. The filtered samples were stored in the dark at 4 °C for up to 1 week before $[Cu^{2+}]$ analysis by ligand exchange/DPCSV and Cu-ISE. All sampling and filtration devices, bottles, and membrane filters that contacted samples were washed with 0.01 M HNO_3 and rinsed with bidistilled water before use.

The samples were tested for Cu adsorption onto container walls and for speciation changes during storage. Measurements made initially and after 5 days indicated no variation in the total concentration or complexation of dissolved copper. The lack of Cu adsorption may have been due to the high level of Cu complexation by organic ligands, which should compete with adsorption sites for binding copper and thereby hold the copper in solution (*3*). Total dissolved copper concentrations were measured directly in acidified sample filtrates by graphite furnace atomic absorption spectrometry. A comparison of such measurements with those by ICP-MS have shown good agreement.

Ligand Exchange/DPCSV Determination of $[Cu^{2+}]$. Details of the ligand exchange/DPCSV method have been previously described (*3*, *13*, *16*, *17*). The method is based on ligand exchange equilibria between an added well-characterized ligand [catechol, 8-hydroxyquinoline (oxine), or tropolone] and natural ligands in the water sample. Following sample equilibration, the Cu complexes formed with the added ligand are measured by adsorptive differential pulse cathodic stripping voltammetry (DPCSV). The $[Cu^{2+}]$ and Cu complexation parameters are determined from equilibrium calculations, based on the following mass balance expression for the total copper concentration ($[Cu_t]$):

$$[Cu_t] = [Cu^{2+}] + [Cu_{in}] + \sum[CuL_i] + \sum[CuR_i] \quad (1)$$

$$= [Cu^{2+}](1 + \alpha_{in} + \sum K_i[L_i] + \alpha_R) \quad (2)$$

$[Cu_{in}]$, $\sum[CuL_i]$, and $\sum[CuR_i]$ are the concentrations of copper complexes with inorganic ligands, natural organic ligands, and added ligand, respectively. α_{in} is the inorganic complexation coefficient, equal to the ratio of the concentrations of inorganic complexes to free cupric ions, as calculated from the major ion composition and pH of the sample. K_i represents the conditional stability constant of the 1:1 Cu complex with a natural organic ligand L_i, and $[L_i]$ is the concentration of free (uncomplexed) ligand, equal to the total ligand concentration minus $[CuL_i]$. The product $K_i[L_i]$ is defined as the Cu-complexing coefficient of ligand L_i. α_R is the Cu-complexing coefficient for binary and ternary complexes with R and equals $\sum[CuR_i]/[Cu^{2+}]$.

Under the working conditions in our lake water samples, the term $(1 + \alpha_{in})$ in eq 2 is negligible and can be ignored. α_R values for catechol and oxine at the different sample pH values were determined from equilibrium calculations utilizing stability constants taken from Martell and Smith (*20*). The complexing coefficients of tropolone were determined by Cu-ISE measurement in UV-irradiated lake water combined with equilibrium calculations (see below).

The concentration of Cu complexes with the added ligand, $\sum[CuR_i]$, is measured selectively by DPCSV; it is proportional to the peak reduction current. Thus, $[Cu^{2+}]$ in the presence of the added ligand can be computed:

$$[Cu^{2+}] = \sum[CuR_i]/\alpha_R = i_p/(S\alpha_R) \quad (3)$$

where i_p (A) is the peak current and S (A M^{-1}) expresses the sensitivity. The concentration of copper present as natural species ($[Cu^{2+}] + [Cu_{in}] + \sum[CuL_i]$), which we will refer to henceforth as $[Cu_{nat}]$, can also be computed. It equals the overall total copper concentration minus the concentration of Cu complexes with the added ligand:

$$[Cu_{nat}] = [Cu_t] - \sum[CuR_i] = [Cu_t] - i_p/S \quad (4)$$

The sensitivity of the DPCSV method had to be calibrated by Cu titration for each individual type of added ligand in each sample. It was determined from the slope of the titration curve of reduction current vs added copper at high Cu concentrations where essentially all of the copper was present as complexes with the added ligand. Although the sensitivity slope (S) varied for each sample and added ligand type, it was independent of the concentration of added ligand over the working range for that ligand; i.e., 0.4−1.6 mM catechol, 0.1−0.4 mM tropolone, and 0.1−0.4 μM oxine (ref 3 and unpublished data). In addition to yielding the sensitivity, S, the titrations of the samples with copper also yielded relationships between $[Cu^{2+}]$ and the total concentration of natural Cu species ($[Cu_{nat}]$).

The addition of the competing ligand binds a portion of the copper in the sample and thereby reduces both $[Cu_{nat}]$ and $[Cu^{2+}]$. Thus, to determine the ambient pCu of the sample, one must be able to correct back to the $[Cu^{2+}]$ that was present before the addition of the competing ligand. In cases where we had complete titration curves, the initial ambient $[Cu^{2+}]$ was determined by interpolation of the relationship between $[Cu^{2+}]$ and $[Cu_{nat}]$. However, in cases where several different concentrations of the same competing ligand were used, we determined complete Cu titration curves only at a single ligand concentration. At the other ligand concentrations, CSV measurements were made only in single

TABLE 1. Ambient pCu of Lake Water Samples Determined by Ligand Exchange and DPCSV with Different Concentrations of Catechol, Oxine, or Tropolone

lake, date	depth (m)	$[Cu_t]$ (M)	working pH	added compd	ligand concn	ambient pCu
Greifen, Oct 25, 1994	2.5	2.30×10^{-8}	8.0	catechol	0.8 mM	15.15
					1.0 mM	15.13
					1.2 mM	**15.17**[a]
				oxine	0.2 μM	15.13
					0.3 μM	14.77
					0.4 μM	**14.97**
					0.5 μM	14.87
				tropolone	0.1 mM	14.95
					0.2 mM	15.25
					0.4 mM	**15.23**
Greifen, Oct 13, 1993	2.5	1.26×10^{-8}	8.0	catechol	1.0 mM	15.25
					1.2 mM	15.30
					1.4 mM	**15.22**
					1.6 mM	15.15
Sempach, Oct 11, 1994	2.5	1.35×10^{-8}	7.9	catechol	1.0 mM	15.13
					1.2 mM	**15.07**
					1.4 mM	15.10
				oxine	0.1 μM	15.06
					0.2 μM	**15.13**
					0.3 μM	15.11
					0.4 μM	14.84
				tropolone	0.4 mM	**15.25**
Lucerne, July 22, 1991	20	9.44×10^{-9}	7.8	catechol	0.5 mM	**14.32**
					1.0 mM	**14.40**
Lucerne, June 30, 1994	2.0	1.16×10^{-8}	7.8	catechol	0.4 mM	13.82
					0.6 mM	**13.74**
					0.8 mM	13.81
					1.0 mM	13.85

[a] Boldface values were determined from interpolation of $[Cu^{2+}]$ titration curves; all other values were determined from single-point DPCSV measurements at different concentrations of the added ligands (see eq 5).

subsamples without copper additions. For these "single point" determinations, the measured cupric ion concentration in the presence of added ligand, designated here as "ambient $[Cu^{2+}]^*$", was corrected back to the initial ambient $[Cu^{2+}]$ from

$$\text{ambient } [Cu^{2+}] = \text{ambient } [Cu^{2+}]^* + \sum [CuR_i]\, d[Cu^{2+}]/d[Cu_{nat}] \quad (5)$$

where $d[Cu^{2+}]/d[Cu_{nat}]$ is slope of the $[Cu^{2+}]$ vs $[Cu_{nat}]$ relationship in the vicinity of ambient $[Cu_{nat}]$ determined at the one ligand concentration for which a complete titration curve was determined. The correction was small, an average of only 0.08 pCu units for 18 determinations (pCu is defined as the negative log of $[Cu^{2+}]$) (Table 1).

The working conditions and procedures for the catechol addition method were taken from Van den Berg (*13*) and Xue et al. (*18*). Those for the oxine and tropolone methods were taken from Van den Berg (*16*) and Donat and Van den Berg (*14*), respectively.

All subsamples used for Cu complexation measurements were buffered by a mixture of HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) and NaOH to maintain pH constant at or near ambient lake water values. For the Swiss lake water samples, a pH buffer stock solution (1 M HEPES and 0.5 M NaOH) was added to give a final pH of 7.8−8.0 (depending on the sample) and a final concentration of 5−6 mM HEPES. For Lake Orta water, the working pH was kept constant at 7.0 or 7.2 by a buffer prepared with different ratios of HEPES and NaOH.

To obtain DPCSV titration data for a sample, a series of subsamples were spiked with different Cu concentrations and a single concentration of added ligand. A series of others received no added copper, but several different concentrations of added ligand. Aliquots of 25 mL were pipetted into a series of 50-mL high-density polyethylene beakers, and then 150 μL of HEPES buffer stock solution was added to each beaker. For the oxine and tropolone methods, the sample aliquots were amended with the copper and competing ligand additions, and then were allowed to equilibrate at 22 ± 2 °C overnight. For the catechol method, the pH-buffered subsamples containing the copper additions were equilibrated overnight without added ligand. They were purged with suprapure N_2 gas for 5 min to remove O_2, amended with additions of catechol and equilibrated for 5 min under an N_2 atmosphere prior to Cu adsorption onto the handing mercury drop electrode. This procedure was necessary because catechol is degraded by O_2 oxidation.

DPCSV analysis was performed on the equilibrated samples. For the oxine and tropolone methods, the equilibrated subsamples containing added ligand were purged with N_2 for 5 min to remove O_2, exposed to a hanging mercury drop electrode for 2.25 min to allow Cu adsorption, and then scanned cathodically to generate curves for reduction current vs voltage. For the catechol method, the N_2-purged samples containing added ligand (see above) were exposed to the mercury electrode to allow Cu adsorption for the same time period as used for the other two ligands.

The DPCSV analyses were conducted with a hanging mercury drop electrode, an Ag/AgCl reference, and a graphite counter electrode. The electrodes were held in either a Model VA 663 stand linked to a E506 polarograph or in a VA stand linked to a 646 VA processor, all made by Metrohm. Potential scanning parameters were as follows: initial potential of −50 mV; pulse height of 50 mV; scan rate of 5 or 10 mV s^{-1}, depending on the sample. The stripping peaks in the lake water samples occurred at −270 to −320 mV for Cu−catechol complexes, −330 to −380 mV for oxine complexes, and −240 to −290 mV for tropolone complexes. For the lower pH samples from Lake Orta, the Cu−catechol reduction peaks were shifted to more positive potentials.

An experiment was conducted with the tropolone method to examine the effect of ligand exchange equilibration time

on measured pCu values. In this experiment, three series of 25-mL subsamples of Lake Sempach water were spiked with 0, 3.2, and 15.7 nM of Cu, respectively, and then buffered to pH 7.9. The three series were allowed to equilibrate overnight at room temperature, and then tropolone was added to each subsample at a final concentration of 0.4 mM. Following this addition, the three series of subsamples were equilibrated for periods of 4 min, 7 h, and 24 h before analysis by DPCSV.

ISE Measurement of [Cu^{2+}]. Free cupric ion concentrations and relationships between [Cu^{2+}] and the total concentration of natural copper species measured by the ligand exchange/DPASV methods were compared with those determined with an Orion Cu-ISE (Model 94-29). The Cu-ISE was coupled to an Ag/AgCl reference electrode (Orion Model 90-01). The procedures used for Cu-ISE titrations and [Cu^{2+}] analysis were similar to those previously described by Sunda and Hanson (*7*).

For Lake Orta water, the ambient copper concentration was sufficient for direct measurements of the ambient [Cu^{2+}]. For samples from Lakes Greifen and Lucerne, however, the ambient copper concentration was too low for reliable measurement. For these samples, the water was titrated with $CuCl_2$, and [Cu^{2+}] was measured over a range of elevated copper concentrations. To maintain constant pH, all samples received the same HEPES/NaOH buffers as used in the ligand exchange/DPCSV analyses of [Cu^{2+}]. pH was monitored with a glass electrode throughout the titrations and was maintained constant to within 0.01 pH unit with small additions of HCl or NaOH. Free cupric ion concentrations were determined via the Nernst equation from the difference between the electrode potential measured in the sample and that in standard solutions of 10^{-6} to 10^{-3} M $CuCl_2$ and 0.01 M KNO_3 at pH 5.5−5.8. Under these conditions, all of the copper in the standard solutions was present as free cupric ions. The electrode potential is proportional to the log of the free cupric ion activity, and to determine [Cu^{2+}], the difference in activity coefficients between the 0.01 M ionic strength standards and that in the pH-buffered lake water samples was factored into our calculations. This activity coefficient adjustment was minor for the Swiss lake water samples because their ionic strengths (∼0.008 M, as determined from the measured major ion composition and the concentration of ionized HEPES buffer) were similar to that (0.01 M) for the standards. Activity coefficients in samples and standards were determined from the extended Debye−Huckel equation (*21*).

Prior to [Cu^{2+}] measurement, the Cu-ISE, reference electrode, composite pH electrode, and borosilicate glass measurement beaker were exposed for 15 min to a chelating solution containing 1 mM ethylenediamine and 0.6 mM HEPES buffer at pH 8.3 and measured pCu of 16. Exposure to the chelating solution removes adsorbed Cu^{2+} from surfaces, including the Cu-ISE membrane surface, and improves electrode behavior at low copper concentrations (*7*). The system was then rinsed with distilled water and exposed to a rinse portion of the lake water sample for 30 min prior to introduction of the sample to be analyzed. Ambient pCu was determined after a 1-h exposure of this sample to the Cu-ISE. The Swiss lake water samples were then titrated with $CuCl_2$, and the electrode potential was measured as a function of increasing copper concentration (*7*).

The cupric ion electrode was used to measure the stability constant (β_2) for the formation of Cu(tropolone)$_2$ complexes in Lake Greifen water. This measurement was necessary because β_2 values are not available in the literature, although formation constants are available for 1:1 tropolone complexes with Cu, Mg, and Ca (*20*). The measurements were made in a lake water sample that had been irradiated with UV light to photooxidize organic complexing ligands. The ionic strength of this water was 0.0055 as based on the measured major ion composition: [Ca^{2+}] = 1.4 mM, [Mg^{2+}] = 0.63 mM, [Na^+] = 0.50 mM, [K^+] = 0.08 mM, alkalinity = 3.62 mM. A sample of this water containing 0.12 mM tropolone and 0.01 mM Cu at 25 °C and pH 7.9 yielded a value for log $\alpha_{Cu-trop}$ of 5.85. From this value we computed a conditional log β_2 value of 13.85 at the ambient ionic strength, [Ca^{2+}], [Mg^{2+}], and pH (7.9). From equilibrium calculations that take into account the extent of tropolone binding to Ca^{2+}, Mg^{2+}, and H^+ [based on published stability constants (*19*)], we compute log β_2 values of 15.45 at the ambient ionic strength (0.0055 M) and 15.65 at infinite dilution ($I = 0$). These β_2 values along with published stability constants (*20*) were used to compute [Cu^{2+}] in tropolone/DPASV determinations of free cupric ion concentration in lake water samples.

Modeling Cu Complexation Data. Relationships between [Cu^{2+}] and the total concentration of natural Cu species and resultant relationships between [Cu^{2+}] and the concentration of copper complexes with natural organic ligands were modeled with equilibrium mass action theory. In these models, we assumed that copper forms discrete 1:1 complexes with a limited number of natural organic ligands (2−3 depending on the titration curve). Values for the total concentration and conditional stability constant for each hypothetical ligand were determined with the FITEQL computer program (*22*). Natural waters probably contain a wide range of different ligands, however, rather than the 2−3 we obtained from our models; thus, the ligand concentrations and conditional constants we derived probably represent only average values for different groups of natural ligands. The modeled values of K_i and total ligand concentration obtained are not independent of each other and are dependent on the portion of the overall relationship between bound copper and [Cu^{2+}] (the detection window) determined by the method used (*23*).

Results and Discussion

[Cu^{2+}] Determination in Lake Water by Ligand Exchange/DPCSV. Catechol, oxine, and tropolone were used as competing ligands in conjunction with DPCSV to determine [Cu^{2+}] in lake water samples. Several different concentrations of each ligand were used in these determinations. Potential scans of the lake water samples in the absence of added ligand at pH 7.5−8.2 showed no significant interfering peaks within the region of the peak potentials for the Cu chelates with the added ligands. Lake water with added catechol showed an interfering peak near −100 mV (apparently due to reduction of uncomplexed catechol); but this peak was well removed from that for Cu−catechol chelates, which occurred at −270 to −320 mV. The reduction potential for Cu−tropolone was −255 ± 35 mV, and tropolone by itself yielded two interfering reduction peaks centered at −110 and −390 mV, respectively. At low ratios of total Cu to tropolone concentrations, the peak for Cu−tropolone chelates was significantly affected by these interferences. The interference was overcome by using low tropolone concentrations (0.1−0.4 mM) and by initiating the reduction scan at a more negative potential (−150 mV). Cu−tropolone adsorption onto the electrode surface was still performed at −50 mV, after which the potential was switched to −150 to initiate the reduction scan. Because of the necessity of adding low tropolone concentrations, the method sometimes had insufficient sensitivity for reliable measurements at low [Cu^{2+}].

The use of oxine had the opposite problem: too low a detection window resulting from too high a Cu affinity constant. Because of its high binding affinity, much lower oxine concentrations had to be added (0.1−0.6 μM) to obtain acceptable α_R values. Even so, oxine analysis of [Cu^{2+}] could not be made in the lake water samples at Cu concentrations greater than about 30 nM, limiting the upper edge of the [Cu^{2+}] detection window.

Despite the above limitations, relationships between [Cu^{2+}] and [Cu_{nat}] determined with the three added ligands in water

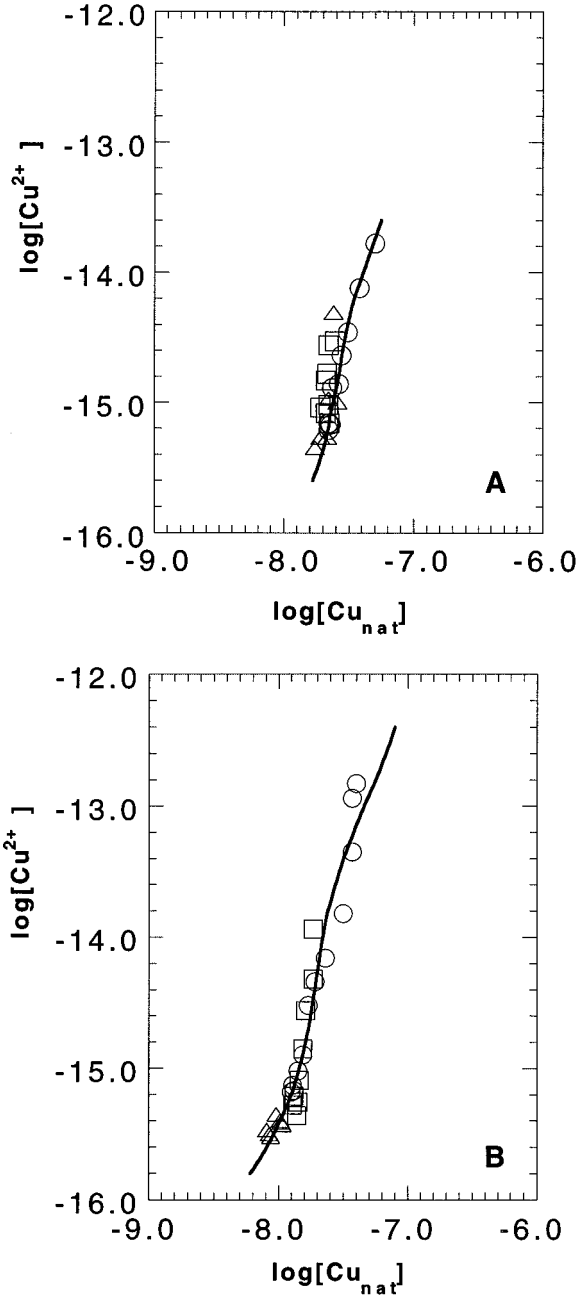

FIGURE 1. Relationships between log [Cu^{2+}] and log [Cu_{nat}] (the total concentration of natural copper species) in lake water samples, as determined from Cu titrations. The data were determined by ligand exchange equilibria and DPCSV utilizing different added ligands: catechol (○), oxine (□), and tropolone (△). The continuous curves were fitted to the catechol data by FETEQL using a two-ligand model. The water samples were collected from a depth of 2.5 m from Lake Greifen on October 25, 1994 (A), and from Lake Sempach on October 11, 1994 (B).

samples from Lakes Greifen or Sempach showed good agreement (Figure 1A,B). The average error about the modeled curves in Figure 1 for all three added ligands at lower [Cu_{nat}] (1×10^{-8} to 4×10^{-8} M) and [Cu^{2+}] (10^{-16} to 10^{-14} M) was less than 0.2 pCu unit, the same as the error observed for different concentrations of a single added ligand. Within the upper edge of the analytical range for oxine (pCu 13−14), deviations were larger, as would be expected.

Measured ambient pCu values in lake water samples determined with different ligands and different concentrations of each ligand also show good agreement (Table 1). For each sample examined, the measured pCu values showed standard deviations about the mean of <0.2 pCu unit.

Reliable measurements of pCu in the water sample should be independent of the added ligand or its concentration, provided an appropriate detection window is maintained. This reliability of measurement, however, will occur only if important criteria are met: the concentration of Cu complexes with added ligand can be properly measured, Cu−ligand exchange equilibrium is reached, Cu equilibria with the added ligand can be accurately calculated, and the relationship between [Cu^{2+}] and [Cu_{nat}] is not altered by the addition of the competing ligand. To meet these criteria, the thermodynamic constants used in equilibrium calculations must be correct; there can be no formation of mixed complexes between the added ligand and natural organic ligands; and there can be no significant, unaccounted for metal competition effects. If the above criteria are not met, the measured [Cu^{2+}] will vary with different added ligands and will decrease with the concentration of ligand added (*4*). The agreement among pCu values determined with different ligands and ligand concentrations indicates that such biasing effects did not occur, supporting the conclusion that the observed pCu values are fundamentally correct.

Our results indicate a lack of bias in pCu measurement due to competition by other trace metals. This lack of metal competition effects may be due to the much higher concentrations of added or natural ligands relative to those of competing trace metals, low affinity of natural ligands for competing trace metals, or preferential binding of copper and competing metals (e.g., zinc) by different subsets of natural ligands as appears to occur for copper and zinc in seawater (*24*). In a previous study in Lake Greifen water, added zinc was found to have no effect on pCu values measured by the catechol/DPCSV method, indicating that zinc does not competitively displace copper from natural organic chelates (*25*).

Based on the above results, catechol appears to be a good choice for routine determination of [Cu^{2+}] by ligand exchange and DPCSV in most samples of lake water in the pCu range of ~11−15.3 at pH 8. It gave no obvious interfering peaks and yielded good analytical sensitivity at added concentrations of 10^{-4}−10^{-3} M. However, at very low ambient copper concentrations or very high levels of complexation, catechol does not have sufficient binding strength to measure ambient [Cu^{2+}], and a much stronger competing ligand, such as oxine, is needed. But with oxine, the upper end of the [Cu^{2+}] analytical range is limited, minimizing the usefulness of this ligand in studying Cu complexation by weaker organic ligands. Tropolone is not as good as the other two ligands at determining ambient [Cu^{2+}] in lake water samples due to the higher level of interfering reduction peaks, which lowers sensitivity and limits the detection window (working range) for [Cu^{2+}] measurement.

Examination of Ligand Exchange Kinetics. Although catechol has good sensitivity and a favorable working titration range for lake water analysis, its instability toward oxidation by O_2 is potentially problematic. The theory of [Cu^{2+}] analysis by ligand exchange requires that samples be measured at equilibrium. But since the samples could not be exposed to oxygen and bubbling for long periods with N_2 was impractical, the samples were equilibrated with catechol for only a short time (5 min) in our experiments. The agreement between our [Cu^{2+}] results with catechol and those with tropolone and oxine, where equilibration times were much longer (14−18 h), suggests that the short equilibration time with catechol was not a problem. This conclusion is also supported by previous tests in Lake Greifen water containing added catechol where no variations in Cu−catechol reduction peak heights were observed in deoxygenated samples for equilibration times of 3−120 min (*3*). A similar rapid equilibration was found here in Cu titrations of Lake Sempach water, in which

TABLE 2. DPCSV Peak Currents and pCu Values Determined for Different Tropolone Equilibration Times in Lake Sempach Water ([Tropolone] = 0.4 mM, pH = 7.9)

$[Cu_t]$ (M)	time after tropolone added	ip (A) by DPCSV for [Cu−tropolone]	$[Cu_{nat}]$ (M)	pCu
1.38×10^{-8}	5 min	4.50×10^{-9}	8.66×10^{-9}	15.27
	7 h	5.00×10^{-9}	8.12×10^{-9}	15.13
	24 h	4.49×10^{-9}	8.67×10^{-9}	15.34
	av	4.67×10^{-9}	8.47×10^{-9}	15.25
	SD	0.29×10^{-9}	0.31×10^{-9}	±0.10
	relative error	6.2%		
1.66×10^{-8}	5 min	5.80×10^{-9}	1.04×10^{-8}	15.16
	7 h	6.60×10^{-9}	0.96×10^{-8}	15.01
	24 h	5.50×10^{-9}	1.07×10^{-8}	15.25
	av	5.95×10^{-9}	1.02×10^{-8}	15.15
	SD	0.57×10^{-9}	0.06×10^{-8}	±0.12
	relative error	9.6%		
2.92×10^{-8}	5 min	1.88×10^{-8}	1.36×10^{-8}	14.75
	7 h	2.30×10^{-8}	1.01×10^{-8}	14.58
	24 h	2.03×10^{-8}	1.23×10^{-8}	14.79
	av	2.07×10^{-8}	1.20×10^{-8}	14.72
	SD	0.21×10^{-8}	0.18×10^{-8}	±0.11
	relative error	10.3%		

TABLE 3. pCu in Lake Orta Samples Determined by Catechol Exchange and DPCSV and by ISE

station	$[Cu_t]$ (M)	pH	pCu by DPCSV	pCu by ISE
central basin	7.08×10^{-8}	7.0	9.75	9.4
south basin	6.45×10^{-8}	7.2	9.82	9.9

the samples were equilibrated with added tropolone for periods of a few minutes to 24 h (Table 2). No variation in measured pCu was observed for the different equilibration times.

Comparison of pCu Measured by Ligand Exchange and DPCSV and by ISE. Ambient pCu measured by catechol/DPCSV and those measured by Cu-ISE were compared in surface samples from Lake Orta, an Italian lake with high total copper concentrations resulting from inputs of industrial pollutants. The copper concentrations in the two Lake Orta samples were 65 and 71 nM (Table 3), 4−8 times higher than values in the three Swiss lakes (see Table 1). The ambient pCu measured by DPCSV was 9.8 in both samples, in reasonably good agreement with values of 9.4 and 9.9 measured in the same samples by ISE. The ratio of ambient $[Cu_{nat}]$ to $[Cu^{2+}]$ in the Lake Orta samples measured by DPCSV was ~400, considerably lower than the ratios (10^5−10^6) observed in Swiss lake samples at the same copper concentration and slightly higher pH (7.8−8.0 for the Swiss lakes vs 7.0−7.2 for Lake Orta). Previously published measurements in Lake Orta water indicate that $[Cu^{2+}]$ increases with increasing $[H^+]$ due to hydrogen ion competition (*18*), as found in other freshwater samples (*7*). Thus, some of the lower level of Cu complexation in the Lake Orta samples at equivalent concentrations of natural copper species $[Cu_{nat}]$ is due to their lower pH. But much of the difference is due to lower concentrations or binding affinities of natural Cu complexing ligands in the Lake Orta water (*18*).

Relationships between $[Cu^{2+}]$ and $[Cu_{nat}]$, the total concentration of natural copper species, were measured by catechol/DPCSV and Cu-ISE in a sample from Lake Lucerne collected on July 22, 1991, and another from Lake Greifen sampled on October 13, 1993. The long overlapping titration curves for the two samples are plotted in Figure 2A,B in terms of log $[Cu^{2+}]$ vs log$[Cu_{nat}]$. Good agreement was observed between the two methods at copper concentrations ≥ 40 nM, but the ISE measurements of $[Cu^{2+}]$ deviated upward from the DPCSV values at the ambient copper concentrations

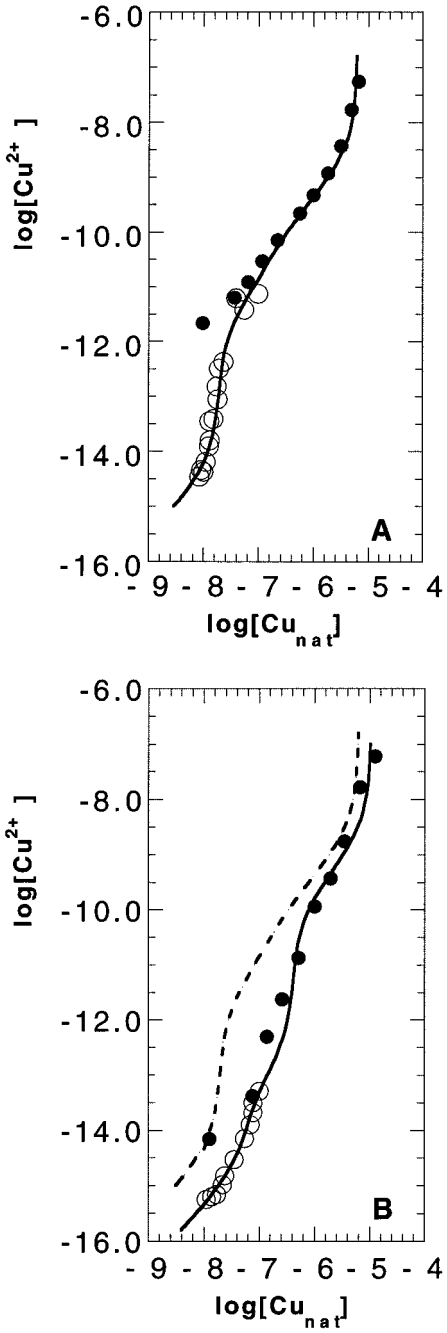

FIGURE 2. Copper titration data for log $[Cu^{2+}]$ vs log $[Cu_{nat}]$ in lake water samples. $[Cu^{2+}]$ was determined by catechol exchange and DPCSV (○) and by Cu-ISE (●). The solid curves give modeled relationships calculated by fitting all the data measured by DPCSV plus that measured by ISE at $[Cu_t] \geq 40$ nM to a three-ligand model with the parameters listed in Table 4. The dashed line in B replicates the modeled curve in A for visual comparison of titration results. The samples were collected on July 22, 1991, at 20 m depth from Lake Lucerne (A) and on October 13, 1993, at 2.5 m depth from Lake Greifen (B).

(9.4 and 12.6 nM). At the ambient $[Cu_{nat}]$, the deviation in measured pCu values between the two methods was 1.0 pCu unit for the Lake Greifen sample, but much higher (2.6 pCu units) for the Lake Lucerne sample.

The discrepancy between the two methods at the low, ambient copper concentrations is due to non-ideal electrode behavior resulting from a lack of equilibration between the electrode membrane and the bulk solution. Similar problems with the Cu-ISE at Cu concentrations below ~30−100 nM (depending on the sample) have been observed previously

TABLE 4. Conditional Stability Constants and Ligand Concentrations for Two- and Three-Ligand Models Obtained from pCu Titration Data Determined by DPCSV and by ISE

	Lucerne July 22, 1991 pH 7.8		Greifen Oct. 13, 1993 pH 8.0		Greifen Oct. 25, 1994 pH 8.0	Sempach Oct. 11, 1994 pH 7.9
method	DPCSV	DPCSV + ISE	DPCSV	DPCSV + ISE	DPCSV	DPCSV
initial [Cu_{nat}] (M)	9.44×10^{-9}		1.26×10^{-8}		2.30×10^{-8}	1.35×10^{-8}
ambient pCu	14.41		15.19		15.16	15.11
[Cu_{nat}] range (M)	8.53×10^{-9}–9.82×10^{-8}	8.53×10^{-9}–2.29×10^{-5}	1.10×10^{-8}–9.97×10^{-8}	1.10×10^{-8}–1.26×10^{-5}	2.16×10^{-8}–5.01×10^{-8}	1.28×10^{-8}–3.97×10^{-8}
log K_1	14.3	14.3	14.6	14.6	15.8	15.5
log K_2	11.0	10.8	13.0	12.5	13.2	12.6
log K_3		8.6		8.6		
total [L_1] (nM)	19.0	18.5	56.9	56.9	25.6	19.0
total [L_2] (nM)	110	100	150	350	110	100
total [L_3] (nM)		6.0		10.0		

for natural river water samples (*7*). A major factor in this lack of equilibration may be the release of Cu^{2+} at the electrode surface from oxidation of the CuS, a major component of the ISE solid membrane. Such oxidative release can result in a much higher steady-state [Cu^{2+}] at the electrode surface than occurs in the bulk solution at low copper concentrations. The presence of specific oxidants (other than O_2) or slow reaction kinetics between Cu^{2+} released at the electrode surface and organic complexing ligands can further exacerbate the problem. Since CuS oxidation rates and Cu^{2+} chelation kinetics may differ from one sample to the next, the sensitivity limit of the electrode may also vary to some extent, as observed previously (*7*) and in the present study (Figure 2A,B).

Because the Cu-ISE and the Cu−catechol/DPCSV methods have quite different analytical working ranges, meaningful comparisons for the two methods can only be made at intermediate [Cu_{nat}] or [Cu^{2+}] where the detection windows for the two methods overlap. The agreement between pCu values measured by the two methods within this overlapping [Cu_{nat}] range of ∼40−100 nM for the Swiss lake samples and at the ambient [Cu_{nat}] (65−71 nM) in the Lake Orta samples (Table 3) provides further support for the validity of both methods within their respective working ranges.

Modeling of Organic Complexation in Lake Water Samples. Since the Cu-ISE and the catechol exchange/DPCSV methods have different analytical ranges, combining the Cu complexation data obtained with both methods provides a more complete description of the organic complexing characteristics of the sample than can be obtained with either method alone. In constructing our composite titration curves, we used all of the catechol/DPCSV data and all Cu-ISE data except those at the ambient copper concentrations. A three-ligand organic complexation model was required to fit the long composite titration curves for the two lake water samples that ranged from a pCu of 14 or 15 to a pCu of 7. The ligand concentrations and conditional stability constants obtained are listed in Table 4.

Although we could fit the data for the long composite titration curves to discrete three-ligand models, the water may in fact contain a large mixture of ligands in which those with high stability constants are relatively scarce while those with lower stability constants are much more numerous. In that case, the conditional constants and ligand concentrations listed in Table 4 would be average values for different classes of ligands possessing different ranges of stability constants.

A comparison of the composite curves of [Cu^{2+}] vs [Cu_{nat}] for the two samples revealed a higher degree of copper complexation in the Lake Greifen sample than in the Lake Lucerne sample throughout the titration range (Figure 2B). This difference, however, was much smaller at high Cu concentrations than at low or intermediate concentrations. These differences were also apparent from the results of the organic complexation models, which required three ligands or ligands classes to adequately describe the titration data. The first of these ligand classes (L_1), which accounts for most of the Cu binding at the ambient [Cu^{2+}] in the samples, had similar conditional stability constants in the Lake Greifen and Lake Lucerne samples (log K_1 = 14.6 and 14.3, respectively). But the concentration of this ligand class in the Lake Greifen sample (57 nM) was three times that in the Lake Lucerne sample (18.5 nM). An even wider discrepancy was observed between the two samples for the intermediate strength ligand class, L_2, whose concentration (350 vs 100 nM) and conditional affinity constant ($10^{12.5}$ vs $10^{10.8}$ M^{-1}) were both considerably higher in the Lake Greifen sample. By contrast, the conditional constant for the weakest ligand class, L_3, was the same ($10^{8.6}$ M^{-1}) in the two lake water samples, and the concentration of this ligand was only 1.7-fold higher in the Lake Greifen sample.

The higher level of copper complexation by high- to intermediate-strength ligands in the Lake Greifen sample may reflect the higher productivity in this eutrophic lake and associated higher release of organic ligands by phytoplankton or other microorganisms (*18*). Copper complexation by these ligands has been found to seasonally covary with primary productivity in Lake Greifen (*3*) and to decrease below the euphotic zone of the eutrophic Lake Sempach (*18*), further supporting this hypothesis. By contrast, our results show that the lowest affinity ligands, which may represent Cu binding by ubiquitous humic materials, show much less variation between the Lake Greifen and Lake Lucerne samples (Table 4), suggesting that these more abundant, less reactive ligands are much less affected by productivity variations. A similar situation occurs in seawater where copper is complexed by two classes of organic ligands. The first is present at low concentrations of ∼1−10 nM with conditional stability constants of 10^{12}−10^{13} M^{-1}, similar to constants for the intermediate strength ligand class, L_2, in the Lake Greifen and Lake Sempach samples (Table 4). The second ligand class is present at higher concentrations (∼5−100 nM) with conditional constants of 10^{9}−10^{10} M^{-1} (*4*−*6*, *10*, *12*−*14*). In the central North Pacific, the high affinity ligand class was present only in the upper 200 m, in waters recently exposed to phytoplankton growth, but was absent in deeper waters (*6*). By contrast, the low affinity ligand class had similar concentrations between the surface and a depth of 1400 m. These data are consistent with the release of strong ligands by phytoplankton in surface waters but indicate little or no algal release of the weaker ligands, which have residence times in the ocean of tens to hundreds of years (*6*).

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