Comparison of the Complexation of Cu and Cd by Humic or Fulvic Acids and by Ligands Observed in Lake Waters

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(Accepted 29 March 1999)

Abstract. The complexation of Cu and Cd by ligands observed in filtered unfractionated lakewaters is compared to the complexation by humic and fulvic acids. Complexation parameters (conditional stability constants and ligand concentrations) of Suwannee River fulvic acids (FA), purified peat humic acids (HA) and of ligands in lakewater samples have been determined using the same methods (ligand-exchange and CSV (cathodic stripping voltammetry) or ASV (anodic stripping voltammetry)), and the same titration ranges of Cu, Cd and organic carbon concentrations. The performance of the used techniques is first evaluated in FA and HA suspensions, and gives comparable results with the literature values for the same materials, according to published models (5-site model, NICA model) and parameters. Model calculations using the WHAM model for FA and HA (Tipping, 1994) are also presented. The comparison of titrations of FA and HA with Cu and Cd with those of lakewater samples indicates that stronger ligands than FA and HA are present at low concentrations in the lakewaters. Specific strong ligands occur in particular in eutrophic lake waters, whereas in a lake with higher metal concentrations and low biological productivity the ligands more closely match the fulvic acid characteristics.

Key words: Cd complexation, Cu complexation, humic-metal complexation, humic substances, lake water, natural ligands

1. Introduction

The complexation of copper and cadmium by naturally occurring ligands is a subject of great environmental relevance, because the availability and toxicity of these metals to organisms strongly depend on their speciation (Brand et al., 1986; Bruland et al., 1991; Morel et al., 1991; Sunda and Guillard, 1976; Sunda and Huntsman, 1995). Biological and chemical interactions of these metals are related to the concentrations of free aquo Cu or Cd ions, and not to total metal concentrations. Different approaches have been used to determine the complexation of copper and cadmium by natural ligands. On one hand, numerous studies of the Cu or Cd complexation in seawater and freshwater samples have demonstrated that especially Cu and to a somewhat lesser extent Cd are strongly complexed in seawater, as well as in freshwater (Achterberg et al., 1997; Bruland, 1992; Coale

and Bruland, 1990; Donat et al., 1994; Moffett, 1995; Muller, 1996; Sunda and Huntsman, 1991; Xue et al., 1996; Xue and Sigg, 1993, 1998). Only few studies however have attempted to specifically isolate and characterize these strong Cu-complexing ligands in natural water samples (Biber et al., 1996; Donat et al., 1997; Gordon et al., 1996; Midorikawa and Tanoue, 1996).

On the other hand, the complexation of Cu and Cd by isolated fulvic and humic acids (FA and HA) has been extensively studied (Bartschat et al., 1992; Breault et al., 1996; Buffle et al., 1977; Cabaniss and Shuman, 1988; Hering and Morel, 1988; Leenheer, 1981; Mantoura et al., 1978; McKnight et al., 1983; McKnight and Wershaw, 1989; Pinheiro et al., 1994; Turner et al., 1986). HA and FA are defined according to standard isolation procedures (Buffle, 1988; Leenheer et al., 1989; Thurman, 1985). They occur ubiquitously in water systems, and constitute from 10–30% of DOC (dissolved organic carbon) in seawater to 70–90% in wetland water, 40–90% of DOC in streams and about 50% of DOC in lakewaters, depending on hardness (Thurman, 1985; Breault et al., 1996).

It has however not been investigated in detail whether the complexation by HA and FA may explain the findings mentioned above on strong complexation in unfractionated water samples. The fulvic acid fraction of DOC was found to be a major constituent of the natural ligands complexing Cu in the case of streams with relatively high Cu concentrations and low pH (Breault et al., 1996). Benedetti et al. (1995) have argued that humic substances would play a predominant role in complexing copper, on the basis of model calculations and of comparisons with measured $[\text{Cu}^{2+}]$ (Xue and Sigg, 1993).

Indirect measurement techniques, involving ligand-exchange and DPCSV (differential pulse cathodic stripping voltammetry) or DPASV (differential pulse anodic stripping voltammetry), are currently widely accepted to determine the concentrations of free metal ions and complexation parameters in seawater (Bruland, 1992; Campos and Van den Berg, 1994; Donat et al., 1994; Donat and Van den Berg, 1992; Moffett, 1995; Moffett et al., 1997; Van den Berg, 1984). We have already applied ligand-exchange/CSV to investigate $[\text{Cu}^{2+}]$ and Cu complexation in Swiss lakes, and ligand-exchange/ASV for $[\text{Cd}^{2+}]$ and Cd complexation (Xue et al., 1996; Xue and Sigg, 1993, 1998; Xue and Sunda, 1997). Our observations show that a class of very strong ligands (L_1) with low concentration governs the Cu and Cd speciation at the ambient level of lakewaters. The occurrence of these strong ligands appears to be linked to biological activity.

In the present study, we compare the complexation of Cu and Cd by FA or HA and by ligands in unfractionated lakewaters, using the same techniques and the same concentration ranges of total Cu and Cd. The performance of the used techniques, involving ligand-exchange and voltammetric measurements, is first evaluated in HA and FA suspensions. The obtained results are compared to literature values. We then attempt to evaluate the relative importance of FA and HA and of more specific ligands for the complexation of Cu and Cd in lake waters, using parameters derived from our measurements, as well as models for FA and

HA available in the literature (Benedetti et al., 1995; Cabaniss and Shuman, 1988; Tipping, 1994).

2. Experimental

2.1. MATERIAL AND SOLUTION OF FA OR HA

Suwannee River fulvic acids (SRFA) were obtained from B. Voelker (Voelker and Sulzberger, 1996); they have been isolated by J. Leenheer according to the method described in Leenheer (1981). This material was chosen because it is representative of freshwater FA, and metal complexation data are available for comparison. Stock solutions of SRFA in water (0.2 mg/mL) were prepared weekly and stored in the refrigerator. Purified peat humic acid (PPHA) was sent by C. J. Milne from British Geological Survey. The initial stock suspension contains 5.28 mg PPHA/mL, which was prepared at Wallingford, U.K., using the standard IHSS dialysis method (Milne et al., 1995). The suspension was stored in the refrigerator till use. The proton and major cations binding parameters, as well as the Cu and Cd complexation characteristics of this PPHA have been determined in other studies (Benedetti et al., 1995; Kinniburgh et al., 1996; Milne et al., 1995).

A SRFA titration solution (500 mL) was prepared a day before voltammetry measurement for Cu or Cd speciation. The solution contained 6 mg FA/L, 0.01 M KNO₃ and 6 mM HEPES (from 1 M HEPES stock solution). The organic carbon concentration of this FA solution (3.23 mg C/L other than the buffer), according to its elemental composition (Leenheer, 1981), is close to DOC in eutrophic lakes. To determine Cu-HA or Cd-HA complexation, a 1 L solution PPHA was prepared one day before voltammetry measurements. The HA solution contained 0.01 M KNO₃, 5 mM HEPES at pH 7.80, and 1.32 mg HA/L for Cu complexation or 4.12 mg HA/L for Cd complexation. The lower HA concentration was used for the Cu complexation because preliminary experiments had indicated irresolvable interferences with higher HA concentrations. All the determinations in the solutions (6 mg/L FA or 1.32 mg/L HA) were carried out both without addition and with addition of different catechol concentrations in order to subtract interfering peaks from total peak currents for labile Cu.

2.2. LAKE WATER SAMPLES

Water samples were taken from Lakes Greifen, Sempach, and Lucerne in Switzerland and from Lake Orta in Italy. These lakes were chosen because they represent different conditions with respect to trophic state and metal loads. Lake Greifen is a highly eutrophic lake with a seasonally anoxic hypolimnion, located north-east of Zurich. Lake Sempach is a eutrophic lake in central Switzerland. The hypolimnion is artificially aerated with oxygen during the summer and the whole lake is mixed by compressed air between fall and spring. Lake Lucerne is a large oligotrophic lake in central Switzerland. Neutral average pH is observed in the lakes Greifen,

Sempach and Lucerne ($\text{pH} = 8.0 \pm 0.3$ in epilimnion samples). Lake Orta is a large subalpine lake in northern Italy, which had been heavily polluted with Cu and ammonium from industrial sources from the 1920's to 1960's. The lake was remediated by liming in 1989/90 and pH 7.0–7.5 is now found in the epilimnion. Major signs of biological recovery were manifested after liming. A detailed description of the lakes has been reported elsewhere (Knauer et al., 1998).

Water samples were taken from the water columns at the deepest points of lakes Greifen and Sempach, at the Kreuztrichter station of lake Lucerne and in the Central basin of Lake Orta. Samples have been collected at various times and depths from Lake Greifen, Sempach and Lucerne. Detailed data from a sampling campaign in spring 1995 are shown (Knauer et al., 1998). The samples were collected with a Go-Flo sampler (General Oceanics, 5 L), transferred to polyethylene bottles, and filtered with $0.45 \mu\text{m}$ Millipore membranes after transport to the laboratory. About 100 ml of the filtrates was immediately acidified to 0.01 M HNO_3 to determine total dissolved metal concentrations by ICP-MS (Perkin-Elmer SCIEX ELAN 5000, detection limit $0.1 \mu\text{g/L}$ for Cu, $0.005 \mu\text{g/L}$ for Cd). Another aliquot was kept at 4°C for speciation within 3 days. All devices in contact with the samples were cleaned with 0.01 M HNO_3 and bidistilled water before use.

2.3. CU COMPLEXATION AND DPCSV DETERMINATION

Details of determination for free cupric ion concentration and complexation parameters have been described elsewhere (Xue and Sigg, 1993; Xue and Sunda, 1997). Briefly, the method is based on ligand-exchange equilibrium between added catechol and natural ligands in samples. The Cu complexes with catechol are measured by adsorptive differential pulse cathodic stripping voltammetry (DPCSV). The sensitivity of the DPCSV method for Cu-catechol complexes must be calibrated by Cu titration for each individual sample. It was determined from the slope of the titration curve at high Cu concentrations where essentially natural ligands were saturated and the concentration of Cu-catechol complexes was proportional to that of the added Cu concentration. When a sample is titrated with Cu, free cupric ($[\text{Cu}^{2+}]$) and total Cu concentrations other than Cu-catechol complexes ($[\text{Cu}_T^*]$, natural species in lake water or humic, fulvic and inorganic complexes in the HA and FA solutions) can be determined at any titration point from equilibrium calculation via the measured Cu-catechol concentration. Then, a data set of $[\text{Cu}^{2+}]$ and corresponding total Cu concentrations $[\text{Cu}_T^*]$ is fitted with a two ligand model, using the chemical equilibrium fitting program FITEQL (Westall, 1982). The conditional stability constants and ligand concentrations are obtained from the fitting parameters.

To obtain DPCSV titration data for Cu complexation in a sample, a series of subsamples were spiked with different Cu concentrations. Aliquots of 25 mL were pipetted into a series of 50 mL high-density polyethylene beakers. 150 μL of HEPES buffer stock solution (1 M HEPES with different concentrations of

NaNO₃) was added to each beaker for lake samples to get 6 mM of final buffer concentration. All the working pH's for the lake samples were close to the natural values. The series was allowed to equilibrate at 22 ± 2 °C overnight.

The optimal concentrations of catechol were adapted for each sample. The titrations of the samples from Lakes Greifen, Sempach or Lucerne were carried out in the presence of 0.8–1.6 mM catechol at pH 7.8, the one for Lake Orta in the presence of 0.1 mM catechol at pH 7.3. The optimal concentrations of catechol were between 0.5–1.0 mM in the FA solutions and 0.1–0.2 mM in the HA solutions at pH 7.5–8.0.

DPCSV analysis was performed with a hanging mercury drop electrode, an Ag/AgCl reference, and a graphite counter electrode. The electrodes were held in a 647 VA stand linked to a 646 VA processor, all made by Metrohm. When the subsamples were exposed to the electrodes, they were purged with suprapure N₂ gas for 5 min to remove O₂, amended with addition of catechol and equilibrated for another 5 min under N₂ atmosphere with stirring. Adsorptive collection onto the hanging mercury electrode was conducted at a potential of –50 mV for 2 min with stirring, and at rest for 15 s. A cathodic scan was then performed to generate a curve for reduction current versus potential, with the following parameters: initial potential of –50 mV; pulse height of 50 mV; scan rate of 10 mV s^{–1}. The stripping peaks occurred at –270 to –320 mV in lake samples, –250 to –270 mV in the SRFA solution and –250 to –290 mV in the PPHA solution at pH 7.8–8.0.

2.4. CD COMPLEXATION AND DPASV DETERMINATION

Determination of Cd complexation is based on ethylenediamine (EN) ligand-exchange and differential pulse anodic stripping voltammetry (DPASV) measurement, details of which are described elsewhere (Xue and Sigg, 1998). With regard to DPASV, inert Cd means the non-reducible complexes and includes strong organic complexes and Cd adsorbed on colloids; labile species include free aqua ions, inorganic and rapidly dissociated (weak) organic complexes. The Cd-EN complexes are fully labile. When EN is added in excess compared to natural weak organic ligands, and ligand-exchange equilibrium is achieved in a sample, the labile (weak) organic complexes are mainly constituted by Cd-EN complexes, thereby, the contribution to the electrolysis current is basically due to free ions, inorganic and EN complexes. Therefore, [Cd²⁺] can be calculated from the mass balance of measured labile Cd concentration, inorganic and EN complexing coefficients. The corresponding total concentration of Cd including inert complexes other than Cd-EN complexes [Cd_T^{*}] (natural species in lake waters or FA, HA and inorganic complexes in the FA and HA solutions) is obtained from the difference between the original total Cd and Cd-EN complexes. With the assumption of a one-ligand model for 1 : 1 complexes, the titration data sets of [Cd²⁺] and [Cd_T^{*}] could be fitted by Scatchard plots, and conditional stability constants and available ligand concentrations were obtained.

To obtain DPASV titration data for Cd complexation in a sample, a series of subsamples was spiked with Cd in a similar way as described above for Cu, then a given concentration of EN (0.2–1 mM) was added to each subsample. For comparison, other series were prepared without addition of EN. The slopes of the titration curves (peak current vs. total Cd concentration) in the higher concentration ranges were used to calibrate for labile Cd.

DPASV analysis was performed with the same instrument as for DPCSV. When the subsamples were exposed to the electrode, they were purged with suprapure N₂ gas for 5 min. Then, Cd was accumulated on the hanging mercury electrode at -1.1 V for 5 min with 2900 rps stirring, and the potential scan was initiated after 15 s rest. Scanning pulse height was 50 mV, rate 10 mV/s. The stripping peak potential was measured at $-(580-610)$ mV.

2.5. DATA EVALUATION AND MODELS

The data obtained in our study on complexation of Cu and Cd by HA, FA and by ligands in lake waters have all been fitted by the simplest model, i.e., by a discrete 1-ligand or 2-ligand model, which is only valid in the concentration range and at the pH used in the experiments. A 1-ligand model was used if it gave a satisfactory fit of the data.

To compare with data in the literature, calculations were performed with the models that have been used by other authors. Binding of Cu by the Suwannee river fulvic acids has been described by a discrete 5-site ligand model (Cabaniss and Shuman, 1988). Binding of Cu and Cd by the PPHA has been described by the NICA model (non-ideal competitive adsorption model (Benedetti et al., 1995)), which takes into account a bimodal distribution of binding sites and a distribution of binding constants around mean values, as well as the competition with protons. The WHAM model developed by Tipping (Tipping, 1994) is a widely used model to evaluate complexation of metals by HA and FA under natural conditions. The WHAM model considers two major types of binding sites, together with 8 different acidity constants. It includes a database for complexation constants of various metals with HA and FA, which has been developed on the basis of numerous literature values. However, the literature values for Cu or Cd were mostly obtained under higher metal concentrations than those used in the present study, as well as higher than those typically encountered in lakes.

3. Results

3.1. COMPLEXATION OF CU AND CD BY FA AND HA

Interference peaks from Cu-FA or Cu-HA complexes were observed to occur at a potential close to that of the Cu-catechol complexes. The stripping potential for Cu-catechol was located around $-(270 \pm 20)$ mV at pH 7.8–8.0, and the interfering peak potential of Cu-FA or Cu-HA just overlapped. The peak potentials of Cu-FA

and Cu-HA shifted to more positive values at lower pH. The interference peaks did however not exceed 15% of the total peaks in the titration range of $[\text{Cu}_T] = 1 \times 10^{-8}$ – 2×10^{-7} M under the working conditions. With higher peak heights and wider peak areas, the PPHA gave stronger interferences than the SRFA at the same concentration. For this reason, no higher HA concentrations were used in the experiments. This kind of interferences was not observed in the lake samples at pH 8.0 ± 0.2 .

The titration curves of SRFA and PPHA solutions with Cu are plotted in Figure 1a for FA and 1b for HA in terms of $\log[\text{Cu}^{2+}]$ vs. $\log[\text{Cu}_T^*]$. The titration curves obtained at different catechol concentrations within the optimal range were in good agreement (Figure 1a). The data from different concentrations of catechol in a sample could be combined to compute the conditional stability constants and ligand concentrations by FITEQL with a two-ligand model (Table I). The curves in Figure 1 are calculated with the conditional stability constants and ligand concentrations listed in Table I. The agreement between data obtained at different concentrations of catechol indicates that the technique with catechol-exchange and DPCSV is fundamentally correct to determine $[\text{Cu}^{2+}]$ and the Cu complexation parameters under the working conditions (Xue and Sunda, 1997).

Titration curves of FA and HA solutions with Cd are plotted in Figure 2. The complexation parameters were fitted to a one-ligand model (Table II). The titrations of both solutions were carried out in the absence and in the presence of 0.6 mM or 1 mM EN. The results of titrations with different EN concentrations were in agreement. The extent of complexation determined by DPASV may be underestimated due to the electrochemical lability of some weaker complexes. At lower pH, labile Cd strongly increased and almost all Cd was labile at pH < 7.2 .

3.2. COMPLEXATION OF CU AND CD BY LIGANDS IN LAKE WATER

The average complexation parameters of Cu in the lakes Greifen, Sempach, Lucerne and Orta are listed in Table III (Knauer et al., 1998; Xue et al., 1996; Xue and Sigg, 1993). The results from the sampling campaign in April–May 1995 are given in Table I. The free $[\text{Cu}^{2+}]$ concentrations at the ambient level were about 6–7 orders of magnitude lower than the total dissolved Cu concentrations in the Lakes Greifen, Sempach and Lucerne. In lake Orta with higher concentration of ambient Cu and lower pH (7.3), the extent of Cu complexation was much weaker, with the ratio $[\text{Cu}^{2+}]/[\text{Cu}_T] \approx 10^{-3}$. Within a narrow titration window at higher concentrations, the titration data in Lake Orta could be fitted by a one-ligand model (Table I). Of course, stronger ligands with lower concentrations may be masked by the higher ambient Cu concentrations. A comparison of the complexation parameters from Table I and Table III indicates that the samples from the euphotic zones during a spring algal bloom in 1995 are representative for the respective lake.

The complexation parameters of Cd from the sampling campaign in April–May 1995 are listed in Table II; some additional results for comparison are given in

Table I. Cu complexation parameters by SRFA or PPHA and by ligands in lake waters

		FA solution SRFA 6.0 mg/L	HA solution PPHA 1.32 mg/l	Lake water Greifen 29 Apr. 95	Lake water Sempach 25 Apr. 95	Lake water Lucerne 4 May 95	Lake water Orta 10 May 95
DOC	mg/L	3.23	0.69	2.8	2.8	1.2	0.8
Chlorophyll a in lakes	$\mu\text{g/L}$			5.5	16.9	2.5	2.9
Titration range of Cu*	M	1×10^{-8} – 2×10^{-7}	1×10^{-8} – 2×10^{-7}	1.4×10^{-8} – 2.2×10^{-7}	7.9×10^{-9} – 2.1×10^{-7}	4.7×10^{-9} – 2.1×10^{-7}	8.2×10^{-8} – 2.8×10^{-7}
Lake sample pH				7.7	7.8	7.7	7.3
Working pH		8.0	7.8	7.8	7.8	7.8	
[Cu _T] in lakes	M			1.4×10^{-8}	7.9×10^{-9}	4.7×10^{-9}	8.2×10^{-8}
Ambient pCu	For lakes			15.5	14.6	14.5	
log K_1		14.1	12.5	15.5	13.8	14.3	
log K_2		11.7	10.3	12.6	12.1	11.6	
[L ₁]	nmol/L	27.3	30.0	30.0	70.0	12.9	
[L ₁]	mol(g C) ⁻¹	8.46×10^{-6}	4.35×10^{-5}	1.07×10^{-5}	2.50×10^{-5}	1.08×10^{-5}	
[L ₂]	nmol/L	250	200	100	100	200	
[L ₂]	mol(g C) ⁻¹	7.74×10^{-5}	2.90×10^{-4}	3.57×10^{-5}	3.58×10^{-5}	1.67×10^{-4}	
Working pH		7.5	7.5				7.3
Ambient pCu							10.3
log K_1		13.3	12.2				
log K_2		11.1	9.8				10.3
[L ₁]	nmol/L	15.3	30.1				
[L ₁]	mol(g C) ⁻¹	4.74×10^{-6}	4.36×10^{-5}				
[L ₂]	nmol/L	119.4	220				150
[L ₂]	mol(g C) ⁻¹	3.70×10^{-5}	3.19×10^{-4}				1.88×10^{-5}

* Titration range of Cu reflects the working window, in which the titration data set was used to obtain the fitting parameters.

Table II. Cd complexation parameters by SRFA or PPHA and by ligands in lake waters

		FA solution SRFA 6.0 mg/L	HA solution PPHA 1.32 mg/L	Lake water Greifen 29 Apr. 95	Lake water Sempach 25 Apr. 95	Lake water Lucerne 4 May 95	Lake water Orta 10 May 95
DOC	mg/L	3.23	2.15	2.8	2.8	1.2	0.8
[Cd _T] in lakes	M			7.6×10^{-11}	5.8×10^{-11}	8.5×10^{-11}	7.2×10^{-10}
Titration range of Cd	M	1.8×10^{-9} – 2.9×10^{-8}	8.9×10^{-9} – 1.8×10^{-8}	6×10^{-10} – 9×10^{-9}	5×10^{-10} – 9×10^{-9}	6×10^{-10} – 9×10^{-9}	7.2×10^{-10} – 7×10^{-9}
Lake water pH				7.7	7.8	7.7	7.3
Working pH		8.0	8.0	7.8	7.8	7.8	7.3
Ambient pCd	in lakes			12.1	11.8	11.5	9.2
log <i>K</i>		9.4	8.8	10.2	10.4	10.2	8.2
[L]	nmol/L	1.6	7.0	6.0	2.4	1.6	3.0
[L]	mol(g C) ⁻¹	4.8×10^{-7}	3.3×10^{-6}	2.14×10^{-6}	8.57×10^{-7}	1.33×10^{-6}	3.75×10^{-6}

Table III. Average Cu and Cd complexation parameters and general characteristics of lake waters (epilimnion 2–5 m)

	Lakes	Greifen	Sempach	Lucerne	Orta
Primary productivity	$\text{gC m}^{-2} \text{y}^{-1}$	500	350	150	
DOC	mg/L	3.6	4.0	1.0	1.1
pH		7.6–8.3	7.6–8.3	7.6–8.2	7.0–7.5
Cu Complexation ¹		1990–1993 average $n = 10$	1994 average $n = 4$	1991 average $n = 4$	June 1994 range $n = 2$
Working pH		7.9	7.9	7.8	7.0–7.5
$[\text{Cu}_T]$	M	$(1.6 \pm 0.5) \times 10^{-8}$	$(6.4 \pm 2.9) \times 10^{-9}$	$(9.1 \pm 2.1) \times 10^{-9}$	7.0×10^{-8}
pCu		14.8 ± 0.4	15.0 ± 0.3	13.8 ± 0.4	9.8–10.6
$\log K_1$		14.6 ± 0.5	15.1 ± 0.5	13.9 ± 0.4	
$\log K_2$		12.6 ± 0.5	12.8 ± 0.3	11.6 ± 0.3	9.5–10.6
$[\text{L}_1]$	nM	54 ± 22	27 ± 9	21 ± 6	
$[\text{L}_2]$	nM	366 ± 87	195 ± 87	197 ± 65	233
Cd Complexation ²		25 Oct. 1993 depth 2.5 m	26 Feb. 1996 depth 2.5 m	24 June 1996 depth 2.5 m	
Working pH		8.2	8.2	8.0	
$[\text{Cd}_T]$	M	8.0×10^{-11}	2.7×10^{-11}	8.9×10^{-11}	
pCd		12.1	12.2	11.6	
$\log K$		10.1	10.3	10.3	
$[\text{L}]$	nM	4.0	2.0	1.8	

¹ All the data for Cu complexation are taken from reference Xue et al., 1996, those for Lakes Greifen, Sempach, and Lucerne are average values from 2–5 m depth samples, those for Lake Orta only from one sample with two different working pH.

² The data for Cd complexation are presented here only from one additional sample in each lake other than those collected in the Spring 1995, adapted from Xue and Sigg, 1998.

Figure 1a. Titration curves of fulvic acid (FA = 6.0 mg/L) with Cu, obtained by ligand-exchange with catechol and DPCSV, in terms of $[\text{Cu}^{2+}]$ (M) in function of total Cu (except Cu-catechol complexes) $[\text{Cu}_T^*]$ (M). The lower curve is measured at pH 8, with 0.5 mM catechol (open circles), 0.8 mM catechol (open squares) and 1 mM catechol (squares with a cross). The upper curve (open triangles) is measured at pH 7.5 with 0.5 and 0.8 mM catechol. The lines are the fitted curves with the parameters shown in Table I.

Table III (Knauer et al., 1998; Xue and Sigg, 1998). The free $[\text{Cd}^{2+}]$ concentrations were only about one order of magnitude lower than total dissolved Cd. The conditional stability constants of Cd complexes by ligands in the neutral lakes were similar ($\log K = 10.2\text{--}10.4$), but the ligand concentrations were higher in the eutrophic lakes than that in the oligotrophic lake Lucerne. The complexation of Cd in Lake Orta is also much weaker than in the other lakes, again due to higher metal concentration ($[\text{Cd}_T] 7.2 \times 10^{-10}$, pCd 9.2), lower pH and weaker biological activity. Although some uncertainty concerning Cd speciation may occur in lake waters because of the DPASV lability of weaker organic complexes, a larger fraction of non-labile complexes was observed in the lake samples in comparison to the FA and HA solutions. 90–98% of total dissolved Cd were complexed by natural organic ligands stronger than EN in neutral lake waters (Xue and Sigg, 1998). This

Figure 1b. Titration curves of humic acid (HA = 1.32 mg/L) with Cu, obtained by ligand-exchange with catechol and DPCSV, in terms of $[\text{Cu}^{2+}]$ (M) in function of total Cu (except Cu-catechol complexes) $[\text{Cu}_T^*]$ (M). The lower curve is measured at pH 8 (open circles). The upper curve (open squares) is measured at pH 7.5. The lines are the fitted curves with the parameters in Table I.

fact means that interferences of weaker ligands in lake waters, if present, must be less than 10%.

4. Discussion

4.1. COMPARISON OF FA AND HA COMPLEXATION WITH OTHER STUDIES

Our results on Cu complexation with SRFA are compared in Figure 3a with those obtained by Cabaniss and Shuman with an ion selective electrode (ISE) in a higher Cu concentration range ($[\text{Cu}_T] > 10^{-7}$ M and $[\text{Cu}^{2+}] > 10^{-10}$ M). Using the parameters of the 5-site model by Cabaniss and Shuman, we calculated a titration curve at pH 8.0 to compare with our curve in terms of pCu vs. $\log \{\text{CuFA}\}$ (Cu bound per mg C, Figure 3a). Using the model calculations is simply a convenient way to plot the data by different studies. We plotted the curves only in the respect-

Figure 2. Titration curves of fulvic acid (FA = 6.0 mg/L) and humic acid (HA = 4.12 mg/L) with Cd obtained by ligand-exchange with EN and DPASV, in terms of $[\text{Cd}^{2+}]$ (M) in function of total Cd (except Cd-EN complexes) $[\text{Cd}_T^*]$ (M) at pH 8. The lines are the fitted curves with the parameters shown in Table II.

ive working ranges. A comparable extent of complexation is obtained from both curves, although stronger ligands may be more important at lower concentrations. A comparison of the ligand concentrations shows however that only less than 1% of the totally available binding sites (1.6×10^{-2} mol/gC (Cabaniss and Shuman, 1988)) have been titrated here.

For PPHA, our data are compared to those determined by Benedetti et al. (1995), that have measured Cu-HA complexation with ISE, using the same HA material and a similar working window. The titration curve in Figure 3a was calculated based on the extended NICA model and the parameters given by Benedetti et al., 1995. The data on Cu complexation obtained here are thus comparable to those obtained by other techniques. This agreement indicates that the speciation technique of ligand-exchange and DPCSV gives reliable results in the presence of fulvic or humic acids.

To further compare our results with literature data, the WHAM model with universal data base (Tipping, 1994) was used to calculate relations of $[\text{Cu}^{2+}]$ and FA or HA bound Cu in the range of our study, as shown in Figure 3a. The curve calculated by WHAM for FA is located above that by CSV around 10^{-8} M total dissolved Cu and the calculated curve for HA is close to the CSV curve in this range. The slopes of the curves are however different over a wider range. The comparison indicates this model is unable to accurately predict metal binding by

Figure 3a. Comparison of the calculated complexation of Cu by FA or HA obtained in this study with literature data (pH 8). $[\text{Cu}^{2+}]$ (M) is plotted in function of bound Cu $\{\text{CuFA}\}$ or $\text{CuHA}(\text{mol}(\text{mgC})^{-1})$. The complexation by FA and HA (CSV) is calculated with the parameters in Table I and compared to the FA model by Cabaniss and Shuman (1988) (FA (C + S)) and to the HA-NICA model (HA (NICA)) according to Benedetti et al. (1995), as well as to the WHAM model. Except for the WHAM model, all curves are plotted in the respective working windows.

FA or HA under our conditions. This discrepancy is probably due to the data base included in the WHAM model, which relies mostly on studies performed at higher metal concentrations.

On the other hand, we must realize that each technique has its own optimal working window, and that an extrapolation with fitting parameters may lead to unreliable results and may not be directly comparable with other techniques. For example, extrapolating the NICA model curve down to about 10^{-15} M $[\text{Cu}^{2+}]$ (Figure 3a), would give similar results as those obtained in the neutral lakes (Benedetti et al., 1995). The measured complexation by FA or HA using CSV gives however higher $[\text{Cu}^{2+}]$ at the same total concentrations (Figure 3a). In the present study, therefore, we compare complexations of FA or HA and of ligands in lake waters in the same working window, using the same technique.

Complexation of Cd by FA or HA may be underestimated in this study, because DPASV labile Cd may include some weak organic complexes besides EN. The comparison of our Cd titration data to those from ISE determination and the extended NICA model (Figure 3b) illustrates that the Cd-HA complexes are partly

Figure 3b. Comparison of the calculated complexation of Cd by HA obtained in this study with literature data. $[\text{Cd}^{2+}]$ (M) is plotted in function of bound Cd $\{\text{Cd-HA}\}(\text{mol (mgC)}^{-1})$. The complexation by HA is calculated with the parameters shown in Table II (ASV) and compared to the NICA model (NICA) according to Benedetti et al. (1995).

labile by DPASV and that a weaker complexation by HA is obtained using ASV in comparison to results by ISE. Electrochemical availability of Cd-HA at low pH has also been demonstrated by Pinheiro et al (1994).

4.2. COMPARISON OF CU TITRATION CURVES OF FA OR HA AND OF LIGANDS IN LAKE WATERS

In the following, we compare titration curves of FA and HA, with those of lake waters, calculated on the basis of the conditional stability constants and ligand concentrations, that have all been obtained with the same technique and in the same concentration range. The differences in complexation are visible as shift of the titration curves. This type of comparison is more reliable than a direct comparison of the stability constants or ligand concentrations.

Titration curves of FA and HA solutions with Cu, as well as of the samples from Lakes Greifen and Sempach are plotted in Figure 4a. All the curves were calculated with the parameters listed in Table I. To match the DOC in lake waters, the ligand concentrations of either FA or HA were normalized to 2.8 mg C/L; in one curve a mixture of 2.6 mg C/L of FA and 0.26 mg C/L of HA is assumed. In the lower range of total Cu concentrations, close to ambient Cu level in the lakes, the curves for HA and FA all lie over those for lake samples. Higher $[\text{Cu}^{2+}]$ thus occur in the HA or FA solutions than in the lake samples at the same $[\text{Cu}_T]$ and DOC. This comparison shows that the complexation by ligands in the lakewater samples was stronger than that by FA or HA at the ambient concentrations. However, the

Figure 4a. Comparison of the complexation of Cu measured in lakewaters (L. Greifen and L. Sempach, DOC = 2.8 mg/L) to the complexation by FA (2.8 mg C/L), HA (2.8 mg C/L) or a mixture of FA+HA (2.6 mg C/L FA + 0.24 mg C/L HA) at pH 7.8. The curves for $[\text{Cu}^{2+}]$ (M) in function of total Cu (M) are calculated with the parameters listed in Table I.

titration curves of ligands in the lake samples and of FA or HA approach each other closer at higher copper concentrations, indicating that HA or FA may play a role at higher Cu concentrations as weaker ligands with higher concentrations.

A sample from Lake Orta is compared to FA (0.8 mg C/L, pH 7.3–7.5) in Figure 4b, using the parameters from Table I. Although the titration of FA solution was carried out with lower total Cu concentrations relative to that of Lake Orta, in this case, the Cu complexation in Lake Orta appears to be close to the complexation by FA, indicating again that FA are more important at higher Cu concentrations.

Bound Cu per mg organic carbon $\{\text{CuL}\}$ was also calculated to clearly compare binding of Cu by ligands in lake samples and by humic substances. The fitted curves for $\log\{\text{CuL}\}$ ($\text{mol}(\text{mg C})^{-1}$) as function of $\log[\text{Cu}^{2+}]$ are shown in Figure 5, within the respective titration window. At higher free cupric concentration ($\log[\text{Cu}^{2+}] > -12$), all the ligands with lower concentrations would be saturated. Because the titrations were carried out only in the lower concentration range, weaker ligands with higher concentrations could not be detected and included in the fitting parameters. In this range of lower concentrations encountered in natural

Figure 4b. Comparison of the complexation of Cu measured in Lake Orta (DOC = 0.8 mg/L, pH 7.3) to the complexation by FA (0.8 mg C/L, pH 7.5). The curves for $[\text{Cu}^{2+}]$ (M) in function of total Cu (M) are calculated with the parameters listed in Table I.

waters, the ligands in the lake waters bind more Cu than HA and FA, and the ligands in the eutrophic lakes bind more than in the oligotrophic lake. Again, all curves approach each other closely at higher free Cu^{2+} concentrations. The curve for the oligotrophic lake Lucerne is even closer to the curves for HA and FA than those for the eutrophic lakes.

All the comparisons indicate that the complexation by ligands in the lakewaters is stronger than that by FA or HA at the ambient Cu concentrations, but that it may be similar to that by FA and HA at higher Cu concentrations.

4.3. COMPARISON OF CD TITRATION CURVES OF FA OR HA AND OF LIGANDS IN LAKE WATERS

The Cd titration curves of FA, of FA+ HA and of the neutral lake waters (Figure 6) were calculated with the fitting parameters listed in Table II and normalized to 2.8 mg C/L, either as FA or as mixture of FA and HA. In the figure, a curve calculated by the WHAM model is also shown, assuming a mixture of FA and HA (2.6 mg C/L FA + 0.24 mg C/L HA). The titration curves of humic substances, that have been determined by ASV or respectively modeled with WHAM, are similar, especially at higher Cd total concentrations, in contrast to the Cu complexation. This similarity may be due to the data base used by the WHAM model, in which

Figure 5. Calculated bound Cu per mg organic carbon CuL (mol (mgC)^{-1}) in function of $[\text{Cu}^{2+}]$ (M), using the parameters in Table I for L. Greifen, L. Sempach, L. Lucerne, FA and HA.

the data for Cd are probably collected from studies using similar techniques as the present study. In the range close to ambient total Cd concentrations, ligands in neutral lake waters exhibit stronger complexation and result in lower $[\text{Cd}^{2+}]$ at the same $[\text{Cd}_T]$ than FA and HA. Like for Cu, the Cd titration curves of ligands in the lake samples and of FA or HA approach each other closer at higher Cd concentrations, indicating that FA or HA may play a role at higher Cd concentrations as weaker ligands with higher concentrations. Figure 7 shows Cd bound per mg C and also illustrates stronger binding of Cd by the ligands in the neutral lake waters. The Cd complexation by lake Orta water with higher [Cd] and lower pH was again closer to that by humic substances (Figure 8). The WHAM model curve was plotted in Figure 8 assuming a mixture of FA and HA (0.72 mg C/L FA + 0.08 mg C/L HA).

Also the difference in lability of complexes in lakewaters and FA or HA implies that stronger complexing ligands with lower concentrations occur in lakewaters, which are not present in FA or HA solutions. These results thus all show that strong ligands binding Cd, other than HA or FA, exist at ambient Cd concentrations of lake waters and that HA or FA may play a role as weaker ligands at higher concentrations.

5. Conclusion: Nature and Origin of Ligands in Lake Waters

The results presented here indicate the presence in lake waters of ligands exhibiting a high affinity and selectivity for the complexation of Cu and Cd. These ligands appear to be better ligands for Cu and Cd than isolated humic and fulvic acids, although the low concentrations of Cu and Cd used in the present study would allow the detection of the most specific binding groups of HA and FA. These findings imply that these highly specific ligands are not extracted by the usual methods for

Figure 6. Comparison of the complexation of Cd measured in lakewaters (L. Greifen and L. Sempach) to the complexation by FA (2.8 mg C/L), HA (2.8 mg/L) or a mixture of FA + HA (2.6 mg C/L FA + 0.24 mg C/L HA) at pH 7.8. The curves for $[Cd^{2+}]$ (M) in function of total Cd (M) are calculated with the parameters in Table II. WHAM indicates a model calculation according to the WHAM model, assuming a mixture of FA + HA (2.6 mg C/L FA + 0.24 mg C/L HA).

Figure 7. Calculated bound Cd per mg organic carbon $\{Cd_L\}$ ($\text{mol}(\text{mgC})^{-1}$) in function of $[Cd^{2+}]$ (M), using the parameters in Table II for L. Greifen, L. Lucerne, FA and HA.

Figure 8. Comparison of the complexation of Cd measured in Lake Orta to the WHAM model (pH 7.3). WHAM indicates a model calculation according to the WHAM model, assuming a mixture of FA + HA (0.72 mg C/L FA + 0.08 mg C/L HA).

the extraction of HA and FA, and probably differ in their structures from HA and FA. Ligands with high affinity for Cu are likely to involve amino groups, which are only minor parts of the functional groups of FA and HA. Further work needs to be done to characterize the range of molecular weights and the structures of these ligands.

Furthermore, these specific ligands are present at low concentrations (about 1–300 nM) and thus represent only a tiny fraction of DOC in a typical sample. If the number of specific Cu or Cd binding ligands of a lake water sample is related to DOC, these concentrations (1×10^{-6} – 1×10^{-4} mol (g DOC) $^{-1}$) are 1–3 orders of magnitude lower than the typical total number of binding groups in HA or FA (1 – 10×10^{-3} mol (g C) $^{-1}$).

With regard to the origin of these ligands, the differences observed between different lakes indicate that the occurrence of strong ligands may be linked to biological productivity. The specific ligands show stronger complexation for Cu or Cd in the eutrophic lakes than in the oligotrophic lake, as mentioned here in the result section and in other studies (Xue et al., 1996; Xue and Sigg, 1998; Xue and Sunda, 1997). No specific ligands were detected in Lake Orta, with low biological productivity and higher metal concentrations. All these observations indicate that these specific ligands are probably of recent biological origin.

The relative role of fulvic and humic acids and of more specific ligands needs to be carefully assessed in various aquatic systems. It is likely that FA and HA play an important role for metal complexation in systems with high DOC concentrations

and relatively high metal concentrations as e.g., in Breault et al. (1996), especially if DOC mostly originates from soils or wetlands. In systems with high biological productivity and relatively low DOC, such as the eutrophic lakes presented here, the more specific ligands may be more relevant.

References

- Achterberg, E. P., Van den Berg, C. M. G., Boussemart, M., and Davison, W. (1997) Speciation and cycling of trace metals in Esthwaite Water: A productive English lake with seasonal deep-water anoxia. *Geochim. Cosmochim. Acta* **61**, 5233–5253.
- Bartschat, B. M., Cabaniss, S. E., and Morel, F. M. M. (1992) Oligoelectrolyte model for cation binding by humic substances. *Environ. Sci. Technol.* **26**(2), 284–294.
- Benedetti, M. F., Milne, C. J., Kinniburgh, D. G., Van Riemsdijk, W. H., and Koopal, L. K. (1995) Metal ion binding to humic substances: Application of the non-ideal competitive adsorption model. *Environ. Sci. Technol.* **29**, 446–457.
- Biber M. V., Gülaçar F. O., and Buffle, J. (1996) Seasonal variations in principal groups of organic matter in a eutrophic lake using pyrolysis/GC/MS. *Environ. Sci. Technol.* **30**, 3501–3507.
- Brand, L. E., Sunda, W. G., and Guillard, R. R. L. (1986) Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* **96**, 225–250.
- Breault, R. F., Colman, J. A., Aiken, G. R., and McKnight, D. (1996) Copper speciation and binding by organic matter in copper-contaminated streamwater. *Environ. Sci. Technol.* **30**, 3477–3486.
- Bruland, K. W. (1992) Complexation of cadmium by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* **37**, 1008–1017.
- Bruland, K. W., Donat, J. R., and Hutchins, D. A. (1991) Interactive influence of bioactive trace metals on biological production in oceanic waters. *Limnol. Oceanogr.* **36**, 1555–1577.
- Buffle, J. (1988) *Complexation Reactions in Aquatic Systems: An Analytical Approach*. Ellis Horwood.
- Buffle, J., Greter F., and Haerdi, W. (1977) Measurement of complexation properties of humic and fulvic acids in natural waters with lead and copper ion-selective electrodes. *Anal. Chim. Acta* **118**, 29–44.
- Cabaniss, S. E. and Shuman, M. S. (1988) Copper binding by dissolved organic matter: I. Suwanee River fulvic acid equilibria. *Geochim. Cosmochim. Acta* **52**, 185–193.
- Campos, M. L. A. L. and Van den Berg, C. M. G. (1994) Determination of copper complexation in sea water by cathodic stripping voltammetry and ligand competition with salicylaldoxime. *Anal. Chim. Acta* **284**(3), 481–496.
- Coale, K. H. and Bruland, K. W. (1990) Spatial and temporal variability in copper complexation in the North Pacific. *Deep-Sea Res.* **37**, 317–336.
- Donat, J. R., Kango, R. A., and Gordon, A. S. (1997) Evaluation of immobilized metal affinity chromatography (IMAC) for isolation and recovery of strong copper-complexing ligands from marine waters. *Mar. Chem.* **57**, 1–10.
- Donat, J. R., Lao, K. A., and Bruland, K. W. (1994) Speciation of dissolved copper and nickel in South San Francisco Bay: A multimethod approach. *Anal. Chim. Acta* **284**, 547–571.
- Donat, J. R. and Van den Berg, C. M. G. (1992) A new cathodic stripping voltammetric method for determining organic copper complexation in seawater. *Mar. Chem.* **38**, 69–90.
- Gordon, A. S., Dyer, B. J., Kango, R. A., and Donat, J. R. (1996) Copper ligands isolated from estuarine water by immobilized metal affinity chromatography: Temporal variability and partial characterization. *Mar. Chem.* **53**, 163–172.
- Hering, J. G. and Morel, F. M. M. (1988) Humic acid complexation of calcium and copper. *Environ. Sci. Technol.* **22**, 1234–1237.

- Kinniburgh, D. G., Milne, C. J., Benedetti, M. F., Pinheiro, J. P., Filius, J., Koopal, L. K., and Van Riemsdijk, W. H. (1996) Metal ion binding by humic acid: Application of the NICA-Donnan model. *Environ. Sci. Technol.* **30**, 1687–1698.
- Knauer, K., Ahner, B., Xue, H. B., and Sigg, L. (1998) Metal and phytochelatin content in phytoplankton from freshwater lakes with different metal concentrations. *Environ. Toxicol. Chem.* **17**, 2444–2452.
- Leenheer, J. A. (1981) Comprehensive approach to preparative isolation and fraction of dissolved organic carbon from natural water and wastewaters. *Environ. Sci. Technol.* **15**(5), 578–587.
- Leenheer, J. A., McKnight, D. M., Thurman, E. M., and MacCarthy, P. (1989) Structural components and proposed structural models of fulvic acid from the Suwannee River. In R. C. Averett, J. A. Leenheer, D. M. McKnight, and K. A. Thorn (eds.), *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*, Vol. Open-File Report 87-557. U.S. Geology Survey, pp. 331–360.
- Mantoura, R. F. C., Dickson, A., and Riley, J. P. (1978) The complexation of metals with humic materials in natural waters. *Estuarine Coastal Mar. Sci.* **6**, 387–408.
- McKnight, D. M., Feder, G. L., Thurman, E. M., Wershaw, R. L., and Westall, J. C. (1983) Complexation of copper by aquatic humic substances from different environments. *Sci. Total Environ.* **28**, 65–76.
- McKnight, D. M. and Wershaw, R. L. (1989) Complexation of copper by fulvic acid from the Suwannee River – Effect of counter-ion concentration. In R. C. Averett, J. A. Leenheer, D. M. McKnight, and K. A. Thorn (eds.), *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*, Vol. USGS Open File Report, 87–557, U.S. Geology Survey, pp. 63–69.
- Midorikawa, T. and Tanoue, E. (1996) Extraction and characterization of organic ligands from oceanic water columns by immobilized metal ion affinity chromatography. *Mar. Chem.* **52**, 157–171.
- Milne, C. J., Kinniburgh, D. G., De Wit, J. C. M., Van Riemsdijk, W. H., and Koopal, L. K. (1995) Analysis of proton binding by a peat humic acid using a simple electrostatic model. *Geochim. Cosmochim. Acta* **59**(6), 1101–1112.
- Moffett, J. W. (1995) Temporal and spatial variability of Cu complexation by strong chelators in the Sargasso Sea. *Deep-Sea Res.* **42**, 1273–1295.
- Moffett, J. W., Brand, L. E., Croot, P. L., and Barbeau, K. A. (1996) Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. *Limnol. Oceanogr.* **42**(5), 789–799.
- Morel, F. M. M., Hudson, R. J. M., and Price, N. M. (1991) Limitation of productivity by trace metals in the sea. *Limnol. Oceanogr.* **36**, 1742–1755.
- Muller, F. L. L. (1996) Interactions of copper, lead and cadmium with the dissolved, colloidal and particulate components of estuarine and coastal waters. *Mar. Chem.* **52**, 245–268.
- Pinheiro, J. P., Mota, A. M., and Gonçalves, M. L. S. (1994) Complexation study of humic acids with cadmium(II) and lead(II). *Anal. Chim. Acta* **284**, 525–537.
- Sunda, W. G. and Guillard R. R. L. (1976) The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. Mar. Res.* **34**, 511–529.
- Sunda, W. G. and Huntsman, S. A. (1991) The use of chemiluminescence and ligand competition with EDTA to measure copper concentration and speciation in seawater. *Mar. Chem.* **36**, 137–163.
- Sunda, W. G. and Huntsman, S. A. (1995) Regulation of copper concentration in the oceanic nutricline by phytoplankton uptake and regeneration cycles. *Limnol. Oceanogr.* **40**, 132–137.
- Thurman, E. M. (1985) *Organic Geochemistry of Natural Water*. Kluwer Academic Publishers.
- Tipping, E. (1994) WHAM – A chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. *Comput. Geosci.* **20**(6), 973–1023.

- Tipping, E. and Hurley, M. A. (1992) A unifying model of cation binding by humic substances. *Geochim. Cosmochim. Acta* **56**, 3627–3641.
- Turner, D. R., Varney, M. S., Whitfield, M., Mantoura, R. F. C., and Riley, J. P. (1986) Electrochemical studies of copper and lead complexation by fulvic acid. 1. Potentiometric measurements and a critical comparison of metal binding models. *Geochim. Cosmochim. Acta* **50**, 289–297.
- Van den Berg, C. M. G. (1984) Determination of copper in sea water by cathodic stripping voltammetry of complexes with catechol. *Anal. Chim. Acta* **164**, 195–207.
- Voelker, B. M. and Sulzberger, B. (1996). Effects of fulvic acid on Fe(II) oxidation by hydrogen peroxide. *Environ. Sci. Technol.* **30**, 1106–1114.
- Westall, J. C. (1982) *FITEQL, A Program for the Determination of Chemical Equilibrium Constants from Experimental Data*. Chemistry Department, Oregon State University.
- Xue, H. B., Oestreich, A., Kistler, D., and Sigg, L. (1996) Free cupric ion concentrations and Cu complexation in selected Swiss lakes and rivers. *Aquat. Sci.* **58**, 69–87.
- Xue, H. B. and Sigg, L. (1993) Free cupric ion concentration and Cu(II) speciation in a eutrophic lake. *Limnol. Oceanogr.* **38**, 1200–1213.
- Xue, H. B. and Sigg, L. (1998) Cd speciation and complexation by natural organic ligands in freshwater. *Anal. Chim. Acta* **363**(3), 249–259.
- Xue, H. B. and Sunda, W. G. (1997) Comparison of $[\text{Cu}^{2+}]$ measurements in lake water determined by ligand exchange and cathodic stripping voltammetry and by ion-selective electrode. *Environ. Sci. Technol.* **31**, 1902–1909.