COPPER SPECIATION IN ESTUARIES AND COASTAL WATERS

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Abstract.

The goals of this dissertation are to better understand the sources and the Cu binding ability of ligands that control Cu toxicity in estuaries and harbors, where elevated Cu concentrations have caused documented toxic effects on microorganisms, fish, and benthic fauna. I modified and improved a commonly used approach to determine metal speciation (competitive ligand exchange adsorptive cathodic stripping voltammetry, CLE-ACSV). Using this new approach to chemical Cu speciation and an old approach to physical Cu speciation (filtration), I show that riverine humic substances, filtrable, recalcitrant and light absorbing molecules from degraded plant material, can account for all of the Cu binding in the Saco River estuary. This finding directly supports the hypothesis that terrestrial humic substances might be the most important source of Cu ligands for buffering Cu toxicity in coastal locations with freshwater inputs. However, fieldwork in coastal waters with large inputs of both Cu and suspended colloids (Boston Harbor, Narragansett Bay, and two ponds on Cape Cod) shows that some Cu present in these samples is inert to our competitive ligand exchange method for at least 48 hours. These results support the hypothesis that a significant fraction of the Cu present in these samples is physically sequestered in colloidal material, with the remaining fraction complexed by humic substances. Previous studies of Cu speciation were not able to distinguish between strongly complexed Cu and inert Cu, and our analytical approach should be used further to determine the role of colloids in Cu speciation in all natural waters.

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Table of Contents.

Chapter 1. Introduction

The toxicity of high concentrations of Cu to microorganisms and other species is of concern in polluted estuaries and nearshore waters. The toxicity of Cu is related to the concentration of free Cu, [Cu^{2+}], and not to the concentration of total Cu. Cu is distributed amongst the "free" form (usually <1% of total Cu) and Cu ligands (>99%), so that ligands control Cu toxicity. The goals of the work in this dissertation are to better understand sources and the Cu binding ability of ligands that control Cu toxicity in streams and harbors, where elevated Cu concentrations have caused documented toxic effects on microorganisms, fish, and benthic fauna.

Using competitive ligand exchange adsorptive cathodic stripping voltammetry (CLE-ACSV), I conducted field work that directly supports our hypothesis that terrestrial humic substances are important Cu ligands in coastal locations with freshwater inputs. Our previously published work (Appendix D) shows that terrestrial humic substances, dissolved, recalcitrant and light absorbing molecules from degraded plant material, bind Cu strongly enough in seawater to possibly account for much of the Cu binding in near-shore areas. This work suggested that terrestrial humic substances might account most or all of the Cu binding in estuarine and coastal samples. We now show in Chapter 3 that the ligands in the Saco River can account for all of the Cu binding in a sample at high salinity (27‰) further downstream in the estuary. The binding ability of the Saco riverine ligands in the 27‰ sample can be modeled as that of a very reasonable concentration of humic acid extract.

We also show that the binding ability of humic substances strongly depends on pH and salinity, but the pH dependence decreases with increasing salinity, which makes it

difficult to understand the effects of pH and salinity on riverine ligands. Therefore it will be difficult to predict the contribution of terrestrial humic substances to Cu binding in estuarine samples without investigating directly the change in their binding behavior upon mixing with seawater.

Also Chapter 3, I show in actual coastal field samples that the new calibration method we developed for CLE-ACSV (Appendix D) is needed to accurately measure the Cu binding ability of humic substances and therefore of field samples containing humic substances or any mixture of stronger and weaker Cu ligands. Traditional methods underestimate the importance of humic substances and overestimate [Cu^{2+}].

I was able to do accurate work on the effects of pH and salinity on riverine ligands because I re-calibrated the added ligand for CLE-ACSV, salicylaldoxime, for changes in salinity and pH (Chapter 2 and Appendices A, B, and C). These new results can be used to accurately assess Cu speciation and [Cu^{2+}] in mid-estuarine points and at a wide range of values of pH.

I also developed a new metal speciation approach that tests whether any Cu is "kinetically inert" in coastal areas (Chapter 4). With this approach, I show that 10 to 80% of the Cu in coastal polluted areas (Boston Harbor, Narragansett Bay, and two ponds on Cape Cod) is "non-exchangeable" to 1 mM salicylaldoxime, so that it must either be inert to ligand change or have a conditional stability constant of greater than 10^{17}. We argue that this "non-exchangeable" Cu is inert on a 48-hour time scale based on kinetic arguments using the stability constant of 10^{17} and fastest possible formation rate of the "non-exchangeable" Cu-ligand complex. This 48-hour time scale is comparable to the mixing time of some estuaries and to the equilibration times used for CLE-ACSV, so that

this finding has important implications for Cu fate and toxicity and offers clues as to the identity of these inert Cu compounds.

This chapter describes the results of methods development to accurately determine the Cu binding ability of natural ligands in samples of varying pH and ionic strength with competitive ligand exchange adsorptive cathodic stripping voltammetry (CLE-ACSV).

Because colloids are common in coastal and estuarine systems, and theoretically can physically sequester Cu in their interiors, I speculate that colloids may account for the bulk of the inert Cu that I found. This hypothesis is bolstered by the fact that some kinetically inert Cu is not removed by a 0.2 μm filter but is partially removed by a 0.02 μm filter, consistent with colloid filtration behavior (Chapter 4). I cannot rule out several other types of very strong Cu ligands that might be also be inert. However, my finding that some inert Cu is likely colloidal may be an important milestone in determining the relationship between physical and chemical speciation of Cu, and is applicable to other metals and even organic pollutants.

I show that interpretations of linearized Cu titration data would interpret inert Cu as strong Cu ligands with a known strength, which actually the strength of this inert Cu is unknown. I present an example of this type of data interpretation that shows that the strongest ligand class reported in the many Cu speciation studies that use this approach includes any inert Cu that was present in the samples. Therefore these types of interpretations may distort the data and not represent well the binding ability of the ligands actually present in the sample. This has serious implications for determining the contributions of different ligand sources to the sample.

Finally, I attempted to measure the Cu binding ability of sewage ligands from sewage treatment plants on three Massachusetts streams (Chapter 5). If ligands in sewage bind Cu, which is also released in sewage due to leaching from Cu drinking pipes then the toxicity of Cu to freshwater organisms such as daphnia (water fleas) and trout may be decreased. I have preliminary results that suggest that Cu is strongly bound by ligands of unknown identity in sewage. However, analytical difficulties prevented me from completing a survey of Cu speciation upstream and downstream of three sewage treatment plants, which would show the relative roles of riverine and sewage ligands on reducing the impact of Cu on stream ecology.

Chapter 2. Calibration of salicylaldoxime for pH and salinity

2.1. Introduction

CLE-ACSV is a two-part process: first, a known concentration of a well-characterized and purified synthetic ligand is allowed to equilibrate with a series of samples containing a range of concentrations of added copper. Salicylaldoxime (SA) is a very strong, well-characterized added ligand which partitions only negligibly into natural organic matter at concentrations typical of rivers and coastal areas (Appendix B). In the presence of SA, Cu species include free Cu, Cu complexed by ligands in the sample, and Cu complexed by SA:

$$[Cu]_T = \Sigma[CuL_i] + \Sigma[Cu(SA)_x] \quad (2.1)$$

where the concentration of free Cu, $[Cu^{2+}]$, is negligibly small (picomolar concentrations compared nanomolar concentrations of total Cu), and where

$$\Sigma[Cu(SA)_x] = [Cu(SA)] + [Cu(SA)_2] \quad (2.2)$$

and Cu(SA) and $Cu(SA)_2$ are the *mono* and the *bis* complexes of copper with SA, respectively (both complexes are important in the range of concentrations of SA used).

The value of $\Sigma[Cu(SA)_x]$ in the samples is analyzed using adsorptive cathodic stripping voltammetry, ACSV, which is discussed in more detail in Appendix D. For ACSV, Cu-SA complexes adsorb to the surface of the mercury drop electrode and Cu^{2+} in the SA complexes on the drop is reduced during a negative potential scan, which produces a peak current with magnitude ip. The relationship between the current peak height, i, and $\Sigma[Cu(SA)_x]$, called the sensitivity, S:

$$\Sigma[Cu(SA)_x] = ip/S \quad (2.3),$$

must be determined in the absence of ligands that compete with SA for Cu, so that any additional Cu added ($\Delta[Cu]_T$) is complexed only by SA:

$$\Delta[Cu]_T \cong \Delta\Sigma[Cu(SA)_x] \quad (2.4).$$

We conduct multiple Cu titrations of each sample to collect data on a range of Cu ligands including those that control Cu speciation at a relevant range of $[Cu]_T$. For each titration point, in the absence of both SA and the Cu complexed by SA, the water sample would have an identical value of $[Cu^{2+}]$ and a theoretical total copper concentration $[Cu]_T^*$ that is given by:

$$[Cu]_T^* = [Cu]_T - [Cu(SA)_x] \quad (2.5),$$

where $[Cu^{2+}]$ is negligible compared to $\Sigma[CuL_i]$. The value of $[Cu^{2+}]$ is calculated from $\Sigma[Cu(SA)_x]$, $[SA]_T$, and the conditional stability constants of the *mono* and *bis* complexes, $K_{Cu(SA)}$ and $\beta_{Cu(SA)2}$:

$$[Cu^{2+}]=\Sigma[Cu(SA)_x]/(K_{Cu(SA)}[SA]_f+\beta_{Cu(SA)2}[SA]_f^2) \quad (2.6)$$

where $[SA]_f$ is the concentration of SA not bound to copper:

$$[SA]_f = [SA]_T - [Cu(SA)] - 2[Cu(SA)_2] \quad (2.7),$$

calculated using EXCEL to solve for the four unknowns in the four equations (Eqs. 2.6 and 2.7 and the two equilibrium mass law expressions for formation of Cu-SA complexes.)

Because no one has yet attempted to determine whether SA, a diprotic acid, binds Cu as HSA^- or SA^{2-} (the two possibilities assuming that only a deprotonated binding site binds Cu strongly), the "Cu(SA)" and "$Cu(SA)_2$" above refer to Cu complexed with "SA", the abbreviation for salicylaldoxime, and do not refer Cu complexed to the "correct" Cu species (such as $Cu(SA)^0$ and $Cu(SA)_2^{2-}$ instead of $Cu(HSA)^+$ and $Cu(HSA)_2^0$). However, because the convention so far in the literature is to use "SA" as an abbreviation for salicylaldoxime, we keep with this convention when describing SA speciation in general

terms, as above. However, for discussion of which species of SA complexes Cu, as we do in the rest of this chapter, we describe Cu-SA speciation with the correct SA species.

Salicylaldoxime was previously calibrated for copper at pH=8.35 (Campos, 1994). To extend the results of Campos (1994) for lower values of pH, we determined the effects of pH on K_{CuSA} and $\beta_{Cu(SA)2}$. In addition, we reassessed K_{CuSA} and $\beta_{Cu(SA)2}$ by obtaining more data at low $[SA]_T$ and low salinity. This method development was necessary for accurate measurement of Cu speciation using CLE-ACSV in a range of salinity and pH values. For example, we used the results of this calibration for determination of Cu speciation in the Saco River Estuary, and for assessing the effects of changes in salinity and pH on the Cu binding ability of riverine Cu ligands (Chapter 3, Chapter 5).

2.2. Methods

2.2.1. Relationships between pH, salinity and "true" stability constants of SA

SA is a diprotic acid (Fig. 2.1), and the two possibilities for stoichiometrically correct complexation reactions of SA with Cu (assuming that Cu binds a charged functional site) are

$$HSA^- + Cu^{2+} \rightarrow Cu(HSA)^+ \quad (2.8a)$$

and

$$2HSA^- + Cu^{2+} \rightarrow Cu(HSA)_2^0 \quad (2.8b)$$

if HSA^- binds Cu, or

$$SA^{2-} + Cu^{2+} \rightarrow Cu(SA)^0 \quad (2.8c)$$

and

$$2SA^{2-} + Cu^{2+} \rightarrow Cu(SA)^{2-} \quad (2.8d)$$

if SA^{2-} binds Cu. The "true" thermodynamic constants for the reactions in Eq. 2.8a-d are defined as a function of the activities of the species, so that

$$K_{CuSA,true} = \frac{\{CuHSA^{+}\}}{\{Cu^{2+}\}\{HSA^{-}\}} \tag{2.9a}$$

and

$$\beta_{CuSA,true} = \frac{\{Cu(HSA)_2^{0}\}}{\{Cu^{2+}\}\{HSA^{-}\}^2} \tag{2.9b}$$

for HSA-, or

$$K_{CuSA,true} = \frac{\{CuSA^{0}\}}{\{Cu^{2+}\}\{SA^{2-}\}} \tag{2.9c}$$

and

$$\beta_{CuSA,true} = \frac{\{Cu(SA)_2^{2-}\}}{\{Cu^{2+}\}\{SA^{2-}\}^2} \tag{2.9d}.$$

for SA^{2-}, where the activity of each species X, {X}, is equal to the activity coefficient, γ_x, multiplied by the concentration of the species,

$$\{X\}=\gamma_X[X] \tag{2.10}.$$

The true stability constants do not change as a function of salinity or pH. Instead, in the case of salinity, the activity coefficients change, and in the cases of pH and salinity, the fraction of $[SA]_f$ that is $[HSA^-]$ changes due to proton or cation competition with HSA^-.

It is convenient to use a conditional stability constant that is dependent on the concentrations, not the activities, of each species, so that ionic strength effects are included in the conditional stability constant. In addition, conditional stability constants are defined for the concentration of $[SA]_f$ and not $[HSA^-]$ or $[SA^{2-}]$. So we substitute Eq. 2.10 into Eqs. 2.9a-d for each activity parameter and separate the activity coefficients

from the concentrations of each value, and also multiply the expression by the ratio of $[HSA^-]$ or $[SA^{2-}]$ to $[SA]_f$, so that

$$K_{CuSA} = K_{CuSA,true} \frac{\gamma_{Cu^{2+}}\gamma_{HSA^-}}{\gamma_{CuHSA^+}} \frac{[HSA^-]}{[SA]_f} = \frac{[CuHSA^+]}{[Cu^{2+}][SA]_f} \quad (2.11a)$$

and

$$\beta_{Cu(SA)_2} = \beta_{Cu(SA)_2,true} \frac{\gamma_{Cu^{2+}}\gamma_{HSA^-}^2}{\gamma_{Cu(HSA^-)_2^0}} \frac{[HSA^-]^2}{[SA]_f^2} = \frac{[Cu(HSA)_2^0]}{[Cu^{2+}][SA]_f^2} \quad (2.11b)$$

for HSA^- complexing Cu, or

$$K_{CuSA} = K_{CuSA,true} \frac{\gamma_{Cu^{2+}}\gamma_{SA^{2-}}}{\gamma_{CuSA^0}} \frac{[SA^{2-}]}{[SA]_f} = \frac{[CuSA^0]}{[Cu^{2+}][SA]_f} \quad (2.11c)$$

and

$$\beta_{Cu(SA)_2} = \beta_{Cu(SA)_2,true} \frac{\gamma_{Cu^{2+}}\gamma_{SA^{2-}}^2}{\gamma_{Cu(SA)_2^{2-}}} \frac{[SA^{2-}]^2}{[SA]_f^2} = \frac{[Cu(SA)_2^{2-}]}{[Cu^{2+}][SA]_f^2} \quad (2.11d).$$

for SA^{2-} complexing Cu. We maintain the convention that the conditional binding constants, which are the constants measured directly in the samples, are written with no additional subscript, as that is the way they are presented in other chapters.

We measured and modeled the conditional stability constants of SA using these relationships between the true and conditional stability constants for each theoretical complex. First we modeled pH dependence of SRC(SA) to show that Cu is complexed by HSA^- and not SA^{2-}. Then we measured and modeled the salinity dependence of the correct Cu-SA complexation reaction assuming that only ionic strength effects were important. With these results, we can interpolate the binding strength of the mono and bis Cu-SA complexes in the range of pH and salinity of our experiments.

2.2.2. Calibration of SA for pH

First, we need to determine the relationship between $[HSA^-]$ and $[SA]_f$ (if the reactions in Eqs. 2.9a and b correctly describes Cu-SA complexation) or the relationship between $[SA^{2-}]$ and $[SA]_f$ (if the reactions in Eqs. 2.9c and d correctly describes Cu-SA complexation. If the concentrations of SA species other than the acid-base speciation of SA are negligibly small, The speciation of SA not bound to Cu can be described as:

$$[SA]_f=[SA^{2-}]+[HSA^-]+[H_2SA] \quad (2.12).$$

The conditional acid dissociation constants of H_2SA and HSA^- are

$$K_{a1} = K_{a1,true}\frac{\gamma_{H_2SA}}{\gamma_{HSA^-}} = \frac{\{H^+\}[HSA^-]}{[H_2SA]} \quad (2.13)$$

and

$$K_{a2} = K_{a2,true}\frac{\gamma_{HSA^-}}{\gamma_{SA^{2-}}} = \frac{\{H^+\}[SA^{2-}]}{[HSA^-]} \quad (2.14),$$

respectively. Because a pH meter measures the activity of H^+, $\{H^+\}$, we do not separate the concentration and activity coefficients of H^+ as we do for the SA species. Substituting variables in Eq. 2.13 and 2.14 with Eq 2.12 and rearranging gives the pH dependence of $[HSA^-]$ as a function of $[SA]_f$

$$\frac{[HSA^-]}{[SA]_f} = \frac{1}{10^{pKa_1-pH}+1+10^{-pKa_2+pH}} \quad (2.15)$$

and $[SA^{2-}]$ as a function of $[SA]_T$

$$\frac{[SA^{2-}]}{[SA]_f} = \frac{1}{10^{pKa_1+pKa_2-2pH}+10^{pKa_2-pH}+1} \quad (2.16).$$

These relationships are substituted into Eqs. 2.11a-d to calculate the conditional stability constants as a function of pH.

2.2.3. Measurement of SRC(SA)

To measure K_{CuSA} and $\beta_{Cu(SA)2}$ as a function of salinity, we conducted a series of competitive ligand exchange experiments with SA and EDTA, whose conditional binding constants with Cu and with other cations are well-known, in mixtures of ligand-free UV-SW and DDW. In these samples,

$$[Cu]_T \approx [CuEDTA] + \Sigma[Cu(SA)_x] \quad (2.17),$$

(we can neglect competition by weaker Cu ligands such as carbonate and hydroxide at the concentrations of SA and EDTA used.) Substitution of the Cu-ligand complexes with their mass law equivalents, followed by separation of $[Cu^{2+}]$ from all expressions on the right side of Eq. 2.8, gives

$$[Cu]_T = [Cu^{2+}](K_{CuEDTA}[EDTA]_f + K_{CuSA}[SA]_f + \beta_{Cu(SA)2}[SA]_f^2) \quad (2.18).$$

The "side reaction coefficient" of SA and EDTA is proportional to the amount of total Cu which each chelator complexes at equilibrium and is defined as:

$$SRC(SA) = K_{CuSA}[SA]_f + \beta_{Cu(SA)2}[SA]_f^2 \quad (2.19).$$

Replacing Eq. 2.19 for variables in Eq.2.18, rearranging and dividing Eq. 2.6 by Eq. 2.18 and canceling out the variable $[Cu^{2+}]$ that appears in both numerator and denominator, we obtain a relationship between total Cu and Cu complexed by SA in the presence of EDTA:

$$\frac{[Cu(SA)_x]}{[Cu]_T} = \frac{SRC(SA)}{K_{CuEDTA}[EDTA]_f + SRC(SA)} \quad (2.20).$$

The peak height obtained from CLE-ACSV analysis is proportional to $\Sigma[Cu(SA)_x]$ (Eq. 2.3). The ratio of $\Sigma[Cu(SA)_x]$ to $[Cu]_T$ is proportional to the ratio of the peak height of a sample equilibrated with EDTA, ip, to that equilibrated without EDTA, ip_0, so that

$$\frac{ip}{ip_0} = \frac{SRC(SA)}{K_{CuEDTA}[EDTA]_f + SRC(SA)} \quad (2.21).$$

Eq. 2.21 is solved for SRC(SA). $[EDTA]_f$ is simply equal to the added $[EDTA]_T$ since $[Cu]_T << [EDTA]_T$ in all cases. Calculated K_{CuEDTA}, and values of ip and ip_0 measured over a range of EDTA are used to obtain an average value of SRC(SA) for each sample type.

2.2.4. Calculation of K_{CuEDTA}

We calculated K_{CuEDTA} at different salinities (in the range of salinities used here, K_{CuEDTA} is independent of pH) using equilibrium constants from Morel and Hering (Morel, 1993) and average cation concentrations in seawater from Millero and Sohn (Millero, 1991). We made corrections for ionic strength effects using the Davies Equation, including the ionic strength of the seawater matrix (0.72 M) and that of the 0.02 M buffer (0.02 M) (Appendix A). The calculated value of K_{CuEDTA} at a salinity of 35‰ is $10^{10.06}$ (in agreement with Campos ,1994) and at 1‰ is $10^{11.60}$. The value of K_{CuEDTA} is not pH dependent in seawater or at lower salinity.

2.2.5. Experimental setup.

To calibrate SA at different values of pH and salinity, we ran a series of CLE-ACSV experiments to measure ip_0 and ip in the absence and presence of EDTA (1-10 or 10-100 μM), 20 nM Cu, and 0.02 M buffer. For the pH experiments, the sample matrix was UV-SW with buffers (boric acid (EM Science, Suprapur®, pH range of 7.5-8.2), HEPES (OmniPur, pH 7-8), and MOPS (Sigma, pH 6-7)) adjusted to different values of pH, and SA concentrations were 50 or 500 μM. For salinity experiments, the matrix was UV-SW (35‰) or UV-SW diluted with DDW (for S=1 or 2‰) at pH = 8.0 (boric acid buffer), and SA concentrations were 1, 5, 10, 25, or 50 μM SA. Samples were equilibrated for 24 to 72 hours (48 hours for the pH experiments) in Teflon® bottles before analysis.

2.2.6. Voltammetric analyses

We used differential pulse ACSV with a PAR 303A static mercury drop electrode and an EG&G PAR 394 analyzer. Instrument settings were as follows: adsorption potential, -0.08 V (versus Ag/AgCl electrode); scan range, -80 to -600 mV; scan rate, 20 mV/s; drop time, 0.2 s; pulse height, 25 mV. Adsorption times for ACSV ranged from 10 seconds to 4 minutes, depending on the sensitivity needed. We verified that the signal was linear with respect to adsorption time for longer adsorption times, indicating that sensitivity-reducing "surfactant effects" were insignificant. Values of ip less than 1 nA were discarded, and for each value of ip/ip_0 less than 0.95 and greater than 0.05, SRC(SA) was calculated with Eq. 16. An average value of SRC(SA) was obtained from at least three measurements of ip/ip_0. pH was measured after voltammetric analysis to +/- 0.03 pH units.

2.3. Results.

2.3.1. SRC(SA) and salinity.

Salicylaldoxime was previously calibrated for copper at pH=8.35 (Campos, 1994). To determine the effect of salinity on K_{CuSA} and $\beta_{Cu(SA)2}$, Campos et al. measured SRC(SA) at salinities of 1, 10, 20, and 35‰ for 25 μM SA. In addition, Campos et al. determined the values of K_{CuSA} and $\beta_{Cu(SA)2}$ at 1, 2, and 25 μM SA to obtain information about the relative importance of *mono* and *bis* Cu-SA complexes at salinity of 35‰.

We measured ip/ip_0 using five concentrations of EDTA in samples with salinities of 1, 2, or 35‰, each at 1, 5, 10, 25, or 50 μM SA (black squares in Fig. 2.2.) A model fit of the average SRC(SA) obtained from the five values of ip/ip_0 in each experiment is also shown (black lines). Where there are multiple lines (1 psu, 1 μM SA; 33 psu, 50 μM

SA), more than one EDTA titration of SA was conducted on separate days. Results were identical for 24 hour and three-day equilibration times. Plots outlined in heavier frames are data for which Campos(1994) conducted experiments at identical concentrations of SA and salinity values.

Using Excel, we modeled competitive ligand exchange between EDTA, SA, and Cu for each experiment, using our calculated values of K_{CuEDTA} with the values of K_{CuSA} and $\beta_{Cu(SA)2}$ reported by Campos (1994), adjusted for pH (see Section 2.3.3). We calculated the ratio of ip/ip_o expected (Eq. 2.21) with concentrations of EDTA from 10^{-7} to 10^{-3} M. Results of this model of the Campos data are presented as dashed lines in Figure 2.2. The plots with thicker frames are conditions for which Campos obtained data; they did not report any experiments at low [SA] and low salinity. Our values of ip/ip_0 agree with those calculated from Campos (1994) at 35‰, but are significantly greater for lower salinity samples, especially at low [SA].

2.3.2. Cu-SA *mono* and *bis* speciation.

To determine K_{CuSA} and $\beta_{Cu(SA)2}$ for each experiment, using Sigmaplot 4.0, we fit values of SRC(SA) using the equation

$$\log SRC(SA) = \log([SA]_f 10^{\log K} + [SA]_f^2 10^{\log \beta}) \qquad (2.22).$$

The results of this best fit are shown in Fig. 2.3. The best fits gave values and relative errors for $\log K_{CuSA}$ and $\log \beta_{Cu(SA)2}$ for each salinity. Using these constants, we calculated new best-fit relationships for the dependency of $\log K_{CuSA}$ and $\log \beta_{Cu(SA)2}$ on salinity:

$$\log K_{CuSA} = (10.90 \pm 0.03) - (0.90 \pm 0.02) \log S \qquad (2.23a)$$

and

$$\log \beta_{Cu(SA)2} = (15.49 \pm 0.08) - (0.62 \pm 0.07) \log S \qquad (2.23b).$$

Values of $\log K_{CuSA}$ and $\log\beta_{Cu(SA)2}$ obtained for each salinity experiment are shown as circles in Fig. 2.4 (with salinity on a linear scale) along with their best fits (Eqs. 2.23.a and b, heavy black lines). The values reported by Campos (adjusted for pH with Eqs. 2.23a and b) are shown as squares, and their corresponding best fits as light lines. Our values of $\log\beta_{Cu(SA)2}$ agree very well with theirs over the range of salinities, but our results show that $\log K_{CuSA}$ is significantly higher than what they report.

2.3.3. Calibration of SRC(SA) for variation in pH.

We measured values of SRC(SA) at different pH values at salinity = 35‰ (Fig. 2.5) to determine whether the relationship between (SRC)SA and pH is best modeled with Eqs. 2.11 a and b (HSA^- complexes Cu) or 2.11 c and d (SA^{2-} complexes Cu) (Fig 2.6). In this way we deduce whether HSA^- or SA^{2-} complexes Cu, and we can interpolate the conditional Cu-SA stability constants to any value of pH.

The reported conditional proton dissociation constants at an ionic strength of 1.0 and 0.5 M are $pK_{a1} = 8.85$ and $pK_{a2} = 11.39$ and $pK_{a1} = 8.84$ and $pK_{a2} = 11.46$, respectively (Martell, 1993). We used the value for 0.5 M ionic strength because the actual ionic strength of these seawater samples, 0.63 M, is close to 0.5 M, and the values of the conditional proton dissociation constants do not change much from 0.5 to 1.0 M ionic strength. Plugging these values into Eqs. 2.15 and 2.16, we calculated the new ratios of HSA^- or SA^{2-} to $[SA]_T$ in the range of pH values from 6 to 9. Then, we substituted these ratios into Eqs. 2.15 and 2.16 in Eqs. 2.11a-d and divided by the ratios of HSA^- or SA^{2-} to $[SA]_f$ for pH=8.0:

$$K_{CuSA,pH=X} = K_{CuSA,pH=8.0} \frac{\left([HSA^-]/[SA]_f\right)_{pH=x}}{\left([HSA^-]/[SA]_f\right)_{pH=8.0}} \qquad (2.24a)$$

and

$$\beta_{Cu(SA)_2,pH=X} = \beta_{Cu(SA)_2,pH=8.0} \frac{\left([HSA^-]/[SA]_f\right)^2_{pH=x}}{\left([HSA^-]/[SA]_f\right)^2_{pH=8.0}} \quad (2.24b),$$

or

$$K_{CuSA,pH=X} = K_{CuSA,pH=8.0} \frac{\left([SA^{2-}]/[SA]_f\right)_{pH=x}}{\left([SA^{2-}]/[SA]_f\right)_{pH=8.0}} \quad (2.24c)$$

and

$$\beta_{Cu(SA)_2,pH=X} = \beta_{Cu(SA)_2,pH=8.0} \frac{\left([SA^{2-}]/[SA]_f\right)^2_{pH=x}}{\left([SA^{2-}]/[SA]_f\right)^2_{pH=8.0}} \quad (2.24d),$$

where the value of the conditional stability constant at pH=8.0 is the value we measured for S=35‰ at pH=8.0 (Section 2.3.2). Because we are at constant salinity, the activity coefficients remain constant and are included in the conditional stability constants above.

In Fig. 2.6a, the thin dashed and dotted lines for both 50 and 500 μM SA represent the calculated SRC(SA) for the *mono* complex and SRC(SA) for the *bis* complex, respectively, and the solid lines represent total SRC(SA) calculated using values of K_{CuSA} and $\beta_{Cu(SA)2}$ adjusted for pH with Eqs. 2.24a and b (assuming HSA^- complexes Cu) and either 50 or 200 μM SA. The same is true for the dashed, dotted, and solid lines in Fig. 2.4b, except SRC(SA) was calculated using Eqs. 2.24b and c (assuming SA^{2-} complexes Cu). Clearly the model assuming HSA^- complexes Cu best fits the data, and Eqs. 2.8a and b correctly describe the reaction of Cu with SA to make the *mono* and *bis* complexes.

In samples with low ionic strength (ie. low salinity samples), the pH calculation will change slightly (see Eqs. 2.13 and 2.14). The pK_{a1} for SA at 1‰ is calculated with the Davies Equation to be 8.90, 0.06 log units greater than that reported an ionic strength of

0.5 M. The change in the value of pK_{a2} will not significantly affect the calculations for the ratio of $[HSA^-]$ to $[SA]_T$ at the pH range of interest (6 to 9). Because ionic strength effects change our pH calculations negligibly at different salinities, we used the same values of pK_{a1} and pK_{a2} at 0.5 M ionic strength for all salinities (Chapter 3 and Chapter 5.) However, more precise interpolations could be completed for very low salinities using pK_{a1} values calculated for lower salinities.

2.3.4. Ionic strength.

We modeled the changes in $\log K_{CuSA}$ and $\log\beta_{Cu(SA)2}$ as a function of salinity (Fig. 2.4) as if ionic strength effects (and not competition by Ca^{2+} or another cation with Cu^{2+} for SA) were the cause for their change. We used the appropriate activity coefficients for Cu^{2+}, HSA^-, and H^+ calculated using the Davies Equation. Then, we used activity coefficients calculated with the Davies Equation to predict the ratio of the values of $[HSA^-]/[SA]_T$ using Eqs. 2.13 and 2.14. We calculated the new values of K_{CuSA} and $\beta_{Cu(SA)2}$ expected at 1 and 2‰ using Eqs. 2.11a and b. The values calculated are shown in Fig. 2.4 as black dots. Ionic strength corrections do not fit the salinity dependence of $\log K_{CuSA}$ and $\log\beta_{Cu(SA)2}$ well.

There are at least two possible reasons for the fact that the ionic strength predictions do not fit well the measured data: one theoretical and one analytical. The theoretical reason is that something else besides ionic strength controls SA speciation as a function of salinity; this could include Ca^{2+} or another cation. The NIST database does not offer any measured values of Ca-SA species, so we are unable to predict whether Ca-SA is important. However, we argue that it is unlikely that Ca^{2+} or another cation is important at a salinity of 35‰. Because we are able to model the pH effects on SA speciation so well at S=35‰ (see Fig. 2.6), H^+ must dominate SA speciation here. If Ca^{2+} is also

important, then it would be necessary to invoke an SA complex that includes both H^+ and Ca^{2+}. . The ionic strength predictions do not fit the Campos (1994) data well either, suggesting that SA speciation is more complicated than assumed.

2.4. Conclusions.

We show that Eqs.2.23.a and b can be used to interpolate the approximate values of K_{CuSA} and $\beta_{Cu(SA)2}$ between 1 and 35‰. Values of $\log\beta_{Cu(SA)2}$ from agree well with those shown previously in Campos (1994), but we found $\log K_{CuSA}$ to be considerably greater than what they report (Fig. 2.4). Because our extensive data on SRC(SA) at low [SA] are reproducible and consistent in trend (Fig. 2.2), and Campos and co-workers obtained limited low [SA] low salinity data, we believe that our values are correct.

The values of K_{CuSA} and $\beta_{Cu(SA)2}$ can also be independently adjusted for changes in pH using Eqs. 2.15 (for the case in which HSA^- complexes Cu) at salinity = 35 ‰, and, we assume, at lower salinity values.

Predicted ionic strength effects cannot account for the changes in $\log K_{CuSA}$ and $\log\beta_{Cu(SA)2}$ with salinity. Eqs. 2.23a and b are not appropriate for extrapolating K_{CuSA} and $\beta_{Cu(SA)2}$ for salinities below 1‰ because we have shown that the effect of cation competition (other than SA acid/base speciation) on K_{CuSA} and $\beta_{Cu(SA)2}$ may also be important. The distinction is important for freshwater samples because below 1‰, the value of $[Ca^{2+}]$ and other cations varies widely with stream and watershed chemistry, while the 0.02 M buffer necessary for CLE-ACSV will dominate ionic strength effects. The distinction is also important because our extrapolations of pH dependence at lower salinity assume that H^+, and not another cation, dominates SA speciation. Because we see both a pH effect at

salinity = 35‰ and a "salinity effect" that is greater than that explicable by ionic strength effects, the speciation of Cu and SA may not be as straightforward as we assume in this chapter. The assumptions of this chapter effect the results and interpretations of field data in Chapters 3 and 5.

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(2) Morel, F. M. M., Hering, J.G. *Principles and Applications of Aquatic Chemistry*; John Wiley & Sons, Inc.: New York, 1993.

(3) Millero, F. J. M. L. S. *Chemical Oceanography*; CRC Press, Inc.: Boca Raton, 1991.

(4) Martell, A. E., R. M. Smith, R. J. Motekaitis. Texas A&M University: College Station, 1993.

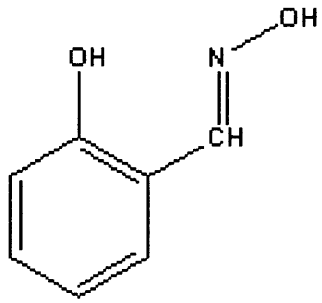
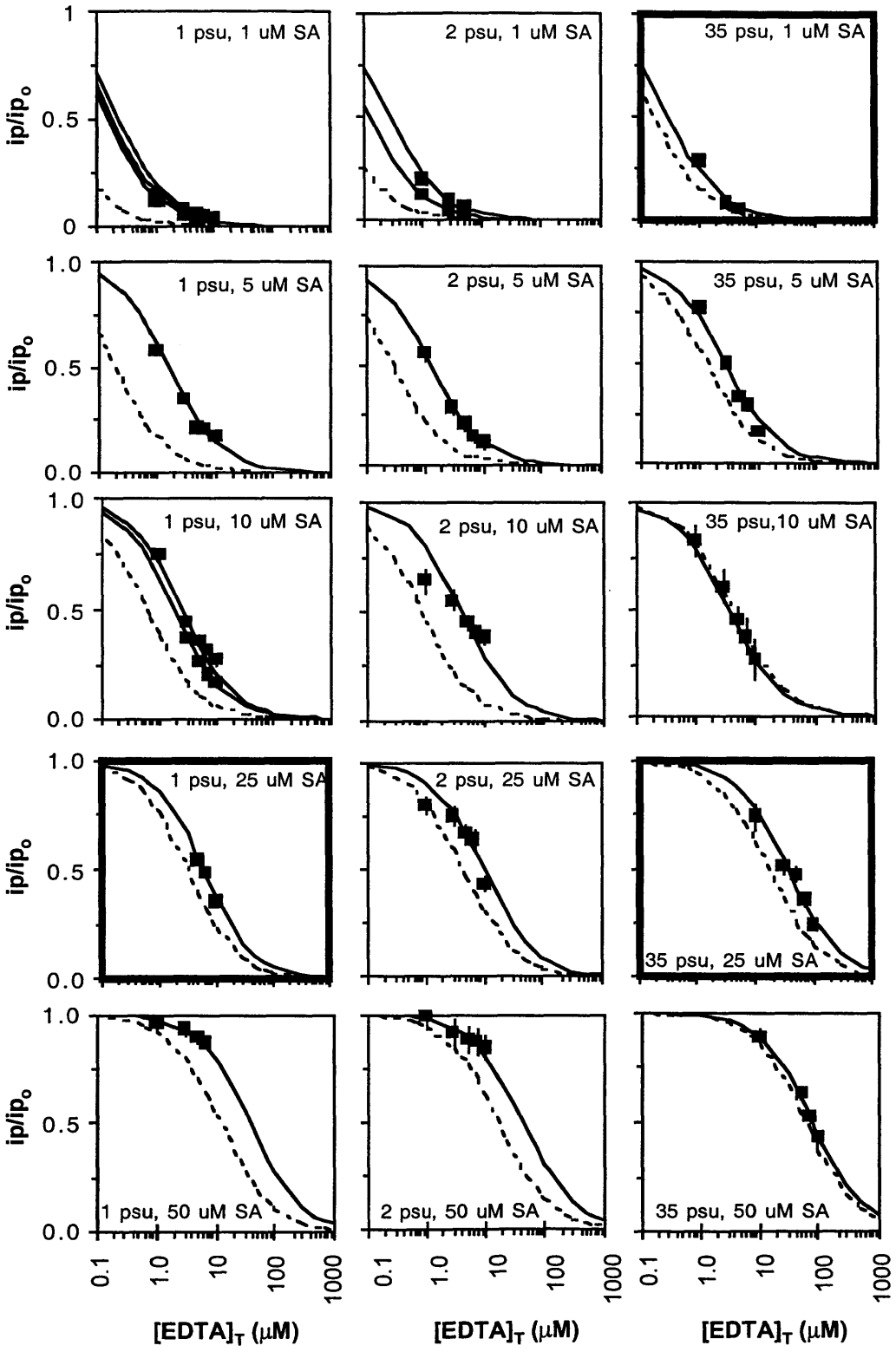


Fig. 2.1. Molecular structure of salicylaldoxime (SA).

Figure 2.2. Ratios of peak height measured in the presence of EDTA to peak height in the absence of EDTA for the calibration of SA dependence on salinity. Raw data (■) modeled with the average of all values of SRC(SA) obtained from each experiment (black lines). Where more than one line is present, there was more than one experiment conducted (always on different days with different samples). Ratios are also modeled based on the values of SRC(SA) reported by Campos and coworkers (1994) (dashed lines.) Samples with thick frames are combinations of [SA] and salinity for which Campos and coworkers conducted actual experiments.


1 psu, 1 uM SA
2 psu, 1 uM SA
35 psu, 1 uM SA
1 psu, 5 uM SA
2 psu, 5 uM SA
35 psu, 5 uM SA
1 psu, 10 uM SA
2 psu, 10 uM SA
35 psu,10 uM SA
1 psu, 25 uM SA
2 psu, 25 uM SA
35 psu, 25 uM SA
1 psu, 50 uM SA
2 psu, 50 uM SA
35 psu, 50 uM SA
ip/ip$_o$
[EDTA]$_T$ (μM)
0.1
1.0
10
100
1000
0.0
0.5
1.0
1

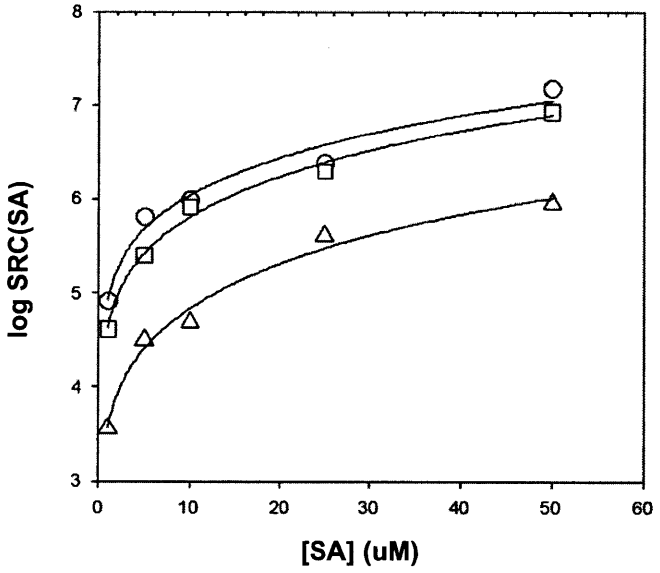


Fig. 2.3. Measured values of SRC(SA) for salinities of (O) 1‰, (□) 2‰, and (Δ) 35‰, and the best fit of each dataset determined using Sigmaplot 4.0 and Eq. 2.22. The results of the best fits are shown in Eqs. 2.23a, b.

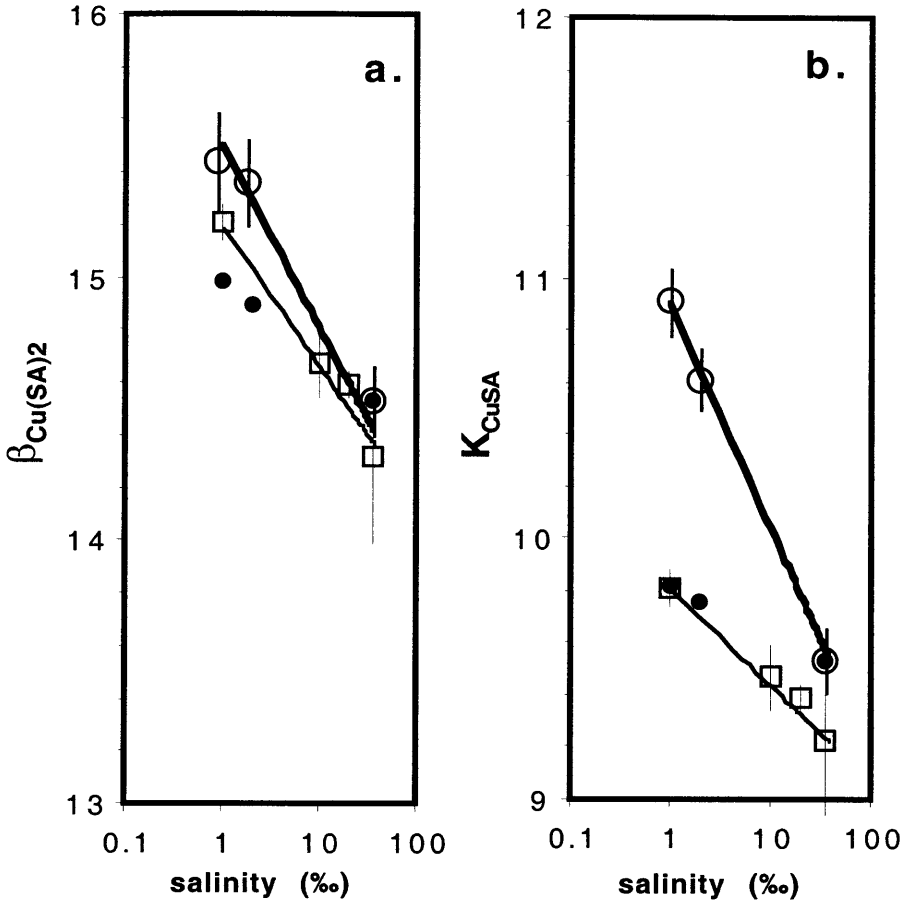


Figure 2.4. Values of log $\beta_{Cu(SA)2}$ and log K_{CuSA} as a function of salinity measured in this work (O) and from Campos et al (1994) (□). Best fit lines are plotted from Eqs. 23.a and b. The values of log $\beta_{Cu(SA)2}$ and log K_{CuSA} at 1 and 2‰ are predicted using ionic strength arguments from the modeled value of log $\beta_{Cu(SA)2}$ (gray dots) and log K_{CuSA} (black dots) at 35‰, using the Davies Equation and the reported value of pK_{a1} for SA (NIST).

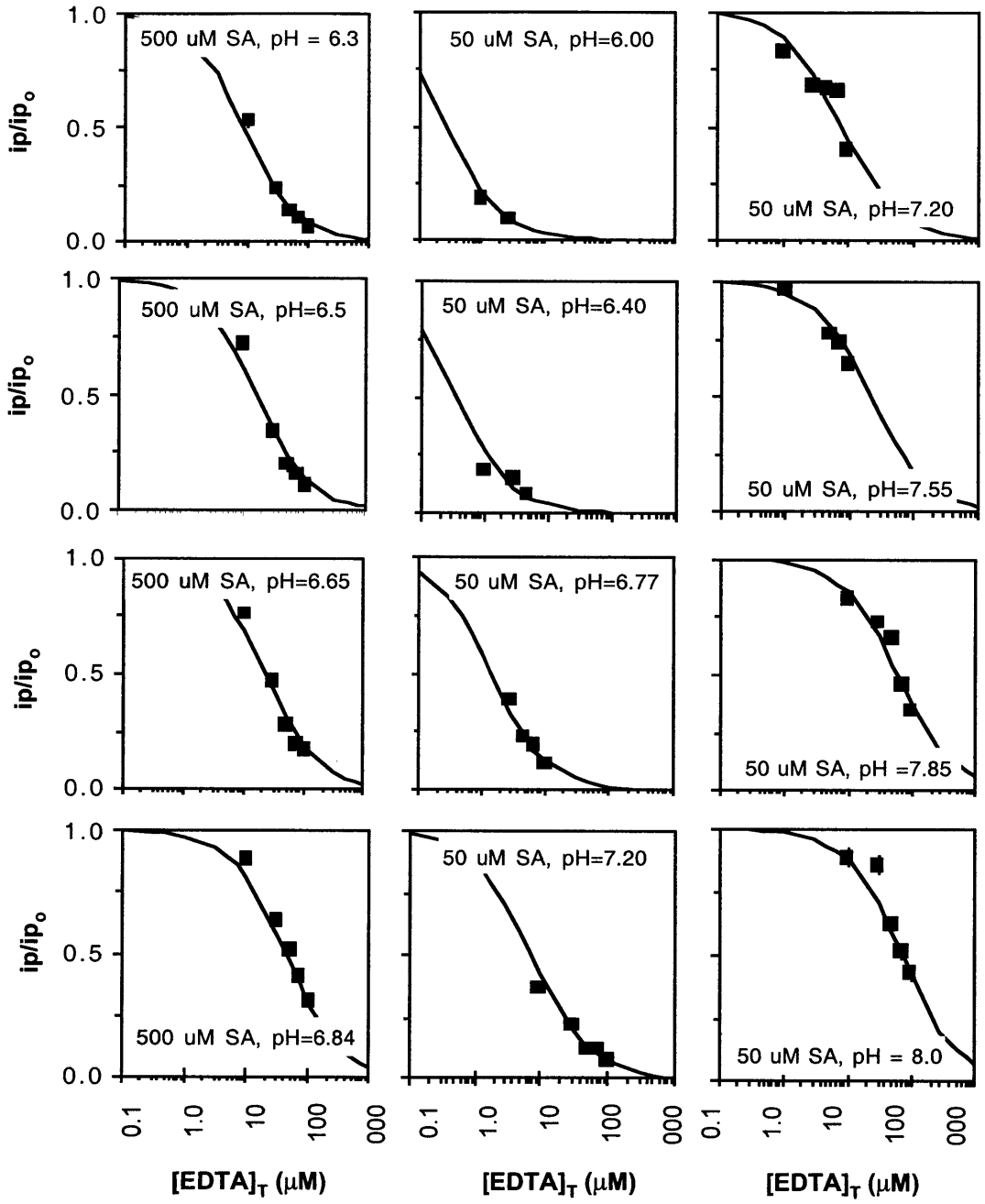


Figure 2.5. Ratios of peak height measured in the presence of EDTA to peak height in the absence of EDTA for the calibration of SA dependence on salinity. Raw data (■) modeled with the average of all values of SRC(SA) obtained from each experiment (black lines).

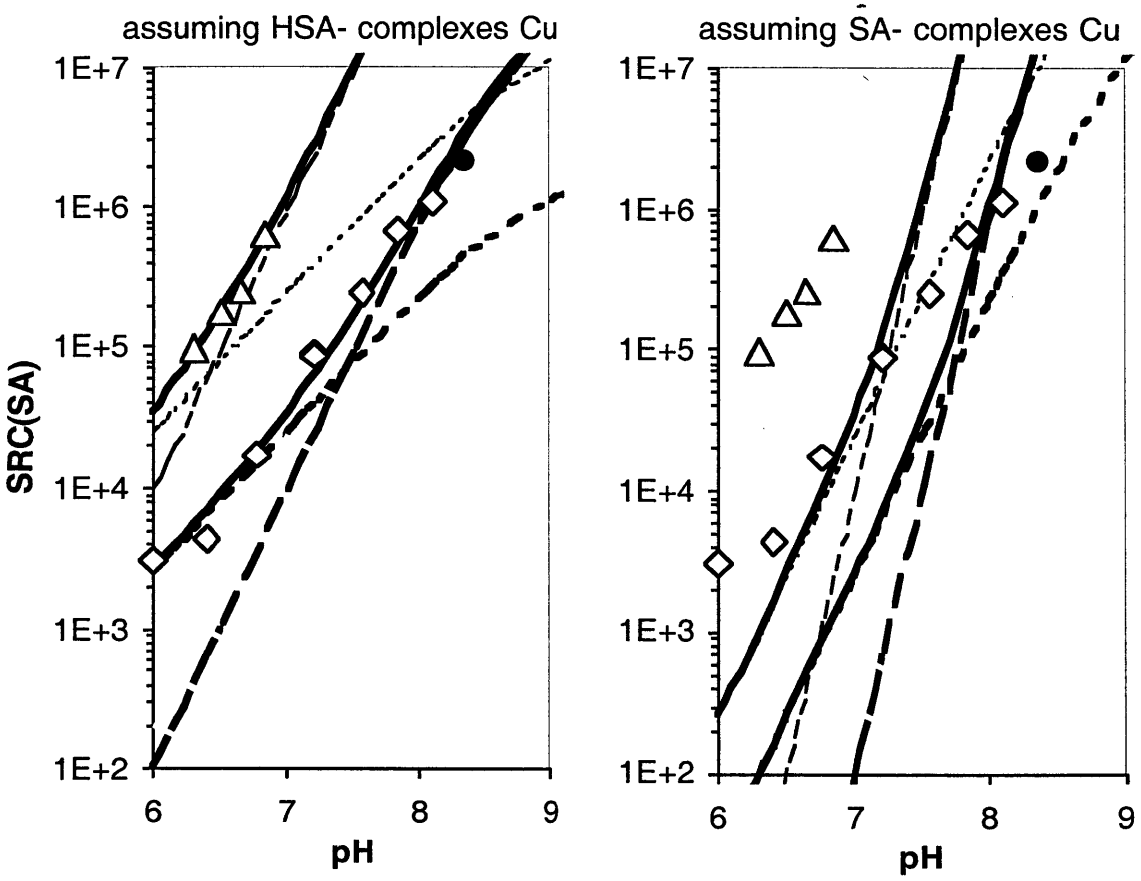


Figure 2.6. The side reaction coefficient of salicylaldoxime measured at 50 μM SA (◊) or 500 μM SA (Δ). The side reaction coefficent is modeled using the stability constants determined in Fig. 3 and assuming that either HAS- or SA- complexes Cu (see Eqs. 18.a and 18.b). The SRC(SA) from Campos et al, calculated for 50 μM SA at pH 8.35, is also shown (●).

Chapter 3. The contribution of riverine Cu ligands to Cu speciation in the Saco River estuary

Abstract

Riverine ligands have been proposed to be important to Cu speciation in estuaries and near-shore samples. A few field studies have been attempted to determine whether riverine ligands behave conservatively in a salinity gradient, but these studies did not conclusively show that riverine ligands were important at high salinities and pH values of the estuaries. We analyzed Cu speciation along the salinity gradient at the mouth of the Saco River, ME, to quantify pH and salinity effects on the riverine ligands. We show that riverine ligands, which may be dominated by terrestrial humic substances, can account for all the Cu ligands even at the sample taken at a salinity of 27‰, even after dilution and changes in binding ability due to pH and salinity effects.

3.1. Introduction

The toxicity of high concentrations of Cu to plankton (Sunda, 1976; Hall, 1997) and other species is of concern in polluted estuaries and near-shore environments. The toxicity of Cu is related to the concentration of free Cu, $[Cu^{2+}]$, and not to the concentration of total Cu, $[Cu]_T$. Cu is distributed amongst the "free" form (usually <1% of $[Cu]_T$) and Cu ligands (>99%), so that

$$[Cu]_T=[Cu^{2+}]+\Sigma[CuL_i] \quad (3.1),$$

where $\Sigma[CuL_i]$ is the sum of Cu complexed to all the different ligands of type i present in the water column. The extent to which ligands successfully compete with each other for

Cu depends on the relative concentration, $[L_i]$, and conditional binding strength K_{CuLi}, of each, so that Eq. 3.1 can also be expressed as

$$[Cu]_T=[Cu^{2+}]+K_{CuL1}[L_1]_f[Cu^{2+}]+K_{CuL2}[L_2]_f[Cu^{2+}]+\ldots+K_{CuLn}[L_n]_f[Cu^{2+}] \quad (3.2)$$

where, for this example, ligands of two different sources (and as many as n sources) are present, and $[L_i]_f$. is the concentration of the ligand of that source not complexed to Cu, or

$$[L_i]_f=[L_i]_T-[CuL_i] \quad (3.3)$$

In order to predict $[Cu^{2+}]$ and therefore the potential toxic impact of Cu, one needs to know the concentrations and strengths of all the Cu ligands present in the water column.

There are several possible sources of ligands to an estuary, and from the point of view of the estuary, they can be roughly be divided into the two categories of autochthonous (produced in situ or entrained from bottom sediments) and allochthonous (imported from rivers or terrestrial sources). Autochthonous ligands include microbially produced ligands (Moffett, 1997; Leal, 1999) and sediment sources of humic substances and sulfide (Skrabal, 2000). Allochthonous sources include microbially produced ligands, terrestrial humic substances (Kogut, 2001; Xue, 1999), sewage effluent and surface runoff (Sedlak, 1997), and riverine dissolved and colloidal sulfides stabilized by complexation with Cu (Rozan, 1999d). While these two categories share some ligand types (e.g., microbial ligands, reduced sulfide, and humic substances), they serve to distinguish between ligand sources whose importance may depend on estuarine dynamics, such as upwelling, nutrients loads and Cu concentrations, and those that depend on other factors, such as watershed characteristics, river flowrate, and sewage input (Rozan, 1999d). If we want to be able to predict $[Cu^{2+}]$ on a long term basis, we must know how the relative inputs of autochthonous and allochthonous ligands change with seasonal or watershed use changes, for example.

Terrestrial humic substances may account for a large fraction of the total Cu speciation in estuaries with large riverine inputs. Humic substances contain a spectrum of Cu binding strengths that can be modeled as organic acids and other ligands with varying strengths e.g., (Bartschat, 1992) or as two or more arbitrary bins of ligands with average conditional binding constants (Kogut, 2001). Because humic substances have a spectrum of binding sites with varying conditional binding constants, we refer to this spectrum as their "Cu binding ability" over a range of Cu concentrations. The Cu binding ability of terrestrial humic extracts increases with increases in pH and decreases with increased ionic strength effects (Cabaniss, 1988). However, competition with Ca and Mg were not observed to be significant in several studies with SRFA and SRHA (Hering, 1988; Cabaniss, 1988). These studies were conducted at $[Cu]_T$ higher than the usual ambient $[Cu]_T$ found even in polluted estuaries, but it is likely that the stronger ligands in humic substances are affected by changes in pH and salinity as well. In field samples, an increase in Cu binding upon increase of pH from 8.2 to 9.0 was reported for an estuarine sample (Sunda, 1991). Any study that attempts to quantify the importance of terrestrial humic substances (or riverine ligands in general) to Cu speciation in estuaries and near-shore areas must take into account the effects of salinity and pH on the binding ability of these ligands.

Previous studies to assess the importance of riverine ligands to Cu binding in estuaries show in general that Cu is more strongly bound in low salinity samples than in high salinity samples (van den Berg, 1987; van den Berg, 1986; van den Berg 1990; Apte, 1990; Gardner, 1991). For the first four studies, the pH was held constant at 7.7 – 8.0 in attempts to maintain the conditional stability constants of these ligands constant and measure the concentrations of ligands along the salinity transect and therefore determine whether riverine ligands behaved conservatively. All four found conservative dilution of

ligands (or in the case of the van den Berg, 1986 study, large variability in ligand concentrations) along the transect. In the fifth study (Gardner, 1991), ligand binding constants and concentrations were obtained at different values of pH and salinity, and it was suggested that the net effect of pH (increasing ligand strength) and salinity effects (decreasing ligand strength) on riverine ligands was null, so that ligand concentrations appeared conservative in the salinity gradient. However, because all these voltammetric studies employed competitive ligand exchange adsorptive cathodic stripping voltammetry (CLE-ACSV) or ASV calibrated with "internal calibrations", which can significantly underestimate the importance of humic substances (Moffett, 1997; Kogut, 2001; Voelker, 2001), they likely underestimated the binding ability of riverine ligands. Further, the conditional stability constants of the ligands were allowed to co-vary with ligand concentrations, which may have affected the accurate determination of ligand concentrations (Voelker, 2001). We have successfully developed a new external calibration method that accurately assesses the Cu binding ability of humic substances and of mixtures of stronger and weaker ligands (Kogut, 2001) at different values of pH and salinity (Chapter 2), so that we would be able to avoid all of these problems with previous attempts to determine the importance of riverine ligands in estuaries.

The goal of this study is to increase our understanding of the Cu binding behavior of natural riverine ligands, specifically humic substances, as they travel through an estuary, so that the relative importance of terrestrial and other sources of ligands to estuaries and coastal waters can be assessed more accurately. We analyzed Cu speciation with CLE-ACSV and our new calibration method in samples taken from a salinity transect in the Saco River estuary, ME. Iron, which has frequently been shown to flocculate with high molecular weight humic substances, is conservative in this river (Mayer, 1982a), suggesting that the settling of iron and therefore also the settling of humic substances to

sediments is negligible. The Saco River is not heavily polluted with Cu and other metals, making it a good site to assess the importance of humic substances in the absence of reduced sulfides, which would likely not be stabilized in high concentrations without high concentrations of metals. The Saco River also has relatively little development near its mouth, so that issues with sewage effluent and anthropogenic chelators are also minimized.

3.2. Methods

3.2.1. Sample Collection

Samples were collected along the salinity gradient in the Saco River in October, 2001, during an incoming tide from a small aluminum boat with an electric outboard motor. Samples for Cu titrations were collected in acid-cleaned Teflon® bottles just under the surface of the water, from the bow when underway to minimize any contamination from the boat. These samples were "quick frozen" in liquid nitrogen to minimize coagulation of organic matter due to "freezing out" and subsequently stored in a –4°C freezer for three months. Comparison of a riverine sample immediately after sampling and after three months of cold storage shows that this method of sample preservation does not alter the Cu binding ability of the water. Salinity and ambient pH were monitored during sampling using a Hydrolab Minisonde. Salinity was measured again before measurement with a portable conductivity meter (VWR Scientific) calibrated with UV-SW (salinity=35 psu) (Table 3.1.) The pH of each sample was measured again after adjustment with buffer and CLE-ACSV analysis using a pH meter (Orion). Samples to be analyzed for total organic carbon (TOC) and color absorbance were collected in precleaned DOC-free

amber bottles, acidified to pH 3 with phosphoric acid, and stored at +4°C for three months.

3.2.2. Reagents

Buffers were made by adjusting 1.0 M boric acid (EM Science, Suprapur®, pH range of 7.5-8.2), HEPES (OmniPur, pH 7-8), and MOPS (Sigma, pH 6-7)) with concentrated ammonia (J.T. Baker) or hydrochloric acid (J.T. Baker) until the desired pH was reached. UV oxidized Sargasso seawater (UV-SW) was collected in August 1997 with trace metal clean methods and subjected to at least six hours of ultraviolet light oxidation with a medium pressure mercury lamp (Ace Glass, 1000 W). Deionized distilled water (DDW) was from a Millipore Q-H_2O system. Fresh 1 mM primary and 1 μM secondary copper standards were made every day by serial dilutions of an Aldrich atomic absorption standard solution ($CuSO_4$ in 1% HNO_3, 10 000 ppm Cu) with DDW; the final pH of the secondary copper standard was about 6. Salicylaldoxime (Aldrich) was purified by repeated filtering and recrystallization from solutions containing 1 mM EDTA (Campos, 1994). SA was dissolved in DDW for a final stock solution concentration of 0.025 M. SA stock solutions were replaced monthly and refrigerated when not in use. Acid-cleaned Teflon® or polycarbonate containers were used for all reagents and experiments to minimize adsorption of copper and ligands to bottle surfaces and leaching of phthalate plasticizers into the samples.

3.2.3. Filtering

Cu speciation samples were syringe-filtered through acid cleaned 0.2 μm polycarbonate membrane filters (Nucleopore, 47 mm filter diameter) sandwiched in acid-cleaned

polycarbonate filter holders. This filtration protocol does not add significant concentrations of Cu or strong Cu ligands (Chapter 4).

3.2.4. Total organic carbon and color

Samples for total organic carbon (TOC) measurements were acidified to pH=3 with phosphoric acid (J.T. Baker) and TOC was measured using a Shimadzu TOC-5000 analyzer calibrated with diluted solutions of a 1mg/ml potassium acid phthalate standard (VWR Scientific). Absorbance of the sample at 350 nm (with the absorbance at 500 nm subtracted) was measured with Hewlett-Packard 8543 UV-visible photospectrometer.

3.2.5. Total Cu.

Samples from each filter fraction were acidified to pH < 2 with concentrated nitric acid (J.T. Baker Instra-Analyzed) and irradiated with ultraviolet light from a mercury lamp (Ace Glass, 1000 W) for at least six hours in quartz 125 ml tubes. Samples were then brought to circumneutral pH and analyzed with CLE-ACSV. With all ligands destroyed, $[Cu]_{T,0}$ in the original sample is $[Cu(SA)_x]$ in the sample, which is measured as described below.

3.2.6. CLE-ACSV

CLE-ACSV is a two-part process: first, a known concentration of a well-characterized and purified synthetic ligand is allowed to equilibrate with a series of samples containing a range of concentrations of added copper. Salicylaldoxime (SA) is a very strong, well-characterized added ligand which partitions only negligibly into natural organic matter at concentrations typical of rivers and coastal areas (Appendix B). In the presence of SA,

Cu species include free Cu, Cu complexed by ligands in the sample, and Cu complexed by SA:

$$[Cu]_T = [Cu^{2+}] + \Sigma[CuL_i] + \Sigma[Cu(SA)_x] \quad (3.4)$$

where

$$\Sigma[Cu(SA)_x] = [Cu(SA)] + [Cu(SA)_2] \quad (3.5)$$

and $Cu(SA)$ and $Cu(SA)_2$ are the *mono* and the *bis* complexes of copper with SA, respectively (both complexes are important at the concentrations of SA used).

The value of $\Sigma[Cu(SA)_x]$ in the samples is analyzed using adsorptive cathodic stripping voltammetry, ACSV, which is discussed in more detail in Appendix D. For ACSV, Cu-SA complexes adsorb to the surface of the mercury drop electrode and Cu^{2+} in the SA complexes on the drop is reduced during a negative potential scan, which produces a peak current with magnitude ip. The relationship between the current peak height, i, and $\Sigma[Cu(SA)_x]$, called the sensitivity, S:

$$\Sigma[Cu(SA)_x] = ip/S \quad (3.6),$$

must be determined in the absence of ligands that compete with SA for Cu, so that any additional Cu added ($\Delta[Cu]_T$) is complexed only by SA:

$$\Delta[Cu]_T \cong \Delta\Sigma[Cu(SA)_x] \quad (3.7).$$

S depends on both instrument settings (e.g. adsorption time and potential) and the sample matrix. The "internal calibration", a method commonly used to determine the sensitivity, assumes that for each titration, ligands in the sample are "titrated out" at higher $[Cu]_T$ and therefore Eq. 3.7 applies to this region of the titration. We found that this assumption is false for samples which contain humic substances or other mixtures of heterogeneous ligands, and we developed a more robust technique we call an "overload" titration (Kogut, 2001). For the overload titration method, at least two Cu titrations (with different and large concentrations of SA) that appear identical prove that SA at those

concentrations outcompetes all of the natural ligands present in the sample, so that Eq. 3.7 applies to this titration. The sensitivity of one overload titration is then used to determine titrations at lower [SA] ("speciation titrations") which are designed to allow the ligands in the sample to compete effectively with SA and therefore reveal information about the binding ability of those ligands.

We need correction factors to extrapolate S from overload titrations with high [SA] to speciation titrations with low [SA] because S depends somewhat on SA (see Appendix C). The correction factors are obtained by comparing S at high and low [SA] in sub-samples that have been UV-irradiated to destroy all ligands that could compete successfully with low [SA]. The correction factors depend in part on instrument settings as well as sample salinity and pH, may be related to changes in Cu-SA speciation on the surface of the mercury drop during CSV analysis (Campos, 1994; Appendix D), and range in value from 0.3 to 1.0 in the samples we have analyzed. We successfully used correction factors with overload titrations to relate peak heights to $\Sigma[Cu(SA)_x]$ in humic extract solutions (Appendix D).

3.2.7. Setup for Cu overload and speciation titrations.

For overload and speciation Cu titrations, 10.0 ml of the sample was pipeted directly into the electrode's Teflon® sample cup. A buffer adjusted to the pH desired was added for a final concentration of 0.02 M. SA (1, 3, 5, or 10 μM for speciation titrations and 100 μM for overload titrations and $[Cu]_{T,0}$ analysis) was also added to the sample at this time. Copper from the secondary standard was added sequentially after each titration point for a range of added $[Cu]_T$ from 10 to 200 nM. Before each voltammetric analysis, samples were allowed to equilibrate for 3 minutes. Overlapping overload titrations at 25 and 50

μM SA for the 0.5% Saco River sample show that 25 μM SA outcompetes all natural ligands for Cu (data not shown.) Overlapping titrations with different SA show that 3 minutes is adequate time for equilibration amongst Cu, SA, and the ligands present (see Results).

3.2.8. Cu speciation titrations.

We conduct multiple Cu titrations of each sample to collect data on a range of Cu ligands from those that control Cu speciation at a relevant range of $[Cu]_T$. For each titration point, in the absence of both SA and the Cu complexed by SA, the water sample would have an identical $[Cu^{2+}]$ and a theoretical total copper concentration $[Cu]_T^*$ that is given by:

$$[Cu]_T^* = [Cu]_T - \Sigma[Cu(SA)_x] = [Cu^{2+}] + \Sigma[CuL_i] \cong \Sigma[CuL_i] \quad (3.8),$$

where $[Cu^{2+}]$ is negligible compared to $\Sigma[CuL_i]$. The value of $[Cu^{2+}]$ is calculated from $\Sigma[Cu(SA)_x]$, $[SA]_T$, and the conditional stability constants of the *mono* and *bis* complexes, $K_{Cu(SA)}$ and $\beta_{Cu(SA)2}$:

$$[Cu^{2+}]=\Sigma[Cu(SA)_x]/(K_{Cu(SA)}[SA]_f+\beta_{Cu(SA)2}[SA]_f^2) \quad (3.9)$$

where $[SA]_f$ is the concentration of SA not bound to copper:

$$[SA] = [SA]_T - [Cu(SA)] - 2[Cu(SA)_2] \quad (3.10),$$

calculated using EXCEL to solve for the four unknowns in the four equations (Eqs. 3.9 and 10 and the two equilibrium mass law expressions for formation of Cu-SA complexes.) The presentation of Cu speciation data in plots of $[Cu^{2+}]$ versus $[Cu]_T^*$ shows the binding ability of the sample in the range of $[Cu]_T^*$ for which data was collected. Where more or stronger ligands are present in the sample, $[Cu^{2+}]$ is lower at any single value of $[Cu]_T^*$. The data can be modeled with FITEQL or another modeling program as concentrations of one or more ligands each with an average K_{CuLi}, but these

types of fits might not provide meaningful constants and should be used carefully (Voelker, 2001).

3.3. Results

3.3.1. Cu speciation in Saco River estuary

We hypothesize that riverine ligands in general are a major source of ligands to estuaries and coastal areas, even after dilution and potential decrease of their binding ability by cation competition and ionic strength effects. Because we further hypothesize that terrestrial humic substances can dominate Cu speciation in rivers and estuaries, we measured TOC and light absorption at 350 nm as potential tracers of humic substances in each sample of the Saco River estuary (Fig. 3.2). Non-conservative behavior indicates a source or sink of TOC in estuary, or that the system is not at steady state (Fig 3.1). .Absorbance of the samples at 350 nm shows similar behavior to that of the TOC and appears to be roughly proportional to TOC.

We determined the Cu binding ability of each sample using our new SA constants corrected for pH and salinity. Fig. 3.3(a-g) shows $[Cu^{2+}]$ as a function of $[Cu]_T$* in each sample, along with the corresponding salinity and pH (after the addition of buffer) for that sample. The pH at which Cu speciation was determined (controlled with added buffer) is usually within 0.2 pH units of the original pH of the sample. Heavy black lines in Fig. 3.3(b-g) represent the Cu binding ability of the ligands in the riverine end member (salinity = 0.5‰) (Fig. 3.3a) as if only dilution with seawater were important for decreasing their impact and no ocean sources of ligands are important. To do this, we

multiplied the concentration of ligands ($\Sigma[CuL_i]$) measured at each point in the titration by the dilution factor,

$$\text{dilution factor} = \frac{\text{salinity}_{\text{ocean}} - \text{salinity}_0}{\text{salinity}_{\text{ocean}} - \text{salinity}_1} \quad (3.11),$$

where $\text{salinity}_{\text{ocean}}$ is 33‰ (S in the Gulf of Maine varies from 32 to 34‰ depending on depth (http://www.csc.noaa.gov/crs/cruises/may96me/), salinity_0 is 0.5‰ and salinity_1 is the salinity of each estuarine sample. This method produces the same result as "diluting" the ligands (if we had ligand concentrations) and calculating a new $[Cu^{2+}]$ from the new $\Sigma[CuL_i]$: a shift to the left on a plot of $[Cu^{2+}]$ versus $[Cu]_T$*. The light dashed lines represent the calculated Cu binding ability of the ligands assuming that the content of ligands normalized to TOC remains constant within the estuary (for this to be the case, the TOC added in the estuary must have similar Cu binding characteristics to that in the riverine end member.) Dilution alone underestimates the loss in binding ability of these ligands (solid line), while normalization to TOC makes the difference between actual and predicted Cu binding slightly larger (although the difference is within experimental uncertainty.) The apparent decrease in Cu-binding ability with increase in salinity may indicate removal of ligands within the estuary. However, the total effect of increasing pH and increasing salinity within the estuary may be to decrease the ligands' binding ability. The data in Figure 3.3 do not allow us to distinguish this effect from removal of ligands.

3.3.2. Cu binding ability of riverine ligands with changes in pH and salinity

To test to what extent changes in pH affect the binding ability of the ligands in the sample at different salinities, we compared Cu titrations in samples identical except for pH. At low salinity (0.5‰), there is roughly a one-to-one inverse relationship between $[Cu^{2+}]$ and $[H^+]$ at any value of $[Cu]_T$* (Fig. 3.4). The pH effect decreases with an

increase in salinity, with practically no change in binding ability with an increase of over 1 pH unit at salinity=27‰.

3.3.3. The binding ability of riverine ligands diluted to S=27‰

The most straighforward way to test if the riverine ligands can account for the Cu binding ability of the 27‰ sample is to dilute the 0.5‰ sample to 27 ‰ and titrate the diluted sample with Cu at the same pH. When the 0.5‰ sample and 20.4‰ sample are adjusted to salinity=27‰ and pH=8.0 using UV irradiated Sargasso seawater (S=35‰) and boric acid buffer, the Cu binding ability of each sample is indistiguishable from that of the 27‰ sample (Fig. 3.5).

3.3.4. Internal calibration.

To evaluate the accuracy of the internal calibration when applied to Cu speciation data in the Saco River, we reinterpreted the raw titration data for the 0.5 and 27.6‰ Saco River samples at pH=8.0 (Fig. 3.6) employing internal calibrations (using the last three points in the titration (white symbols) for which the response of current peak height to added $[Cu]_T$, e.g. (Coale, 1988; Moffett, 1997). Values of $[Cu^{2+}]$ and $[Cu]_T$* recalculated with S obtained from internal calibrations (gray and white symbols) and "overload" titrations (black) symbols) show that internal titrations generally underestimate $[Cu]_T$* and overestimate $[Cu^{2+}]$ (Fig. 3.7).

3.4. Discussion

3.4.1. Internal calibration.

We argued previously that humic substances can interfere with the determination of ligand concentrations and conditional binding constants (Voelker 2001; Chapter 4). We show here that internal calibrations are not appropriate for determining the binding behavior of samples from the Saco River, which may contain humic substances or a heterogeneous mixture of ligands. The internal calibrations depend on the assumption that weaker ligands do not bind Cu in the presence of the concentration of the added ligand in the titration, but the weaker ligands in Saco River do compete with 1 and 5 μM SA.

Interestingly, the two titrations interpreted with internal calibrations happen to overlap in both cases shown here (Fig 3.7), which would lead the analyst to conclude that internal calibrations are safe to use for these samples. However, this overlap is an artifact of the fact that the difference in S of the1 uM titrations between internal and overload titrations are so much less than the difference for the 5 μM SA titrations (see Table 3.2). If the value of S determined by internal calibration is 40% of that of the "real" (ie overload) sensitivity, then the calculated value of $\Sigma[Cu(SA)_x]$ is overestimated by a factor of 2.5 (Eq. 3.6), as is $[Cu^{2+}]$ (Eq. 3.8). If S is overestimated by only 79%, then $\Sigma[Cu(SA)_x]$ and $[Cu^{2+}]$ are overestimated by a factor of only 1.3. The extent to which the internal calibration underestimates $[Cu]_T$* (or [CuLi] is less straightforward. At higher levels of $[Cu]_T$ in the titration where the relationship between current peak height and added $[Cu]_T$ appears linear, Eq. 3.7 is assumed to be valid, and the concentration of additional natural ligands titrated is "forced" to zero.

We previously recommended that any study of Cu speciation designed to determine the Cu binding ability of riverine and near-shore samples use overload titrations, using humic extract speciation data, artificial datasets, and coastal samples to show the potential and real magnitude of the problem (Kogut, 2001, Voelker, 2001, Chapter 4). We here again show with field data from the Saco River that the issues we discuss are similar to what we expected for humic substances.

3.4.2. Total organic carbon and color

We measured total organic carbon (TOC) in the estuary in order to possibly correlate TOC concentrations to the Cu binding ability of unfiltered samples. Total organic carbon was non-conservative through the Saco River estuary (Fig. 3.2), and it appears that there was a mid-estuarine source of TOC at or upstream of the salinity=10‰ location which increased TOC by factor of 1.3 to 1.5. DOC has been observed to be conservative in estuaries, but it is impossible to tell if such is the case for the Saco River estuary because of the large TOC input in the middle of the estuary. Researchers frequently measure dissolved organic carbon (DOC, the concentration of total organic carbon in a 0.2 μm filtrate), filtered possibly in order to sterilize the DOC samples or to avoid blocking the capillary tubes in the TOC analyzer. We found that matter removed with a 0.2 μm filter was responsible for some Cu binding in Waquoit Bay, MA (salinity=21‰) (Chapter 4), which justifies TOC measurements as a more likely indicator of the Cu binding ability of riverine samples. In addition, samples were not turbid, so that DOC was probably a large fraction of TOC.

Color, which is a tracer of terrestrial humic substances, also appeared to have a mid-estuarine source. Color was roughly correlated to TOC, suggesting that TOC was dominated by terrestrial humic substances.

3.4.3. Riverine ligands.

The Cu binding ability of the 0.5‰ sample, diluted to 27‰ with UV-SW and adjusted to pH=8.0, overlaps with that of 27‰ sample, suggesting that the riverine ligands can account for all the Cu binding at salinity = 27‰ within the uncertainty of the measurements (Fig. 3.5). If other ligands significantly contributed to the Cu binding ability of the 27‰ sample, then [Cu^{2+}] measured for this sample would be lower than the adjusted 0.5‰ sample. The same experiment conducted with a mid-estuary point (20‰) shows that the ligands present at mid-estuary ligands can account for all the Cu binding ability at 27‰ as well (Fig. 3.5). Based on these two points, riverine ligands dominate Cu speciation at both salinity = 20‰ and 27‰, and any mid-estuary source of ligands, such as sediments or microbial production, does not appear to be important in the Saco River estuary during this sampling period. The estuary is only one mile long; so the residence time of riverine ligands in the estuary is probably too short to allow for significant ligand destruction by microbial degradation or sunlight. The data do not rule out removal of riverine ligands and subsequent addition of ligands from other mid-estuary sources, but it is unlikely that the addition would substitute ligands of similar (within 10%) binding ability as the riverine ligands at that pH and salinity.

3.4.4. Humic substances and the Saco riverine ligands

There are several points that support our hypothesis that the riverine ligands are dominated by humic substances. The lack of clear inflection points in the Cu speciation

data for all samples indicates that a complex mixture of ligands of different strengths bind Cu, consistent with Cu titrations of humic substances (Kogut, 2001). The 0.5‰ sample, when diluted to 27‰ and adjusted to pH=8, can be modeled as 0.4 C/l humic carbon based on our SRHA titrations (Fig. 3.5). This concentration of SRHA is reasonable compared to TOC in the Saco River estuary even in the 27‰ sample. If riverine TOC is diluted conservatively, we predict that roughly 0.5 mg C/L of the 0.8 mg/l TOC measured at 27‰ is riverine TOC (using Eq. 3.4), with the difference of 0.3 mg/l contributed from ocean and estuary sources. For seven nearby rivers in Massachusetts, the humic material collected on an XAD-8 column was 50-70% of total DOC (Breault, 1996). The modeled 0.4 mg/l C SRHA is about 80% of the riverine TOC, somewhat larger than the upper limit of the percentages measured but still reasonable. We assume for this argument that DOC is a large fraction of TOC; if DOC is signficantly less than TOC in the Saco River estuary, then humic substances would have to account for even more of the DOC. We also assume that humic substances from the Massachusetts Rivers bind Cu similarly to those in the Saco River estuary, but while humic substances from different sources may bind Cu differently at low Cu, they have been shown not to at higher Cu (Cabaniss, 1988). Finally, Breault and coworkers (1996) showed that the "fulvic" fraction (removed with an XAD-8 column) could account for all of the Cu binding by organic matter measured in Massachusetts rivers. Although they compared relatively weak binding by the fulvic fraction and riverine samples, their results support our hypothesis that reasonable concentrations of humic acid extract can be used to explain riverine Cu speciation data in the absence of any sign of another specific source of strong ligands (such as inflection points in the riverine titration data, Fig. 3.3-3.5)

If riverine humic substances dominate Cu binding in the 27‰ sample, salinity effects, partially masked by pH effects (which work in the opposite direction), must account for

the remaining difference between the actual data and the data predicted based on conservative dilution alone (Fig.3.3). The finding that salinity effects are of greater magnitude than pH effects differs from results obtained in an England estuary, where salinity and pH effects on riverine ligands appeared to cancel each other and the net result was an apparent behavior of conservative dilution of riverine ligands (Gardner, 1991). However, the authors used internal calibrations, which likely introduced errors in their quantification of ligand concentrations, especially in the low salinity samples. At any rate, because the pH effect varies with salinity (Fig. 3.4), and therefore any salinity effects would also change with changes in pH, it is difficult to quantify and predict the effects of salinity on the ligands as they travel through the estuary. These issues are complicated further by the fact that ionic strength effects on humic substances are likely not straightforward. For example, macromolecules such as humic substances can change molecular configuration (and potentially hinder access to binding sites) in response to local increases in their hydrophobicity when protons or cations neutralize negatively charged binding sites, (Schwarzenbach, 1993), without necessarily causing removal by flocculation and settling.

Flocculation and subsequent settling of humic substances is the only other reason for non-conservative decrease in binding ability of humic substances as they are transported through an estuary. That high molecular weight humic substances (>0.45 μm) flocculate and settle in several rivers (Sholkovitz, 1978) has been used to support assumptions that terrestrial humic substances are not important in coastal areas, but this assumption does not necessarily apply to the bulk pool of riverine humic substances. Conservative behavior of low molecular weight humic substances (<0.01 μm) has also been shown in these same rivers (Sholkovitz, 1978). Riverine Fe was conservative in the Saco River estuary (Mayer, 1982a) and Fe colloids at low salinity (0-10‰) could not be

ultracentrifuged (Mayer, 1982b), implying that the flocculation of Fe (and, we argue, any associated humic substances that co-flocculate with Fe) did not cause subsequent removal. However, because we saw significant inputs of TOC and color (at 350 nm) at 10 and 20‰, we cannot show that TOC and humic material was not removed in the Saco River estuary in our field study. A field study that combines filtration of samples or extraction of humic material on XAD columns with comparison of riverine ligand binding in mixtures of seawater and appropriate pH could show whether we can relate humic concentrations and ligand behavior (Breault, 1996).

Why didn't we see other ligand sources in the Saco River? We did not need the presence of ligands autochthonous to the estuary to explain Cu binding in the 27‰ sample. But microbial activity in October might be low in the Saco River, so that our results do not argue against the importance of microbially produced ligands in the spring and summer in the Saco River. Reduced sulfides are potentially important in the Saco River estuary because the muddy banks smelled sulfidic. But any sulfide present in the water column in concentrations above $[Cu]_T$ (about 3-6 nM) would not be stabilized by complexation with Cu against rapid oxidation (Rozan, 1999), so we think that sulfide did not contribute significantly to Cu binding at higher Cu concentrations. Our copper speciation data at values of $[Cu]_T$* below 6 nM, where sulfide could be stabilized by complexation with Cu, are too sparse in most samples (because of purposely low deposition times to avoid surfactant effects) to make a statement about the role of sulfide at low $[Cu]_T$. Finally, we cannot explain why an increase in DOC and color at 20 and 27‰ did not correspond with an increase in Cu ligand ability, although the addition expected would almost be within our analytical uncertainty (compare Fig. 3.3.e and f).

Other estuaries have different Cu ligand sources and might have different Cu speciation that what we saw in the Saco River in October. In warmer, more nutrient-rich estuaries, microbial ligands may play a role in buffering Cu toxicity (Leal, 1999; Moffett, 1997), and the Saco River itself might have an important microbial input in the spring and summer. In estuaries with higher Cu, sulfide may be stabilized by Cu and therefore play a larger role in Cu speciation, as it appeared to in Cu polluted Connecticut rivers (Rozan, 1999d). In estuaries with sewage and runoff inputs of Cu ligands such as those reported in the San Francisco Bay (Sedlak, 1997), Cu also might be more tightly bound than it would be by riverine humic substances alone.

Finally, our measurements of Cu speciation in estuarine samples at lower pH and salinity are dependent on the assumption stated in Chapter 2 that the salinity effect at lower pH is the same as that measured at pH = 8.0. Preliminary calibration experiments at pH = 6.5 and salinity = 1 and 2‰ suggest that we overestimated the salinity effect at low pH when we assumed that the salinity effect at pH = 6.5 is the same as that at pH = 8.0 (Chapter 2, Eqs. 2.23a,b). If we overestimated the salinity effect on K_{CuSA} and $\beta_{Cu(SA)2}$ at low pH then we overestimated K_{CuSA} and $\beta_{Cu(SA)2}$ at low salinity and pH and therefore Cu speciation data at low salinity and pH would be shifted upwards (due to an increase in calculated $[Cu^{2+}]$, Eq. 3.9).

If we overestimated the salinity effect on K_{CuSA} and $\beta_{Cu(SA)2}$ at low pH, our interpretation of the estuarine trends shown in Figs. 3.3 and 3.4 would also change. For Fig. 3.3, only the Cu titration of low salinity samples at pH = 7.0 would shift upwards. The solid black lines, based on the titration data in the 0.5‰ sample, might lie on or above the titration data for the other samples at higher salinity and pH. If the lines do lie above the titration data for other samples, this would suggest that riverine ligands cannot account for all the

binding in those samples. In Fig. 3.4, the titration data in the 0.5‰ sample at low pH (6.5, 7.0, and 7.7) would be shifted upwards as well. This shift upwards would cause the dependence of the Cu binding ability of the riverine ligands on pH at low salinity to appear larger and therefore cause the trend in decreasing effect of pH on Cu binding ability with increasing salinity more dramatic.

3.5. Conclusions.

We show that riverine ligands dominate Cu speciation throughout the entire Saco River estuary (up to a salinity of 27.6‰). We can model the Cu binding ability of the riverine ligands in the 27.6‰ sample as similar to that of a reasonable concentration of riverine humic extract at 35‰, allowing for variation in the fraction of TOM that is humic substances. That humic substances dominate Cu speciation in the Saco River estuary emphasizes the importance of considering the affect of humic substances on the determination of the concentrations and conditional stability constants of proposed specific ligands from other sources. An allochthonous source of terrestrial Cu ligands can serve to buffer Cu concentrations in estuaries with large Cu inputs in the absence of other significant ligand inputs to an extent greater than usually recognized in estuarine Cu speciation studies emphasizing other ligand sources.

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Table 3.1. Measurements in the Saco River estuary: salinity, pH (ambient), TOC, color, and total unfiltered Cu.

Sample number	Salinity (ppt)	pH	TOC (mg C/L)	Color (absorbance units)	$[Cu]_T$ (nM)
1	0.5	7.2	3.2	0.043	2.5
2	0.7	7.3	3.4	0.056	3.9
3	1.0	7.2	3.1	0.054	6.2
4	2.2	7.0	3.1	0.050	4.9
5	10.1	7.1	3.1	0.046	2.6
6	20.4	7.5	2.4	0.040	2.5
7	27.6	7.8	0.8	0.016	4.0

Table 3.2. Comparison of determined sensitivities obtained with overload and internal calibrations. S is measured in peak height (nA) per nM $\Sigma Cu(SA)_x$ (Eq. 3.6).

Sample and titration	S (overload) (nA/nM)	S (internal) (nA/nM)	Ratio S(internal) to S(overload)
0.5‰			
1 μM SA	0.29	0.12	0.41
5 μM SA	0.32	0.25	0.79
27.6‰			
1 μM SA	0.19	0.16	0.80
5 μM SA	0.26	0.23	0.88

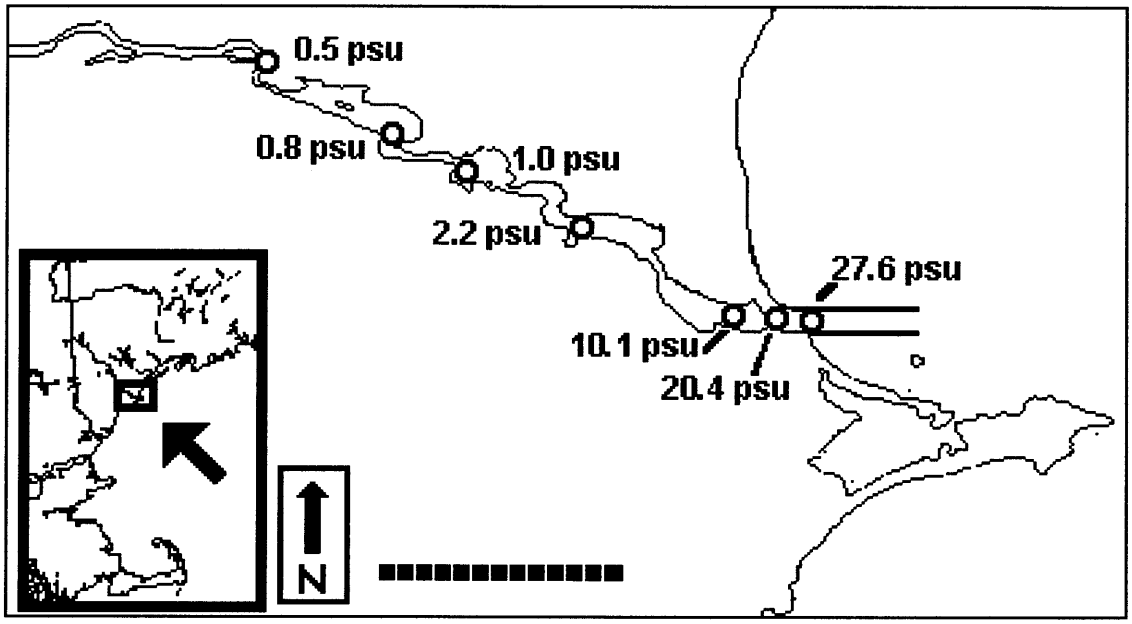

Fig. 3.1. Map of the Saco River Estuary, including sampling locations and salinities measured.

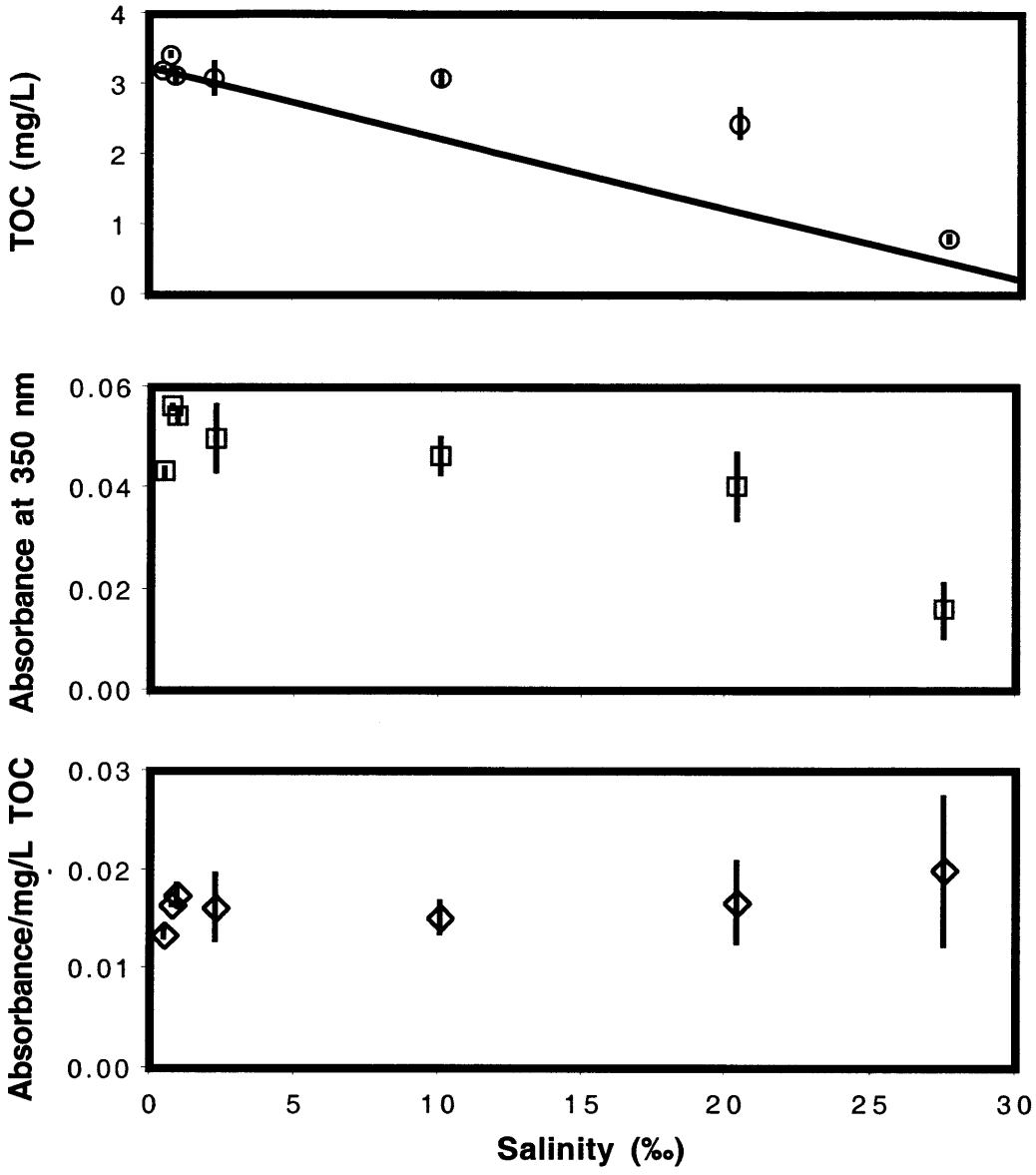

Figure 3.2. Concentations of TOC, absorbance at 350 nm, and ratios of absorbance to TOC versus salinity for the seven Saco River estuary samples.

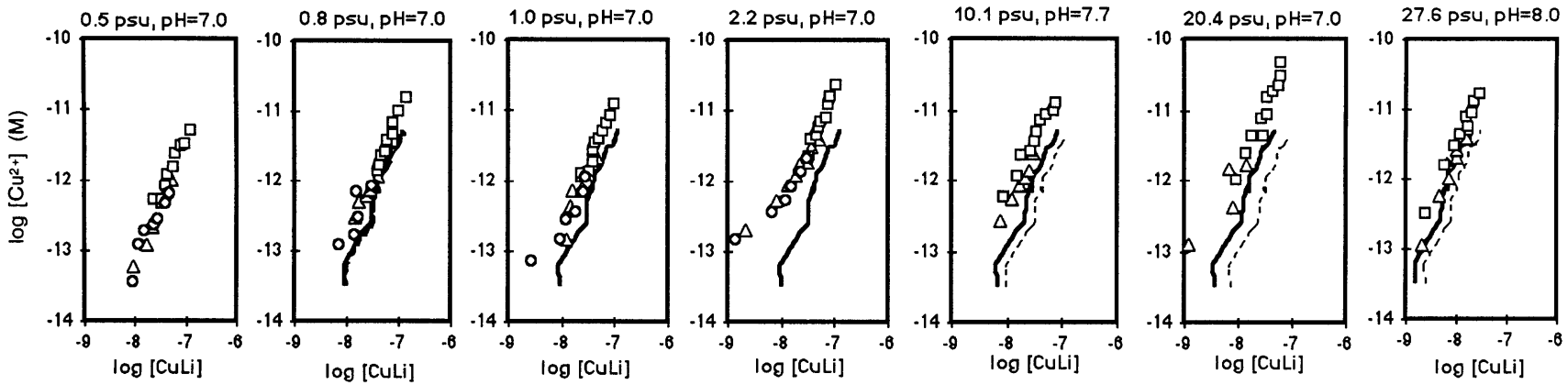

Fig. 3.3. The Cu binding ability of samples collected in the Saco River Estuary. Heavy solid lines represent the Cu binding ability expected if the Cu binding ligands of the 0.5 ‰ salinity sample were diluted by ligand-free seawater to the salinity of each of the other samples (using Eq. 3.11). Dashed lines represent the Cu binding ability expected if the Cu binding ligands of the 0.5 ‰ sample are normalized to measured TOC concentrations (Fig. 3.2).

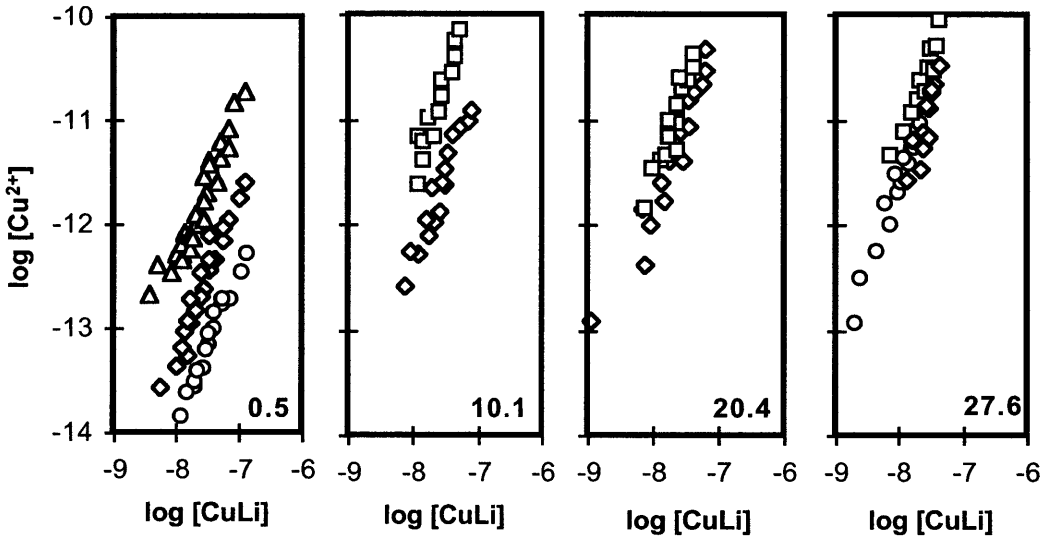

Fig. 3.4. The copper binding ability of ligands in Saco River estuary samples (0.5, 10.1, 20.4, and 27.6 ‰) at four values of pH (△, 6.5; □, 7.0; ◊, 7.7; ○, 8.0.)

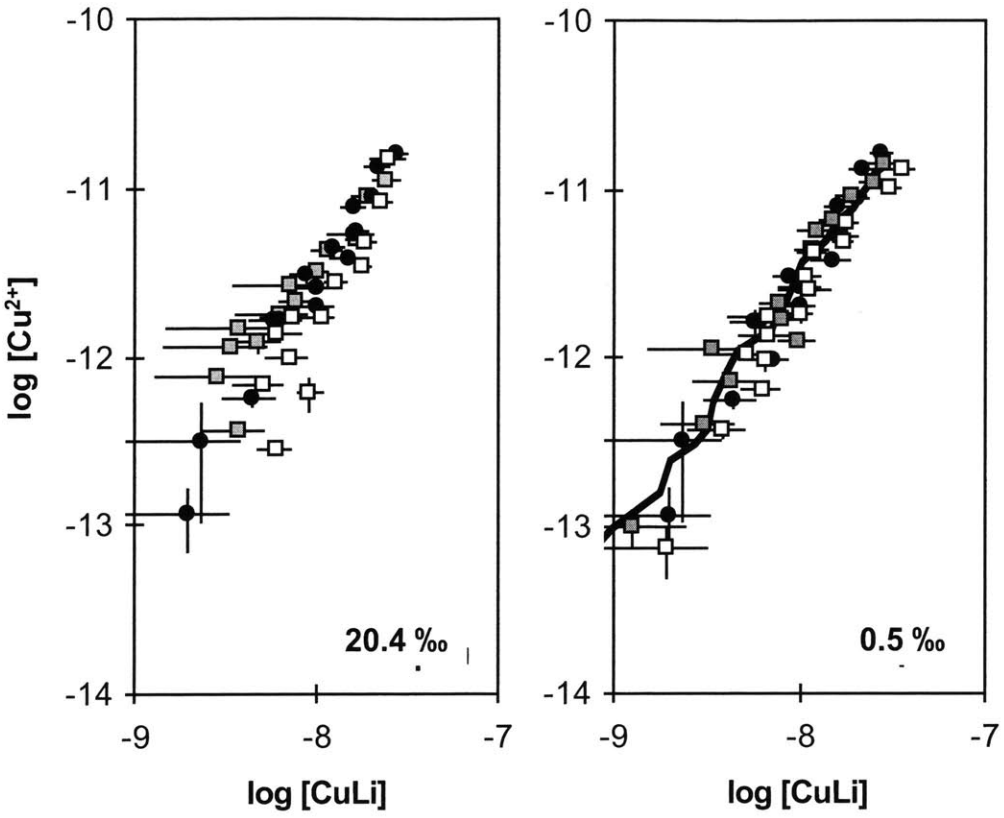

Fig. 3.5. The Cu binding ability of the filtered (gray squares) and unfiltered (white squares) 20.4‰ and the 0.5‰ samples when adjusted to pH = 8.0 and salinity 27.6‰ with UV irradiated seawater, and compared to the Cu binding ability of the 27.6‰ sample at pH=8.0 (●). Line in 0.5‰ sample represents the Cu binding ability of 0.4 mg C/l Suwannee River Humic Acid in UV-irradiated seawater (see text and Appendix D).

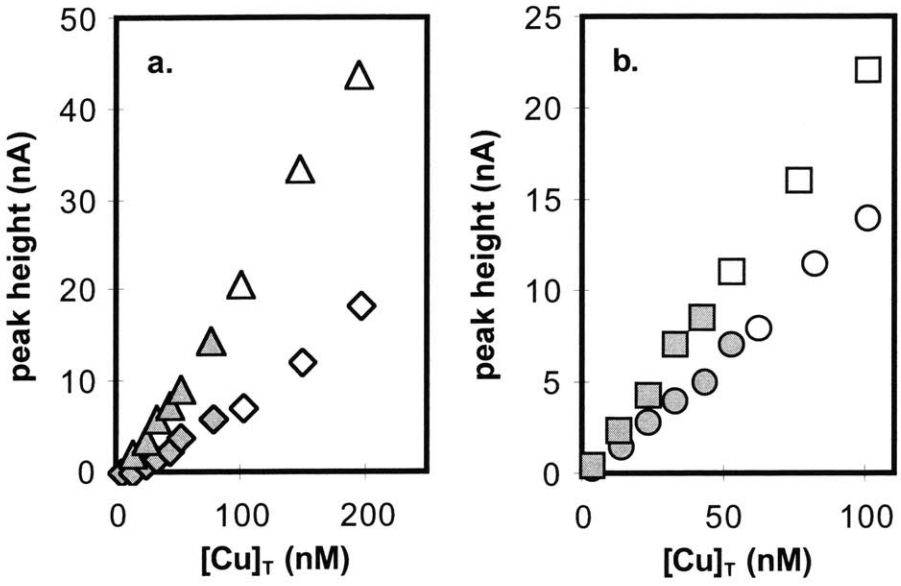

Figure 3.6. a). Raw data for a) titrations (diamonds,1 μM SA; triangles, 5 μM SA) of the 0.5‰ sample and b) for titrations (circles,1 μM SA; squares, 5 μM SA) of 27.6‰ Saco River estuary samples, both at pH = 8.0. White symbols are used for internal calibrations (results shown in Fig. 3.7.)

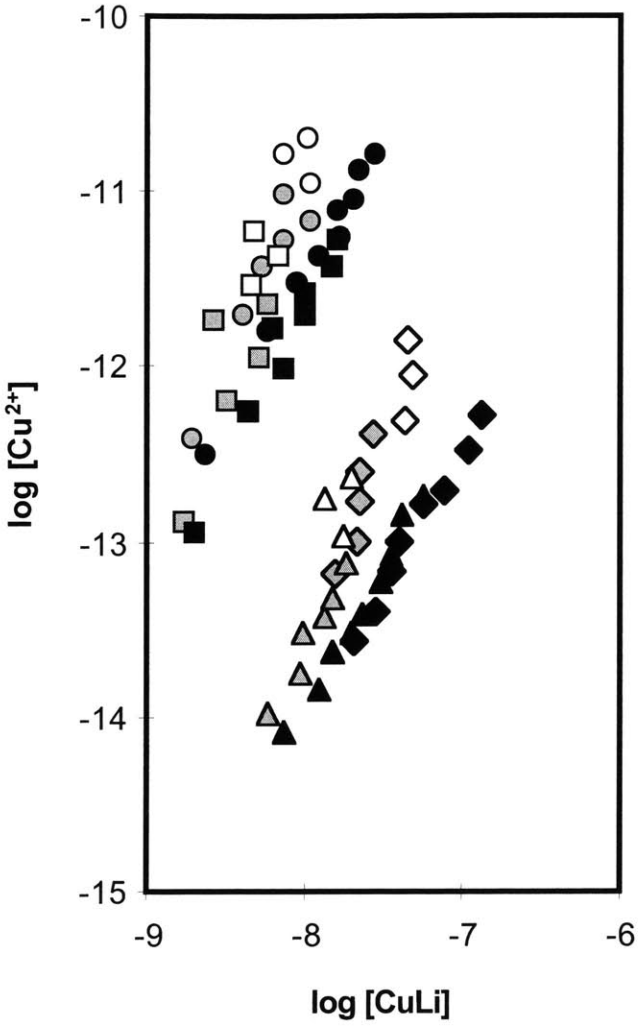

Figure 3.7. The titrations in Fig. 3.6 (0.5 and 27.6‰ Saco River samples at pH=8.0) interpreted with internal calibrations through the white symbols. Black symbols are the same data interpreted with overload titrations (see text.)

Chapter 4. Kinetically inert Cu in coastal waters

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Abstract.

Many studies have shown that Cu and other metals present in natural waters are mostly bound by unidentified compounds interpreted to be strong ligands which bind metals reversibly. However, commonly applied analytical techniques are not capable of distinguishing strongly but reversibly bound metal from metal bound in kinetically inert compounds. In this work we use a modified Competitive Ligand Exchange Adsorptive Cathodic Stripping Voltammetry (CLE-ACSV) method combined with size fractionation to show that all of the apparently strongly bound Cu in samples from five New England coastal waters (1 to 18 nM, 10 to 60% of total Cu) is actually present as kinetically inert compounds. In three of the five samples examined by ultrafiltration, a significant portion of the 0.2 um-filtrable inert Cu was retained by a 0.02 um pore size filter, suggesting that at least some of the Cu was kinetically inert because it was physically sequestered in colloidal material. The rest of the ambient Cu, and Cu added in titrations, was reversibly bound in complexes that could be modeled as having conditional stability constants of 10^{10} to 10^{12}. The Cu binding ability of these complexes was equivalent to that of seawater containing reasonable concentrations of humic substances from terrestrial sources, (0.15-0.45 mg C/l). Both the inert compounds and the reversible ligands are important for determining $[Cu^{2+}]$ at ambient Cu levels.

4.1. Introduction

Elevated copper concentrations due to anthropogenic inputs can be toxic to microorganisms and algae in coastal areas (*e.g.* Sunda, 1976; Hall, 1997). In coastal areas, the concentration of "free" Cu ($[Cu^{2+}]$) is a small but variable fraction of total dissolved Cu ($[Cu]_T$) because most Cu is complexed by ligands (L_i) in the water column, so that

$$[Cu]_T=[Cu^{2+}]+\Sigma[CuL_i] \quad (4.1),$$

where $\Sigma[CuL_i]$ is the sum of all the Cu complexes with different types of ligands. Although the mechanisms of Cu toxicity to sensitive organisms such as phytoplankton are not completely understood, $[Cu^{2+}]$ is generally thought to be a better predictor of Cu toxicity than $[Cu]_T$ ((Sunda, 1976; Meyer, 1999; Anderson, 1978).

To predict $[Cu^{2+}]$ it is necessary to understand the sources and behavior of the strong Cu ligands controlling Cu speciation. It is usually stated that if we knew the conditional stability constants (K_{CuLi}) and total concentrations ($[L_i]_T$) of these ligands, we could determine $[Cu^{2+}]$ as a function of total Cu using the equation:

$$K_{CuLi}= [CuL_i]/([Cu^{2+}][L_i]_f) \quad (4.2),$$

in which $[CuL_i]$ is the concentration of copper bound to ligands of class i, and

$$[L_i]_f = [L_i]_T - [CuL_i] \quad (4.3).$$

However, we do not know whether all Cu ligands complex Cu reversibly in natural waters, so this equilibrium approach may not be applicable in all situations. We need to understand the identity and fate of Cu complexes, as well as the extent to which ligands bind Cu reversibly, to better predict the processes controlling $[Cu^{2+}]$ in natural waters. .

A number of studies have focused on determining the sources and chemical identities of strong Cu ligands in coastal waters. In seawater systems and laboratory cultures,

phytoplankton and other microorganisms have been shown to produce ligands. For example, two species of marine microalgae, *Synechococcus sp.* and *Emiliania huxleyi*, have been observed to excrete strong copper-complexing ligands when copper stressed in culture, most likely as a defense mechanism against copper toxicity (Moffett, 1996; Leal, 1999). Strong Cu ligands not attributable to plankton may also exist in near-shore systems. Ligands in estuarine sediment pore waters could represent a significant source of strong ligands to Chesapeake Bay if they are stable in oxic waters (Skrabal, 2000). Similarly, Sedlak et al. (1997) found that treated sewage effluents and creeks are a source of strong ligands to South San Francisco Bay. These ligands are not necessarily organic; Rozan et al. (1999d, 2000) have shown that sulfide compounds resist oxidation in oxic waters and could account for 10 to 60% of the total strong copper complexes in Connecticut rivers. Copper titrations of Suwannee River humic and fulvic acids show that humic substances can be responsible for moderately strong Cu binding in near-shore waters with terrestrial humic inputs (Kogut, 2001).

There is substantial evidence that some of the compounds binding Cu in coastal waters are colloidal. For example, Cu speciation studies combined with ultrafiltration in Galveston Bay, Texas showed that the "colloidal" fraction of organic matter contained stronger ligands than the "ultrapermeate" of molecular size < 1 kDa (Tang, 2001). In Narragansett Bay, Rhode Island and in two rivers in England, a significant portion of the strong Cu ligands were retained by ultrafilters (1 kDa and 3 kDa, respectively, Wells, 1998; Muller, 1996a). Colloidal Cu species could belong to several of the "ligand" categories discussed in the previous paragraph. For example, humic substances are expected to be partially retained by 1-3 kDa ultrafiltration membranes (Averett, 1989), and Rozan and Benoit (1999d) found evidence of colloidal metal sulfide species in Connecticut rivers.

The presence of strongly bound colloidal Cu species also raises the possibility that the Cu is bound in kinetically inert forms. For example, micelles and colloidal humic substances could physically trap Cu in hydrophobic interior "microenvironments" (Gustafsson, 1997). Colloidal "sea onions", composed of layers of organic matter and metal and with diameters as small as 10 nm, have also been proposed as a form of inert metal (Mackey, 1994). The colloidal physical sequestration hypothesis was invoked previously to explain the very slow release (hours to days) of Cu from Newport River estuary samples upon acidification to pH 2 (Sunda, 1991). If some concentration of Cu in the sample is kinetically inert ($[Cu]_{inert}$) due to colloids, then Eq. 4.1 should be modified so that

$$[Cu]_T=[Cu^{2+}]+\Sigma[CuL_i]+[Cu]_{inert} \quad (4.4)$$

The apparent strength and specificity for Cu of ligands observed in many natural waters has been cited as evidence that they are compounds of biological origin, perhaps "designed" specifically for the purpose of complexing Cu, but the possibility that the Cu appears to be strongly bound because it is kinetically inert is seldom considered (Mackey et al, 1994).

A simple way to show that inert Cu is absent from a natural water sample is to perform competing ligand exchange with high concentrations of a very strong competing ligand; if all of the Cu is released from the natural compounds and bound by the competing ligand, none of the Cu is inert. If some Cu is "non-exchangeable" in competitive ligand exchange, then that fraction of the Cu is either irreversibly bound (and perhaps physically sequestered) or reversibly complexed by ligands too strong to exchange Cu with the competing ligand. We are aware of only two studies in which close to all of the Cu present in a coastal water sample was shown to be exchangeable. In Vineyard Sound, almost all Cu was exchangeable (Moffett, 1997), and in the Tamar Estuary (S=33.8 ‰),

about 95% of 11.8 nM total Cu was exchangeable (van den Berg, 1990). Both of these works used competing ligand exchange with a range of competing ligand binding strengths and concentrations to more fully characterize the speciation of Cu in the samples.

Many studies report concentrations and conditional binding constants of the strongest ligands found from manipulated data without showing the raw data or discussing release of all Cu from the natural ligands. The most common approaches to obtaining Cu speciation data at low (ambient) Cu levels, anodic stripping voltammetry (ASV) and competing ligand exchange adsorptive cathodic stripping voltammetry (CLE-ACSV) with a single competing ligand strength, are not capable of distinguishing inert Cu from strongly bound Cu. Furthermore, as discussed in Voelker et al. (2001), if a mixture of ligands is present in the water sample, reported total ligand concentrations and binding constants represent a somewhat arbitrary blending of contributions from different types of ligands. For example, in a sample that contains both inert Cu and humic substances, concentrations of strong ligands and conditional stability constants extracted from titration data do not accurately reflect the properties of either of these ligand types. The problem is worsened by internal calibration errors introduced by neglecting the presence of weaker ligands in the sample. These considerations raise the concern that the strongest ligand class reported in previous studies includes any inert Cu.

The goal of this study was to determine the extent to which kinetically inert Cu is important in polluted coastal waters, where high concentrations of colloidal material and Cu may make it especially likely that significant concentrations of inert Cu are present. We determined Cu speciation within the range of Cu typical for polluted coastal areas using a combination of filtration and CLE-ACSV. We measured the concentration of Cu

non-exchangeable to CLE using very large concentrations of the competing ligand salicylaldoxime (SA) (Kogut, 2001). In addition, we filtered the samples with 0.2 and 0.02 μm pore size filters to determine the concentrations of non-exchangeable Cu in each size fraction. Because we found that weaker binding sites in humic substances (or any mix of weaker Cu ligands) can interfere with the common internal calibration method for CLE-ACSV, we used a new calibration method which is accurate even in near-shore samples where humic substances and other weak ligands may be present (Voelker, 2001; Campos, 1994). We show that our approach to obtaining and interpreting CLE-ACSV speciation data is necessary for accurate characterization of Cu speciation in near-shore waters.

4.2. Methods

4.2.1. Sample Collection

Samples were collected in December, 2001 and January, 2002 about 20 meters offshore of the University of Rhode Island's Narragansett campus, in Narragansett Bay, RI; near docks on Waquoit Bay and Eel Pond on Cape Cod; and 100 meters offshore in Quincy Bay and Dorchester Bay, Boston Harbor (Fig. 4.1). An additional sample was collected from the Saco River estuary, Maine, for use as a low Cu control sample. Offshore samples were collected either from a polyethylene kayak or an aluminum rowboat. One liter acid-cleaned Teflon® bottles were filled with sample water just below the surface and upcurrent of the dock or boat to minimize contamination. UV oxidized Sargasso seawater (UV-SW) was collected in August 1997 with trace metal clean methods and subjected to at least four hours of ultraviolet light oxidation with a mercury lamp (Ace Glass, 1000 W). Acid-cleaned Teflon® or polycarbonate containers were used for all

reagents and experiments to minimize adsorption of copper and ligands to bottle surfaces and leaching of phthalate plasticizers into the samples.

To make buffered UV-SW, 1 ml of UV-oxidized 1 M boric acid (EM Science, Suprapur®), adjusted to pH of 8.2 with 0.35 M ammonia, was added to one liter of UV-SW for a final buffer concentration of 1 mM. Deionized distilled water (DDW) was from a Millipore Q-H_2O system. Fresh 1 mM primary and 1 μM secondary copper standards were made every day by serial dilutions of an Aldrich atomic absorption standard solution ($CuSO_4$ in 1% HNO_3, 10,000 ppm Cu) with DDW; the final pH of the secondary copper standard was about 7. Salicylaldoxime (Aldrich) was purified by equilibration with EDTA and repeated filtering and recrystallization (Norrman, 1993). SA was dissolved in DDW for a final stock solution concentration of 0.025 M. SA stock solutions were replaced monthly and refrigerated when not in use.

4.2.2. Filtration of Cu speciation samples

We chose the conventional (and convenient) 0.2 μm filter and 0.02 μm ultrafilter pore sizes. Samples were syringe-filtered through 0.2 μm polycarbonate membrane filters (Nucleopore, 47 mm filter diameter) sandwiched in polycarbonate filter holders. Subsamples of this 0.2 μm filtrate (dissolved fraction) were filtered again through 0.02 μm pore size inorganic membrane cartridge filters (Anatop-25, 22 mm filter diameter). The filters were acid-cleaned and rinsed with DDW before use.

We tested the filters for addition and sorption of Cu and ligands by filtering a solution of 10 nM Cu and 100 μM SA added to UV-SW (which has about 1.5 nM Cu). SA was added to minimize sorption of dissolved Cu to the filters that would not occur in the

presence of natural Cu ligands. Subsamples of the filtrates were UV-irradiated for 6 hours to destroy any ligands leached off the filter that might complex Cu and decrease the concentration of Cu measurable by CLE-ACSV. Total Cu after both sets of treatments was measured by CLE-ACSV (see Section 4.2.6). The total Cu concentrations in the filtrates and ultrafiltrates after both treatments were indistinguishable from the expected concentration of 11.5 nM (Fig. 4.2). Contamination from the filters of Cu and ligands strong enough to outcompete 100 μM SA was therefore negligible.

4.2.3. Dissolved organic matter and salinity

Samples for dissolved organic carbon (DOC) measurements were filtered using pre-combusted glass syringes and polysulfone cartridge filters, acid washed and rinsed with DDW according to the recommended procedure (Campos, 1994). Samples were acidified to pH=4 with phosphoric acid (J.T. Baker) and concentrations of DOC were measured using a Shimadzu TOC-5000 analyzer calibrated with diluted solutions of a 1mg/ml potassium acid phthalate standard (VWR Scientific). Salinity was measured with a portable conductivity meter (VWR Scientific) calibrated with UV-SW (S=35 psu).

4.2.4. CLE-ACSV

Competitive Ligand Exchange Adsorptive Cathodic Stripping Voltammetry (CLE-ACSV) is an ideal method with which to investigate Cu speciation because the method can be adjusted to measure both stronger and weaker Cu ligands. CLE-ACSV is a two-part process: first, a known concentration of a well-characterized and purified synthetic "added ligand", AL, is allowed to equilibrate with a series of samples containing a range of added copper. Salicylaldoxime (SA) is a very strong, well-characterized ligand whose Cu binding strength has been calibrated with EDTA (Chapter 2) and which is not

expected partition into organic matter (Appendix B). In the presence of SA, Cu species include free Cu, Cu reversibly complexed by ligands in the sample, any kinetically inert Cu, and Cu complexed by SA:

$$[Cu]_T = [Cu^{2+}] + \Sigma[CuL_i] + [Cu]_{inert} + \Sigma[Cu(SA)_x] \quad (4.5)$$

where

$$\Sigma[Cu(SA)_x] = [Cu(SA)] + [Cu(SA)_2] \quad (4.6)$$

and $Cu(SA)$ and $Cu(SA)_2$ are the *mono* and the *bis* complexes of copper with SA, respectively (both complexes are important at the concentrations of SA used). By adding large concentrations of SA, one can decrease $\Sigma[CuL_i]$ to values much lower than ambient $\Sigma[CuL_i]$, so that natural ligands in the sample release Cu to maintain thermodynamic equilibrium amongst Cu and ligands at the same total Cu concentration (Eq. 4.5). However, if inert Cu is present, then $\Sigma[Cu(SA)_x]$ can only be as great as the difference between $[Cu]_T$ and $[Cu]_{inert}$.

4.2.5. Overload titration

We have developed a new protocol for distinguishing "surfactant effects" from complexation when calibrating the CLE-ACSV technique (Voelker, 2001; Campos, 1994). For ACSV measurements, the relationship between the current peak height, I, and $[Cu(SA)_x]$, is called the sensitivity, S:

$$[Cu(SA)_x] = I/S \quad (4.7)$$

S depends on both instrument settings (e.g. adsorption time and potential) and the sample matrix. To avoid problems with using external and internal calibrations to determine S in the presence of compounds that may sorb to the mercury drop surface and hinder the simultaneous sorption of $Cu(SA)_x$ (the surfactant effect), we developed a technique called an "overload" titration (Kogut, 2001). The overload titration is similar to the frequently

used internal calibration in that the sensitivity is measured directly in the water sample to be analyzed. However, a concentration of SA is used that is shown to be great enough to outcompete all of the natural ligands present in the sample so that no apparent decrease in sensitivity due to Cu complexation by natural ligands can occur. Any additional Cu added ($\Delta[Cu]_T$) is complexed only by the added ligand SA, so that:

$$\Delta[Cu]_T \cong \Delta\Sigma[Cu(SA)_x] \quad (4.8)$$

The slope of I versus $[Cu]_T$ should then be equal to the sensitivity S. In order to show that $[SA]_T$ is sufficiently high to outcompete all exchangeable natural ligands, the analyst conducts titrations at two or more different concentrations of SA; if the titration slopes are identical, then no exchangeable natural ligands are competing with SA at those concentrations. Since overload titrations yield no information on exchangeable Cu ligands, speciation titrations with lower concentrations of SA also need to be performed on the same sample.

Correction factors are needed to extrapolate sensitivity from overload titrations to speciation titrations because the sensitivity of measurements conducted with 1-10 uM $[SA]_T$ (speciation titrations) is not the same as with 25 or higher uM $[SA]_T$ (overload titration). We and others hypothesized that the change in SA speciation (i.e. the ratio of $[Cu(SA)]$ to $[Cu(SA)_2]$) resulting from an increase in $[SA]_T$) affects the sensitivity because only $Cu(SA)_2$ is reduced at the mercury drop during the ACSV step (Campos, 1994; Kogut, 2001). We compared the slopes of standard curves obtained with varying $[SA]_T$ in UV-SW and determined reproducible correction factors (as percent of the sensitivity measured using the 25 μM $[SA]_T$ titration) of 55% (± 3%), 78% (± 4%), and 92% (± 5%) for 1, 3, and 5 μM SA, respectively (Appendix D). The sensitivity does not change appreciably for SA concentrations greater than 25 μM, so that no correction factors are needed to compare overload titrations (for this work, titrations with greater

than 25 μM $[SA]_T$). Correction factors differ negligibly for samples at 21 psu, the lowest salinity of samples examined here (Kogut, 2001).

The use of overload titrations for calibration requires that the surfactant effect, if any, be independent of $[SA]_T$. We have observed that this is not necessarily the case in samples exhibiting strong surfactant effects. However, in the samples considered in this work, overload titrations with $[SA]_T$ of 25 μM consistently exhibited the same slope as titrations of UV irradiated samples with the same $[SA]_T$, indicating that no surfactant effect was present and that the external calibration in UV irradiated samples was therefore also valid. Nevertheless, the overload titrations were indispensable for showing the lack of a surfactant effect. In addition, use of overload titrations and correction factors allowed us to determine the sensitivity of several Cu speciation titrations simultaneously. Finally, overload titrations were also used to determine the concentration of non-exchangeable Cu, as discussed below.

4.2.6. Total Cu

Samples from each filter fraction were acidified to pH < 2 with concentrated nitric acid (J. T. Baker Instra-Analyzed) and irradiated with ultraviolet light from a medium pressure mercury lamp (Ace Glass, 1000 W) for at least six hours (ten hours for unfiltered samples) in quartz 125 ml tubes. We assume that direct UV irradiation combined with hydroxyl radical formation from nitrate destroy both organic matter and inorganic matrices (e.g. sulfides) within six hours. Samples were then brought to circumneutral pH and prepared for voltammetric analysis as discussed below. With all ligands destroyed, $[Cu(SA)_x]$ should be equal to $[Cu]_T$ in the sample. Values of $\Sigma[Cu(SA)_x]$ measured after six and ten hours of UV irradiation agreed within error.

4.2.7. Exchangeable and non-exchangeable Cu after 48 hours

We used competing ligand exchange with very high concentrations of the competing ligand SA to determine whether significant concentrations of non-exchangeable Cu, which we believe to be kinetically inert Cu species (see discussion section), were present in our samples. For each sample, we added as much SA as is feasible (the maximum $[SA]_T$ is a function of the solubility of SA in a concentrated stock solution) to outcompete as many exchangeable Cu ligands in the sample as possible. SA from standard solutions was added to each 30 ml sample for $[SA]_T$ concentrations of 0.001 M (for January 2002 samples only), 500 μM, or 50 μM. The samples were allowed to equilibrate in the dark at 4°C and subsamples were taken for ACSV analysis after 24 and 48 hours. Exchangeable Cu was determined as the concentration of $\Sigma[Cu(SA)_x]$ at the ambient $[Cu]_T$ of the sample, measured using standard additions of Cu . The concentration of non-exchangeable Cu was then calculated as the difference between the concentration of total Cu (determined in UV-irradiated samples) and exchangeable Cu,

$$[\text{non-exchangeable Cu}] = [Cu]_T - [\text{exchangeable Cu}] \quad (4.9).$$

4.2.8. Cu speciation titrations

For determination of the binding ability of exchangeable ligands, $[SA]_T$ must be low enough so that it does not outcompete the exchangeable ligands of interest and high enough so that the natural ligands do not complex all of the added Cu. For each titration point, in the absence of both SA and the Cu complexed by SA, the water sample would have an identical $[Cu^{2+}]$ and a theoretical total copper concentration $[Cu]_T^*$ (Moffett, 1997; Kogut, 2001) that is given by:

$$[Cu]_T^* = [Cu]_T - \Sigma[Cu(SA)_x] = [Cu^{2+}] + \Sigma[CuL_i] + [Cu]_{inert} \cong \Sigma[CuL_i] + [Cu]_{inert} \quad (4.10),$$

where $[Cu^{2+}]$ is negligible compared to $\Sigma[CuL_i]$. The value of $[Cu^{2+}]$ is calculated from $\Sigma[Cu(SA)_x]$, [SA], and the conditional stability constants of the *mono* and *bis* complexes, $K_{Cu(SA)}$ and $\beta_{Cu(SA)2}$:

$$[Cu^{2+}] = \Sigma[Cu(SA)_x]/(K_{Cu(SA)}[SA]+\beta_{Cu(SA)2}[SA]^2) = \Sigma[Cu(SA)_x]/SRC(SA) \quad (4.11)$$

where [SA] is the concentration of SA not bound to copper:

$$[SA] = [SA]_T - [Cu(SA)] - 2[Cu(SA)_2] \quad (4.12),$$

calculated using EXCEL to solve for the four unknowns in the four equations (Eqs. 4.11 and 4.12 and the two equilibrium mass law expressions for formation of Cu-SA complexes.)

Cu speciation titrations were calibrated with a 25 μM $[SA]_T$ overload titration sample on the day of speciation titration analysis. Because the sensitivity of CLE-ACSV in the filtered and unfiltered samples were identical for all samples (based on comparisons of the 50 and 500 μM SA overload titrations), 25 μM SA overload titrations were conducted only on the unfiltered samples, but used to determine the sensitivity of both filtered and unfiltered samples.

4.2.9. General titration setup

For all Cu titrations ($[Cu]_T$ determinations, overload titrations at 50 and 500 μM SA for "non-exchangeable" Cu, Cu speciation titrations, and overload titrations at 25 μM SA for calibration of speciation titrations), the sample analyzed was a 10.0 ml aliquot of the sample pipetted directly into the electrode's Teflon® sample cup. Boric acid buffer, adjusted to pH=8.0 with ammonia, was added for a final concentration of 0.02 M. SA was also added to the sample at this time, except for the 50 and 500 μM SA titrations, where SA had been added 24 or 48 hours previous to ACSV analysis. Copper from the

secondary standard was added sequentially to the sample after each titration point's voltammetric analysis of $\Sigma[Cu(SA)_x]$, for a range of $[Cu]_T$ from 0 to 300 nM for Cu speciation titrations, and 0 to 50 nM for all other titrations. Before each analysis, samples were allowed to equilibrate for 3 minutes for the speciation titrations, and 1 minute for all other titrations. Cu speciation data from titrations with different concentrations of SA which overlap show that 3 minute equilibration times are adequate for these samples (see Results.)

4.2.10. Voltammetric analyses

$\Sigma[Cu(SA)_x]$ in the samples was analyzed using adsorptive cathodic stripping voltammetry. This analysis consists of two steps: an adsorption step, during which $Cu(SA)_x$ complexes are sorbed to a mercury drop electrode, and a potential scan in the negative direction, during which the current produced by the reduction of the copper in the sorbed complexes is measured. We used differential pulse ACSV with a PAR 303A static mercury drop electrode and an EG&G PAR 394 analyzer. Instrument settings were as follows: adsorption potential, -0.08 V (versus Ag/AgCl electrode); scan range, -80 to -600 mV; scan rate, 20 mV/s; drop time, 0.2 s; pulse height, 25 mV. The time for the adsorption step was 0 seconds (December sampling) or 10 seconds (January sampling). Because there is additional time for adsorption during the potential scan up to the potential at which Cu-SA complexes are reduced, the "effective" adsorption time is about 3 or 13 seconds. It was deemed necessary to use such short adsorption times to eliminate surfactant effects. The reduction of Cu in the $Cu(SA)_2$ complex produced a well-defined peak at -330 to -400 mV in the potential scan (Campos, 1994; Kogut, 2001).

4.2.11. Modeling Cu speciation with FITEQL

Two different methods were used to generate model fits for the December data. In both kinds of model fits, average non-exchangeable Cu concentrations were determined *a priori* from overload titrations (50 and 500 μM $[SA]_T$) of the 24 and 48 hour equilibrated samples, and the data points from overload titrations were not used in determining additional model parameters. Non-exchangeable Cu was then included in equilibrium calculations as Cu complexed to a ligand with an extremely large stability constant (log K = 40).

The first kind of model (Model 1) assumed the presence of one or several ligands with fixed conditional stability constants (the justification for this is discussed extensively in Voelker, 2001), in addition to non-exchangeable Cu. FITEQL (Westall, 1982) was then used to determine the best fit total ligand concentrations. The raw data ($\Sigma[Cu(SA)_x]$ versus $[Cu]_T$) was entered into FITEQL together with estimates of precision and accuracy of $\Sigma[Cu(SA)_x]$ measurements. FITEQL fits thus minimized the difference between observed and modeled $\Sigma[Cu(SA)_x]$ values.

The second kind of model fit (Model 2) assumed that a spectrum of ligands with the same properties as Suwannee River humic substances (SRHA) (from Kogut, 2001) was present in addition to "non-exchangeable" Cu. The only fitting parameter for the "exchangeable" ligands in this model was the concentration, in mg/l, of humic substances. Since FITEQL could not be applied for this type of fit, we used Sigmaplot 4.00's non-linear curve fit routine to minimize the difference between $[Cu]_T$* of the model and of the data as a function of $[Cu^{2+}]$.

4.3. Results.

4.3.1. Cu speciation

We measured both salinity and DOC in the samples in December 2001, and salinity only in January 2002 (Table 4.1.) Salinity ranged from 28 to 29‰ for all samples except Waquoit Bay, which was sampled near the Childs River and had significant freshwater input. Concentrations of DOC in these samples ranged from 1.8 to 3.0 mg C/l.

We measured Cu speciation in filtered and unfiltered samples from five coastal locations collected during December 2001, using a large range of $[SA]_T$ (1 to 500 μM) to obtain as much speciation information as possible. Figures 4.3a-e show the variation in $[Cu^{2+}]$ as a function of $[Cu]_T^*$ for the filtered samples. Each titration data point corresponds to a measurement of $\Sigma[Cu(SA)_x]$ and $[Cu]_T$, from which the quantities $[Cu]_T^*$ and $[Cu^{2+}]$ can be calculated using Eqs. 4.10 and 4.11. Speciation titrations with different $[SA]_T$ (1, 3, 5, and 10 μM) that agree at one value of $[Cu]_T^*$ suggest that Cu, ligands, and SA are at equilibrium at that point and that all other assumptions associated with ACSV analysis are also valid. The original $[Cu]_T$ values in the filtered samples are indicated as vertical dashed lines intersecting the x-axis at the corresponding values of $[Cu]_T^*$.

Significant concentrations of Cu (1 to 20 nM) did not exchange with SA even at the largest concentrations of SA used (50 and 500 μM). For these samples, the concentration of non-exchangeable Cu did not change significantly between 24 and 48 hours (24 hour data not shown). The highest concentrations of both total Cu (30 to 40 nM) and non-exchangeable Cu (15 to 20 nM) were found in Eel Pond and Waquoit Bay (Fig. 4.3a,b). Smaller concentrations of both total and non-exchangeable Cu were found in samples from the more open coastal locations (Fig 4.3c-e).

We compared different model fits of the titrations of the December samples to determine whether the data for the exchangeable Cu are consistent with a humic-like spectrum of weaker ligands, and whether there is any evidence for the presence of more strongly bound (but exchangeable) Cu in any of the samples (Table 4.2 and lines in Fig. 4.3(a-e)). We attempted to fit all five data sets to a one-ligand model (Model 1a; log K = 10; thin line) and a three-ligand model (Model 1b; log K values 13, 11.5, and 10; medium line). For all samples, FITEQL failed to converge when all three ligand concentrations were used as fitting parameters, because one of the three ligands always failed to contribute significantly to the fitting result. We therefore generated the two-ligand fits shown in Table 4.2 by setting the insignificant ligand's concentration at zero. In all five samples, Model 2, for which we assumed that only a humic-like spectrum of ligands was present in addition to non-exchangeable Cu, yielded good results (Model 2; heavy line in Fig. 4.3(a-e)). In the samples from Eel Pond, Quincy Bay, and Dorchester Bay, the fits assuming the presence of a single weak ligand (Model 1a) looked significantly worse than the fits assuming a humic-like spectrum. The differences between each model and the data were insignificant compared to the uncertainty associated with the Cu speciation measurements, especially at $[Cu]_T$* values close to $[Cu]_{inert}$.

We also measured Cu speciation in unfiltered samples taken in December 2001. For most of the samples, Cu speciation in the unfiltered subsamples was similar to that of the filtered samples (data not shown). In Waquoit Bay, some exchangeable ligands were removed by filtration with a 0.2 μm filter (Fig. 4.4.)

4.3.2. Internal calibrations and ligand "best fits"

In order to determine the extent to which weaker ligands interfere with internal calibrations in near-shore samples, we re-interpreted the raw "speciation titration" data for the filtered December sample for Eel Pond using internal calibrations. Fig. 4.5 shows the raw titration data at 1, 5, and 10 μM SA for these two samples. The last three points of each titration (white symbols), which appear to have a linear response of current peak height to added $[Cu]_T$, were used to determine the sensitivity of the titration (Coale, 1988; van den Berg, 1992; Moffett, 1997). The sensitivity of the titrations determined with internal calibrations were 81, 88, and 96%, respectively, of those determined with overload titrations.

Values of $[Cu^{2+}]$ and $[Cu]_T^*$ recalculated with S obtained from internal calibrations (gray and white symbols) and overload titrations (dotted symbols) show that internal calibrations can lead to underestimates of $[Cu]_T^*$ and overestimates of $[Cu^{2+}]$ (Fig. 4.6). At higher $[Cu]_T^*$ in each titration, the internal calibration "defines out of existence" weaker ligands that bind Cu and therefore causes large errors in both variables. As we stated earlier, overload titrations at 50 and 500 μM SA produced the same slope S, thereby proving that ligands in the sample bind Cu negligibly and that Eq. 4.8 is applicable.

To complete the common approach to interpretation of CLE-ACSV titrations, we fit the Eel Pond Cu data interpreted with internal titrations to obtain ligand "class" concentrations ($[L_i]_T$) and K_{CuLi} with the Langmuir linearization technique (as described in Miller, 1997). Langmuir linearizations of the 1 μM SA, 5 and 10, 50, and 500 μM SA titrations, shown in Fig. 4.7, fit the manipulated data very well. But, the same linearizations, shown in Fig. 4.6, fail to fit the raw data at $[Cu]_T^*$ lower than about 9.7

nM, the concentration of non-exchangeable Cu in the sample. The non-exchangeable ligand must have a $K_{CuL}>10^{16.6}$ to fit the raw data of the 500 μM titration (double dotted dashed line in Fig. 4.6; Table 4.3).

Values of $[L]_T$ and K_{CuL} obtained from the Langmuir linearizations are similar to those reported for other coastal samples (Table 4.3). The concentration of $[L]_T$ decreases with increasing $[SA]_T$ until a constant (within uncertainty) value of $[L]_T$ is approached (but K_{CuLi} continues to increase dramatically). The trend in $[L]_T$ suggests the presence of non-exchangeable Cu, but the use of Langmuir linearizations (or other linearization techniques) can mislead the analyst to believe that K_{CuLi} is measureable.

4.3.3. Filtration

To further constrain the size of both exchangeable ligands and non-exchangeable Cu, we filtered samples collected from the same sites in January 2002 with 0.2 and 0.02 μm filters (Fig. 4.8a-e). We modified the Cu speciation approach used for the December samples by using a larger concentration of SA (1 mM) and omitting Cu titrations at lower [SA] to obtain information only on whether the Cu already present in the samples was non-exchangeable or exchangeable. In addition, we used a longer adsorption time to increase our sensitivity. As with the December samples, the concentrations of non-exchangeable Cu did not change between 24 and 48 hours for these samples.

Significant concentrations of non-exchangeable Cu were found in nearly all of the samples, with the possible exception of the Narragansett Bay ultrafiltered sample. Cu in the January 2002 Waquoit Bay in particular was almost 80-90% non-exchangeable,

compared to about 40% in the December Waquoit Bay sample. For the other samples, roughly 40 to 60% of the Cu was non-exchangeable, similar to the December samples.

Neither non-exchangeable nor exchangeable Cu was found to be primarily associated with any particular size fraction in all samples from January 2002. For example, non-exchangeable Cu in both the Waquoit Bay (Fig. 4.8b) and Eel Pond (Fig. 4.8a) samples was only partially removed by the 0.02 μm filter. In Narragansett Bay, non-exchangeable Cu was partially retained by the 0.2 μm filter with most or all of the remainder completely retained by the 0.02 μm filter. A significant fraction of the exchangeable Cu was also retained by the 0.2 μm filter, especially in the Eel Pond and Narragansett Bay samples (Figure 4.8a,c). No significant fraction of "exchangeable" Cu was retained by the 0.02 μm filters in any of the samples

4.4. Discussion

4.4.1. Kinetically inert Cu

Our results show that a significant fraction of the ambient Cu in the coastal waters we examined was present in non-exchangeable form. Non-exchangeable Cu could be non-exchangeable for two reasons. First, it could be bound in a form that does not release Cu to SA to a significant extent within 48 hours even though formation of the $Cu(SA)_x$ complexes is thermodynamically favored. We propose that a 48 hour equilibration time qualifies as a time span for which we could consider such complexes "kinetically inert", based on comparison to typically used equilibration times for CLE-ACSV and for physical transport times in coastal waters. The second possibility is that the Cu is bound so strongly that negligible formation of $Cu(SA)_x$ should occur at equilibrium. We used the highest concentration possible of a very strong added ligand, but even 1 mM SA ($SRC(SA) = 10^{8.5}$, see Eq, 4.11 for definition of SRC(SA)) is not strong enough to rule out

very strong ligands with conditional binding constants greater than about $10^{17.3}$ (estimated as shown for Fig. 4.6, except using the January titrations with 1 mM SA) present at concentrations equal to or lower than that of "non-exchangeable" Cu for each sample.

However, we can show that Cu complexed by dissolved ligands with a binding constant greater than $10^{17.3}$ should also not dissociate to a significant extent within 48 hours, and therefore also fits our definition of "kinetically inert." Following the approach of Witter and Luther (Witter, 1998), the conditional binding constant of CuL, in this case with respect [Cu'], the total concentration of inorganically complexed Cu, is related to the rate constants of complex formation (k_1) and dissociation (k_2):

$$K'_{CuL}=k_1/k_2 = \frac{[CuL]}{[Cu']_f[L]_f} \qquad (4.14),$$

If L is a small molecule, the maximum possible value of k_1 is the diffusion-limited rate constant of ~10^{10} $M^{-1}s^{-1}$, which would only apply if all of the species making up Cu' could react with all of the species making up "L_f" to form CuL at a diffusion-limited rate. Given that K'_{CuL} is greater than $10^{15.9}$, (equivalent to a K_{CuLi} defined with respect to $[Cu^{2+}]$ of $10^{17.3}$), we use Eq. 4.14 to determine that k_2 must be less than $1.3x10^{-6}$ s^{-1}, so that at most 20% of the complex would dissociate in 48 hours. While in the presence of 1 mM SA an associative ligand exchange mechanism is possible, so that attaining equilibrium in a 48 hours competing ligand exchange experiment would not be impossible, such a mechanism is much less likely to occur for ligands present at much lower concentrations in natural waters (Hering, 1990). In summary, then, we argue that non-exchangeable Cu, as defined by our 1 mM SA ligand exchange experiments, must be kinetically inert on time scales of at least 48 hours, whether it is tightly bound to small molecules or present in physically sequestered colloidal forms.

It is possible that inert Cu was present in many of the natural water samples examined by previous studies, whose methods would not have been able to distinguish strongly but reversibly bound Cu from kinetically inert Cu. In addition, the total strong ("L1") ligand concentrations reported in previous studies could include contributions of both kinetically inert Cu compounds and reversibly bound Cu compounds. To illustrate these points, we interpreted the data we obtained from titrations at lower SA concentrations (1, 5, 10, and 50 and 500 μM SA, or SRC(SA) = $10^{3.5}$, $10^{4.3}$, $10^{4.8}$, $10^{6.0}$, and $10^{7.9}$) using a common linearization technique to extract values of $[Li]_T$ and K_{CuLi} from the data (Table 4.3). The speciation data from any one of these titrations would be interpreted by these techniques as Cu binding by a single strong ligand of measurable K_{CuLi}. When a small SRC(SA) was used, the observed value of $[Li]_T$ included contributions of weaker ligands, while at higher SRC(SA), the observed $[Li]_T$ approached the concentration of kinetically inert Cu, while K_{CuL} increased with increasing SRC(SA). The trends of decreasing $[Li]_T$ and increasing K_{CuLi} observed with increasing SRC of the added ligand have been recognized previously (van den Berg, 1990; Bruland, 2000), but not in the context of kinetically inert Cu. The "correct" SRC(SA) for quantifying only inert Cu depends on the concentration of the other ligands present in the sample.

In general, only a lower limit on the value of K_{CuLi} of the strongest ligand can be obtained from titration data unless there are speciation data at values of $[Cu]_T$* smaller than the concentration of the total ligand concentration (Fig. 4.6). This is equivalent to showing that there is no non-exchangeable Cu. In most published studies, raw data are not shown and the range of $[Cu]_T$* examined is not stated explicitly, so that it is impossible to tell if the strongest Cu ligands in the sample were actually too strong to be measured directly by the method employed (or kinetically inert). To avoid such ambiguities, we recommend reporting titration data in the form of log$[Cu^{2+}]$ versus log$[Cu]_T$* plots, or at least

reporting K_{CuL1} values as lower limits and admitting the possibility that kinetically inert Cu is present, unless experiments were performed to show that all Cu is exchangeable to a sufficiently high concentration of added ligand.

While our data represent a more complete look at Cu binding compounds in five NE coastal sites, our findings are mostly consistent with the earlier work performed at these sites. Wells et al. found significant concentrations of Cu bound by ligands with $K_{CuL}>10^{11}$ with respect to Cu' (Wells, 1998), which could include both inert Cu and some of the more strongly bound exchangeable Cu. However, a significant fraction of the strongly bound Cu was found to be colloidal (size cut-off 8 kDa) only in the upper Narragansett Bay, but not in the lower bay, closer to where our samples were collected. Bruland et al. (2000) reported the presence of a 0.2 um filtrable ligand with a K_{CuL1} of $10^{15.7}$ in a Narragansett Bay sample, implying that a significant fraction of Cu remained "non-exchangeable" at an SA concentration of 55 μM. However, if only weaker ligands and inert Cu were present in this sample, the L_T they observed with increasing SRC(SA) should have approached a limiting value (as in our Table 4.3 for Eel Pond) instead of decreasing; it is unclear whether this difference in their result and ours can be attributed to experimental uncertainty or whether other ligand types were also present in their sample. In Waquoit Bay, a previous study observed that 25 μM SA could not outcompete about 8 nM Cu ($K_{CuL}>10^{14.1}$) (Moffett, 1997).

Our finding of kinetically inert Cu in the particulate and colloidal size fractions of these coastal samples supports the hypothesis that colloidal material physically sequesters a large fraction of the total Cu, thereby rendering it kinetically inert to ligand exchange. We never found an excess of non-exchangeable ligands over $[Cu]_T$, and, in the Saco River estuary ($[Cu]_T$ = 4 nM), we did not find significant concentrations of non-

exchangeable Cu (data not shown). These results suggest that the existence of inert Cu is dependent upon stabilization by previous incorporation of Cu into colloids such as micelles and colloidal humic substances (Gustafsson, 1997) and "sea onions" (Mackey, 1994). Although it is difficult to place a lower limit on the size range for material that can physically sequester copper, we think it is possible that colloids that pass through a 0.02 μm filter may still sequester Cu. If this is true, then physical sequestration could account for the kinetically inert Cu we found in all of our size fractions.

Sulfides are also candidates for the formation of kinetically inert Cu. Reported conditional stability constants of "simple" 1:1, 1:2 and 2:3 Cu sulfide complexes indicate that 1 mM SA should easily be able to outcompete sulfide for Cu (Al-Farawati R., 1999; Zhang, 1994; Luther, 1996). However, there is growing evidence for the existence of Cu-sulfide species that are either kinetically inert or far more stable. Rozan and coworkers (1999d, 2000) have reported significant concentrations of Cu-sulfide complexes in rivers (13-60% of total 0.2 um filtrable Cu), some of them colloidal (> 3 kD), whose quantities they inferred from measuring the release of sulfide upon acidification in a pH range (5.0 to 2.8) where only Cu-sulfide compounds should dissociate. These sulfide-releasing compounds persisted on a time scale of several weeks in an oxygenated river water sample, under conditions where one would expect dissociation of "simple" Cu-sulfide complexes to occur, based on reported equilibrium constants. A more recent work also presents mass spectroscopic evidence for the existence of kinetically or thermodynamically stable Cu-sulfide clusters in oxygenated river waters (Rozan, 2000). In addition, Luther et al. (1996) observed formation of Cu-S compounds inert to dissociation at pH < 2, which they attributed to partial Cu(II) reduction and formation of polysulfide species upon titration of micromolar concentrations of Cu(II) with HS^- (Luther, 2002). While it is not clear whether any of these Cu-S compounds would be

able to exchange Cu with 1 mM SA, these studies suggest that it is likely that kinetically inert Cu-S species are formed in sulfidic environments which can persist for some time in oxygenated systems.

Finally, microbial ligands are also a candidate for kinetically inert Cu. Croot and coworkers (2000) found that some of the Cu added to filtered cultures of cyanobacteria formed compounds electrochemically inert to reduction even at very negative potentials. Phytoplankton are known to exude hydrogen sulfide (Walsh, 1994), so formation of the Cu-S compounds discussed in the previous paragraph is a possibility, although it is not clear whether such compounds will form at the low concentrations of sulfide expected to be present in phytoplankton cultures. Another class of compounds known to be produced by microorganisms are thiols (Leal, 1999), but titration data of Cu bound by thiol compounds shows that these compounds will release Cu to SA instead of forming non-exchangeable species. Since the studies examining formation of strong ligands in phytoplankton cultures use the same titration and data interpretation techniques as the studies of natural water samples, the same ambiguities regarding the presence or absence of kinetically inert Cu species exist. Further studies using the techniques discussed in this work are needed to determine whether Cu compounds inert to ligand exchange are actually formed in phytoplankton cultures. However, it does not seem likely that microbial exudates are responsible for formation of colloidal (>0.2 μm) kinetically inert Cu species.

4.4.2. Exchangeable ligands

The exchangeable ligands can be modeled as reasonable concentrations of a terrestrial humic acid (Model 2 in Table 4.2). It is generally assumed that humic substances from

different sources do not differ greatly in their metal-binding abilities. If the humic substances present in our field sites are able to complex Cu similarly to SRHA in seawater (Fig. 4.3a-e), they would need to be present at concentrations of approximately 0.15 to 0.45 mg C/l (calculated from the model output in Table 4.2 using a ratio of carbon weight to total weight of approximately 0.5 (Aiken, 1985) to account for the exchangeable ligands in these samples. DOC concentrations in these samples range from 1.5 to 2.8 mg /l (Table 4.1). In Waquoit Bay, the sample of lowest salinity (21 psu), highest DOC, and highest concentration of "exchangeable" ligands, humic substances are most likely to be the only important exchangeable ligands. But concentrations of "humic-like" ligands were still substantial (about 30% of that of Waquoit Bay) at the Narragansett Bay site, which is well-flushed with seawater. Work using lignin as a tracer of terrestrial humic substances shows that they account for up to 20% of total DOM even miles offshore (salinity > 30‰) (Moran, 1991), so a terrestrial humic material could still account for this binding, although an autochthonous input of ligands from microorganisms or nearby sediments might be important as well. There was no correlation between DOC and modeled humic concentrations, consistent with our expectation that humic carbon does not make up a constant fraction of DOC in coastal waters. In a related study, we show that riverine inputs account for all of the exchangeable ligands present in the Saco River estuary, up to a salinity of 27‰ (Chapter 3).

Our data also indicate that particulate species represent a part of the pool of exchangeable ligands in some systems. Some of the weaker ligands present in the December Waquoit Bay sample were removed with the 0.2 μm filter (Fig. 4.4), but not in the other December samples. In the samples collected in January 2002, 10 to 60% of the exchangeable Cu is lost by 0.2 um filtration in all samples, indicating binding by particulate species.

In summary, while we have shown that humic substances could account for all of the exchangeable ligands we observed in our samples, we cannot rule out that other types of exchangeable ligands are also present. Our model comparisons (Table 4.2) show that there is little difference in the ability of various models to fit our titration data, and a humic-like spectrum of ligands in not necessarily present in our samples.

4.5. Conclusions

A much larger data set is needed to determine whether Cu speciation in our systems and other coastal waters can be accounted for by a simple hypothesis, for example, a single source of non-exchangeable Cu and a riverine source of exchangeable ligands. All of our samples were collected in winter, when microbial populations are relatively small and sulfide fluxes from sediments are also expected to be lower. Further seasonal fieldwork in locations of spring and summer microbial blooms and sulfate reduction will show if these sources contribute to kinetically inert Cu or weaker ligands, and whether the exchangeable "L1" strength ligands reported during other summer studies (e.g. Bruland 2000; Moffett, 1997) are distinct from the Cu binding compounds we found in this study. As we show in this study, distinguishing between "inert" and strongly bound exchangeable Cu, and using calibration techniques capable of accurately quantifying weaker ligands, will be crucial for making reliable analyses of spatial and temporal variability in concentrations of different types of Cu-binding compounds.

That the Cu does not exchange with high concentrations of SA even after 48 hours does not imply that this Cu should be exempt from regulation by the Environmental Protection Agency, for example. One might argue that this Cu is not toxic because it is not bioavailable. However, because we do not know the identity of the inert Cu complexes,

we cannot predict the fate and transport of the Cu complexes during the 48 hour time span. For example, the ligands might degrade in sunlight or be destroyed by microorganisms and therefore release Cu more quickly than we would predict based on our experiments equilibrated under dark and (for the 0.2 and 0.02 μm filtered samples) likely sterile conditions.

Another important unresolved question is how formation of inert Cu compounds affects $[Cu^{2+}]$ in coastal systems. If a substantial portion of the total Cu in the water is not bound in inert compounds, the concentration and binding characteristics of the exchangeable ligands will be more important for determining $[Cu^{2+}]$ than the exact quantity of inert Cu. However, a number of studies have observed a total strong ligand concentration very close to that of total Cu in coastal samples (e.g. Moffett, 1997; Muller, 1998). If the strong binding reported in these studies is actually due to formation of inert Cu compounds, determining the circumstances under which the concentration of inert Cu approaches $[Cu]_T$ will be critical for predicting the behavior of $[Cu^{2+}]$ in natural waters.

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Table 4.1. Sampling dates and locations, with measured salinity, DOC, total, and "non-exchangeable Cu (in nM) in the unfiltered and filtered samples. Inert Cu reported is the average measured after 24 and 48 hours. ("na" means that the measurement was not taken.)

Sample date and location	**Salinity (‰)**	**DOC (mg/l)**	**unfiltered**		**filtered 0.2 μm**		**filtered 0.02 μm**	
			$[Cu]_T$	$[Cu]_{inert}$	$[Cu]_T$	$[Cu]_{inert}$	$[Cu]_T$	$[Cu]_{inert}$
December 27, 2001								
Eel Pond	27	1.8	32	14	26	9.7	na	na
Waquoit Bay	21	2.3	42	21	32	13.9	na	na
Quincy Bay	28	2.3	8.8	5.0	8.1	4.3	na	na
Dorchester Bay	28	3.0	10.5	3.5	8.1	2.6	na	na
Narragansett Bay	28	2.1	14	5.9	13	5.2	na	na
January 4, 2002								
Eel Pond	27	na	37	18.5	27	17	21	11
Waquoit Bay	12	na	17	13	14	11	8.5	4.8
Quincy Bay	28	na	10	3.5	7.7	3.5	7.5	3.5
Dorchester Bay	28	na	12	6.0	7.8	2.5	7.7	3.5
Narragansett Bay	28	na	13	7.5	7.0	3.5	4.5	0.7

Table 4.2. Results of three different models of exchangeable ligands in December 2001 samples. In all three models, the following concentrations of non-exchangeable Cu were determined independently: Eel Pond, 9.7 nM; Waquoit Bay, 13.9 nM; Quincy Bay, 4.3 nM; Dorchester Bay, 2.6 nM; and Narragansett Bay, 5.2 nM.

Model 1a: 1-Ligand fit, log K fixed at 10				WSOS/DF[1]
	L_T (nM)			
Eel Pond	226±28			0.88
Waquoit Bay	421±61			0.79
Quincy Bay	192±31			0.36
Dorchester Bay	223±51			0.62
Narragansett Bay	93±22			0.13
Model 1b: 2-ligand fit				
	L1 (nM) log K 13.0	L2 (nM) log K 11.5	L3 (nM) log K 10.0	
Eel Pond	0	27±7	75±41	0.28
Waquoit Bay	0	9±12	311±157	0.79
Quincy Bay	18±8	0	76±53	0.04
Dorchester Bay	8±3	0	102±60	0.09
Narragansett Bay	2±2	0	81±27	0.10
Model 2: humic-like spectrum of ligands				
	mg/l humic acid [2]			
Eel Pond	0.61±0.03			0.54
Waquoit Bay	0.92±0.04			0.80
Quincy Bay	0.49±0.03			0.16
Dorchester Bay	0.53±0.05			0.31
Narragansett Bay	0.31±0.02			0.14

[1] Weighted sum of squares divided by degrees of freedom. A measure of goodness of fit; this is the parameter FITEQL attempts to minimize when determining a best fit.

[2] 1 mg/l SRHA corresponds to 1.36 nM L1T (log K 13.0), 15.5 nM L2T (log K 11.5) and 238 nM L3T (log K 10.0) (Voelker and Kogut, 2001).

Table 4.3. Results of interpretation of the filtered December 2001 Eel Pond Cu titration data with internal calibration and Langmuir linearization (see Fig. 4.6).

Langmuir linearization	log SRC(SA)	log K_{CuLi}	$[L_i]$ (nM)
1 μM SA	3.5	12.1	29.3
5 μM SA	4.3	12.6	12.6
10 μM SA	4.8	13.7	13.7
50 μM SA	6.0	14.9	10.9
500 μM SA	7.9	16.6	9.5

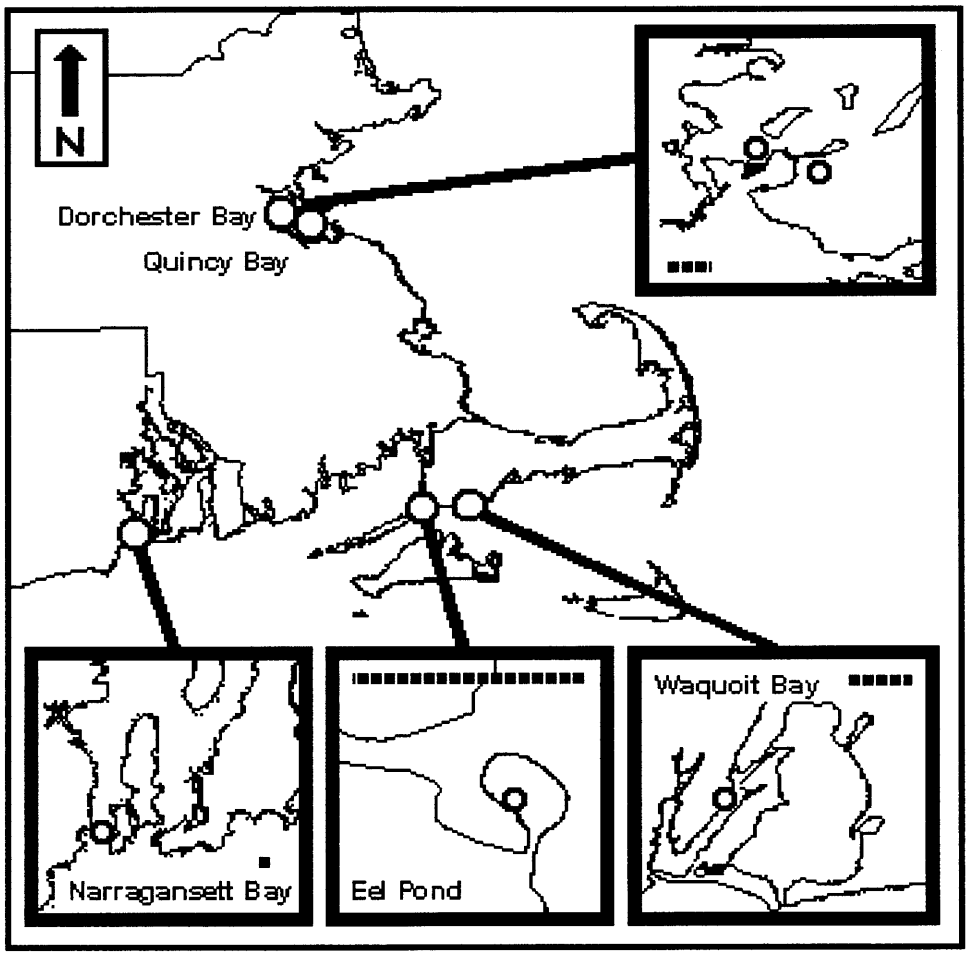

Fig. 4.1. Map of the five sampling sites. Striped bars represent a distance of 1 mile in each site close-up.

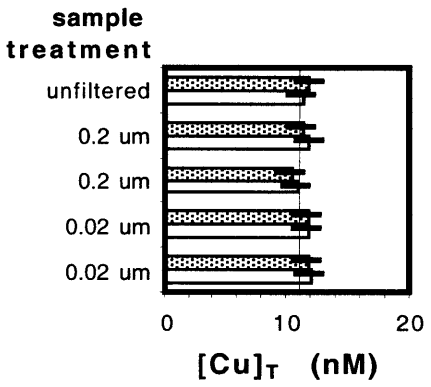

Fig.4.2. Measurement of $[Cu]_T$ before and after filtration through 0.2 and 0.02 μm pore size filters. Solid line represents expected $[Cu]_T$. of 11.6 nM. Stippled and empty bars represent Cu measured with CLE-ACSV before and after UV oxidation, respectively.

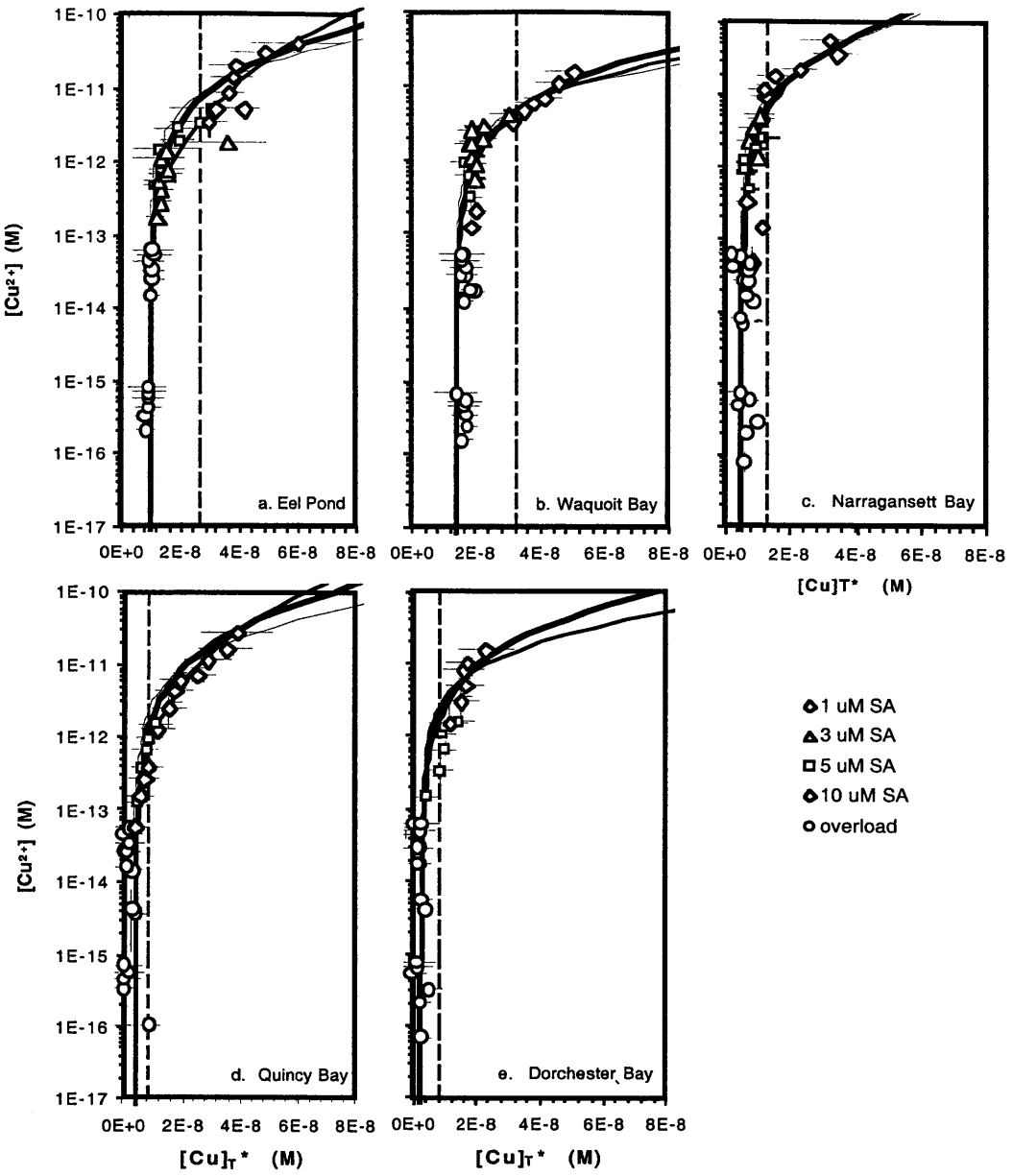

Fig.4.3. Cu speciation in filtered samples collected in December 2001. See key for data point symbols. Vertical dashed lines represent total filtered Cu, determined in UV-oxidized samples. Solid light, medium, and heavy lines represent Models 1a,b and 2 (one-ligand, two-ligand, and humic acid models, respectively; model parameters shown in Table 4.2.)

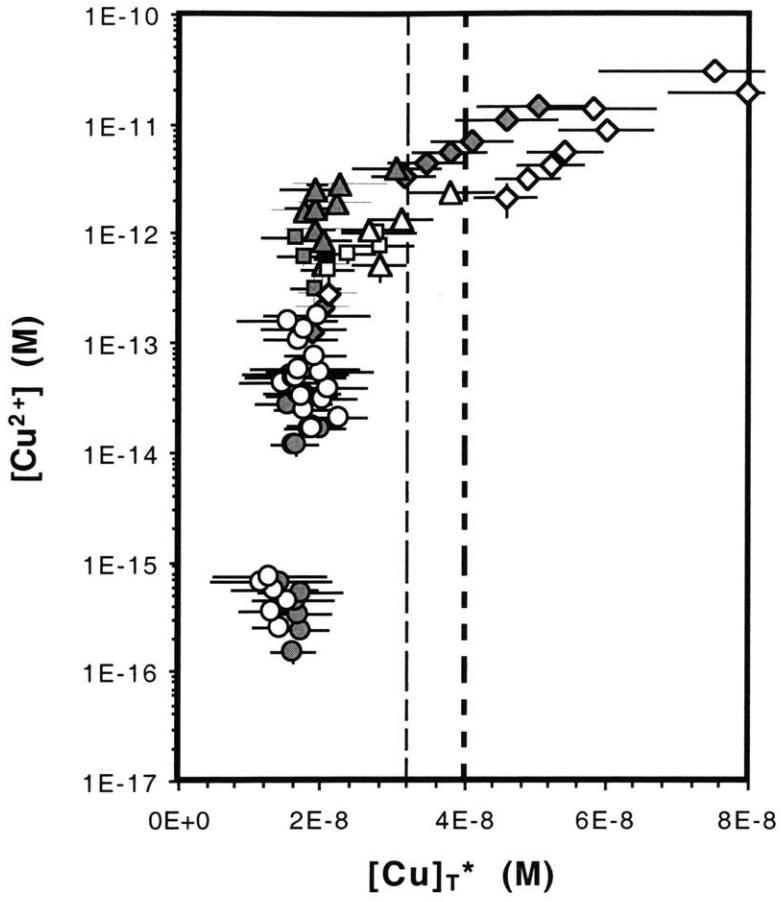

Fig. 4.4. Cu speciation in both filtered (gray) and unfiltered (white) subsamples for Waquoit Bay (December 2001). Heavy and light vertical dashed lines cross the x-axis at the concentration of total Cu measured in the unfiltered and filtered samples, respectively.

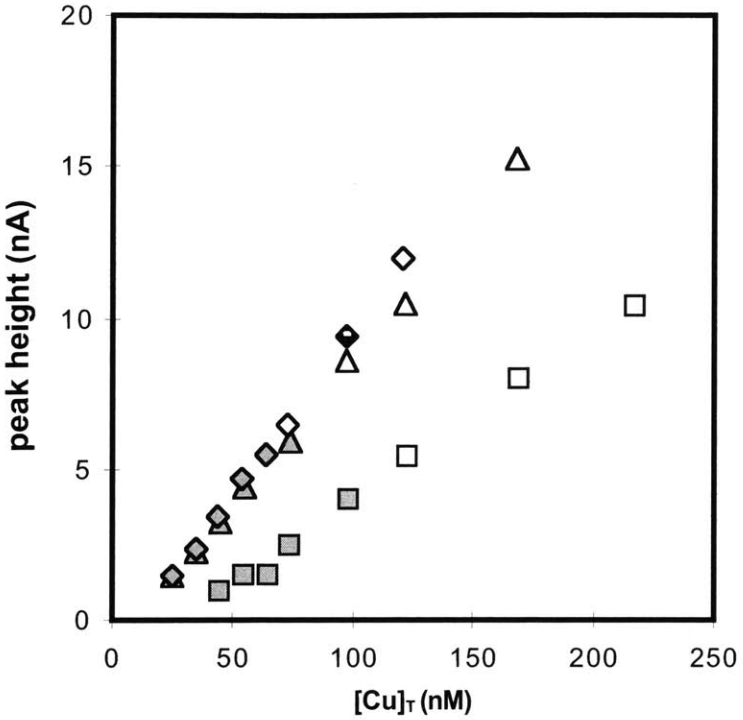

Fig. 4.5. The raw titration data for the filtered December 2001 Eel Pond sample. See Fig 4.6 for symbols. The last three points of each titration were used for internal calibrations.

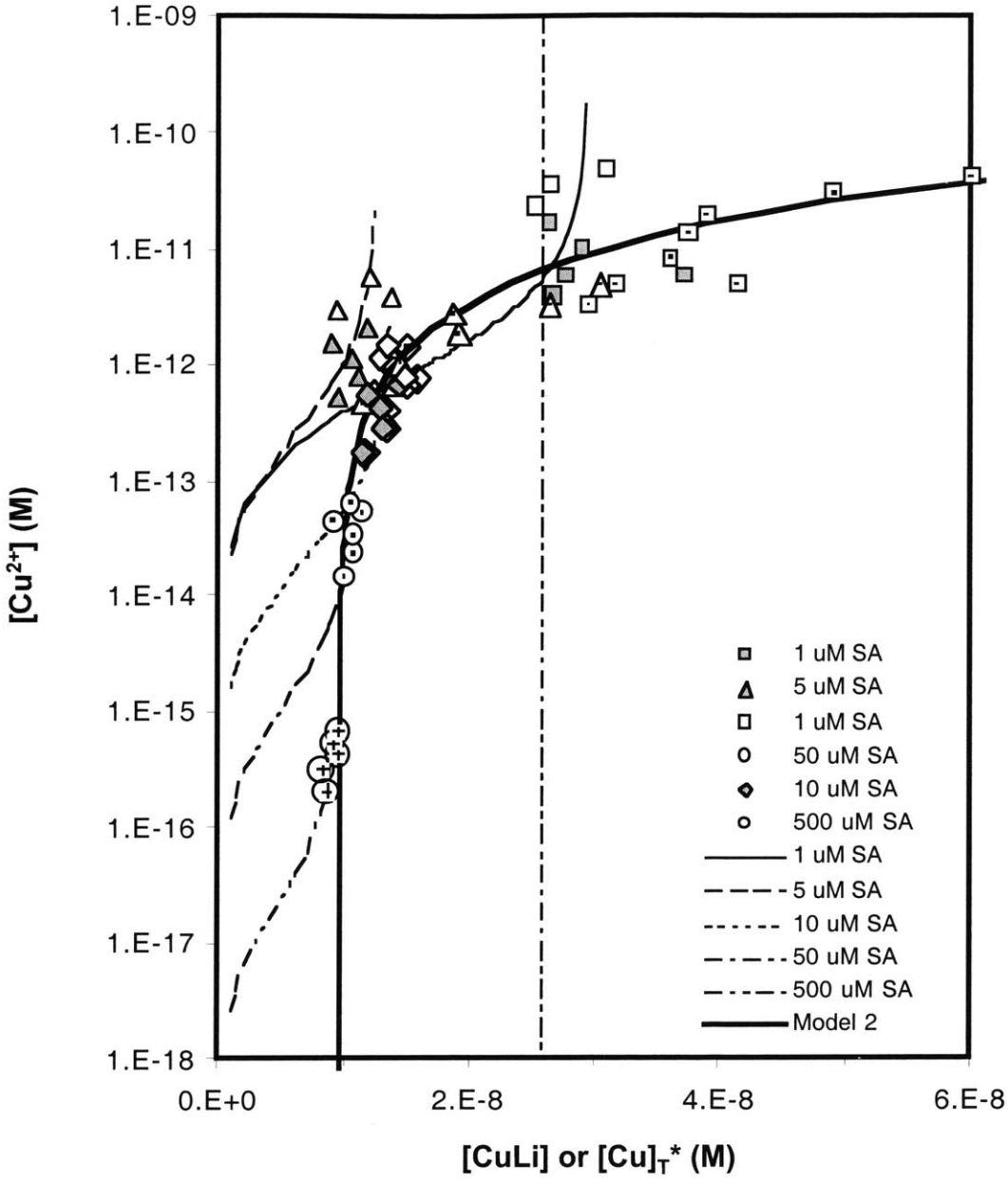

Fig. 4.6. Comparison of reinterpretation of the filtered Eel Pond titrations with internal (gray symbols, with white symbols representing points used for calibration) and overload titrations (dotted symbols). See key for [SA] used and for results of Lanmuir linearizations (light lines). Also included as a heavy solid line is Model 2 (Table 4.2).

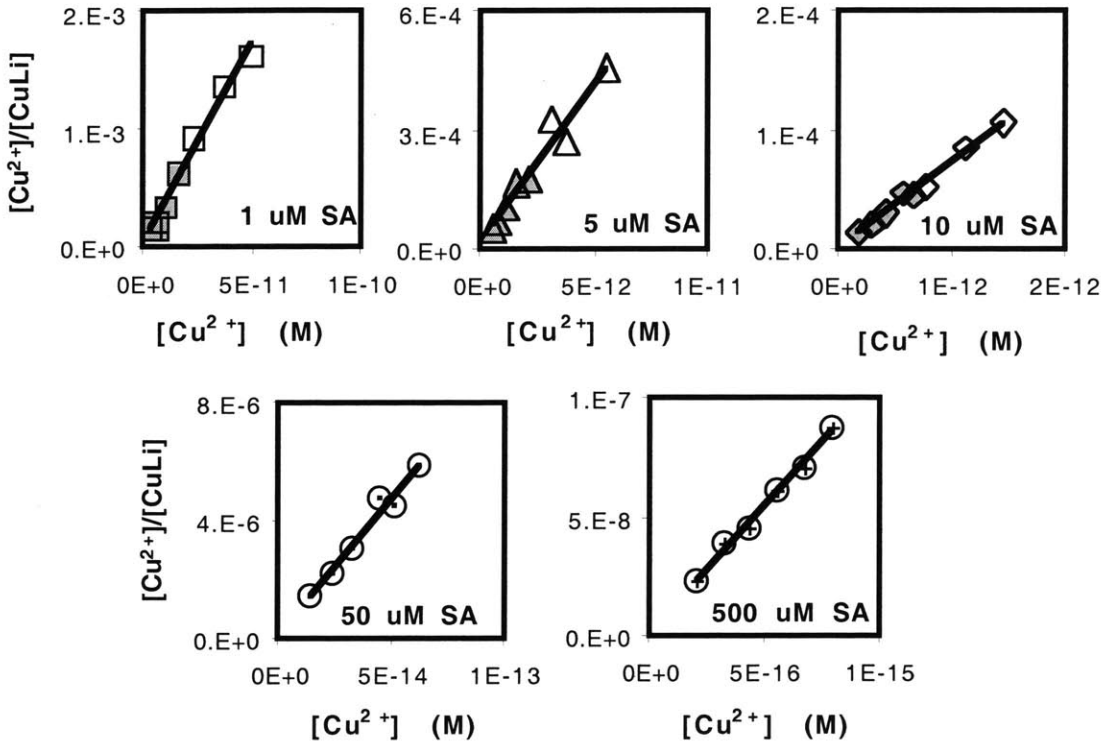

Fig. 4.7. Langmuir linearizations for internal (1, 5, and 10 μM SA) and overload (50 and 500 μM SA) titrations, used to model data in Fig. 4.6.

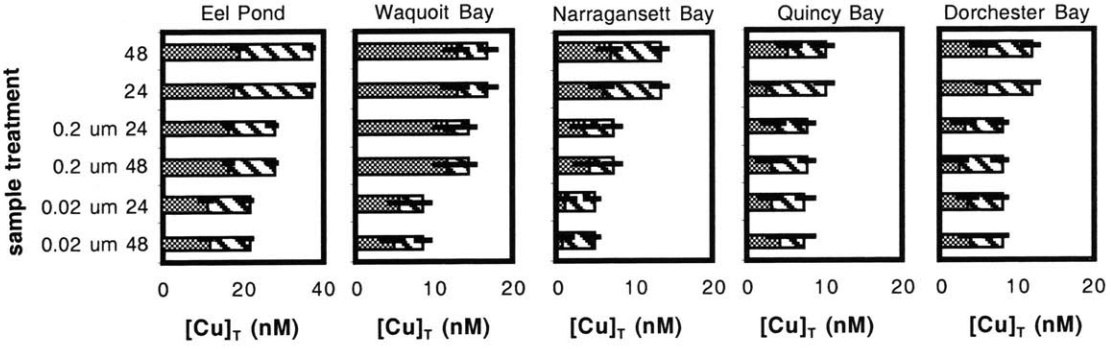

Fig. 4.8a-e. "Exchangeable" (striped bars) and "non-exchangeable" (stippled bars) Cu for January 2002 samples.

Chapter 5. Cu Speciation in the Taunton River Watershed

5.1. Introduction.

5.1.1. Cu speciation in freshwater systems

Total Cu concentrations can be much greater than 100 nM in polluted streams, but Cu ligands can reduce $[Cu^{2+}]$ to sub-nanomolar concentrations. Cu in rivers is present in several species, including $[Cu^{2+}]$, generally considered the most toxic form of Cu, and Cu complexed by Cu ligands, which is generally considered to be not immediately bioavailable and therefore not toxic. This theory is supported by the fact that the toxicity of Cu to fish and microorganisms is related to the concentration of free Cu ($[Cu^{2+}]$) and not to $[Cu]_T$ (DiToro, 2000). However, $[Cu^{2+}]$ and the binding ability of Cu ligands in rivers are difficult to measure, and therefore we do not know enough about Cu speciation in rivers to be able to predict Cu speciation and therefore Cu toxicity.

We do have some examples of what controls Cu speciation in several freshwater systems. Humic substances accounted for a large fraction of the total Cu binding in a Swiss lake (Xue, 1999). Research in Massachusetts streams shows that fulvic acids (extracted with a XAD-8 column) could account for all the Cu binding by dissolved natural organic matter, with EDTA, a common anthropogenic chelator, accounting for the remainder of Cu ligands (Breault, 1996). Cu sulfide complexes (Rozan, 2000) can resist oxidation in aerobic waters for several days and could account for 10 to 60% of the total strong copper complexes observed in several rivers in Connecticut (Rozan, 1999d). In addition, research done in coastal and seawater samples suggests that phytoplankton produce Cu ligands in response to elevated Cu concentrations (Moffett, 1997; Leal, 1999); this theory could apply to phytoplankton in freshwater systems as well.

5.1.2. POTW effluent as source of Cu

Sewage is a big source of Cu to rivers, so most of the concern with Cu toxicity is in rivers with large inputs of Cu from sewage sources. Publicly owned treatment works (POTW) release treated sewage effluent with high concentrations of Cu due to the high rate of leaching of Cu pipes into drinking water. The leaching of Cu from pipes can be minimized by increasing the pH of the drinking water to decrease Cu solubility. Nevertheless, POTW's are often out of compliance for Cu releases into the river, based on the current method of determining effluent standards based on Site Specific Water Quality Standards promulgated by the Environmental Protection Agency under the Clean Water Act.

5.1.3. Sewage as source of Cu ligands

The role which sewage plays in Cu speciation is unknown because no one has directly investigated the Cu binding ability of treated sewage released into freshwater systems. .Sedlak (1997) found that treated sewage effluents and creeks carrying polluted runoff could be a source of strong ligands to South San Francisco Bay. POTW's could also be a source of Cu ligands to rivers; sewage is likely to be an important source of EDTA, suggested to complex a significant fraction of total Cu in Massachusetts streams (Breault, 1996). Treated sewage may contain Cu ligands besides EDTA, such as bacterial and algal exudates (Rudd, 1984).

There would be a strong seasonal dependence of the role that POTW's play in contributing Cu ligands to rivers because river water discharge rates can vary by an order of magnitude from winter to summer. POTW discharges, which remain roughly constant throughout the year, may be only a small fraction of total river water discharge in the

spring, but a large fraction of total river discharge in the summer. Therefore, POTW ligand contributions will most likely be more important during the summer, and may be especially important during droughts.

5.1.4. Goals of Research

We sampled the treated effluent of three POTW's in the Taunton River Watershed and river sites upstream and downstream of each POTW to determine $[Cu]_T$ and the Cu binding ability of the treated sewage.

In addition, we sampled river sites roughly 0.2 to 0.5 miles upstream and downstream of the POTW to measure total Cu and the Cu binding ability of the river. Knowing $[Cu]_T$ and the Cu binding ability in each sample, we can attempt a mass balance of Cu and ligands upstream and downstream of the POTW (before and after the addition of POTW ligands) to determine if the POTW added significant concentrations of Cu and ligands to the river.

Finally, we sampled from March (high river discharge) to September (low river discharge) to explore whether seasonal hydrological patterns had an effect on the relative importance of the impact on rivers of Cu and Cu ligands released by POTW's.

5.2. Methods.

5.2.1. Site description

Town River, located in southeastern Massachusetts, is about 14 miles long and drains a small area (approximately 100 square miles) into the Taunton River, which flows into the Atlantic Ocean. The watershed is a patchwork of low-density settlement and fields, with

some wetlands and forested areas. The upstream sampling site (Site 7) is about 500 feet upstream of the effluent outlet of the Bridgewater POTW. Site 8 is about 1/2 mile downstream of the POTW, and the next site (Site 9, downstream of the confluence of the Town and Matfield rivers) is about 1/4 mile downstream of Site 8. Approximate distances are based on maps; because the river meanders, exact mileages are more difficult to determine.

The publicly owned treatment works are enhanced secondary treatment plants built in the 1950's (and retrofitted with enhanced treatment since then). Treatment of wastewater includes screening and settling suspended particles and a nitrification/denitrification step to remove Biological Oxygen Demand (BOD). These steps are followed by chlorination and aeration (K. McLaughlin, personal communication). The treated sewage water when discharged was clear and nearly odorless (it smelled of algal growth). Based on its color, the effluent does not contain high concentrations of terrestrial humic substances, but likely contains bacterial and algal products and synthetic chelators that are not degraded by the bacteria, as well as colorless organic matter.

5.2.2. Sample collection

Samples were collected by wading into the stream and dipping bottles below the surface upstream of our position in the water. Care was taken to minimize sediment disturbance and to allow disturbed sediment particles to wash downstream before sampling. During low flow or backwater conditions, we sampled from the fast flowing portion of the stream. Stream pH was measured in the stream with a multimeter.

5.2.3. Water mass balance for the Town River

River discharge in the rivers in the Taunton River watershed varies widely on a seasonal basis as well as on a day-to-day basis, depending on rainfall and watershed and river characteristics. Determining the average discharge for any one river is important for two reasons. First, it is important to know the average discharge of the river relative to that of the POTW in order to do mole balance calculations on Cu and Cu ligands. Second, sampling should be conducted during "average flow conditions" to capture "average" Cu and Cu ligand data for that part of the year.

A USGS station is located at Bridgewater, on Taunton River about 2 miles downstream of the confluence of the Town and Nemasket Rivers, with stream discharge data uploaded continuously to the USGS website (http://waterdata.usgs.gov/ma/nwis/). The USGS station websites shows current water flow and the "median daily streamflow" based on 53 years of record by USGS. The 53 year average shows that the streamflow, or discharge, can vary from 4000 liters/s (in April) to 200 liters/s (in September). Heavy rainfall can also temporarily increase discharge by up to a factor of ten, and even smaller storms can increase discharge by a factor of two. It often takes almost a week for discharge to return to the median daily value.

We calculated the average summer discharge of the Town River (in Watershed "C"), upstream of the USGS station and near POTW "C", by measuring discharge with a flowmeter and streambed dimensions. On June 11, 2001, during a period of median discharge for that date in the Taunton River, depth in Town River itself varied from 0.3 to 3 meters. On this date, flow speeds were measured to be 15 cm/s in the fast shallow (2 feet) sections to 5 cm/s in deeper (10 feet) sections. The width of the river is relatively constant at 5 to 7 meters at any depth. Because the banks of the river are nearly vertical,

the cross section of the river is roughly rectangular. We calculated discharge as the flow speed multiplied by the cross sectional area (depth times width) of the stream. The discharge of the Town River was approximately 700-900 liters/s on this day.

In order to extrapolate average summer and winter discharges from the calculation of June discharge of the Town River near POTW "C", we multiplied the Town River June discharge by the ratio of March to June and September to June flows for the 53-year average for the Taunton River stream gauge. We extrapolate an average March discharge of 2300 liters/s and an average September discharge of 230 liters/s.

The Bridgewater POTW (POTW "C") releases about 1.44 million gallons/day or 5.5×10^6 liters/day. This release remains relatively constant throughout the year (Ken Heim, personal communication.) During spring floods and storms, the ratio of wastewater discharged from Bridgewater POTW may be only a small portion (less than 3%) of the total Town River water. However, during the summer months, when river flow decreases to a trickle, the ratio of wastewater to upstream water may be as high as 30% (and greater during extreme droughts). While concentrations of Cu in the POTW effluent must be very high in order to affect riverine Cu concentrations during winter and spring, during late summer drought low flow conditions, riverine Cu concentrations are more likely to increase significantly downstream of the POTW.

5.2.4. Materials.

Deionized distilled water (DDW) was from a Millipore Q-H_2O system. Fresh 1 mM primary and 1 μM secondary copper standards were made every day by serial dilutions of an Aldrich atomic absorption standard solution ($CuSO_4$ in 1% HNO_3, 10 000 ppm Cu)

with DDW; the final pH of the secondary copper standard was about 7. The added ligand for competitive ligand exchange adsorptive cathodic stripping voltammetry (see below) was salicylaldoxime (SA) , purified by equilibration with EDTA and repeated filtering and recrystallization. SA was dissolved in DDW for a final stock solution concentration of 10^{-4} M. SA stock solutions were replaced every three months and refrigerated when not in use. A solution of1 M boric acid (EM Science, Suprapur,, pH range of 7.5-8.2), HEPES (OmniPur, pH 7-8), or MOPS (Sigma, pH 6-7), adjusted to pH of 6-8 depending on the sample, was added to one liter of UV-SW for a final pH buffer concentration 0.02 M.

5.2.5. CLE-ACSV

The goal of obtaining copper titration data is to determine the variation in free copper concentration, $[Cu^{2+}]$, as a function of $[Cu]_T$ in the sample, to obtain information about the Cu ligands in that sample. Cu titrations with Competitive Ligand Exchange Adsorptive Cathodic Stripping Voltammetry (CLE-ACSV) is a two part process. First, for CLE, a known concentration of a well-characterized and purified synthetic ligand is added to a series of samples containing the natural ligands and a range of added copper and allowed to equilibrate. Salicylaldoxime (SA) is used because it is a very strong, well-characterized ligand whose Cu binding strength has been accurately calibrated with EDTA (Campos, 1994); Chapter 2) and which doesn't partition into organic matter (Appendix B). In the presence of SA, Cu species are distributed amongst free Cu, Cu complexed by ligands in the sample, CuL_i, and Cu complexed by SA:

$$[Cu]_T = [Cu^{2+}] + \Sigma[CuL_i] + \Sigma[Cu(SA)_x] \qquad (5.1).$$

ACSV (see below) is then used to measure the concentration of copper complexed with added ligand, $\Sigma[Cu(SA)_x]$ as a function of $[Cu^{2+}]$ during a titration of the sample with Cu.

In the absence of both SA and the Cu complexed by SA, the water sample would have an identical $[Cu^{2+}]$ and a theoretical total copper concentration $[Cu]_T^*$ (Moffett, 1997; Kogut, 2001) that is given by:

$$[Cu]_T^* = [Cu]_T - \Sigma[Cu(SA)_x] = [Cu^{2+}] + \Sigma[CuL_i] \cong \Sigma[CuL_i] \quad (5.2).$$

Therefore, the concentration of Cu complexed by ligands in the sample is determined for each value of $[Cu]_T$. The corresponding value of $[Cu^{2+}]$ is calculated from $\Sigma[Cu(SA)_x]$, [SA], and the conditional stability constants of the *mono* and *bis* complexes, $K_{Cu(SA)}$ and $\beta_{Cu(SA)2}$:

$$[Cu^{2+}]=\Sigma[Cu(SA)_x]/(K_{Cu(SA)}[SA]+\beta_{Cu(SA)2}[SA]^2)=\Sigma[Cu(SA)_x]/SRC(SA) \quad (5.3)$$

where [SA] is the concentration of SA not bound to copper:

$$[SA] = [SA]_T - [Cu(SA)] - 2[Cu(SA)_2] \quad (5.4),$$

calculated using EXCEL to solve for the four unknowns in the four equations (Eqs. 5.3 and 5.4 and the two equilibrium mass law expressions for formation of Cu-SA complexes.)

The variation in $[Cu^{2+}]$ that would be measured as a function of $[Cu]_T^*$ ($[Cu]_T$ in the original sample) can therefore be read directly off a plot of $[Cu^{2+}]$ versus $[Cu]_T^*$ obtained from CLE titrations. Each titration data point corresponds to a measurement of $\Sigma[Cu(SA)_x]$ and $[Cu]_T$, from which the quantity $[Cu]_T^*$ can be calculated directly using Eq. 5.2. Titrations with different [SA] that agree at one value of $[Cu]_T^*$ suggest that Cu, Cu ligands, and SA are at equilibrium at that point.

In order to obtain information about ligand concentrations and conditional binding strengths of the sample, the titration data can be modeled (for example, fitting plots of $[Cu^{2+}]$ versus $[Cu]_T^*$ with FITEQL (Voelker, 2001). However, this approach has several

limitations, including the impossibility of accurately determining the contributions of each ligand "type" in a mixture of two or more ligands, including humic substances (Voelker, 2001). In this chapter, we instead use comparisons of plots of $[Cu^{2+}]$ versus $[Cu]_T$* to show whether Cu titrations of certain ligand types (ie EDTA or humic substances) match titrations of the field data, and whether these ligand types might therefore be used to explain the field data.

5.2.6. Calibration of ACSV with the overload titration.

A relatively new protocol is required for distinguishing "surfactant effects" from complexation when calibrating the CLE-ACSV technique (Kogut, 2001; Voelker, 2001). This protocol contrasts sharply with, and in many cases is preferable to, current protocols for copper titrations of freshwater samples.

For ACSV measurements, the relationship between the current peak height, ip, and $[Cu(SA)_x]$, called the sensitivity, S:

$$[Cu(SA)_x] = ip/S \qquad (5.5)$$

S depends on both instrument settings (e.g. adsorption time and potential) and the sample matrix. To avoid problems with using external and internal calibrations to determine S in the presence of humic substances, we developed a technique called an "overload" titration (Appendix D). The overload titration is similar to the frequently used internal calibration in that the sensitivity is measured directly in the water sample to be analyzed. However, a concentration of SA is used that is high enough to outcompete all of the natural ligands present in the sample so that no apparent decrease in sensitivity due to Cu complexation by natural ligands occurs. Because any additional Cu added ($\Delta[Cu]_T$) is complexed only by the added ligand SA:

$$\Delta[Cu]_T \cong \Delta\Sigma[Cu(SA)_x] \quad (5.6)$$

The slope of ip versus $[Cu]_T$ in this part of the titration should then also be equal to the sensitivity S.

One complication with the overload titration calibration method is that the sensitivity with 1 uM SA in UV-SW is not the same as the sensitivity with 25 uM SA in UV-SW. The change in SA speciation (i.e. the ratio of [Cu(SA)] to $[Cu(SA)_2]$ resulting from an increase in $[SA]_T$) affects the sensitivity because only $Cu(SA)_2$ is reduced at the mercury drop during the ACSV step (Campos, 1994). Correction factors must be determined separately for samples of different pH and salinity or ionic strength, which seem to affect them (Chapter 2).

As a rule of thumb, in the absence of any surfactant effects, we have found so far the sensitivity does not increase at concentrations of SA any greater than 25 or 50 μM; at that point, S remains constant at SA concentrations from 50 μM to at least 2500 μM. In the presence of surfactant effects, sensitivity continues to increase upon the addition of greater than 50 μM SA.

We used [SA] as high as 2500 μM in the Taunton River watershed samples. We note that in order to use 2500 μM SA, we had to add 1 ml of 0.0025 M SA stock solution to a 10 ml sample; therefore, dilution corrections of about 10% were made for the sensitivity when extrapolated to titrations with lower [SA]. To determine that 2500 μM was sufficient to remove all "exchangeable" Cu from the sample, we titrated the sample with SA, measuring the peak height until the change in peak height did not increase with addition of more SA. No increase in peak height proves that there is no increase in $[Cu(SA)_x]$ formed (as well proving as the absence of any surfactant effect). If no

additional $Cu(SA)_x$ has formed, then theoretically, SA has removed all the Cu from natural ligands and $[Cu(SA)_x]$ is equal to $[Cu]_T$.

This approach to finding the minimum concentration of SA to conduct overload titrations does not work if the peak height continues to increase with increasing [SA] even at very high concentrations of SA. If the analyst never finds two overlapping overload titrations at two concentrations of SA, it is impossible to tell whether the increase in peak height is due to increase in sensitivity or in $[Cu(SA)_x]$, and the analyst should look for another calibration method.

5.2.7. Cu titration setup with overload titrations

For overload titrations and Cu speciation titrations calibrated with overload titrations, the sample analyzed was a 10.0 ml aliquot of the sample pipeted directly into the electrode's Teflon® sample cup. Buffer was added for a final concentration of 0.02 M. SA was also added to the sample at this time. Copper from the secondary standard was added sequentially to the sample after each voltammetric analysis of $\Sigma[Cu(SA)_x]$ for a range of $[Cu]_T$ added from 0 to 300 nM (sometimes more or less). Before each analysis, samples were allowed to equilibrate for 3 minutes for the speciation titrations, and 1 minute for all other titrations. Cu speciation data from titrations with different concentrations of SA which overlap show that 3 minute equilibration times are adequate for these samples (see Results.)

5.2.8. Calibration of sensitivity with addition of EDTA

For the summer samples, we attempted to use an alternative calibration method using EDTA as a second added ligand (Sunda, 1991) as an alternative to overload titrations.

Because I was unfamiliar with using CLE-ACSV in freshwaters and was worried about using high SA concentrations (much higher than I had used so far in coastal waters), we switched to a method that did not require overload titrations and high concentrations of SA. For "EDTA titrations", the analyst can use any concentration of SA. In fact, the analyst could avoid correction factors altogether by conducting an EDTA calibration for each speciation titration (each at a different [SA].) Therefore the EDTA calibration method is theoretically more robust than the overload titration method.

This EDTA calibration method depends on the comparison of two Cu titrations of two subsamples at the same concentration of SA, with one titration containing a known concentration of EDTA. Equilibration is obtained for both titrations (at least 48 hours, as EDTA equilibration times are slow (Hering, 1990). For the titration with EDTA, total Cu is distributed amongst Cu, natural ligands, SA, and EDTA, so that

$$[Cu]_T=[Cu^{2+}]_2+[CuL_i]_2+[Cu(SA)_x]_2+[CuEDTA^{2-}] \quad (5.7),$$

where the subscript "2" denotes the titration with EDTA. For the titration without EDTA, the Cu species are those listed in Eq. 5.1. The peak height at any one point for either titration can correspond to only one value of $[Cu(SA)_x]$, which is directly proportional to $[Cu^{2+}]$. There can be only one value of S, the sensitivity, because the sample matrices are identical except for the addition of EDTA, which is unlikely to cause any interference with CLE-ACSV at low concentrations. An addition of EDTA, which complexes a certain concentration of Cu in the sample that would otherwise be complexed by SA or the natural ligands, can provide information about the relationship between peak height and $[Cu(SA)_x]$ in the sample.

For both samples, the one with and the one without EDTA added, at one peak height, the values of S, $[Cu(SA)_x]$, $[Cu^{2+}]$, [and $[CuL_i]$ must be identical, but $[Cu]_T$ in the sample

with added EDTA must be greater than that in the sample without EDTA. To determine the value of S which best fits a set of two titrations (one with and one without EDTA), an initial value of S, based on standard curves in DDW or UVSSW run under identical conditions, is used first. A value of $[Cu(SA)_x]$ is calculated for each data point using this value of S, the peak height ip, and Eq. 5.5. The value of $[Cu^{2+}]$ is calculated for each point from the value of $[Cu(SA)_x]$ and Eq. 5.3. Then, the theoretical concentration of [CuEDTA] in equilibrium with each of these values of $[Cu^{2+}]$ is calculated using calculated stability constants for CuEDTA (Appendix A) and the concentration of Ca^{2+}, which controls EDTA speciation, reported by ENSR (data not shown.) Then, these values of $[CuEDTA]_{calc}$ are added to the values of $[Cu]_T$ for each data point in the titration without EDTA, so that $[Cu]_{T,2}$, the theoretical total Cu concentration that is needed so that all EDTA and SA and natural ligands can be at equilibrium with the calculated values of $[Cu^{2+}]$, is

$$[Cu]_{T,2}=[Cu]_T+[CuEDTA]_{calc} \qquad (5.8).$$

We plotted the peak heights measured for the titration without EDTA, which correspond to the original values of $[Cu(SA)_x]$, versus $[Cu]_{T,2}$, versus $[Cu]_{T,2}$. The titration with EDTA added overlaps the titration without EDTA added if the estimated value of S, which directly affects the calculated value of $[Cu^{2+}]$ and therefore $[CuEDTA]_{calc}$, is just large enough. There is only one value of S that will satisfy this requirement, which is the true value of S.

In practice, the titration points of the imaginary titration with EDTA will lie in between data points of the genuine EDTA titration, so we used a best-fit linear relationship through the data points as a guide to best match between the real and imaginary titrations. While the titrations (consisting of only four points for this work) do appear linear, a decent linear fit in no way suggests that the titration is truly linear. The linear fit is the

best we can do in absence of the real relationship between the points. Given the large scatter in the real data, the use of a linear fit is the smallest source of error with this method (see Results and Discussion).

While an EDTA titration could be used for every speciation titration to determine the value of S direction at each concentration of SA, we continued to determine the value of SA at only one concentration of SA (usually, 200 μM SA). We used the correction factors to extrapolate from one concentration of SA to another for the remaining speciation titrations (at 100, 50 and 10 μM SA). We used 2 μM EDTA for all titrations; we were limited to this concentration because the greatest concentration of total Cu used was up to 1 uM, and the values of $[Cu(SA)_x]$ and [CuEDTA] were sometimes as high as 0.15 μM. Thus the error in our calculations sometimes approached 10%, but this is small compared to the quality of the preliminary data we obtained (see Results and Discussion.)

5.2.9. Cu titration setup with EDTA calibration titrations

The titration setup for EDTA calibration was as follows. For each of five titrations (the 200 μM SA + 2 μM EDTA calibration titration and the four speciation titrations at 200 μM SA, 100 μM SA, 50 μM SA, and 10 μM SA), 30 ml of the sample was pipeted into four 30 ml bottles. SA, EDTA, and 0.2M buffer were added, along with 0, 20, 60, or 100 μM Cu for the speciation titrations and 60, 100, 200, and 400 μM Cu for the EDTA calibration titration (for increased peak height). All samples were equilibrated for 48 hours in the dark at room temperature, and ACSV analysis was conducted on 10 ml aliquots as described below.

5.2.10. Determination of $[Cu]_T$

Samples from each filter fraction were acidified to pH < 2 with concentrated nitric acid (J. T. Baker Instra-Analyzed) and irradiated with ultraviolet light from a medium pressure mercury lamp (Ace Glass, 1000 W) for at least six hours (ten hours for unfiltered samples) in quartz 125 ml tubes. We assume that direct UV irradiation combined with hydroxyl radical formation from nitrate destroy both organic matter and inorganic matrices (e.g. sulfides) within six hours (Chapter 4). Samples were then brought to circumneutral pH and prepared for voltammetric analysis as described below. With all ligands destroyed, $[Cu(SA)_x]$ should be equal to $[Cu]_T$ in the sample.

We ran procedural blanks and standards, including a DDW blank, DDW acid/base (a/b) standards with 50 or 100 nM Cu added, and three UV irradiation standards with 100 nM Cu added in DDW (Fig. 3.1). The acid/base standards were designed to test the recovery of Cu when samples were acidified and then returned to circumneutral pH by addition of ammonia to check for Cu precipitation as $CuCO_3$ in high pH solutions after addition of ammonia and before addition of SA. The UV DDW tests simulated the whole UV-irradation process including acidification, UV-irradition, and addition of ammonia and SA before CSV to determine $[Cu]_T$. Recovery of Cu is 90-98% percent in these samples, indicating that negligible Cu was lost from solution during the irradiation procedure.

5.2.11. Voltammetric Analysis

$\Sigma[Cu(SA)_x]$ in the samples was analyzed using adsorptive cathodic stripping voltammetry. This analysis consists of two steps: an adsorption step, during which $Cu(AL)_x$ complexes are sorbed to a mercury drop electrode, and a potential scan in the negative direction, during which the current produced by the reduction of the copper in

the sorbed complexes is measured. We used differential pulse ACSV with a PAR 303A static mercury drop electrode and an EG&G PAR 394 analyzer. Instrument settings were as follows: adsorption potential, -0.08 V (versus Ag/AgCl electrode); scan range, -80 to -600 mV; scan rate, 20 mV/s; drop time, 0.2 s; pulse height, 25 mV. The time for the adsorption step was 0 seconds. Because there is additional time for adsorption during the potential scan up to the potential at which Cu-SA complexes are reduced, the "effective" adsorption time is about 3 seconds. It was necessary to use short adsorption times to reduce or eliminate surfactant effects. The reduction of Cu in the $Cu(SA)_2$ complex produced a well-defined peak at -330 to -400 mV in the potential scan (Campos, 1994); Appendix D).

5.3. Results and Discussion

5.3.1. Total and dissolved Cu

Cu concentrations in the Taunton River estuary were measured to see the POTW effluent had high concentrations of Cu. Data for total dissolved Cu in March and total Cu in May, 2001, are presented in Figure 5.2. For both dates, Cu is elevated (100-200 nM) in the sewage effluent, and at lower but variable (20-60 nM) concentrations in the rivers.

We wanted to verify that the Cu release by the POTW caused Cu levels in river sites downstream of the POTW outfall to be significantly higher than those upstream. The Cu concentrations in the three watersheds of the Taunton watershed area, marked "A", "B", and "C" to denote the ENSR designation of the POTW associated with each. These figures show the samples in order of sampling location from upstream on left to downstream on right, including the POTW sample sites. Watershed "A" has the highest riverine concentration of total and dissolved Cu in the river downstream of the POTW site. This suggests that large Cu concentrations downstream are a result of the Cu release

of POTW "A" (assuming that dissolved Cu concentrations in POTW "A" are elevated as they are for the May total Cu sampling). This trend of increased downstream Cu concentrations also holds for POTW "B" in the March dissolved Cu data. However, for POTW's "B" and "C" for the May total Cu data, total Cu concentrations downstream of the POTW look similar to those upstream of the POTW, suggesting that POTW effluent does not add significant concentrations of Cu to the river.

The finding that only POTW "A" has a significant effect on the Cu concentrations downstream are consistent with the fact that the effluent flowrate for "A" is also greater than those of "B", and "C", which are roughly constant at 132, 92, and 63 liters/s, respectively (K. Heim, personal communication). However, for a more quantitative assessment of the effect greater POTW flowrates and Cu releases have on the streams, the flowrates of the streams themselves must be taken into account. The flowrates are unknown, and may vary widely depending on rainfall and water retention capability of the wateshed. Also, in spring, POTW releases can be only a small fraction (1% or less) of the total flow (see water mass balance calculations for Town River above.) In summer, when river flowrates are much lower and POTW effluent accounts for a larger fraction of the total river flow, Cu concentrations downstream may be more consistently elevated.

5.3.2. Cu speciation titrations calibrated with overload titrations

Sites were sampled in March, May, and July 2001. The March samples were used to experiment with determining Cu speciation in freshwater samples so that Cu speciation could be determined quickly and accurately in the later samplings, when river discharges

were expected to be lower. March samples were the only ones that were analyzed with overload titration calibrations.

Site 9 (pH adjusted to 7.0) was used to determine the minimum concentration of SA needed to conduct a successful overload titration in this sample (where SA outcompetes all natural ligands for Cu). Fig. 5.3a shows the result of titrating the sample with Cu up to 100 nM added Cu, and then titrating with SA up to 3000 μM SA while holding $[Cu]_T$ constant. The peak heights obtained in the presence of concentrations of SA of 2500 – 3000 μM were identical within analytical error, and so for these concentrations of SA we assumed that SA outcompetes all natural ligands in the sample for Cu. Therefore, concentrations of SA greater than 2500 μM could be used for overload titrations. We obtained the sensitivity S for this raw data using Eq. 5.5 and the point corresponding to ip and $[Cu(SA)_x]$ for 2500 μM SA. The calibrated data (converted from peak height to values of $[Cu(SA)_x]$ using our value of S) are shown in Fig. 5.3b, where the white diamonds correspond to the titrations points at 250 nM Cu and the black diamonds correspond to the titration points with increasing [SA]. The titration points curve slightly to the left because of the dilution of total Cu in the sample due to additions of SA stock solution. The black solid line in Fig. 5.3b is the one to one line, where all Cu is bound by SA ($[Cu]_T = [Cu(SA)_x]$ for all points). We used this titration to show that 2500 μM SA could be used for overload titrations for this Site 9 sample.

In addition, the data points for which [SA] was increased can be used as a "backwards titration". The backwards titration (increasing [SA]) is compared to the "forward" titration (increasing $[Cu]_T$) in Fig. 5.3c. The fact that the "backwards" titration overlies the Cu titration data suggests that SA and the natural ligands complex Cu reversibly, and

that true equilibrium can be assumed for this sample after the three minute equilibration time.

Another titration of the same Site 9 sample at pH = 7.0, conducted the next day as described in Sections 5.2.6 and 5.2.7, is shown in Figure 5.4a and b. This sample closely matches that of experiment of the previous day (shown in Fig. 5.4 as black dots). Agreement suggests that both the unconventional approach of backwards and forwards titrations and our more extensively used overload titration method were successful in this sample, and that Cu ligands in the sample were not degraded (or created) during the one day time span.

Also included in Fig. 5.4b is data for Cu titrations of 6 mg/L Suwannee River Fulvic Acid (SRFA) at pH=7.5 in DDW with 0.01M KNO_3 from Fig. 1a in Xue (1999) (white dots). The binding ability of 6 mg/L SRFA at similar pH is less than that of the Site 9 sample. Instead, 24 mg/L SRFA (or fulvic acid with Cu binding properties similar to that of SRFA) would account for all of the Cu binding in Site 9 (gray dots.) Site 9 (Taunton River) was highly colored and very likely has at high concentrations of fulvic acid, but it is unknown whether it contained 24 mg/L fulvic acid (we did not take these measurements or DOC measurements). However, this crude comparison shows that it is not unlikely that fulvic acid accounts for most of the Cu binding in this sample, allowing for possible effects of differences in fulvic acid character and sample chemistry (proton and Ca^{2+} competition and ionic strength effects).

The appearance of strong ligands in samples from POTW "C" (Figure 5.5) suggests that Cu is already complexed by chelators in the sewage before it enters the river. Ethylenediaminetetraacetic acid (EDTA), an anthropogenic chelator in household

detergents and foods, is routinely found in treated municipal sewage in concentrations of 70 to 20,000 nM (Kari, 1996; Bedsworth, 1999) and is a likely Cu ligand if not complexing Fe or Ni (Bedsworth, 1999). Total Ni and Fe concentrations were unknown in this sample. Nevertheless, assuming that Ca is the only cation that competes with Cu for EDTA, we estimated the concentration of EDTA not complexed by Fe or Ni that would explain this Cu binding. Using calculated stability constants for $CuEDTA^{2-}$ (Appendix A) and a Ca concentration of 0.5 mM (K. Heim, personal communication, 2001). The relationship between EDTA and $p[Cu^{2+}]$ is

$$p[Cu^{2+}]=\log([EDTA]_T-[CuEDTA^{2-}])-\log[CuEDTA^{2-}]+\log K_{CuEDTA2-} \quad (5.9).$$

If 200 nM of EDTA existed in the sewage, and all of it was available to complex Cu, then EDTA would produce a Cu speciation curve similar to the dotted line in Fig. 5f. If 500 nM EDTA existed, then EDTA would produce a Cu speciation curve similar to the solid line. EDTA, at concentrations approaching 500 nM (within the reported range of 70 to 20,000 nM (Kari, 1996),) may dominate Cu speciation in the effluent, but accurate measurements of EDTA, Ca and Ni would be necessary to prove the hypothesis. Likely alternatives to the EDTA hypothesis include other anthropogenic chelators such as NTA, stable Cu sulfide or thiol complexes formed during sewage treatment ((Rozan, 1999d), or biogenic ligands from the biological treatment tanks or from the algae growing in the aeration tank, the last step of the tertiary treatment before effluent release (Rudd, 1984).

5.3.3. EDTA calibration.

For samples collected in May, July, and August, we attempted to develop the more robust calibration method using EDTA. We first tried the EDTA calibration method on a solution of 1 mg/L SRHA in UV-irradiated Sargasso seawater, for comparison of this method to the successful overload titration calibration method used for Suwannee River

Humic Acid at pH=8.2 in seawater (Kogut, 2001). The results of the work are shown in Figure 5.6. Figure 5.6 shows the peak heights measured for titration points of the sample with and without EDTA concentrations. The white squares are the peak heights obtained from the 200 μM SA titration with no EDTA. The dotted line is the guide we used to match the "imaginary" EDTA titration obtained with the peak heights from the SA titration with no EDTA (black squares) with the real EDTA titration (black diamonds.) Titrations of the sample at lower [SA] and without EDTA are shown in Figure 5.6b, where the solid line is the 1 to 1 line as if the sample did not contain any natural ligands. The values of $[Cu(SA)_x]$ are calculated from the sensitivity (adjusted with correction factors if necessary) and the peak heights of each titration. Some titration points have $[Cu(SA)_x]$ greater than the 1 to 1 line, suggesting that the sensitivity was slightly overestimated. Comparison of the EDTA method and the overload titration method (black dots, from Kogut, 2001) (Fig. 5.6c) shows generally good agreement between the two methods.

The EDTA calibration method is much less precise than the overload titration here, and the scatter may be due to mostly to the fact that the EDTA titration points were equilibrated in separate bottles and the overload titrations were conducted sequentially in the same sample cup. We have no acceptable explanation for this (pipeting errors are insignificant; SRHA solutions are homogeneous; Teflon bottles used minimize adsorption of DOM to the surface; bottles were identically cleaned and treated). However, over four years we have consistently observed that there is more scatter in data from parallel overnight titration points than from sequential addition titrations. Still, if direct calibrations are needed, the option to use the EDTA calibration method is better than no data.

The EDTA calibration method was attempted for the May sampling of the Taunton River watershed samples. For two samples, Site 8 (1/2 mile downstream of Site 9) and POTW C, reasonable data were obtained (Figs. 5.7, 5.8). These two titrations show that Cu was bound slightly more strongly in May than in March for both these samples (assuming that Cu speciation in Sites 8 and 9 are comparable).

However, many of the titrations did not produce interpretable data. The troubling trend for the raw data was that the peak heights for all four titration points (with increasing $[Cu]_T$) for any value [SA], with or without EDTA, were roughly equal or had considerable scatter. For example, in Fig. 5.9 (Site 7), it was difficult to determine the sensitivity with such large scatter in the titration curve. For the sake of argument, the sensitivity was assumed to be 0.1 nA/nM $[Cu(SA)_x]$, equal to that of the UV standard curve run the same day, so that Figs. 5.9b and c could be generated. However, meaningful interpretation of these data is still not possible as there is no agreement between titrations with different [SA]. These problems were seen consistently seen in samples collected in August and September as well.

We emphasize that the trouble experienced with the May samples and subsequent samples analyzed with EDTA calibration method was not due to the calibration method but due to problems obtaining raw data. Some other type of interference, perhaps caused by high concentrations of organic matter or other metals, was responsible for the "flat" titrations and seemed to be impossible to avoid. Neither calibration method would have been successful for obtaining information about Cu speciation in the May samples without correction of the mysterious interference.

5.4. Conclusions

We present preliminary data showing that the effluent of one POTW (POTW "C") has the ability to strongly bind Cu. The effluent ligands are of unknown identity and are likely a mixture of different ligands, but we show for the sake of argument that the ligands could be modeled as 500 μM of EDTA, a common anthropogenic chelator, assuming this concentration of EDTA is not complexed by Fe or Ni. Therefore, ligands in the effluent could reduce Cu toxicity to riverine organisms. However, the effluent does not bind Cu as strongly as a riverine site one mile downstream of POTW "C" and sampled at the same time (March 2001). The contribution of effluent ligands to the river during the sampling would be minimal both because the effluent ligands are weaker than the riverine ligands, and the POTW effluent discharge is calculated to be only 3% of the total river discharge on the sampling date.

We had analytical trouble with the samples collected subsequently (summer 2001) which prevented us from obtaining Cu speciation from all POTW's and river sites during low flow conditions. Both calibration methods, the overload titration and the EDTA calibration, were problematic in these samples, although both were used successfully in other samples (the overload titration in March samples and also estuarine samples (Chapter 3) and coastal samples (Chapter 4) and EDTA titrations in 1 mg/L SRHA in seawater at pH=8.2). Our trouble may have been related to interference with CLE-ACSV by surfactants (such as organic matter or detergents) in the effluent and the river, or by other metals. The fact that the interference worsened in the summer data is unfortunate because a mass balance on Cu and Cu ligands would have been most interesting during summer. During summer, effluent is a bigger percentage of riverine discharge (about 30% compared to 3% in the spring) and therefore its contribution of Cu and ligands to the rivers is likely more important.

Had the speciation experiments been successful, a number of experiments could have been attempted to further determine riverine and effluent ligand fate and transport. These experiments include $[Cu]_T$ measurements downstream of the POTW's and ligand degradation studies of the river samples.

Our basic approach to mole balance of both total Cu and Cu ligands, which was not attempted here because of a lack of dependable data, might be used in other freshwater systems as well as seawater systems to determine the importance of different sources and sinks of Cu and ligands. One advantage of the Taunton River watershed sites is that the source (POTW) could be treated as a point source, and transport of ligands and Cu a small distance downstream of the POTW outlet could be treated as a one-dimensional. Non-point sources such as multiple combined sewer overflows would complicate mass balance attempts in harbors. Tidal influences and 'patchiness' of coastal waters will also introduce uncertainty into the mole balance model. Also, Cu ligand sources may be non-point source or created in situ, and it is likely difficult to model and quantify the contributions than two sources of Cu ligands (Voelker, 2001). Therefore streams with specific point inputs may be the best systems in which to first attempt mole balance modeling of Cu and Cu ligands.

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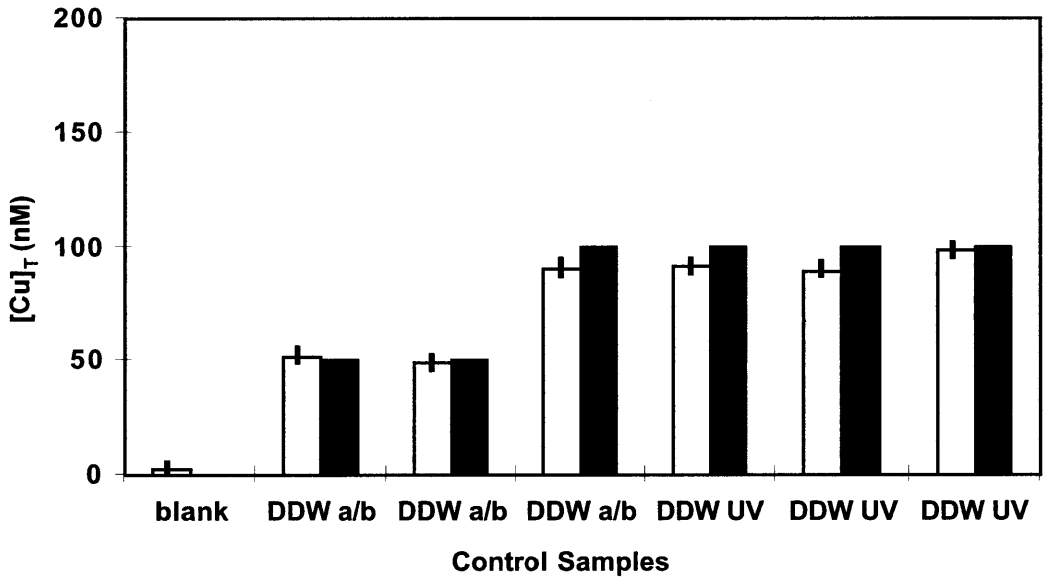

Figure 5.1. Control samples for determining total Cu after treatments (white bars) compared to actual Cu concentrations (black bars). The treatments are acidification and neutralization with base (a/b) treament followed by measurement by CLE-ACSV, and acidification, UV irradiation, and neutralization followed by CLE-ACSV.

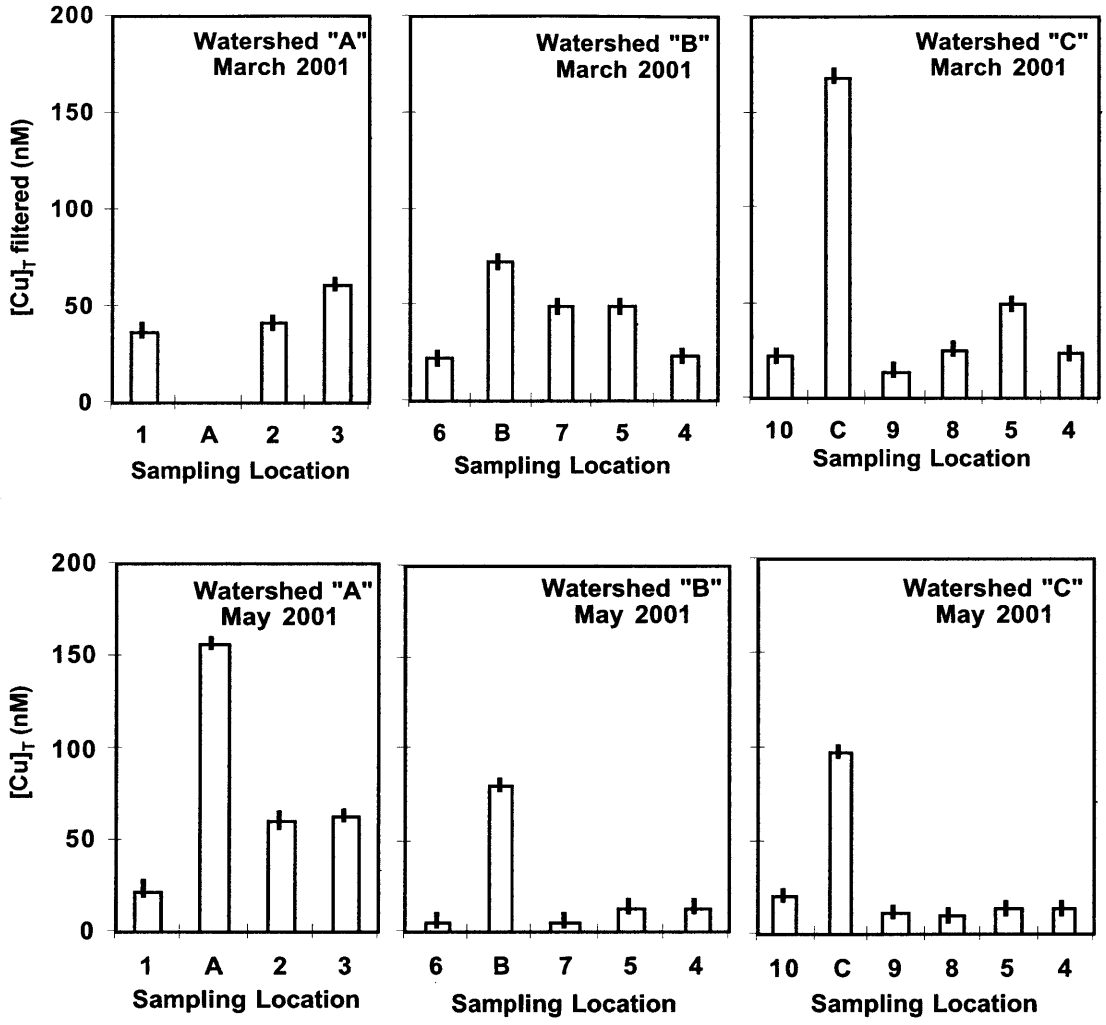

Figure 5.2. Filtered (March 2001) and total (May 2001) Cu concentrations for the three sub-watersheds (A, B, and C) of the Taunton River watershed. in order of sampling location from upstream on left to downstream on right, including the POTW's.

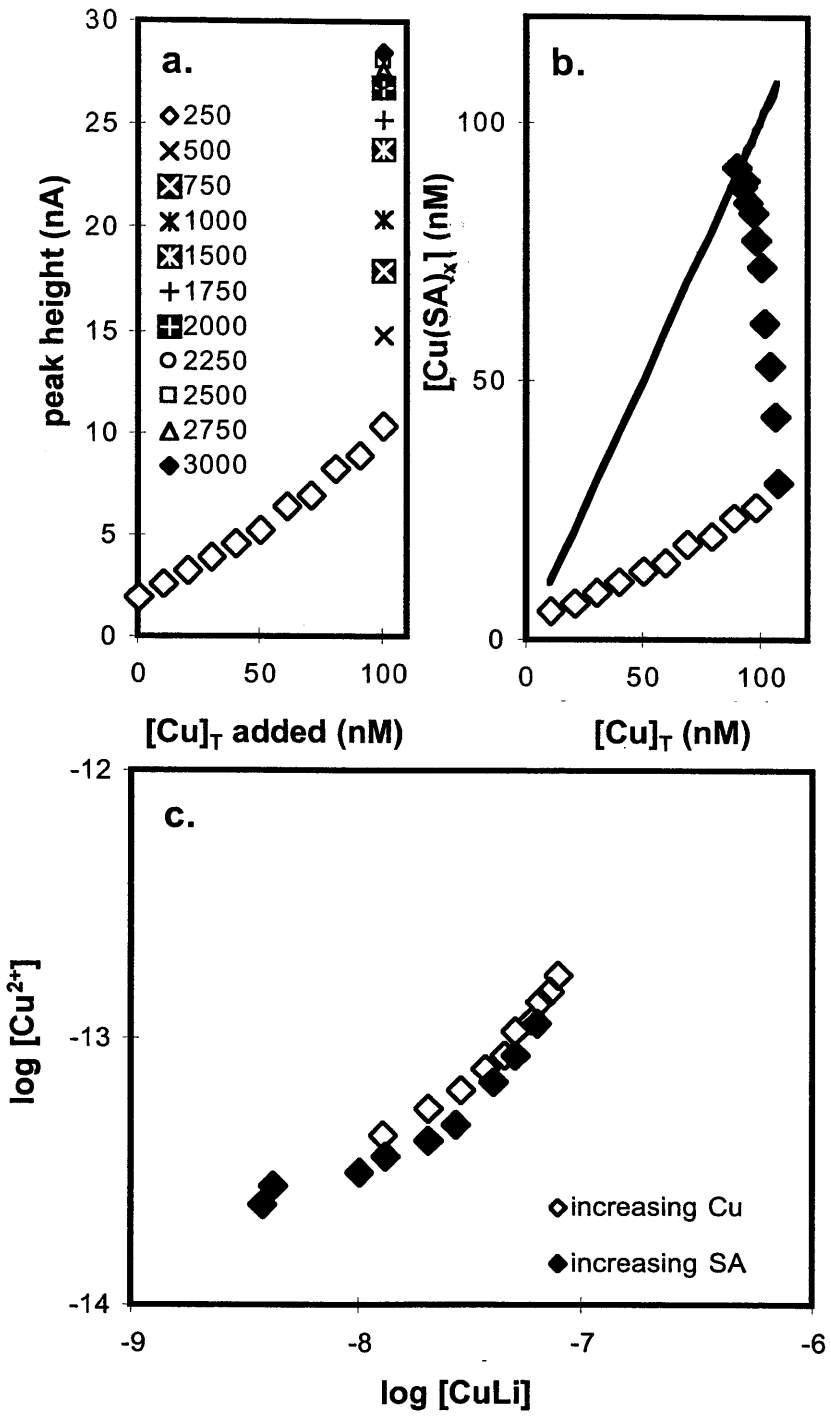

Figure 5.3. Determining Cu speciation in Site 9. a) Raw data for titration of Site 9 with a Cu titration at 250 μM SA followed by a titration with increasing SA ("backwards" titration). b) Values of $[Cu(SA)_x]$ at each titration point using the sensitivity determined with the 2500 μM SA point. C) Cu speciation in Site 9, including 'forwards" titrations (increasing Cu) and backwards titrations (increasing SA). Overlap shows that SA is in equilibrium with natural ligands after a 3 minute equilibration period.

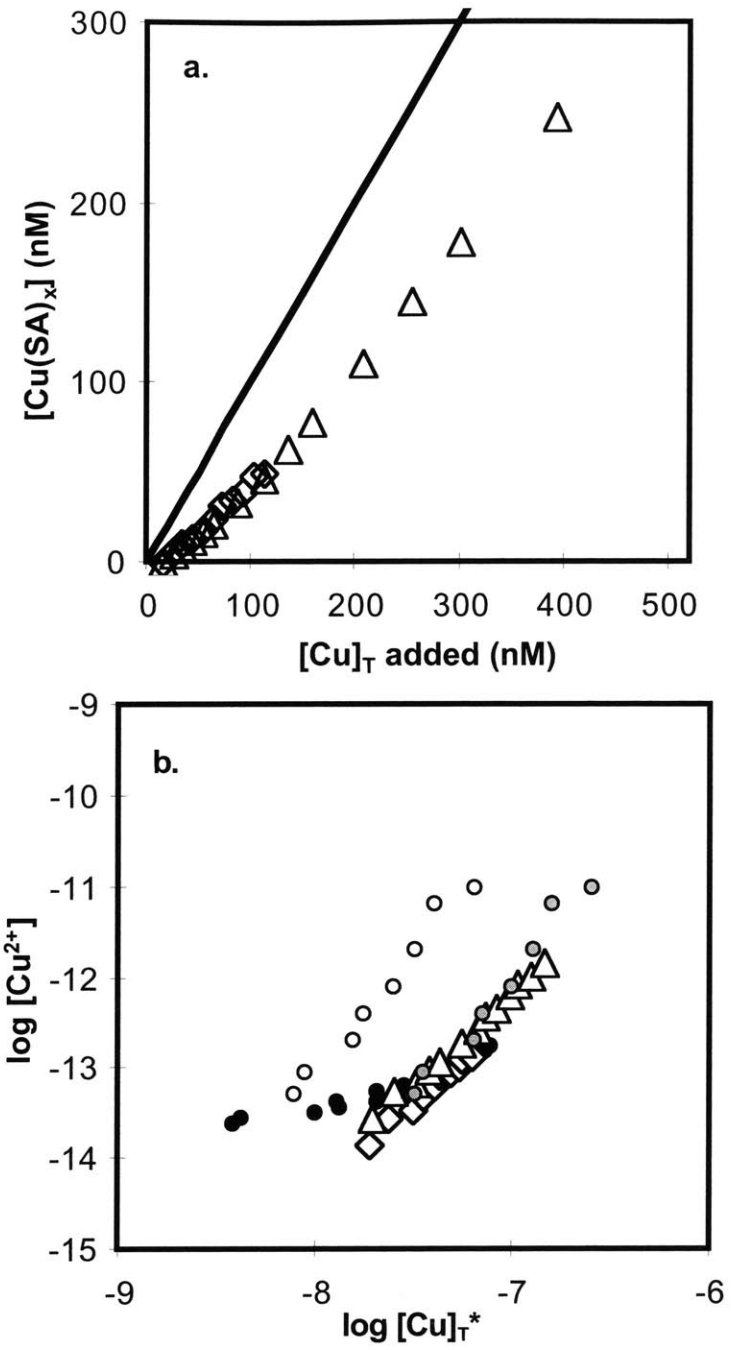

Fig. 5.4. Cu titration and Cu speciation in the same Site 9 sample as in Fig 5.3, using the overload titration calibration method described in Section 5.2.7. Titrations were conducted at 500 (diamonds) and 250 μM SA (triangles). The overload titration (black line) was conducted at 2500 μM S. Also included are the data from the experiment in Fig. 5.3 (●), data eyeballed from Fig. 1a from Xue (1999) which show the binding ability of 6 mg/L SRFA at pH=7.5 (○),and that data extrapolated to 24 mg/L SRFA (gray dots).

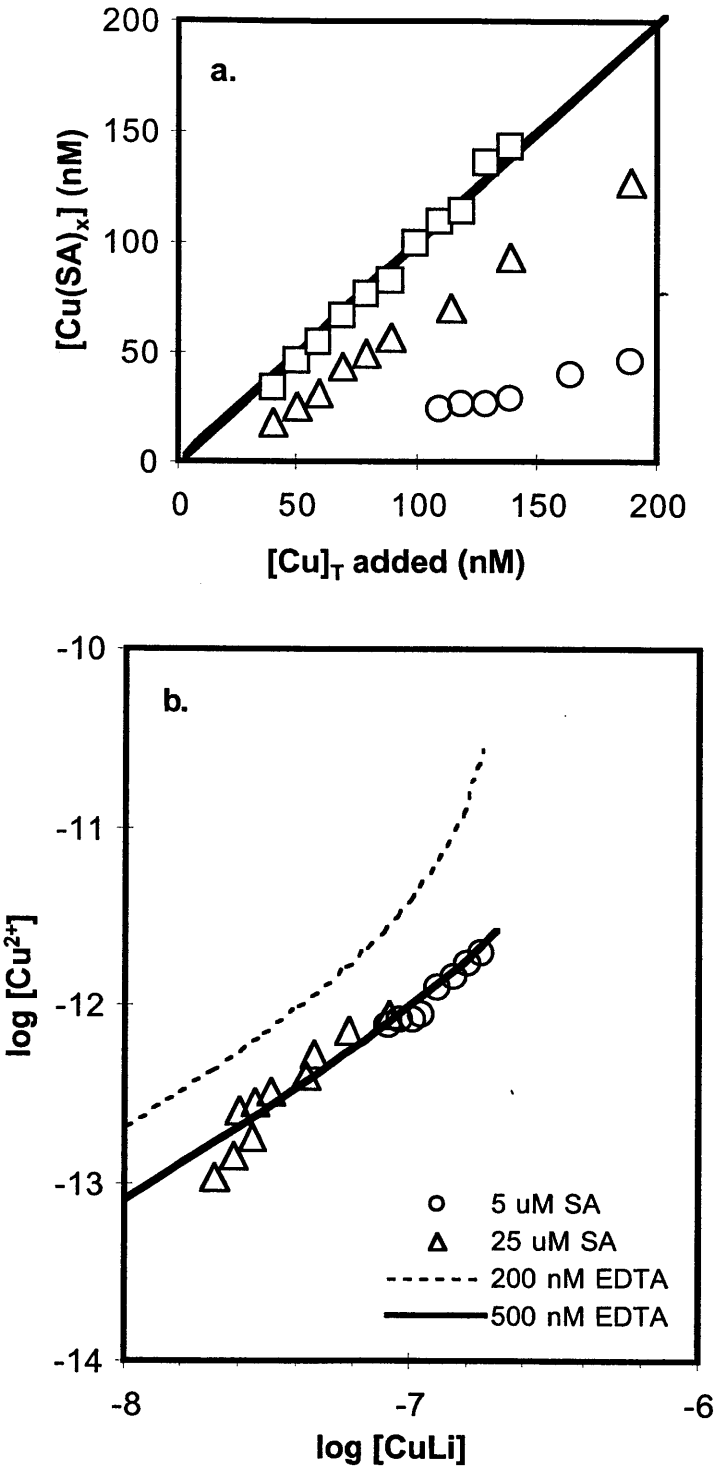

Fig. 5.5. (a) Cu titration and (b) Cu speciation in POTW "C" sample. Circles, triangles, and squares represent titrations conducted at 5, 25, and 50 μM SA, respectively, and the overload titration (black line in (a)) was conducted at 500 μM SA. The Cu speciation of EDTA was modeled as well in (b).

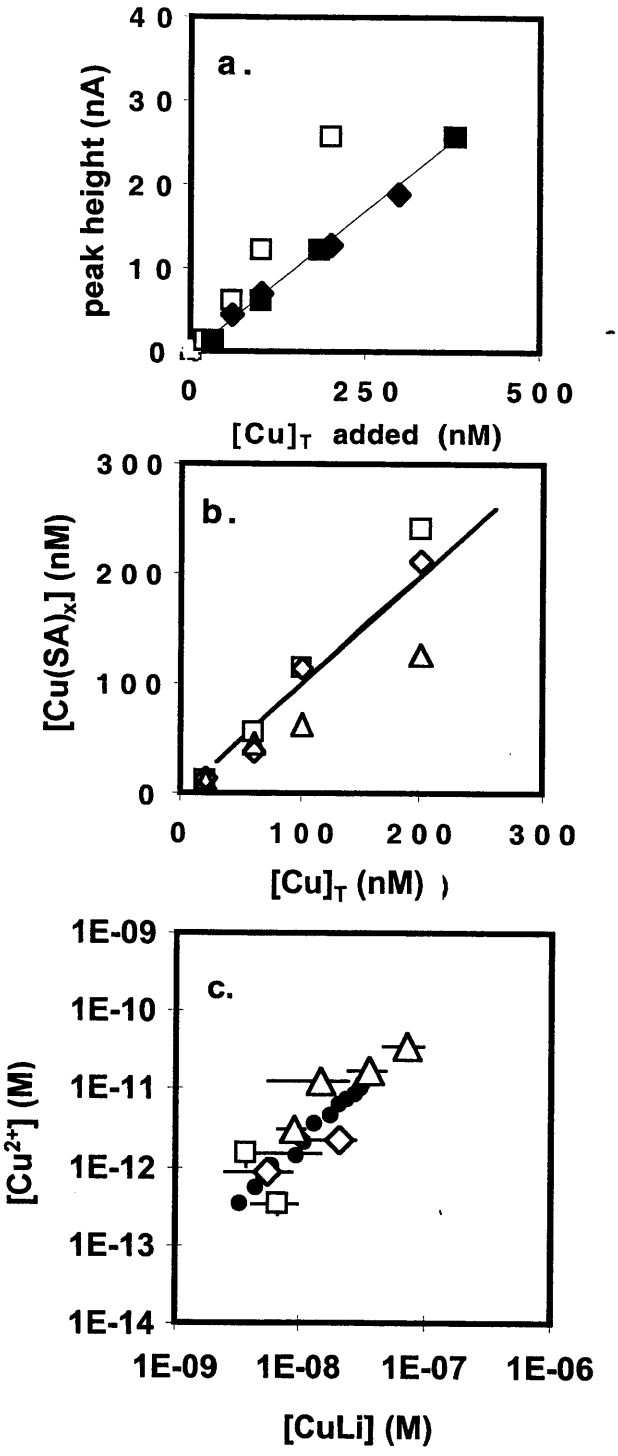

Fig. 5.6. (a) raw data, (b) data calibrated with the EDTA method, and (c) Cu speciation data for 1 mg/L SRHA in seawater, at pH=8.2. Black dots are titrations of 1 mg/L SRHA in seawater, at pH=8.2 conducted with overload titrations (from Kogut, 2001).

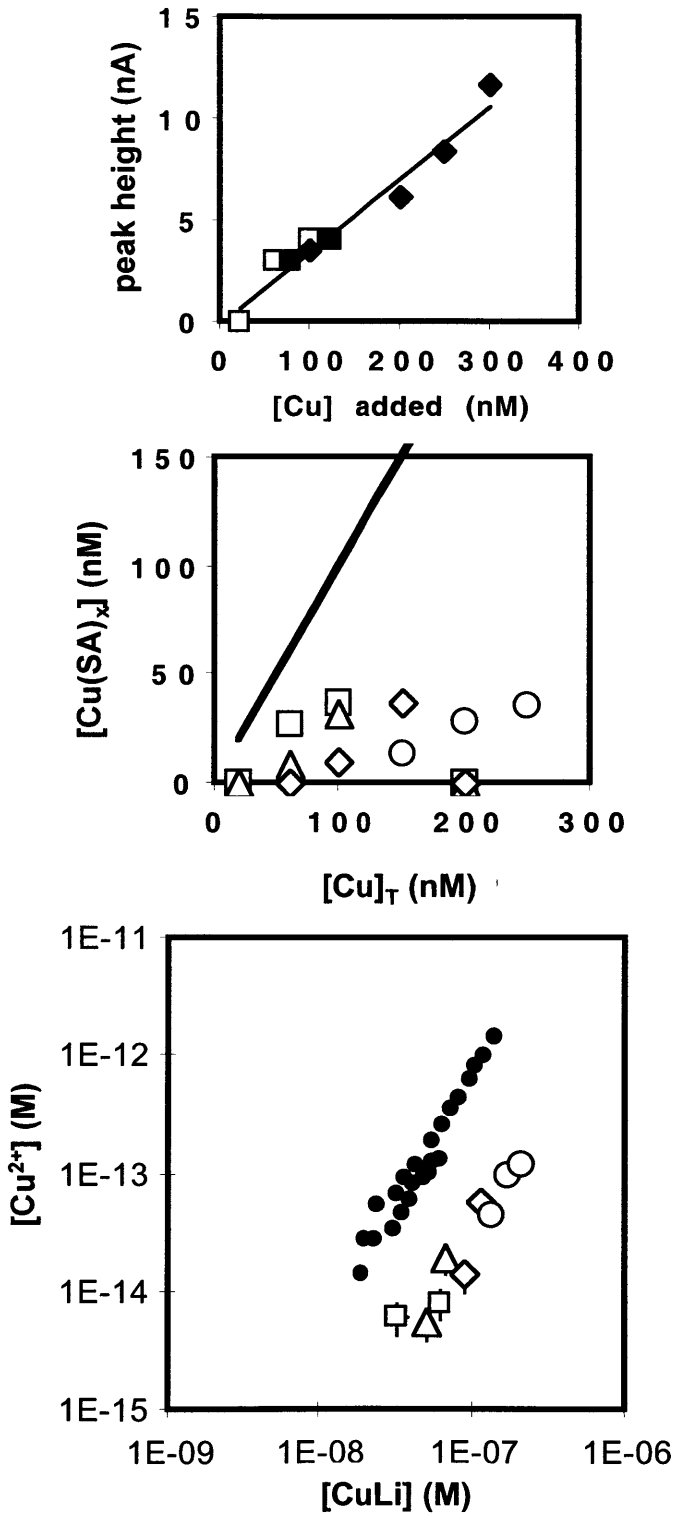

Fig. 5.7. (a) raw data, (b) data calibrated with the EDTA method, and (c) Cu speciation data for Site 9 sampled in May, 2001, at pH = 7. Black dots are data from Fig 5.5.

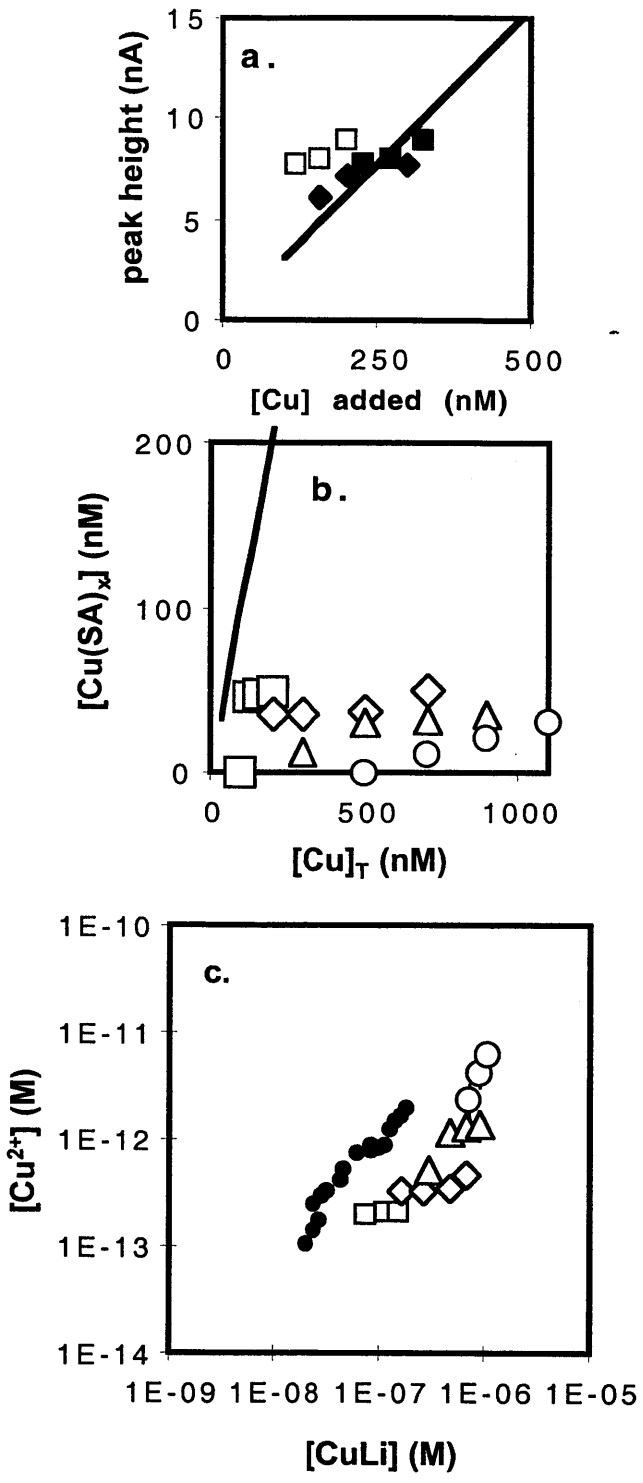

Fig. 5.8. (a) raw data, (b) data calibrated with the EDTA method, and (c) Cu speciation data for Site "C" sampled in May, 2001, at pH = 7. Black dots are data from Fig 5.6.

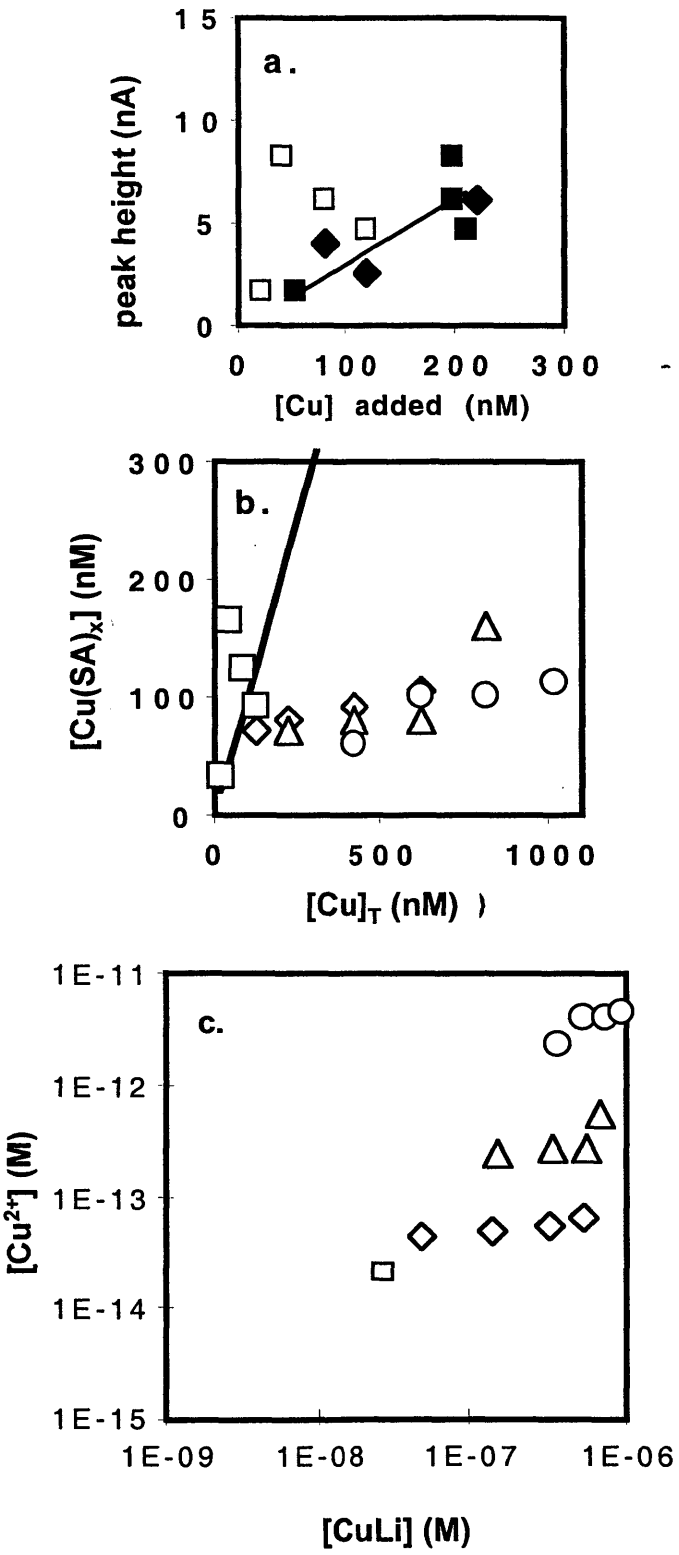

Fig. 5.9. (a) raw data, (b) data calibrated with the EDTA method, and (c) Cu speciation data for Site 7 sampled in May, 2001, at pH = 7.

Appendix A. The Conditional Stability Constant of $CuEDTA^{2-}$

In order to calibrate SA against EDTA in a range of sample salinities and pH, we calculated the change of the conditional, or apparent, stability constant of CuEDTA with changes in Ca^{2+}, Mg^{2+}, H^+, and ionic strength, I. EDTA binds Ca^{2+}, Mg^{2+}, and H^+, and when the concentration of any of these cations is significant compared to $[EDTA]_T$, then a significant fraction of EDTA may be unavailable for binding Cu. Because cation concentrations are relatively high in seawater samples, side reactions of EDTA with these cations must be accounted for in samples of different salinities. In addition, ionic strength is high in seawater samples, so the effect of ionic strength, estimated with the Davies Equation (Morel and Hering, 1993), is also calculated for samples of different salinity.

A.1 Cu-EDTA Complexes.

To simplify calculations of the conditional stability constant of Cu-EDTA complexes, we first determined that $CuEDTA^{2-}$ is the most important Cu-EDTA complex in our working pH range (pH = 6-9). The total of all Cu-EDTA species in seawater is as follows:

$$[Cu\text{-}EDTA]_T=[CuEDTA^{2-}]+[CuHEDTA^{-}]+[CuOHEDTA^{3-}] \quad (A.1),$$

from Morel and Hering (1993). The conditional stability constant of the $CuEDTA^{2-}$ is

$$K_{CuEDTA} = \frac{[CuEDTA^{2-}]}{[Cu^{2+}][EDTA^{4-}]} \quad (A.2).$$

Because the second and third Cu-EDTA complexes include H^+ or OH^-, the formation of these constants requires the release of a proton or hydroxide:

$$Cu^{2+} + H^+ + EDTA^{4-} \rightarrow CuHEDTA^{-} \quad (A.3)$$

or

$$Cu^{2+} + OH^- + EDTA^{4-} \rightarrow CuHEDTA^{3-} \quad \text{(A.4).}$$

The binding strengths of these complexes are pH dependent:

$$K_{CuHEDTA} = \frac{[CuHEDTA^-]}{[Cu^{2+}][H^+][EDTA^{4-}]_f} \quad \text{(A.5)}$$

or

$$K_{CuOHEDTA} = \frac{[CuOHEDTA^{3-}]}{[Cu^{2+}][OH^-][EDTA^{4-}]_f} \quad \text{(A.6).}$$

Table A-1 shows the stability constant for all three Cu-EDTA complexes. When considering only the effect of pH on Cu-EDTA binding, at a pH range from 6 to 9 (our working pH range), the conditional stability constant for each complex, K', where

$$K'_{CuHEDTA} = K_{CuHEDTA} \cdot [H^+] \quad \text{(A.7)}$$

and

$$K'_{CuOHEDTA} = K_{CuOHEDTA^{3-}} \cdot [OH^-] \quad \text{(A.8),}$$

is much less than than K_{CuEDTA}, which is not pH dependent. Therefore $CuEDTA^{2-}$ is by far the most important Cu-EDTA complex in the pH range of 6 to 9.

A.2. Ionic Strength

Because the samples generally had high ionic strength (>0.02 M), we calculated ion strength effects on conditional stability constants. To make ionic strength corrections on all stability constants, we calculated the ionic strength (I) of the sample, whose major ion contributors were salts present in seawater and the 0.02 M buffer. The ionic strength of all samples was calculated as

$$I = 0.5\Sigma(z^2 s) \quad \text{(A.9),}$$

where z is charge number of each species and s is the concentration of the species. Ionic strength, calculated from average ion concentrations in seawater, range from 0.72 M in

seawater to .02 M in seawater diluted to 1‰ (parts per thousand of chlorinity). Because a 0.02 M buffer is added to each sample before analysis, the total ionic strength is the contributions by natural cations plus 0.02 M. The activities of the species were calculated from the Davies Equation,

$$a = \exp(-1.17(z^2)(\frac{I^{0.5}}{(1+I^{0.5})} + 0.3I) \quad \text{(A.10).}$$

All stability constants were multiplied (for free ionic species) or divided (for charged complexes) by the appropriate ion activities.

A.3. Competition of other cations with Cu for EDTA

We calculated the fraction of total EDTA complexed by Ca^{2+}, Mg^{2+}, and H^+ at a range of salinities in order to determine the conditional stability constant of $CuEDTA^{2-}$ in these samples. The thermodynamic stability constant of $CuEDTA^{2-}$, K_{CuEDTA}, is defined for the free concentration of $EDTA^{4-}$, where

$$[EDTA^{4-}] = [EDTA]_T - ([CaEDTA^{2-}] + [MgEDTA^{2-}] + \Sigma_x[H_xEDTA^{x-4}]) \quad \text{(A.11),}$$

assuming that relatively small concentrations of total EDTA are complexed by Cu^{2+}. A new conditional stability constant, $K'_{CuEDTA2-}$, is defined for total EDTA, $[EDTA]_T$, where

$$K'_{CuEDTA} = K_{CuEDTA}\frac{[EDTA^{4-}]}{[EDTA]_T} \quad \text{(A.12).}$$

The value of K'_{CuEDTA} is calculated as a function of Ca^{2+}, Mg^{2+}, and H^+ by substituting in the appropriate complexation equations for each of the additional EDTA complexes in Eq. A.11, dividing out the $[EDTA^{4-}]$ term, and substituting the rearranged Eq. A.11 into Eq. A.12. The conditional binding constant of CuEDTA can then be calculated as

$$K'_{CuEDTA} = \frac{K_{CuEDTA}}{1 + K'_{CaEDTA}[Ca^{2+}]_T + K'_{MgEDTA}[Mg^{2+}]_T + \Sigma_x K_{H_xEDTA^{x-4}}[H^+]^x} \quad \text{(A.13),}$$

where the conditional stability constants of EDTA with Ca^{2+} and Mg^{2+} are in turn calculated with respect to total concentrations of Ca^{2+} and Mg^{2+} and adjusted for ionic strength effects.

To account for the fact that Ca and Mg complex other major anions in seawater, the free concentrations of Ca^{2+} and Mg^{2+}, as a function of complexation by major anions (bicarbonate, carbonate, and hydroxide), were calculated, assuming that complexation by EDTA changed the concentrations of these major cations negligibly because $[EDTA]_T << [Ca^{2+}]_T$ or $[Mg^{2+}]_T$.

Total Na, Ca, Mg, and carbonate concentrations in seawater (35‰) are 0.47 M, 10.3 mM, 52.8 mM, and 2 mM respectively (Millero and Sohn, 1991). We mixed UV-oxidized seawater with distilled, deionized water (DDW), and we assumed that contributions of all ions from DDW were negligible even at 1‰ with the exceptions of H^+ and OH^- (held constant with 0.02M buffer). Total carbonate was calculated using Henry's constant and the acidity constants for carbonate, assuming equilibration of the DDW with air. Values are summarized in Table A-2. The values of $[Ca^{2+}]$ range from 10 mM in seawater to 0.3 mM in seawater diluted with DDW to 1‰.

A.4. Results

Results of our EDTA speciation modeling are included in Table A-2. Ionic strength, calculated from average ion concentrations in seawater, ranged from 0.74 in undiluted seawater to 0.04 M in seawater diluted to 1‰. For both $Ca^{2=}$ and $Mg^{2=}$, the free species was about 66 and 63% of the total concentrations, respectively, with the remainder complexated by SO_4^{2-} and HCO_3^+. However, adding an ion pairing model to better determine the ionic strength effects on the conditional stability constants of $CaSO_4$ and

$CaHCO_3^+$ and of $MgSO_4$ and $MgHCO_3^+$ shows that 90% of total Ca^{2+} is free (with 8% as $CaSO_4$ and 1% as $CaHCO_3^+$) and 89% % of total Mg^{2+} is free (with 10% as $MgSO_4$ and 1% as $MgHCO_3^+$) (Morel and Hering, 1993.) Because we cannot extrapolate these more accurate findings to lower salinity levels, we assumed that Ca and Mg is 100% free. This assumption causes the the conditional stability constant of $CuEDTA^{2-}$ to be underrestimated by only 0.05 log units, an error which is small compared to our uncertainty in calibration of SA (0.1-0.2 log units).

At all values of pH, Ca^{2+} complexed most of the EDTA (roughly 92%) and Mg2+ complexed a smaller fraction (roughly 8%) so that $[EDTA^{4-}]$ was 3e-7% of $[EDTA]_{\%}$ at 35‰ and 2e-6% at 1‰. Concentrations of H^+ species of EDTA were negligible compared to concentrations of $CaEDTA^{2-}$. The overall conditional binding constant of $CuEDTA^{2-}$ varied from $10^{11.60}$ at 1‰ to $10^{10.05}$ at 35‰.

References.

(1) Morel, F. M. M., Hering, J.G. *Principles and Applications of Aquatic Chemistry*; John Wiley & Sons, Inc.: New York, 1993.

(2) Millero, F. J. M. L. Sohn. *Chemical Oceanography*; CRC Press, Inc.: Boca Raton, 1991.

Table A.1. Log values of the conditional stability constants for the three Cu-EDTA complexes as a function of pH.

pH	(unadjusted)	5	6	7	8	9
$CuEDTA^{2-}$	20.5	20.5	20.5	20.5	20.5	20.5
$CuHEDTA^{-}$	23.9	18.9	17.9	16.9	15.9	14.9
$CuOHEDTA^{3-}$	22.6	13.6	14.6	15.6	16.6	17.6

Table A.2. Parameters and results of modeling the conditional stability constant of $CuEDTA^{2-}$ for a range of salinities (valid for pH = 6-9).

Salinity (‰)	$[Ca^{2+}]_T$ (mM)	$[Mg^{2+}]_T$ (mM)	$[CO^{3-}]$ (μM)	Log K'_{CuEDTA}
1	0.295	1.52	66	11.60
10	2.95	15.2	580	10.60
20	5.89	30.4	1700	10.12
35	10.3	53.2	2000	10.05

Appendix B. Approximation of the partitioning of salicylaldoxime into dissolved natural organic matter.

B.1. Introduction.

One assumption of competitive ligand exchange with an added ligand (AL) is that the added ligand concentration dissolved and available to complex copper, $[AL]_{dissolved}$, is equal to the total concentration of AL added ($[AL]_T$). One reason that [AL] would not equal $[AL]_T$ is that AL, an organic molecule, could partition into the natural organic matter in a field sample. This is of particular concern because for Competitive Ligand Exchange Adsorptive Cathodic Stripping Voltammetry, the added ligand is chosen in part for its hydrophobicity. A more hydrophobic ligand adsorbs more readily to the electrode's mercury drop surface, increasing sensitivity of the method. Because concentrations of natural organic matter of rivers and estuaries are often in excess of 10 mg/l, partitioning of the added ligand to organic matter is possible in these types of samples. If the concentration of truly dissolved AL is significantly less than $[AL]_T$, then the copper binding ability of the natural ligands will be overestimated.

The fraction of the added ligand can be predicted by comparing the molecular make-up of the added ligand to that of a similar organic chemical whose hydrophobicity has been measured. Hydrophobicity of organic molecules is quantified by their partitioning in to water and octanol. All chemicals therefore have an octanol/water partitioning coefficient, K_{ow}, where

$$K_{ow} = \frac{\frac{\text{molesAL}}{\text{liter octanol}}}{\frac{\text{molesAL}}{\text{liter water}}} \qquad \text{(B.1).}$$

A value of K_{ow} of 1 predicts that 50% of the added ligand will partition into octanol and 50% into water on a volume/volume basis. The value of K_{ow} can be determined directly by measuring the fractions of $[AL]_T$ which partition into octanol and water. Alternatively, it can be estimated from octanol/water partitioning coefficient of similar organic chemicals and the effects on water solubility of molecular fragments (Handbook of Chemical Property Estimation Methods). This method begins with a parent molecule whose K_{OW} is well known (benzene for organic molecules). Then, fragments with known contributions to K_{ow} are added and subtracted, and their corresponding contribution to or away from hydrophobicity of the added ligand accounted for.

The extent to which an organic molecule partitions into octanol is comparable to the extent to which it will partition into natural organic matter. For a number a different chemicals, the best-fit relationship between K_{om} and K_{ow} was found to be:

$$\log K_{om} = 0.82(\log K_{ow})+0.14 \qquad \text{(B.2)},$$

where the units of K_{om} are mol l water^{-1} kg om^{-1} mol (Schwarzenbach, 1993). The value of K_{om} is generally lower than K_{ow} because natural organic matter is more polar and more charged than octanol under typical conditions (pH range of 5 to 8). The ability of AL to sorb to organic matter does not likely change much for organic matter from different sources, as other nonpolar chemicals are found to sorb in approximately the same manner (with K_{ow} within a factor of 10) for a variety of soils and sediments, (Schwarzenback, p. 272.)

In samples with varying organic matter content, it is more useful to consider x, the fraction of added ligand in the water normalized to the concentration of organic matter in the sample. For this, the value of K_{om} is multiplied by the fraction of organic matter in kg/L,

$$x=K_{om}f_{om} \quad \text{(B.3)}$$

Typical concentrations of organic matter in rivers and coastal areas are 1 to 20 mg/L. Taking an average concentration of 10^{-5} kg/l (10 mg/l), a value of K_{om} of $1x10^5$ means $[AL]_{dissolved}$ is approximately equal to $[AL]_T$. A value of K_{om} less than 10^3 suggests that in a sample with 10 mg/l organic matter, 99% of the AL will be truly dissolved and therefore available to complex copper.

B.2. Calculation of K_{ow} for salicylaldoxime.

For the added ligand salicylaldoxime, SA, whose molecular structure is shown in Fig. 1, an appropriate parent molecule is benzene, with a known K_{ow} of $10^{1.15}$. The appropriate aromatic fragments (and each corresponding contribution to K_{ow}, or f) were added to or subtracted from benzene and its K_{ow}: the cyano group, the phenol fragments, and an aromatic hydrogen fragment:

$$\log K_{ow} = f(-C_6H_5) + f^{\varnothing}(CH{=}NOH) + f^{\varnothing}(-OH) - f^{\varnothing}(-H) \quad \text{(B.4).}$$

The value of K_{ow} for SA is $10^{1.08}$ as calculated using Eq.2 and Table 1. In addition, because the two polar groups, -OH and the nitrogen in CH=NOH are separated by three carbons, K_{ow} is increased slightly by an additional factor which accounts for intramolecular interaction, F_{P3}, where

$$F_{P3}=-0.10(f_1+f_2) \quad \text{(B.5).}$$

The value of K_{ow} for SA is closer to $10^{1.14}$ after this correction.

Using Eq. 2 to relate the octanol/water partitioning to organic matter/water partitioning of SA, SA has a K_{om} of $10^{1.05}$ or approximately 11. This value is much less than the estimated value of 10^3 needed to insure that 99% of the SA is truly dissolved. Therefore, the rough estimations of SA partitioning adequately show that even at very high concentrations of natural organic matter, such as in wetlands or colored rivers, the analyst can assume that all SA is in solution. "Salting effects", or a decrease in water solubility

of SA with an increase in dissolved salts, would increase K_{om} only by a few percent, even in seawater (Schwarzenbach, p. 278).

In areas with increased concentrations of surfactants, such as near sewage outlets, increased care should be taken to insure that SA or other added ligands remain in solution.

References.

(1) Schwarzenbach, R. P., P. M. Gschwend, D. M. Imboden *Environmental Organic Chemistry*; John Wiley & Sons, Inc.: New York, 1993.

Figure B.1. The molecular structure of salicylaldoxime.

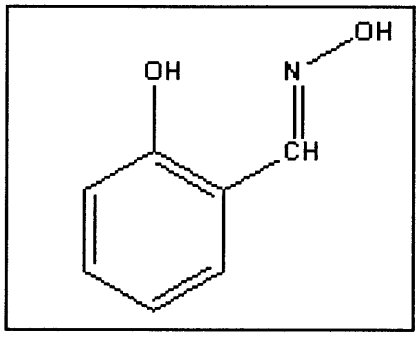

Table B.1. Fragment identities and factors for approximation of K_{ow} for salicylaldoxime.

Fragment identity	**f or $f^{\emptyset}$**
($-C_6H_5$)	1.9
(CH=NOH)	-0.15
(-OH)	-0.44
(-H)	0.23

Appendix C. Correction factors for CLE-ACSV at different values of salinity and pH

We need correction factors to extrapolate S from overload titrations with high [SA] to speciation titrations with low [SA] because S depends somewhat on SA (see Appendix C). The correction factors are obtained by comparing S at high and low [SA] in sub-samples that have been UV-irradiated to destroy all ligands that could compete successfully with low [SA]. The correction factors depend in part on instrument settings as well as sample salinity and pH, may be related to changes in Cu-SA speciation on the surface of the mercury drop during CSV analysis (Campos, 1994; Appendix D), and range in value from 0.3 to 1.0 in the samples we have analyzed.

We determined the correction factors for Saco River by UV-irradiating Saco River samples and adjusting pH to values at which we measured Cu speciation in those samples (Chapter 3). These correction factors are presented in Table C.1.

Table C.1. Correction factors for Saco River Estuary samples.

S ‰	1.1	1.1	20.4	10.1	10.1	27.4	27.4	27.4
pH	7.0	7.7	7.0	7.0	7.5	8.0	7.7	7.0
[SA] (μM)	correction factors							
1	0.33	0.31	0.30	0.27	0.50	0.636	0.38	0.23
3	0.46	0.57	0.45	0.46	0.66	0.873	0.74	0.45
5	0.56	0.76	0.64	0.51	0.93	0.932	0.90	0.76
100	1.00	1.00	1.00	1	1	1	1	1

Appendix D. Strong copper-binding behavior of terrestrial humic substances in seawater.

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Strong Copper-Binding Behavior of Terrestrial Humic Substances in Seawater

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In coastal areas, strong complexation of copper generally reduces its toxicity; our ability to monitor and regulate copper as a toxin therefore depends on our understanding of the sources and sinks of the copper-binding ligands. Terrestrial humic substances (HS) are well-recognized contributors to weak ligand concentrations in aquatic systems. In this work, we show that HS are likely contributors to both stronger and weaker ligand classes controlling copper speciation in coastal areas receiving typical inputs of terrestrial organic matter. We used competitive ligand exchange adsorptive cathodic stripping voltammetry (CLE-ACSV), with the added ligands benzoylacetone and salicylaldoxime, to examine copper binding by terrestrial HS in a seawater matrix, at HS and copper concentrations typical of coastal waters. Copper titration data of 1 mg/L Suwannee River humic acid (SRHA) in seawater could be modeled using conditional stability constants of $10^{12.0}$ and $10^{10.0}$ and total ligand concentrations of 10.4 and 199 nM for a stronger and weaker ligand, respectively. Similar results were obtained for Suwannee River fulvic acid (SRFA). Strong copper binding by SRFA in seawater was weaker than previously reported for a freshwater at similar pH, possibly indicating effects of Ca and Mg competition or ionic strength. Nevertheless, the concentrations and binding strengths of copper ligands we observed are comparable to the range reported in previous coastal speciation studies. In addition, we show that the weaker copper ligands cause internal calibration techniques to significantly underestimate the sensitivity of ACSV in the presence of HS concentrations typical of coastal waters. To address this issue, we demonstrate the use of "overload titrations", using a high enough concentration of added ligand to outcompete all natural ligands as an alternative calibration technique for analysis of coastal samples.

Introduction

Elevated copper concentrations due to anthropogenic inputs can be toxic to microorganisms in coastal areas (e.g., refs *1* and *2*). Total dissolved copper concentrations range from 2 nM in well-flushed coastal areas (*3*) to more than 100 nM in the most heavily impacted areas such as San Francisco Bay (*4*). Copper is up to 99.99% complexed by strong ligands in the water column and not immediately bioavailable, so that toxicity is frequently correlated to the free copper concentration ($[Cu^{2+}]$) and not to total dissolved Cu (*1*, *5*, *6*). To predict and monitor copper's effects as a toxin, we need to understand the sources and fate not only of the metal but also of the ligands controlling the relationship between free copper and total copper.

The copper-binding behavior of a water sample, that is, the variation in $[Cu^{2+}]$ as a function of total copper concentration ($[Cu]_T$) can be calculated if the total concentrations of the ligands present ($[L_i]_T$) and their copper-binding strengths are known. The binding strength of a copper ligand with respect to free copper is represented by its conditional binding constant, K_{CuLi}, defined by the equation

$$K_{CuLi} = [CuL_i]/([Cu^{2+}][L_i] \quad (1)$$

in which $[CuL_i]$ is the concentration of copper bound to ligands (both organic and inorganic) of class L_i, and

$$[L_i] = [L_i]_T - [CuL_i] \quad (2)$$

The speciation of copper in natural waters is described by the equation

$$[Cu]_T = [Cu^{2+}] + \Sigma[CuL_i] \quad (3)$$

where the sum of concentrations of all copper species is the total dissolved copper concentration. Substituting eq 1 into eq 3 and dividing both sides by $[Cu^{2+}]$ relates ligand binding strengths and concentrations to the extent of copper complexation,

$$[Cu]_T/[Cu^{2+}] = 1 + \Sigma(K_{CuLi}[L_i]) \quad (4)$$

The quantity $K_{CuLi}[L_i]$ is called the side reaction coefficient (SRC) of ligand class L_i and is a useful value for comparing copper binding abilities of different ligands. The change in $[L_i]$ with increasing copper concentration can be calculated from eqs 1 and 2:

$$[L_i] = [L_i]_T/(1 + K_{CuLi}[Cu^{2+}]) \quad (5)$$

Usually the concentrations and stability constants of the ligands controlling copper speciation in natural water samples are not known a priori; instead, $[Cu^{2+}]$ is measured as a function of $[Cu]_T$ and the result is described using a one- or multi-ligand model, where $[L_i]_T$ and K_{CuLi} are used as fitting parameters. The strongest ("L_1") ligands found in coastal areas approaching seawater salinity are generally present at nanomolar concentrations and have a conditional copper binding constant of 10^{12} and greater. A more abundant class of weaker ligands, "L_2", with conditional binding constants in the range of 10^8 to 10^{11}, is also observed. The fraction of total copper complexed by inorganic hydroxide and carbonate species in seawater (*7*) is a negligible fraction of $\Sigma[CuL_i]$ in samples that contain L_1-class ligands. Ligands in class "L_1" control $[Cu^{2+}]$ when $[Cu]_T$ is less than $[L_1]_T$ while ligands in class "L_2" control copper speciation when the stronger L_1 class is copper-saturated, that is, when $[Cu]_T$ is greater than $[L_1]_T$. It is unclear to what extent the distinction between L_1 and L_2 class ligands reflects a true difference in the chemical characteristics (e.g., sources, sinks, and chemical reactivity) of these ligands and to what extent it is an arbitrary division.

One identified source of "L_1"-strength copper ligands is planktonic microorganisms. Two species of marine microalgae, *synechococcus sp.* and *emiliania huxleyi*, have been observed to excrete strong copper-complexing ligands when

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copper stressed in culture, most likely as a defense mechanism against copper toxicity (*8*, *9*). Ligands with similar binding strengths ($K_{CuL} = 10^{12}-10^{14}$) are found in the upper mixed layer of the water column of the Northeast Pacific (*10*), the North Sea and Mediterranean Sea (*11*), and the Sargasso Sea (*12*), with concentration versus depth profiles suggesting a plankton source.

L_1-strength ligands that may not be attributable to plankton also exist in coastal systems. Skrabal et al. (*13*) observed that ligands in estuarine sediment porewaters could represent a significant source of strong ligands to Chesapeake Bay if they are stable in oxic waters. Similarly, Sedlak et al. (*14*) found that treated sewage effluents and creeks could be a source of strong ligands to South San Francisco Bay. In addition, copper sulfide and thiol complexes [e.g., $K_{Cu(HS)} = 10^{13}$, $\beta_{Cu(HS)2} = 10^{20}$ (*15*)] can resist oxidation in aerobic waters for several days and could account for 10 to 60% of the total strong copper complexes observed in several rivers in Connecticut (*16*). Resolving the issue of whether L_1-strength ligands come mostly from planktonic or nonplanktonic sources in a given environment is important for understanding not only their environmental fate, but also their ecological significance, since biological production of ligands for copper detoxification must incur some metabolic cost to the organisms producing them.

Terrestrial humic substances (HS) have been discussed as weak ligands in rivers and sewage (e.g., refs *3*, *14*, and *17*) but are usually not considered to be contenders for L_1-strength ligands. In a recent study of humic substances isolated from the Suwannee River and from peat redissolved in freshwaters at pH 8.0 and 7.8, respectively, ligands with conditional binding constants of $10^{14.1}$ at concentrations of 5 nM/mg, and $10^{12.5}$ at concentrations of 23 nM/mg HS were observed (*17*). Similarly, strong copper binding by another peat humic acid was observed at pH 8 (*18*). Typical concentrations of total dissolved organic matter (DOM) in rivers and estuaries are on the order of 2 to 20 mg/L; humic substances probably account for a signification fraction of this DOM [~50% according to Morel and Hering (*19*)]. Although hydrophobic riverine humic substances have been shown to flocculate and settle upon mixing with saline waters (*20*), this process may only be significant in Fe-rich waters. Conservative dilution of DOC (*21*) and colored dissolved organic matter (CDOM) (*22*) have been observed in some estuaries, and work using lignin as a tracer of terrestrial humic substances shows that they account for 60% of the total DOM in a southeastern U.S. estuary (salinity = 18%) and up to 20% of total DOM even miles offshore (salinity > 30%) (*23*). If concentrations of terrestrial humic substances of 1 mg/L and higher can be expected in coastal waters, the study of Xue and Sigg (*17*) suggests that they must be considered as possible sources of L_1-strength as well as L_2-strength copper ligands in coastal waters, unless competition for the ligands by Ca^{2+} and Mg^{2+} ions and ionic strength effects dramatically decrease their copper-binding strengths in seawater.

One study of humic acid isolated from the Suwannee River in Georgia has probed binding sites with conditional binding strengths greater than 10^8 in seawater-like solutions (pH = 8.2; ionic strength = 0.5 M; $[Ca^{2+}] = 10$ mM) (*24*). In this study, the binding ability of a 1 mg/L Suwannee River humic acid solution could be modeled with a three-ligand model as follows: $K_{CuLa} > 10^{11}$; $[L_a]_T = 50$ nM; $K_{CuLb} = 10^{9.2}$; $[L_b]_T = 200$ nM; $K_{CuLc} = 10^{6.6}$; $[L_c]_T = 1800$ nM, where the subscripts a, b, and c denote the strongest, weaker, and weakest ligand classes. The detection limits of anodic stripping voltammetry used in this study prevented probing of binding sites at concentrations lower than 50 nM, leaving open the possibility of stronger binding sites at lower concentrations that could contribute to L_1 in coastal waters.

The purpose of our study is to determine whether humic substances at concentrations typical of estuarine and coastal waters could account for some of the L_1-strength binding observed in these waters. For the first time, we have taken methods developed by oceanographers to study copper speciation at ambient (nanomolar) copper levels in seawater and applied them to 1 mg/L solutions of several humic substance isolates. This study focused on Suwannee River humic acid (SRHA) and Suwannee River fulvic acid (SRFA), chosen because their metal-binding properties and other characteristics have been well studied (*25*) and because we wanted to compare our results to those of the previous works mentioned above (*17*, *24*). We show that these humic substances bind copper strongly in a seawater matrix and propose that humic substances in general may play a previously unrecognized role in the buffering of copper toxicity in coastal areas.

Materials and Methods

Materials. SRHA and SRFA isolated on an XAD-8 column at pH 2 from samples collected at the base of the Okefenokee Swamp in Georgia, USA, were obtained from the International Humic Substance Society (IHSS). In addition, we performed preliminary analyses of Summit Hill humic acid (SHHA), a soil humic acid also from the IHSS.

Acid-cleaned Teflon containers were used for all reagents and experiments to minimize adsorption of copper and ligands to bottle surfaces and leaching of phthalate plasticizers into the samples. Humic substance (HS) stock solutions of 100 mg/L were made every week from freeze-dried humic substances and deionized distilled water (DDW) (Millipore Q-H_2O). UV oxidized Sargasso seawater (UV-SW) was collected in August 1997 with trace metal clean methods and subjected to at least 8 h of ultraviolet light oxidation with a mercury lamp (Ace Glass, 1000 W). To make buffered UV-SW, 1 mL of UV-oxidized 1 M boric acid (EM Science, Suprapur), adjusted to pH of 8.2 with 0.35 M ammonia, was added to 1 L of UV-SW for a final pH buffer concentration of 1 mM. Fresh 1 mM primary and 1 μM secondary copper standards were made every day by serial dilutions of an Aldrich atomic absorption standard solution ($CuSO_4$ in 1% HNO_3, 10 000 ppm Cu) with buffered UV-SW; the final pH of the secondary copper standard was about 7. The added ligand for competitive ligand exchange adsorptive cathodic stripping voltammetry (see below) was either benzoylacetone (bzac) (*26*), or salicylaldoxime (SA) (*27*), both purified by equilibration with EDTA and repeated filtering and recrystallization. Bzac was dissolved in methanol (J. T. Baker, HPLC solvent) for a final stock solution concentration of 10^{-2} M. SA was dissolved in DDW for a final stock solution concentration of 10^{-4} M. SA and bzac stock solutions were replaced every 3 months and refrigerated when not in use.

Competitive Ligand Exchange (CLE) Copper Titration Experiments. Competitive ligand exchange adsorptive cathodic stripping voltammetry (CLE-ACSV) is a two part process: first, a known concentration of a well-characterized and purified synthetic ligand, AL, is added to a series of samples containing the natural ligands and a range of added copper and allowed to equilibrate. ACSV (see next section below) is then used to measure the concentration of copper complexed with added ligand, $\Sigma[Cu(AL)_x]$ as a function of total Cu, where

$$\Sigma[Cu(AL)_x] = [Cu(AL)] + [Cu(AL)_2] \quad (6)$$

and CuAL and $CuAL_2$ are the mono and the bis complex of copper with AL, respectively. At the start of each copper titration experiment, one-half of a 1 L batch of buffered UV-SW was separated for UV-SW standard curves and 0.5 mL of

the HS stock solution was added to the other half for a concentration of 1.0 mg/L HS.

For competitive ligand exchange with SA, copper titrations were set up as one sample to which aliquots of copper were sequentially added. The sample was a 10.0 mL aliquot of the 1.0 mg/L HS solution pipetted directly into the electrode's Teflon sample cup. SA was also added to the sample at this time. Copper from the secondary standard was added sequentially to the sample after each titration point analysis of $[Cu(SA)_x]$ for a range of $[Cu]_T$ from 0 to 180 nM. Before each analysis, samples were allowed to equilibrate for 5 min (SRFA, SRHA) or 15 min (SHHA) (see Results). For bzac titrations, ten to twelve 10.0-mL aliquots of the 1.0 mg/L HS solution were pipetted into individual 30-mL Teflon bottles. Copper from the secondary standard was added to each sample for a range of $[Cu]_T$ from 0 to 200 nM. The samples were equilibrated for 2−3 h, and then bzac was added to each sample and allowed to equilibrate with copper and HS for an additional hour or overnight before analysis.

Voltammetric Analyses. $\Sigma[Cu(SA)_x]$ and $\Sigma[Cu(bzac)_x]$ in the samples were analyzed using adsorptive cathodic stripping voltammetry. This method consists of two steps: an adsorption step, during which $Cu(AL)_x$ complexes are sorbed to a mercury drop electrode, and a potential scan in the negative direction, during which the current produced by the reduction of the copper in the sorbed complexes is measured. We used differential pulse ACSV with a PAR 303A static mercury drop electrode and an EG&G PAR 394 analyzer. Instrument settings were as follows: adsorption potential, −0.08 V (vs Ag/AgCl electrode); scan range, −8 to −500 mV; scan rate, 10 mV/s; drop time, 0.2 s; pulse height, 25 mV. Adsorption times were 10 and 30 s. For both SA and bzac, the reduction of Cu in the $Cu(AL)_2$ complex produced the dominant peak in the potential scan (*27, 28*). Reduction of $Cu(AL)_2$ produced a well-defined peak at −240 to −290 mV for bzac copper complexes and −330 to −400 mV for SA copper complexes. There was no signal caused by the reduction of humic complexes in samples with or without added ligand and copper.

Data Analysis. The goal of obtaining copper titration data is to determine the variation in free copper concentration, $[Cu^{2+}]$, as a function of $[Cu]_T$ in the sample. Competitive ligand exchange is an indirect method for obtaining this information, because the presence of the added ligand changes the speciation of copper in the sample. The new copper speciation equation in the presence of AL, replacing eq 3, is

$$[Cu]_T = [Cu^{2+}] + \Sigma[CuL_i] + \Sigma[Cu(AL)_x] \quad (7)$$

With the values of SRC(AL) [also the "analytical competition strength" (*28*)] used in this study, complexes of copper with inorganic ligands (OH^-, CO_3^{2-}, NH_3) constitute a negligible portion of $\Sigma[CuL_i]$; therefore, all L_i are assumed to be binding sites in either humic or fulvic acid. In the absence of both AL and the Cu complexed by AL, the water sample would have an identical $[Cu^{2+}]$, and a theoretical total copper concentration $[Cu]_T^*$ that is given by:

$$[Cu]_T^* = [Cu]_T - \Sigma[Cu(AL)_x] = [Cu^{2+}] + \Sigma[CuL_i] \cong \Sigma[CuL_i] \quad (8)$$

The variation in free copper concentration that would be measured as a function of $[Cu]_T$ in the absence of added ligand can therefore be read directly off a plot of $[Cu^{2+}]$ versus $[Cu]_T^*$ obtained from CLE titrations (*3, 17, 29*).

Each titration data point corresponds to a measurement of $\Sigma[Cu(AL)_x]$ and $[Cu]_T$, from which the quantity $[Cu]_T^*$ can be calculated directly using eq 8. The value of $[Cu^{2+}]$ is calculated from $\Sigma[Cu(AL)_x]$, [AL], and the conditional stability constants of the mono and bis complexes, $K_{Cu(AL)}$ and $\beta_{Cu(AL)2}$:

$$[Cu^{2+}] = \Sigma[Cu(AL)_x]/(K_{Cu(AL)}[AL] + \beta_{Cu(AL)2}[AL]^2) = \Sigma[Cu(AL)_x]/SRC(AL) \quad (9)$$

where [AL] is the concentration of AL not bound to copper:

$$[AL] = [AL]_T - [Cu(AL)] - 2[Cu(AL)_2] \quad (10)$$

When $[AL]_T$ is much greater than $[Cu]_T$, one can assume that the copper species are negligible in eq 10. However, for titrations using 1 or 3 μM SA and up to 160 nM $[Cu]_T$, this is not a good assumption, so we calculated the exact concentrations of [AL] and $[Cu^{2+}]$ from eqs 6 and 10 and the two equilibrium mass law expressions for formation of Cu-AL complexes, using EXCEL to solve for the four unknowns in the four equations.

Results

Methods Development. During the course of our examination of the copper-binding properties of humic substances in seawater, it became clear that a new protocol was required for distinguishing "surfactant effects" from complexation when calibrating the CLE-ACSV technique. The protocol presented here contrasts sharply with, and in many cases is preferable to, current protocols for copper titrations of coastal samples.

A crucial step in conducting ACSV measurements is to perform calibrations to determine the relationship between the current peak height, I, and $[Cu(AL)_x]$, called the sensitivity, S:

$$[Cu(AL)_x] = I/S \quad (11)$$

S depends on both instrument settings (e.g., adsorption time and potential) and the sample matrix.

Two methods, called "external" and "internal" calibrations, are commonly used to determine S. In an external calibration, copper is added sequentially to UV-SW containing the added ligand. Since no competing natural ligands other than weak carbonate and hydroxide ligands are present in the UV-treated water, AL complexes virtually all of the copper, so that

$$[Cu]_T \cong \Sigma[Cu(AL)_x] \quad (12)$$

A plot of current peak height I as a function of $[Cu]_T$ should therefore yield a straight line with a slope of S. In an internal calibration, copper is added sequentially to the water sample until a linear relationship between peak height and $[Cu]_T$ is observed. The analyst assumes that the reason that the relationship is linear is that at the higher values of $[Cu]_T$, all of the stronger natural ligands present in the sample have been saturated with copper, so that any additional Cu added ($\Delta[Cu]_T$) is complexed by the added ligand:

$$\Delta[Cu]_T \cong \Delta\Sigma[Cu(AL)_x] \quad (13)$$

The slope of I versus $[Cu]_T$ in this part of the titration should then also be equal to the sensitivity S.

Neither of these calibration techniques yields satisfactory results in the presence of humic substances. Since humic substances include hydrophobic dissolved organic compounds that may sorb to the mercury drop surface and decrease the sensitivity by hindering the simultaneous sorption of $Cu(AL)_x$ [the surfactant effect, (*27, 30*)], external calibrations could overestimate the sensitivity. Internal calibrations will underestimate the sensitivity if large concentrations of weaker ligands (such as humic ligands) compete with AL for copper at higher $[Cu]_T$. A decrease in

signal caused by complexation can be difficult to distinguish from one caused by surfactant effects since the observed relationship between peak height and total copper in the latter case may be indistinguishable from the straight line expected in the formed case (this is discussed in more detail in ref *29*). Using MINEQL (*31*) and the three-ligand model for the copper binding properties of SRHA in seawater determined by Hering and Morel (*24*), we calculated that due to complexation effects, an internal calibration in the presence of 1 mg/L SRHA with 1 μM SA would underestimate sensitivity by as much as 40% (*30*) if Cu additions of up to 160 nM were used. The problem worsened slightly when an additional stronger ligand that Hering would not have been able to measure was added to the model calculation. While in theory one could add enough Cu to saturate all of the natural ligands present, this may not be possible in practice, because at high $[Cu(AL)_x]$ concentrations, the current is no longer a linear function of $[Cu(AL)_x]$.

To avoid problems with using either external or internal calibrations in the presence of humic substances, we developed a technique called an "overload" titration, which is similar to an internal calibration in that the sensitivity is measured directly in the water sample to be analyzed; however, a concentration of AL is used that is high enough to outcompete all of the natural ligands present in the sample. In an "overload" titration, eq 13 applies, and peak height versus $[Cu]_T$ should yield a straight line with a slope of S. Since an overload titration does not provide any information on the natural ligands in the sample, other "speciation" titrations with lower concentrations of AL must also be performed. The surfactant effect is assumed to be the same independent of $[AL]_T$. For example, if no surfactant effect is observed with 25 μM SA in the presence of 1 mg/L HS (that is, the sensitivity measured in the presence of HS is the same as that measured in UV-SW), then we assume that the sensitivity of a titration performed at 1 μM SA in the presence of 1 mg/L HS is the same as the sensitivity with 1 μM SA in UV-SW.

One minor complication is that the sensitivity with 1 μM SA in UV-SW is not the same as the sensitivity with 25 μM SA in UV-SW. The change in SA speciation (i.e., the ratio of $[Cu(SA)]$ to $[Cu(SA)_2]$) resulting from an increase in $[SA]_T$) affects the sensitivity because only $Cu(SA)_2$ is reduced at the mercury drop during the ACSV step. We compared the slopes of standard curves obtained with 1–5 μM and 25 μM SA in UV-SW and determined correction factors (as percent of the slopes of the 25 μM SA standard curve) of 55% (± 3%), 78% (± 4%), and 92% (± 5%) for 1, 3, and 5 μM SA, respectively. Since these correction factors were reproducible for different adsorption times and on different days, we used them to extrapolate sensitivities measured at 25 μM SA to lower SA concentrations instead of performing additional UV-SW titrations for each of the SA concentrations used for "speciation" titrations.

We used overload titrations with 25 μM SA to distinguish surfactant effects from complexation effects and determine the sensitivity of titrations with SA. This SA concentration was chosen because MINEQL calculations using Hering and Morel's three-ligand model of SRHA (*24*), plus an additional stronger ligand at a concentration of 10 nM/mg and a K_{CuL} of 10^{12}, indicated that 25 μM SA can outcompete these ligands for copper (*30*). Plots of I versus $[Cu]_T$ obtained using 10 and 30 s adsorption times in 25 μM SA solutions with 1 mg/L SRHA or SRFA yielded straight lines whose slopes were not significantly different from those in 25 μM SA solutions with UV-SW, indicating both that all Cu was bound by SA and that surfactant effects were negligible (Figure 1). Similar results were obtained with 1 mg/L SHHA. The possible exception is the standard curve with 1 mg/L SRFA and 30 s adsorption time; however, the difference between slopes of

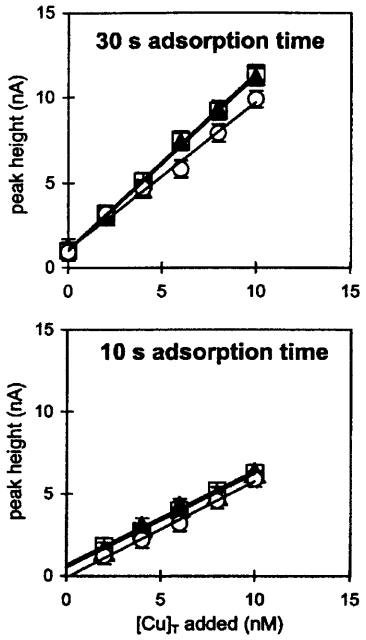

FIGURE 1. Comparison of current peak height versus $[Cu]_T$ added at 25 μM SA in the absence of HS (▲), with 1 mg/L SRFA (○), and with 1 mg/L SRHA (□). Lines represent linear regressions of the data. Lack of any significant difference between slopes of best fits in the presence and absence of HS indicates that surfactant effects are undetectable at adsorption times of 10 or 30 s.

SRFA and UW-SW in Figure 1 are comparable to the one standard deviation in slopes found from repeated determinations of standard curves in UV-SW with 30 s adsorption time. The overload titrations and standard curves were also used to determine $[Cu]_{T,i}$, the concentration of copper in the samples before any addition of copper. The average value of $[Cu]_{T,i}$ in UV-SW was 1.7 ± 0.5 nM. Values in 1 mg/L HS solutions did not differ significantly, indicating that input of Cu from HS stock solutions and contamination were both negligible.

We could not use overload titrations with bzac as the added ligand because 500 μM bzac does not provide a large enough SRC(AL) to outcompete all humic ligands, and bzac is not completely soluble at concentrations much higher than 500 μM (*32*). Since the SA overload titrations indicated that surfactant effects were negligible for 10-s adsorption times, we assumed that they were also negligible for bzac titrations and used UV-SW standard curves (external calibrations) to determine sensitivity.

Copper Titrations of Humic Substances. The times required for copper to equilibrate with the natural ligands and AL were determined for each HS sample prior to titration experiments by monitoring the analytical signal (current peak height) as a function of time, for samples with 1 μM SA and 10 nM total copper. Solutions with 1 mg/L SRFA or SRHA reached equilibrium within 5 min after addition of copper; SHHA reached equilibrium within 15 min. Equilibration times up to 24 h did not result in a change in $[Cu(SA)_x]$ measured. The analytical signal also remained constant with 10 consecutive measurements of the same sample.

Copper titrations of 1 mg/L SRHA clearly show that strong ligands capable of competing with both SA and bzac are present in the humic substance isolates (white symbols in Figure 2) and that internal calibrations would significantly underestimate the sensitivity in all titrations except the one using 3 μM SA. Although the other titrations show a linear

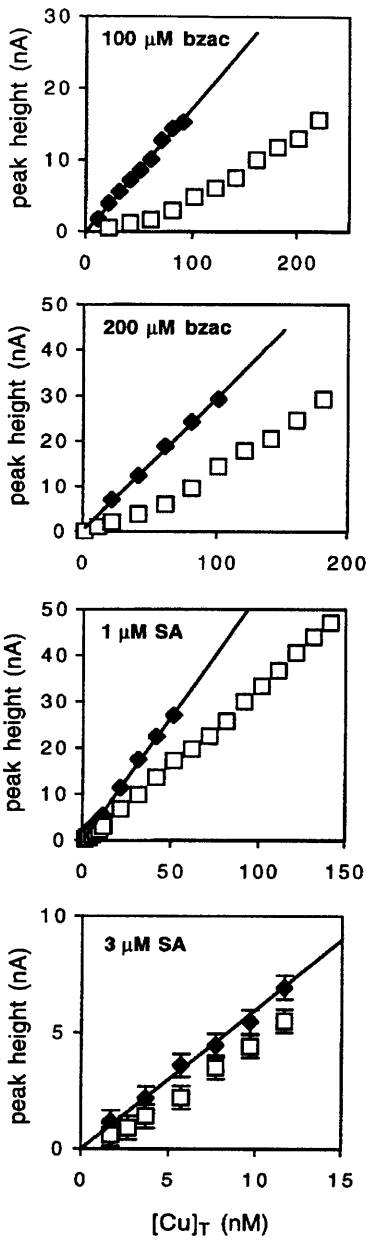

FIGURE 2. Representative raw data (peak height versus total copper) of copper titrations of 1 mg/L SRHA (□). For comparison, data points (◆) and best fit lines of external standard curves (UV standard curves for bzac, overload titrations at 25 μM SA and corrected for SA speciation for SA) are also shown. Raw data for SRFA and SHHA solutions looked similar.

increase in peak height as a function of $[Cu]_T$ at high $[Cu]_T$ values, the slopes of these lines are significantly (10–50%) smaller than the slopes of the lines representing $[Cu(AL)_x] = [Cu]_T$ (solid lines and black diamonds in Figure 2), which were obtained from either overload titrations adjusted with the appropriate correction factor (SA titrations) or from external calibrations (bzac). Similar results were obtained for SRFA. For SHHA, we only conducted one SA titration, which also exhibited the curvature indicating the presence of strong ligands (not shown).

We conducted SRFA and SRHA titrations with several values of SRC(AL) (Table 1), both to probe a wider range of $[Cu]_T^*$ and to be able to compare results from titrations where the ranges of $[Cu]_T^*$ values overlap. Plots of $[Cu^{2+}]$ versus $[Cu]_T^*$ (or $\Sigma[CuL_i]$, which is equivalent; eq 8) obtained from different titrations clearly show the agreement of data obtained in separate titrations with the same value of SRC-(AL) as well as the overlap of data obtained with different values of SRC(AL) (Figure 3a,b). CLE-ACSV with bzac spans the range of $[Cu]_T^*$ between that detectable by CLE-ACSV with SA and that detectable by the ASV method of Hering and Morel (*24*). Error bars were calculated assuming a 10% uncertainty in measured sensitivities, 0.5 nM standard deviation in $[Cu]_{T,i}$, and a 3% variability in determination of the peak height for each measurement. In Figure 3a, two heavy lines corresponding to the model of Hering and Morel (*24*) with the strongest ligand L_a present at 50 nM and with $K_{CuLa} = 10^{11}$ and $K_{CuLa} = 10^{13}$ are shown; the value of $[Cu]_T^*$ at which these two lines converge is the edge of the range of $[Cu]_T$ used in their study. The results of previously published studies of SRFA (*17*) are also shown for comparison (heavy solid line in Figure 3b); these lines are only extended over the range of $[Cu]_T^*$ for which data were obtained.

FITEQL (*33*) is the most convenient way to obtain a multi-ligand description of the combined titration data sets shown in Figure 3. One-ligand models could not obtain satisfactory fits to all of the SRHA and SRFA data points, and three ligand-models would not converge, indicating that a unique determination of three conditional binding constants and three ligand concentrations was not possible. Results of FITEQL fits are shown in Table 2. When K_{CuLi} values were (somewhat arbitrarily) specified for a two- or three-ligand fit of the SRHA data, FITEQL also converged well and produced good fits. Almost identical fits were obtained when different sets of three K_{CuLi} values were specified (Table 2). The results of these models were also plotted in Figure 3a,b as thin dashed and dotted lines.

Representative titrations from Figure 3a are compared to copper titration data from two coastal systems in Figure 4 (*3*). The data overlap at high $[Cu]_T^*$, but some stronger binding behavior in the coastal systems is evident at lower $[Cu]_T^*$.

Discussion

Overload Titrations. We successfully used overload titrations with 25 μM SA to show that surfactant effects were negligible in our 1 mg/L HS solutions. If we had used internal calibrations, we would have underestimated the sensitivity of ACSV by 10 to 50%. Furthermore, we would have discarded the data points used to calculate the slope at greater $[Cu]_T^*$; however, these points provide meaningful copper speciation data that agree closely with data from other titrations (Figures 3a,b).

We recommend the use of overload titrations for precise analysis of copper binding in coastal samples, where similar concentrations of humic substances are likely to be present. However, because field samples might have both ligands stronger than those found in SRHA and SRFA as well as high concentrations of organic matter that cause significant surfactant effects, overload titrations may not necessarily result in straight lines with slopes identical to those of UV standard curves, as in Figure 1. It may be possible to use overload titrations with several values of [AL] to distinguish a decrease in slope due to surfactant effects from one due to copper binding by natural ligands. However, this will only be possible if the relative magnitude of the surfactant effect does not vary with [AL]. We have observed decreases in surfactant effects with increasing [SA] in natural water samples; unless other added ligands behave differently, the use of overload titrations may be limited to verification of an absence of surfactant effects, for example when sufficiently short deposition times are used. One advantage of conducting several overload titrations is that the existence of copper

TABLE 1. Side Reaction Coefficients Used in Titration Calculations[a]

[AL] (μM)	100	200	300	400	500	1	3	5	25
SRC(bzac)	900	2800	5700	9600	14 500				
SRC(SA)						4160	18 500	40 800	704 000

[a] Bzac calibration data ($\beta_{Cu(bzac)2} = 10^{10.5}$) taken from Moffett (*26*). SA calibration data ($K_{CuSA} = 10^{9.5}$, $\beta_{Cu(SA)2} = 10^{15}$) taken from Campos and van den Berg (*27*).

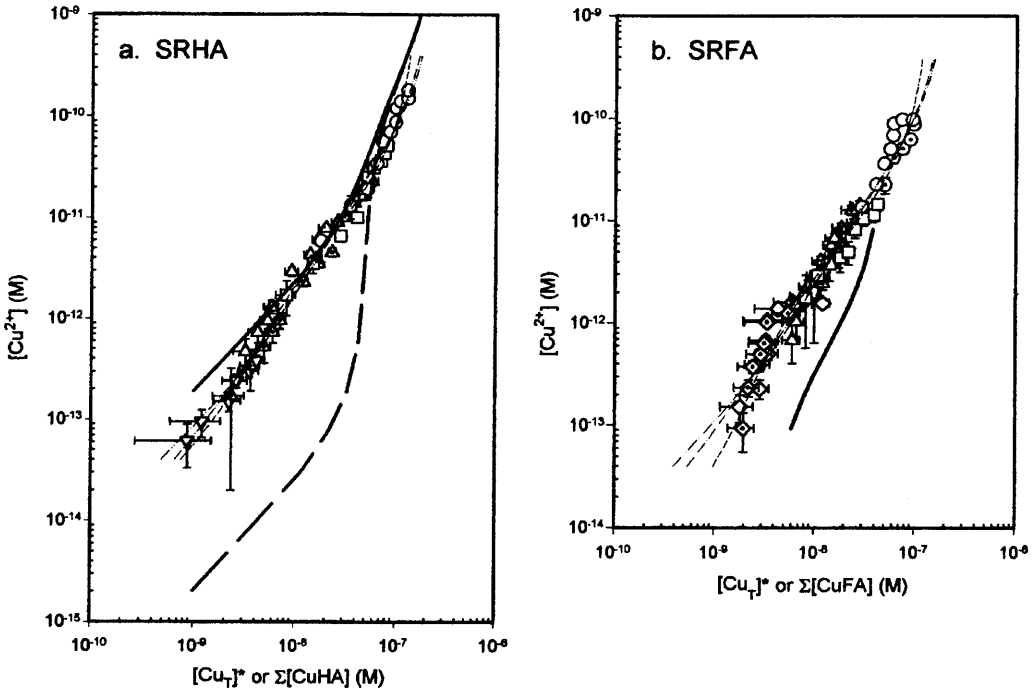

FIGURE 3. Plot of $[Cu^{2+}]$ versus $[Cu]_T^*$ for 1 mg/L SRHA and for 1 mg/L SRFA. Different symbols represent titrations conducted at different SRC(AL): (▽) 3 μM SA; (△) 1 μM SA; (◇) 500 μM bzac; (□) 200 μM bzac; (○) 100 μM bzac. Where two titrations at one value of SRC(AL) are shown, symbols with a dot in the center represent the second titration. Thin lines represent the FITEQL fits of each data set: (- -) model A; (·-·) model B; (-···) model C (see Table 2 for model parameters). (a) The four SRHA titrations presented in Figure 2, plus a second titration with 1 μM SA (obtained with a 10 s deposition time), are compared to ASV data for SRHA in seawater-like solutions (*24*). Heavy lines represent values for K_{CuL_3} of 10^{11} (solid heavy line) and 10^{13} (dashed heavy line) in Hering and Morel's three ligand model of SRHA (see Discussion). (b) Seven representative SRFA titrations are compared to SRFA analyzed in a freshwater matrix (solid heavy line) (*17*), calculated from modeled conditional binding constants and concentrations of ligands/mg SRHA or SRFA within the range of $[Cu]_T^*$ examined by this study.

TABLE 2. Results of Two-Ligand Fits and Three-Ligand Fits Obtained from FITEQL Modeling of All Copper Titrations of Either 1 mg/L SRHA or SRFA Shown in Figure 3[a]

model	K_{CuL1}	$[L_1]_T$	K_{CuL2}	$[L_2]_T$	$KCuL_3$	$[L_3]_T$
			1 mg/L SRHA			
A	$10^{12.74}$	3.4	$10^{10.4}$	137		
B	$10^{13.0}$	1.4	$10^{11.5}$	15.4	$10^{10.0}$	179
C	$10^{12.0}$	10.4	$10^{10.0}$	199		
			1 mg/L SRFA			
A	$10^{13.22}$	2.1	$10^{10.4}$	117		
B	$10^{13.0}$	1.1	$10^{11.5}$	12.0	$10^{10.0}$	160
C	$10^{12.0}$	8.3	$10^{10.0}$	175		

[a] Subscripts 1, 2, and 3 refer to the strongest, weaker (in the case of the three-ligand fits), and weakest ligand classes found. Model A was a two-ligand fit with no other parameters specified; models B and C were conducted with arbitrary fixed values of conditional copper binding constants (in bold.)

species "inert" to exchange with AL, either because they are very tightly bound or because their kinetics of exchange of Cu with AL are slow (*34*), will be indicated by reproducible positive x-intercept values. Kinetically inert copper is possible in complicated coastal systems where colloids and particles are common; the "concentration" of inert copper could be subtracted from the total ligand concentration to find the concentration of exchangeable copper or copper ligands.

When overload titrations are used, sensitivities measured at high AL may need to be adjusted for lower AL using correction factors such as the ones we applied for SA. An increase in sensitivity with an increase in [SA] was also observed by Campos and van den Berg (*27*), who found that the increase in sensitivity was directly proportional to the increase of the ratio of $[Cu(SA)_2]$ to $[Cu(SA)_x]$ in solution, indicating that $[Cu(SA)_2]$ is the electrochemically active species. Our correction factors do not agree with those of Campos and van den Berg, probably because we used a different adsorption potential (−0.08 versus −1.1V), which may alter $[Cu(SA)]/[Cu(SA)_x]$ sorbed on the mercury drop surface, even though $[Cu(SA)]/[Cu(SA)_x]$ in the sample remains constant with one value of [SA].

Copper Binding Behavior of Humic Substances. The copper titrations of 1 mg/L SRHA and 1 mg/L SRFA, plotted as $[Cu^{2+}]$ versus $[Cu]_T^*$ or $\Sigma[CuL_i]$ in Figure 3a,b, show good agreement among titrations from $[Cu]_T^*$ values of approximately 1−100 nM, the range of total dissolved copper

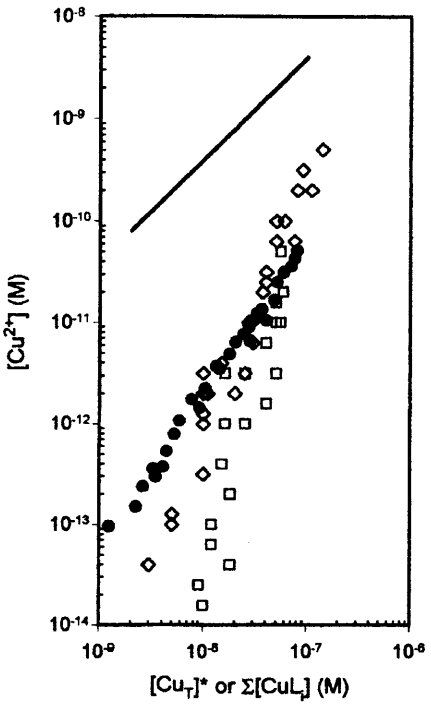

FIGURE 4. Comparisons of titration data of 1 mg/L SRHA (●) and field samples from two estuaries: Waquoit Bay (□), a high DOC harbor (salinity 25‰), and Vineyard Sound (◇), a coastal area (salinity 32‰) (3). Copper speciation expected in seawater from carbonate and hydroxide copper complexation alone (7) is plotted as a solid line.

found in coastal waters. All titrations of each humic substance agree within expected error, verifying that 5 min and 1 h are sufficient for equilibration of SRHA with SA and bzac, respectively, and that the use of UV-SW standard curves for CLE-ACSV with bzac was justified. Comparison of titrations identical except for adsorption times of 10 or 30 s (Δ with or without dot in Figure 3a) provides further verification that surfactant effects were negligible within the expected error of the titrations.

For SRHA, where the CLE-ACSV and ASV data overlap at higher $[Cu]_T^*$ (Figure 3a), the bzac titrations measured a slightly greater binding ability than the previous ASV study (*24*). Possible overestimation of $[Cu^{2+}]$ in the ASV study, due to a contribution of quickly dissociating copper humic complexes to ASV-labile copper, could explain the difference between CLE-ACSV and ASV data.

Below $[Cu]_T^* = 50$ nM, strong ligands in SRHA were analyzed in our study that were not probed previously. For the strongest ligand class for SRHA reported by Hering (*24*), $[L_a]_T$ is given as 50 nmol/mg SRHA, but only a lower limit of K_{CuLa}, 10^{11}, could be determined by their titrations. The heavy lines in Figure 3a, calculated for two possible values of K_{CuLa}, $10^{11.0}$ and $10^{13.0}$, predicting, respectively, stronger and weaker Cu binding than we observed, illustrate that the results of Hering and Morel cannot be used to calculate descriptions of the binding behavior of 1 mg/L SRHA from 1 to 50 nM total copper. Any binding parameters measured at much higher than ambient copper concentrations are irrelevant to calculations of copper speciation, since a small fraction of ligands may bind copper much more strongly than the more abundant ligands that dominate binding at high copper concentrations (*35*). For this reason, it is important that studies report the range of $[Cu]_T$ (or in the case of CLE studies, $[Cu]_T^*$) for which data were obtained (*29*).

With SRFA, we are able to compare the copper binding behavior of the same sample in freshwater (pH = 8.0) (*17*) and seawater (pH = 8.2) matrixes. SRFA titrated in a freshwater matrix binds copper more strongly from 5 to 40 nM total dissolved Cu than SRFA titrated in a seawater matrix, as shown by the relative decrease in $[Cu^{2+}]$ as a function of $[Cu]_T^*$ (Figure 3b). If pH effects alone were responsible for the difference, we would expect to see stronger binding with an increase in pH due to a decrease of H^+ competition. The difference in binding behaviors therefore suggests that increased cation competition or ionic strength effects decrease the binding of copper by low concentration ligands in seawater. (Although neither ionic strength nor concentration of Ca^{2+} and Mg^{2+} of the freshwater were specified in the study of Xue and Sigg, we assume they were much smaller than those of seawater.) This is in contrast to the study of Hering (*24*), which shows that the copper-binding behavior of higher concentration, weaker ligands in SRHA is not affected by the addition of 10 mM Ca^{2+}. However, the coordination chemistry of weaker and stronger ligands is not necessarily the same, so these studies do not contradict each other. That the conditional binding constant of terrestrial fulvic acid can decrease with an increase in cation concentrations has interesting implications for transport of ligands from river to ocean through estuaries, suggesting that comparisons of conditional binding constants and ligand concentrations along salinity transects must be made with care. These results support further research on the copper-binding behavior of riverine ligands, a large proportion of which are likely humic substances, before and after mixing into seawater.

Strong Copper Binding Behavior of HS Ligands. FITEQL modeling of the titration data (Table 2) shows that solutions of 1 mg/L SRHA and SRFA can be modeled as containing 8–10 nM concentrations of ligands with conditional binding strengths within the range of K_{CuL1} (with $K_{CuL1} > 10^{12}$) reported in coastal speciation studies (e.g., refs *3*, *4*, 28, and *36*). We did not conduct enough titrations of SHHA, a soil humic acid, to analyze its ligand content, but its strong binding behavior is similar to that of SRHA and SRFA. If terrestrial humic substances in general have comparable contents of stronger ligands, as has been observed for their weaker ligands (*37*), then it is likely that humic substances make up a significant fraction of L_1-strength copper ligands in coastal areas receiving inputs of terrestrial organic matter.

We conjecture that a random process of humic substance functional groups combining into multidentate copper binding sites accounts for the strong binding behavior of SRHA and SRFA. With an average molecular weight of 1100 Da (*25*), a 1 mg/L solution of SRHA corresponds to concentration of 900 nM. It is reasonable that on the order of one in 90 molecules of SRHA is capable of strongly binding copper with an average $K_{CuL} = 10^{12}$. It is unlikely that these strong ligands are microbially produced ligands isolated together with the humic substances. If any strong microbially produced ligand, with a K_{CuL} of $10^{12.7}$ or greater (*8*), had been present, a more clearly visible inflection point would be expected in the plots of $[Cu^{2+}]$ as a function of $[Cu]_T^*$. Furthermore, nearly equal quantities of the stronger ligands were observed in both the fulvic and the humic acid fractions of the Suwannee River humic substances. It is unlikely that any microorganism-produced ligand isolated on an XAD-8 column would precipitate with SRHA and remain in solution with SRFA in roughly equal amounts.

Interpretations of field research should consider terrestrial humic substances as possible contributors to both L_1 and L_2 strength ligands observed in coastal systems and recognize the role of terrestrial humic substances in decreasing copper toxicity to coastal ecosystems. Figure 4 shows that copper titrations of 1 mg/L SRHA appear similar above 10 nM total

copper to those of two samples from the coast of Cape Cod (*3*); however, some stronger copper binding by the field samples occurs at smaller total dissolved copper. This is similar to the situation found in Swiss lakes (*17*), where humic ligands likely account for some of the copper complexation found in natural systems at total dissolved copper concentrations greater than 50 nM, but ligands stronger than any found in humic substances were found in lake samples at lower copper. However, in another field study, the analysis of copper speciation in a series of samples along a salinity transect in the Severn Estuary suggests that humic substances could control copper speciation even at ambient $[Cu]_T$ (6−56 nM) in this system (*38*). The study found that concentrations of the strongest ligands analyzed (mean value of $K_{CuLl} = 10^{12.1}$, with no trend with salinity) were greatest near the mouth of the river entering the estuary (low salinity) and decreased conservatively as salinity increased, from 200 nM in the river (salinity = 2‰) to 13 nM near the coast (salinity = 33‰). That humic substances were not all removed in the estuary is indicated by the observation that total DOC concentrations (2−4 mg/L in the estuary) also decreased conservatively as salinity increased (*21*). Therefore, the riverine ligands present in this estuary may be humic substances with binding behavior similar to that of SRHA and SRFA. However, the results obtained in the lower salinity waters must be treated with caution, since the authors report seeing considerable "surfactant effects" when using internal calibration (which could at least partially be complexation effects). These different studies indicate that whether humic substances play a dominant role in copper speciation or not depends on whether other sources of strong ligands are also present. In any case, because they are a heterogeneous mixture of ligands that will blur the effects of any truly homogeneous ligands that may also be present, and because they interfere with internal calibrations, humic substances have a significant effect on the interpretation of copper complexation data [this is discussed in detail in Voelker and Kogut (*29*)].

The next step is further investigation of the extent to which rivers and runoff represent sources of strong ligands as well as copper to coastal areas. Do ligand sources autochthonous to a given estuary (e.g., marine phytoplankton) account for more copper binding than allochthonous sources (e.g., terrestrial humic acids)? In other words, are concentrations of strong allochthonous ligands comparable to $[Cu]_T$ in water released to the estuary? Or, is the input of $[Cu]_T$ greater than that of strong allochthonous ligand concentrations, so that autochthonous ligand sources, possibly produced at some cost to marine organisms, are needed to lower $[Cu^{2+}]$ below toxic concentrations? Are there seasonal as well as spatial variations affecting the answers to these questions? Related questions, such as whether allochthonous ligands release copper in saline waters due to cation competition and ionic strength effects, are also potentially important. These questions need to be considered in current discussions regarding both the regulation of releases of copper (and other metals) based on their effects on coastal ecosystems, as well as assessment of ecosystem health of metal polluted areas.

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