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Intercomparison of DPASV and ISE for the Measurement of Cu Complexation Characteristics of NOM in Freshwater

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Complexation by dissolved humic substances has an important influence on trace metal behavior in natural systems. Unfortunately, few analytical techniques are available with adequate sensitivity and selectivity to measure free metal ions reliably at the low concentrations under which they occur in natural waters. In the past, differential pulse anodic stripping voltammetry with a thin mercury film rotating disk glassy carbon electrode (DPASV-TMF-RDGCE) has been used to measure complexation capacities and conditional metal−ligand binding constants in oceanic and estuarine waters. In contrast, few studies have been conducted to validate DPASV's use in freshwaters. The current study compares DPASV and Cu ion-selective electrode (CuISE) methods for measuring free copper, complexation capacities, and conditional binding constants on samples of synthetic and isolated natural organics. Titration data show that DPASV and CuISE are able to measure similar amounts of labile copper when the two methods' detection windows overlap. Furthermore, the greater sensitivity of DPASV allows it to measure free copper at lower concentrations, which correlated well with CuISE data extrapolated into that same region. This strong correlation between these analytical methods provides positive evidence for the use of DPASV (TMF-RDGCE) in measuring free Cu^{2+}, complexation capacities, and conditional binding constants in freshwaters.

Introduction

In freshwaters, complexation by dissolved organic matte (DOM) can dominate trace metal speciation (*1*−*4*). Two factors complicate the quantification of metal−DOM complexation. One is that metal−organic stability constants are highly conditional, varying with properties of the DOM and matrix chemistry that change over time and location. Another is that low concentrations of both trace metals and complexing ligands found in natural systems makes them particularly challenging to assay. A flexible and easy method is needed that will allow measurement of complexing ligands and stability constants at real world concentrations.

Numerous electrochemical techniques have been employed to study metal humic complexation. One analytical technique, differential pulse anodic stripping voltammetry with a thin mercury film deposited on a rotating glassy carbon disk electrode DPASV (TMF-RGCDE), has been successfully used to measure labile metals (i.e., free and readily reversible complexes) at nanomolar levels typically found in seawater (*5*). However, the few validation studies that have been conducted to ascertain the usefulness of this technique have used laboratory solutions containing elevated levels of both DOM and trace metals, which ensured an ISE response (*6*). These high concentrations can also be responsible for the "organic fouling" of the mercury drop (*7*). This study attempted to validate the accuracy of DPASV using concentrations of metals and ligands typically found in natural freshwaters, which also gave the added benefit of significantly decreasing organic adsorption problems.

Two methods have been used to validate analytical techniques used in trace metal complexation studies. One is to measure the complexation of a metal of a well-studied synthetic organic ligand and to compare the measured binding strengths and complexation capacity with known values (*5, 8*). The second method is to compare the results from two or more independent analytical techniques (*4, 9*). Therefore, an overlap in the analytical detection window (*10*) is necessary for a successful validation (*11*).

For validating free metal detection by DPASV, a comparison with an ion-selective electrode (ISE) is the most appropriate method because it is the only technique that directly measures the free metal ions. Most research using ion-selective electrodes has focused on Cu due to its known affinity for organic ligands, its relatively high concentration found in the aquatic environment, its detrimental toxicological effects, and its potential role as a limiting micronutrient. Moreover, of all the ion-selective electrodes, the copper electrode provides the greatest analytical stability and sensitivity. The application of the CuISE on isolated fractions of organic material (i.e., fulvic and humic acids) and synthetic chelating agents has resulted in a large experimental and modeling database for Cu−organic binding (*12*−*14*). Unfortunately, the applicability of CuISE in measuring labile Cu in natural waters has been limited because of its poor sensitivity. This limitation, however, does not preclude its effective use in a comparative study with other complexation analysis techniques when using synthetic river water composed of isolated and preconcentrated natural fulvic acids (FA).

In fact, these two electrochemical methods complement each other. DPASV can detect metals at low natural concentrations, but questions about metal−organic dissociation remain. Potentiometric titrations measure free metals, but they require substantial perturbation of natural conditions (i.e., elevated levels of DOM and metals). Agreement between the two techniques on the amount of free metal present in a sample consisting of a model organic ligand and a metal would greatly increase confidence in the results obtained from DPASV in natural waters.

The goal of this study was to compare two electrochemical techniques, DPASV (TMF-RDGCE) and CuISE, for determining the complexation capacity and binding strengths of aquatic DOM to Cu^{2+}. This was accomplished by conducting Cu^{2+} titrations with both instruments on two well-characterized fulvic acids in identical solution matrixes. The reliability of the comparison depended on the ability of CuISE to measure levels of Me^{2+} that are low enough so as to not saturate the ligand class whose complexation stability fell within the DPASV detection (*11*).

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Methodology

Calculation of Conditional Constants. Riverine trace metals occur in many different chemical forms. For Cu, the mass balance can be expressed as

$$Cu_t = Cu^{2+} + Cu_{in} + \sum CuL_i$$

where Cu_t, Cu^{2+}, Cu_{in}, and CuL_i are the total copper concentration in the sample, the free cupric ion, the inorganically complexed copper, and the copper that was associated with organic ligands.

Under the conditions of these experiments, abundant inorganic species were limited to Cu^{2+}, copper hydroxides, and copper carbonates. Inorganic speciation was calculated for both DPASV and CuISE titration data. For CuISE, the emf signal was standardize to Cu^{2+} after correction for inorganic species, primarily $CuOH^+$ and $Cu(OH)_2$. For DPASV, a labile metal pool is measured that may contain other species besides Cu^{2+}. We assumed that a negligible amount of Cu^{2+} derived from dissociation of metal−fulvic complexes under the condition of low metal levels and high strong ligand binding site concentrations that prevailed in these tests. The use of a RDE also minimizes metal−fulvic acid dissociation. By using a rotation rate of 4000 rpm, the diffusion layer surrounding the electrode is greatly reduced, which allows for only rapidly dissociating species to be detected. Consequently, the measured DPASV-labile metal was corrected only for inorganic side reactions to determine free copper in the sample (*9*). The success or failure of the intercomparison is partially a test of the validity of this assumption. For all speciation calculations, the Davies equation ($I = 0.1$ M, $\gamma_1 = 0.782$, $\gamma_2 = 0.368$; *15*) and the stability constants ($\log K_{CuOH^+} = 6.3$ and $\log K_{Cu(OH)_2} = 11.8$; *15*) were used. Finally, from the Cu mass balance, CuL was determined for each point on the titration curves.

Knowing Cu^{2+}, CuL, and C_t allows the resulting titration curves to be linearly transformed to yield both the conditional stability constant and the complexation capacity of the sample (*16*). The linear transformation was based on a 1:1 complexation formation that is mathematically identical to a Langmuirian adsorption model:

$$[M_T - (M)]/S_T = K_S(M)/[1 + K_S(M)]$$

Using this formulation, both Scatchard plots and van den Berg−Ruzic plots (*17, 18*) were used to transform the data (M_T = total metal, M = free metal) to obtain K_S (conditional binding constants) and S_T (binding site concentration).

Analytical Methods

DPASV and CuISE were compared by performing Cu titrations on separate solutions of ethylenediaminetetraacetic acid (EDTA; DPASV only), ethylenediamine (EN), Suwannee River fulvic acid (SRFA), and Dismal Swamp fulvic acid (DSFA) at pH 5.5. All solutions were adjusted to 0.1 M total ionic strength using $KClO_4$. pH was maintained in the natural organic samples by buffering the solution with 0.1 M MES (2-[*N*-morpholino]ethanesulfonic acid), which is known to have a low affinity for copper. This was confirmed by the low complexation capacity resulting from a DPASV Cu titration of a 0.1 MES sample. Cu titrations were performed over a range of SRFA and DSFA concentrations (1, 10, and 25 mg of C/L). The fulvic acid used in this study was isolated in a procedure similar to that used by McKnight et al. (*1*). Fulvic acid concentrations were verified by total organic carbon analysis using a Shimadzu TOC-5000 analyzer.

DPASV Analysis. The DPASV apparatus and method employed are similar to the one used by Bruland et al. (*19*). The apparatus consisted of a ROTEL RDE and an EG&G 380 polarographic analyzer. The ROTEL system consists of three electrodes: (a) a glassy carbon working electrode (6 mm diameter) active surface, (b) a coiled platinum wire used as the counter electrode, and (c) a coiled silver-coated wire that served as the reference electrode. Both coiled wires were contained in FEP tubing that was filled with an ultrapure saturated KCl solution (the counter electrode was also saturated with AgCl) and isolated from the sample using a fritted porous Vycor tip. The 380 polarographic analyzer allowed for limited adjustment on both the scan width (resolution) and pulse height (sensitivity). The specific settings used for the differential pulse mode were scan rate, 6.66 mV/s; pulse height, 50 mV; step time, 0.3 s; and scan width, 2 mV. For all DPASV measurements, a rotation rate of 4000 rpm was used.

The thin Hg^{2+} film application step was also used as a blank to monitor possible contamination at the low levels of trace metals found in natural environments. First, the working electrode was polished with 0.05 μm Al_2O_3 at a low rotation rate (500 rpm) and then rotated in a weak solution of ultrapure HNO_3 prior to each titration. Furthermore, during each step of each titration, the electrode surface was wiped clean with plain filter paper and rinsed with Nanopure water. Prior to each titration step, a thin Hg^{2+} film was deposited on the electrode by DPASV of a 25-mL sample of deoxygenated Nanopure water spiked with 50 μL of a 5000 μg/L $Hg(NO_3)_2$ solution. The resulting blank voltammogram was used to ascertain the extent of metal contamination. No sample analysis was conducted if metals were detected at concentrations greater than 0.3 nM Cu. If contamination was detected, additional cleaning steps were conducted, and a blank determination was repeated.

For the DPASV determinations, 25-mL aliquots of sample were transferred to acid-cleaned 60-mL FEP Teflon cups. Then, the samples were degassed with oxygen-free N_2 for 15 min and subsequently maintained under a N_2 blanket for the remainder of the titration. Samples were plated at −0.6 V for 480 s, followed by a 30-s quiescent period before the film was stripped by scanning the potential in the positive direction, using the differential pulse mode, to a final potential of −0.1 V. Each spike of each titration was allowed to equilibrate under a N_2 purge for 15 min before DPASV was conducted. The amount of the Cu spike varied depending on the complexation capacity of the sample, but typically ranged from 4 to 31.5 nM. Titrations were carried out until ligand saturation was achieved (typically <250 nM), which was indicated by a change in slope on a plot of current against total metal (*5*).

ISE. An Orion model 9629 Ionplus Series cupric electrode in conjunction with a Mettler DL70-ES titrator was used to measure cupric ion activity during Cu titrations. The CuISE was preconditioned before each titration by (a) polishing the CuS crystal with an Al_2O_3 polishing strip, thereafter rinsing vigorously with water obtained from a Milli-Q ion-exchange water system, and (b) placing it in a 0.025 M trace metal grade H_2SO_4 solution for approximately 20 min, subsequently repeating the Milli-Q water rinse. The CuISE was added to a solution purged with premoistened argon and allowed to equilibrate until the signal stabilized (greater than 30 min). Copper additions during titrations were limited so that the change in ISE signal was <3 mV. The solution was stirred for 30 s after each copper addition, and the measurement was accepted when the drift was less than 0.2 mV/min.

Results

Synthetic Ligands. EDTA has been used to validate DPASV analyses in the past, providing accurate ligand concentration determinations and good agreement between measured and thermodynamically derived binding strengths (*5*). Similar experiments were repeated to test our laboratory apparatus

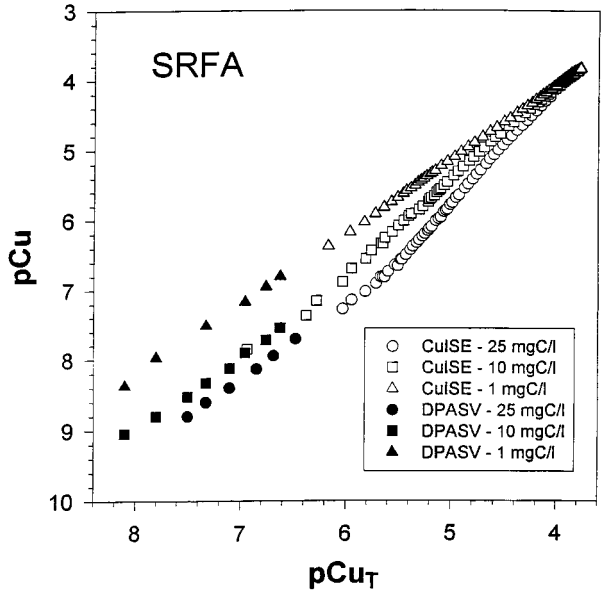

FIGURE 1. Free copper as measured by DPASV and CuISE as a function of total copper for 1, 10, and 25 mg of C/L of SRFA.

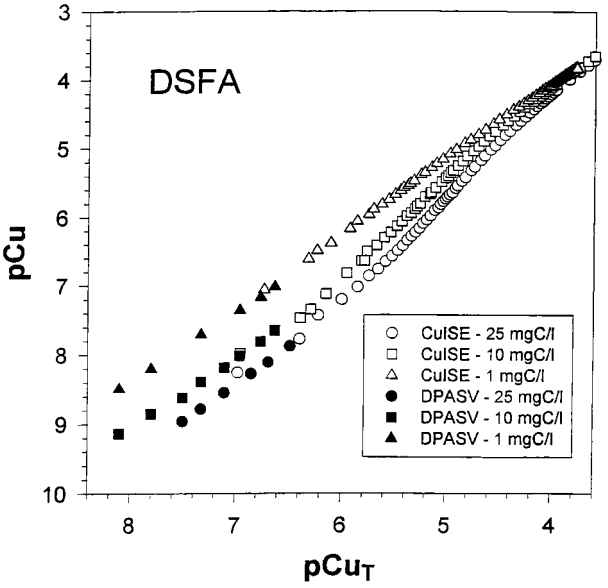

FIGURE 2. Free copper as measured by DPASV and CuISE as a function of total copper for 1, 10, and 25 mg of C/L of DSFA.

and modified DPASV (TMF-RDGCE) method. The Cu titrations were performed in a 100 nM EDTA solution comprised of Nanoapure (Barnstead) water adjusted to pH 8.0 and 2.5 mM Ca. The experiment was repeated three times with an average determined ligand concentration of 105.2 ± 15.7 nM. The experimentally derived binding constant was log $K_{CuEDTA} = 9.1 \pm 0.7$, which is the same as the known value corrected for inorganic side reactions, which accounts for <0.1% of the total Cu.

Titration experiments using EN were conducted using both DPASV and CuISE. EN was chosen because its Cu-binding constants are suitable for study within the detection window of DPASV. Cu titrations were performed on samples containing 20 nM ethylenediamine, which had been adjusted to pH 7.5. The experiment was repeated five times, and the average ligand concentration determined was 18.1 ± 2.1 nM with an average log K_{CuEN} of 8.9 ± 0.2. Again, the ligand concentration agreed within 10% of the total ligand concentration in the sample, and the stability constant was within 2 SD of the known value corrected for inorganic side reactions.

Natural Organics. *Suwannee River Fulvic Acid (SRFA).* Plots of pCu versus pCu$_T$ from both DPASV and CuISE for all levels of DOM show a decrease in the free metal concentration with increasing fulvic acid concentrations (Figure 1). Unfortunately, the upper edge of the DPASV detection window barely overlaps the lower edge of that of CuISE due to binding site saturation at sub-micromolar levels of total copper for DPASV measurements and the insensitivity of CuISE at nanomolar levels of free copper. Nevertheless, the DPASV determined that Cu^{2+} levels appear to fit the trend of Cu^{2+} titration as measured directly by CuISE at lower Cu$_T$ concentrations. The extent of the overlap and degree of concordance of our results are consistent with those reported for CSV by Xue and Sunda (*4*).

Dismal Swamp Fulvic Acid (DSFA). Cu titrations performed using DSFA produced similar plots to those of SRFA (Figure 2). As in the case of SRFA, only a few titration points overlapped from each of the two techniques, but again the titration lines appeared to be contiguous.

Due to the elevated amount of total Cu used for CuISE titrations, Scatchard plots of the CuISE data revealed two binding classes of ligands with the steeper of the two slopes representing complexation by a stronger class of organic ligands (Figure 3). This complexation occurred at low total

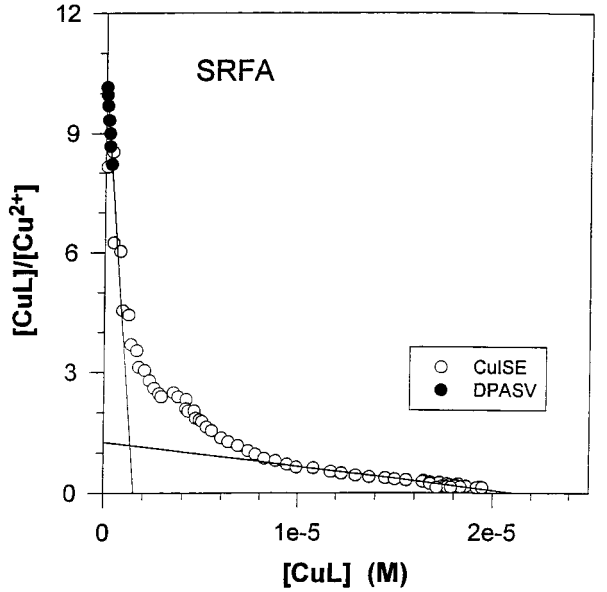

FIGURE 3. Scatchard plots for DPASV and CuISE titrations in 10 mg of C/L of SRFA. Each regression line corresponds to modeling of a separate class of organic binding sites.

Cu and was more representative of the binding measured by DPASV. Therefore, only these CuISE values were used for comparison, which generally encompassed the lowest 6−10 titration data points from CuISE and all of the DPASV points.

Discussion

Although the overlap of data derived from DPASV and CuISE measurements is limited, the two methods agree in this redundant region within measurement uncertainty. For example, Figure 4 is an enlargement of the portion of Figure 3 where the two methods overlap. DPASV data are indicated by filled circles and the solid regression line, which extends to the axis, while CuISE data are shown by open circles and the short dashed regression line. The relatively great scatter of the CuISE measurements in this concentration range leads to a relatively large uncertainty, as is evident from the 95% confidence interval delimited by the dotted line. It is clear from this graphic that the regression for the DPASV data fit

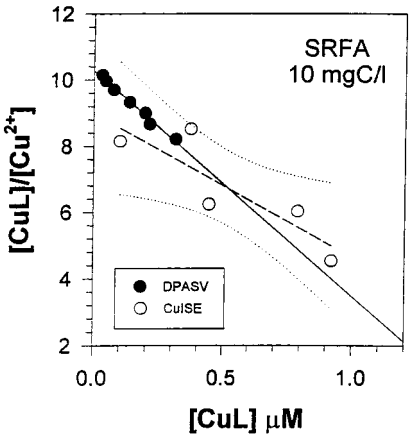

FIGURE 4. Enlargement of the section of Figure 3 where data from the two methods overlap. The regression for the DPASV data (●) overlaps that of the CuISE data (○). Also, the slopes and intercepts of the two regressions are indistinguishable at the $p = 0.05$ confidence level.

TABLE 1. Complexation Capacity and Conditional Stability Constants for SRFA and DSFA Derived from Linear Transformation of the Data[a]

		CuISE		DPASV	
	FA (mg of C/L)	L_T (nM)	log *K*	L_T (nM)	log *K*
SRFA	1	742	5.86	137	7.15
	10	1940	6.46	1146	6.82
	25	3210	6.71	2640	7.08
DSFA	1	1028	5.98	148	6.78
	10	2461	6.45	946	7.02
	25	3065	6.74	2380	6.97

[a] Differences between the two techniques probably reflect the relatively large uncertainty associated with CuISE measurements near its detection limit.

within this area. Furthermore, the difference between the slopes and intercepts of the two regressions are less than two times the combined standard errors, indicating that the lines are not significantly different at the conventional $p = 0.05$ probability level threshold.

It is noteworthy in Table 1 that the abundance of complexation sites calculated from CuISE data is not linearly related to the concentration of DOM and that the binding strengths tend to increase with increasing FA and total Cu added. Neither of these results were expected since complexation capacities should increase linearly and binding constants should remain constant as FA levels are increased. We attributed this effect as a consequence of the great uncertainty of CuISE measurements at the lower level of FA. While the two highest concentrations still do not increase linearly, they are closer to the expect behavior and match the DPASV data better. This and the large scatter of the CuISE data in the low Cu zone suggest that this method was pushed to its useful limit in the region where its detection window overlapped with DPASV.

The model used to calculate the conditional stability constants and complexation capacities may also have influenced the intercomparison. Linear transformation assumes that a 1:1 complexation occurs. To check the validity of this assumption, pCuL was plotted as a function of pCu for the titration of both SRFA (Figure 5) and DSFA (Figure 6). The resulting straight lines with slopes near 1.0 at low Cu_t concentrations showed that a 1:1 metal to organic complexation did occur in this region. However, while the linear regressions representing 1:1 complexation incorporated all

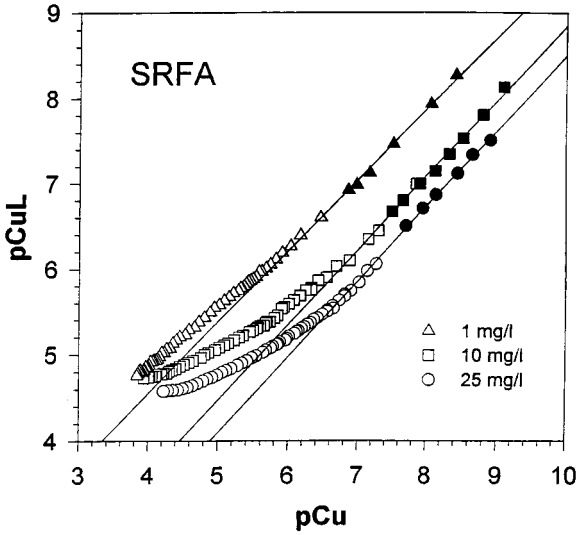

FIGURE 5. Organically bound copper as a function of free copper as measured by DPASV (filled symbols) and CuISE (open symbols) for 1, 10, and 25 mg of C/L of SRFA. Slopes of regression lines reflect complexation ratios at low Cu concentrations.

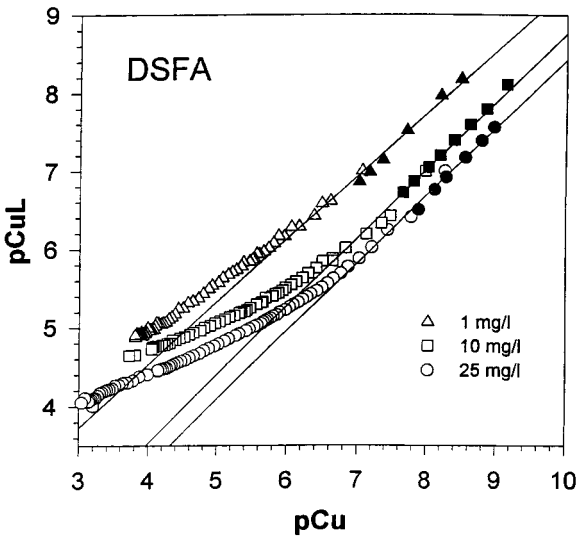

FIGURE 6. Organically bound copper as a function of free copper as measured by DPASV (filled symbols) and CuISE (open symbols) for 1, 10, and 25 mg of C/L of DSFA. Slopes of regression lines reflect complexation ratios at low Cu concentrations.

of the DPASV data, only the lowest measured CuISE values, generally the first 4−5 titration points, had a 1:1 complexation. This is several data points fewer than the 6−10 titration points used for complexation capacity and binding constant calculations of Table 1 and may account for some of the differences observed between the two methods.

The straight lines on Figures 5 and 6 result from a fixed relation between concentrations of CuL and Cu^{2+}. The slopes of the various concentrations of both SRFA and DSFA ranged from 0.81 to 0.89, which was close to the expected 1:1 complexation ratio. Furthermore, changing the FA concentrations should cause the regression lines to be offset vertically by the log of the ratio of the FA concentration. For a change of 1−10 mg of C/L, the vertical offset should be log(10) or 1. For a change of 1−25 mg of C/L, the offset should be 1.40. The observed shifts were close to these expected values (Table 2). This concordance is consistent with the idea that adding FA increases the number of complexation sites without changing their strength significantly. This suggests that DPASV is accurately following the CuISE at low metal

TABLE 2. Expected and Observed Vertical Offsets in the Linear Regression Lines of CuL and Cu^{2+} in Varying Concentrations of FA

fulvic acid	FA ratio	expected offset	observed offset
SRFA	10	1.00	1.13
	25	1.40	1.52
DSFA	10	1.00	1.20
	25	1.40	1.57

concentrations and is not strongly affected by any metal−organic dissociation.

Perhaps one surprising result was the similarity between the two standard fulvic acids tested. The ligand total and binding strengths from DPASV analysis of DSFA showed only minor (<20%) differences as compared to SRFA (Table 1). One possibility is that this is an isolation method artifact since both fulvic acids were separated in a similar manner. On the other hand, this similarity between fulvic acids has been observed in another study, which measured the complexation capacity of natural river waters that were not pretreated before analysis (*20*).

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Literature Cited

(1) McKnight, D. M.; Feder, G.; Thurman, E. M.; Wershaw, R. L. *Sci. Total Environ.* **1983**, *28*, 65−76.
(2) Tipping, E. *Environ. Sci. Technol.* **1993**, *27*, 520−529.
(3) Breault, R.; Colman, J.; Aiken, G.; McKnight, D. *Environ. Sci. Technol.* **1996**, *30*, 3477−3486.
(4) Xue, H.; Sunda, W. G. *Environ. Sci. Technol.* **1997**, *31*, 1902−1909.
(5) Coale, K. H.; Bruland, K. W. *Limnol. Oceanogr.* **1988**, *33*, 1084−1101.
(6) Soares, H. M. V. M.; Vasconcelos, M. T. S. D. *Anal. Chim. Acta* **1994**, *293*, 261−270.
(7) Soares, H. M. V. M.; Vasconcelos, M. T. S. D. *Anal. Chim. Acta* **1995**, *314*, 241−249.
(8) Omanovic, D.; Pizeta, I.; Peharec, Z.; Branica, M. *Mar. Chem.* **1996**, *53*, 121−129.
(9) Donat, J. R.; Loa, K. A.; Bruland, K. W. *Anal. Chim. Acta* **1994**, *284*, 547−571.
(10) Van den Berg, C. M. G.; Nimmo, M.; Daly, P.; Turner, D. R. *Anal. Chim. Acta* **1990**, *232*, 241−249.
(11) Miller, L. A.; Bruland, K. W. *Anal. Chim. Acta* **1997**, *343*, 241−249.
(12) Cabaniss, S. E.; Shuman, M. S. *Geochim. Cosmochim. Acta* **1988**, *52*, 185−193.
(13) Cabaniss, S. E.; Shuman, M. S. *Geochim. Cosmochim. Acta* **1988**, *52*, 195−200.
(14) Hering, J.; Morel, F. M. M. *Environ. Sci. Technol.* **1988**, *22*, 536−549.
(15) Morel, F. M. M.; Hering, J. *Principles and Applications of Aquatic Chemistry*; Wiley: New York, 1993; p 588.
(16) Ruzic, I. *Mar. Chem.* **1996**, *53*, 1−15.
(17) Ruzic, I. *Anal. Chim. Acta* **1982**, *140*, 99−113.
(18) Ruzic, I.; Pecar, J. *Croat. Chem. Acta* **1996**, *70*, 229−245.
(19) Bruland, K. W.; Coale, K. H.; Mart, L. *Mar. Chem.* **1985**, 285−300.
(20) Avdeef, A.; Zabronsky, J.; Stuting, H. H. *Anal. Chem.* **1983**, *55*, 298−304.

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