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KINETICS OF COPPER COMPLEXATION WITH DISSOLVED ORGANIC MATTER USING STOPPED-FLOW FLUORESCENCE TECHNIQUE

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This study provided direct observations on reaction kinetics of Cu and dissolved organic matter (DOM). Stopped-flow method and fluorescence technique were used to measure Cu(II)-DOM complexation over time. Suwannee River reference fulvic acid and water soluble soil organic matter were used as model DOM. Experimental conditions of pH 6, 5×10^{-5} M Cu(II), and 5 mg C/L of DOM were used and the results were to simulate the relevant natural conditions. Both organic ligands reacted fairly rapidly with Cu(II). Reaction half-life of DOM was in the millisecond range. Two types of metal binding sites of organic ligands were identified. Fulvic acid yield forward rate constants: $1 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ and $5.15 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, based on second-order reaction rate model. Water soluble soil organic matter gave forward rate constants: $1.34 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $2.2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. From comparison of kinetic natures, the fast-reacting ligands were comparable to nitrilotriacetic acid (NTA) and slow-reacting ligands were more like salicylic acid.

KEY WORDS: Cu(II) complexation, dissolved organic matter, kinetics, stopped-flow technique

INTRODUCTION

Several studies have reported that the transport and concentration distribution of copper ion are regulated by clay minerals primary due to surface complexation, ion exchange, and surface precipitation¹⁻⁷. These surface reactions noted above are usually modified by the presence of naturally occurring dissolved organic matter (DOM), principally the so called humic substance^{5,8-10}. In mineral/water interface, DOM might occupy the surface reacting sites and render them unavailable to copper ions, thus increasing the transport of copper. In the aqueous phase, DOM complexes with copper, thus altering the speciation and biological availability or toxicity of copper¹¹⁻¹². The complexation reaction between copper and DOM occurs in the water phase as well as mineral/water interfaces. Humic substances appear to be the major metal-complexing ligands and to have played a very significant role in the fate and environmental availability of heavy metals in natural waters^{10,13}. Over the past

years, several studies have been advanced to characterize the nature and properties of humic substances extracted from different soils and aqueous environments, to investigate the binding capacity for metals, and to determine the apparent stability constant of metal-humic/fulvic acids from various sources¹⁴⁻¹⁷. The stability constants of metal-humic/fulvic complexes are useful for modeling the fate of metals in surface and ground water and for understanding trace metal distribution in water treatment processes. Accurate results from model output depend on reliable constants employed in the modeling calculations. Though thermodynamic constants are useful, kinetic information is needed to interpret the reaction mechanism.

Kinetic studies on reactions between metals and DOM have been very limited¹⁸⁻²¹. Plankey and Patterson studied the kinetics of aluminium-fulvic acid complexation in acidic waters, where fulvic acid was isolated from Huntington Watershed of the Adirondack Mountains of New York State¹⁹. Though stopped-flow technique was used to collect kinetic data in their experiment, the fluorescence intensity was not recorded immediately. Under such circumstances, the most important reaction data might not be obtainable. Hering and Morel employed a dual-syringe assembly to mix reagents which were subsequently delivered to the fluorescence detection cuvette in the kinetic study of trace metal complexation²⁰. They reported that the first fluorescence reading was taken nearly 20 seconds after initiating the reaction. For a "fast" reaction, the time required to retrieve kinetic data is extremely important for understanding the reaction mechanisms, especially in the initial stage of the reaction. Laidler suggested that millisecond time scale is needed to carry out kinetic studies on metal complexation²². Copper complexation with DOM has not been fully studied kinetically, and the direct measurement of reaction rate constants has not been reported yet. In this study, a state-of-the-art stopped-flow apparatus with a dead time of 3 millisecond was employed to evaluate the kinetic reaction of copper with fulvic acid and local soil DOM. The objectives of this work are to investigate the rates of complexation reaction between copper and DOM from two sources and to explore their reaction mechanisms. This information is useful in assessing the ecological significance and geological transport of copper ion in natural environments.

BACKGROUND

According to Saar and Weber, the techniques for investigating the complexation of metal ions and DOM can be generally classified into category-separation and nonseparation methods⁹. Separation techniques include liquid chromatography/atomic absorption spectrometry (AAS), ultrafiltration/AAS, and equilibrium dialysis/AAS. Though separation methods are not limited by the type of metal ions being studied, the potential adsorption of metal ions on separating materials and the possibility of shifting equilibria are issues of concern. Nonseparation techniques include electron paramagnetic resonance spectroscopy, ion-selective electrode potentiometry, anodic stripping voltammetry, and fluorescence, etc. While capable of avoiding the adsorbing and equilibria shifting problems, nonseparation methods are

applicable to relatively few specific metal ions. For example, fluorescence is commonly used for paramagnetic metal ions such as Cu, and ion-selective electrode potentiometry depends on the availability of an electrode of a specific metal. Among the various techniques noted above, fluorescence quenching appears to have worked well in an experimental system with very little DOM while still providing satisfactory results. Originally, Ryan and Weber proposed that the fluorescence intensity of a ligand is quenched by complexation with a metal ion and the total fluorescence intensity is contributed from both the free and bound ligands¹⁶. The fluorescence quenching method has been employed successfully by several researchers to determine the conditional stability constants of metal ions complexed with DOM from various sources. Past studies on metal-DOM reactions have indicated that the stability constants of metal-DOM complexes depend on the system conditions such as pH, temperature, DOM concentrations and solution ionic strength. Usually, the stability constant increases as system pH increases or solution ionic strength decreases, and the reported values are somewhere between 10^3 and 10^5 M^{-1} , depending on the specific metal ion studied^{15-16,23}.

Kinetic studies deal with the rate of chemical reactions and how that rate depends on concentrations of reactants and other factors. Kinetic information provides insight into the mechanism of chemical processes. Conventional static systems in which the reactants are introduced into the reacting chamber and the changes in concentrations of both the reactants and products analyzed are limited to "slow" reactions, defined roughly as those with a half-life longer than ten seconds. For "fast" reactions, the techniques generally used for kinetic studies are flow and relaxation methods²². With the advances in detection instrumentation, flow methods are capable of measuring the progress of reactions with half-lives on the order of milliseconds. Reactions with half-lives between microseconds and seconds are studied by relaxation methods such as temperature and pressure jump. Relaxation methods rely on perturbing an established equilibrium and measuring the interested parameter thereafter. The results from relaxation methods need to be evaluated if the reaction under experimental condition is irreversible. Stopped-flow techniques that are capable of mixing two reactants completely within milliseconds are applicable to irreversible reactions and they also have the advantage of monitoring the progress of reaction from the very beginning. Since past kinetic studies on metal-DOM complexation were unable to provide information regarding the dynamic reaction within the first second, this work provided information to fill this "gap" using stopped-flow techniques.

MATERIALS AND METHODS

Materials

The DOM used in this work originated from two sources. Fulvic acid (SRFA) was obtained from the Suwannee River by International Humic Substance Society (IHSS). Water soluble soil organic matter (WSSOM) was retrieved from an andic soil collected from Yangmingshan National Park, Taiwan. The SRFA is well char-

acterized and its isolation method and chemical properties have been reported previously^{24–25}. The andic soil is rich in organic carbon (15.62%) and thus high concentrations of DOM can be extracted. For comparison, organic carbon in SRFA is approximate 53.5% (IHSS). A stock solution of SRFA was prepared by dissolving freeze-dried SRFA in Milli-Q reagent water and filtered through a Whatman 0.45 μm cellulose nitrate membrane. To simulate a near natural condition, stock WSSOM solutions were obtained by adding 2.5 grams of soil to flasks containing 100 or 200 ml Milli-Q water and shaking the flasks (120 rpm) on a temperature-controlled (25°C) reciprocating shaker for 24 hours. At the end of 24 hours, the suspensions were filtered through 0.45 μm membranes and the fresh filtrates were used as WSSOM in this study.

Analytical-grade reagents of NaOH, ClO_4 , NaClO_4 , and $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ were used for solution pH and ionic strength adjustment (except for WSSOM) as well as copper working solution preparation. Solution containing 10 mg C/L of SRFA, 10 mg C/L of WSSOM, and 1×10^{-4} M of Cu(II) were prepared for the kinetic study (the concentrations of reactants in mixing chamber were half of the prepared ones). The concentrations of DOM and Cu(II) used here should not have caused any Cu-DOM coagulation²⁶.

Methods

The organic carbon concentrations of SRFA and WSSOM working solutions were determined by a TOC analyzer (OIC Model 700). Spectrofluorometric measurements were accomplished with an Aminco SLM 8000 spectrofluorophotometer. The fluorescence excitation and emission wavelengths were set at 330 and 450 nm in the SRFA solution and 340 and 440 nm in the WSSOM solution. For kinetic runs with the WSSOM sample, a filter lens was used to enhance the detected fluorescence intensity. Kinetic runs were performed with the same spectrofluorophotometer equipped with stopped-flow apparatus (Aminco FP-052). This kinetic setup is able to reach greater than 98% mixing efficiency at the observation point in less than 1.5 milliseconds; the minimum acquisition time and minimum acquisition rate are 10 milliseconds and 1 point per millisecond, respectively. The fluorescence measurement was extended to 20 to 50 seconds. In equilibrium titration experiments, the system pH was maintained at 6.0 ± 0.1 ²³. In the kinetic runs, the pH value of reagents was preadjusted to 6.0 ± 0.1 . It is believed that the small change in pH has no effect on rate constant²¹. All experiments were performed at 25°C. The copper concentration was measured using atomic absorption spectrophotometry (Perkin Elmer 5000).

Analysis

Saar and Weber reported that the quenching of the fluorescence intensity of fulvic acid is proportional to the degree of metal and fulvic acid complexation²⁷. Ryan and Weber thereafter postulated that the total fluorescence intensity (*I*) is equal to the sum of $\alpha_{\text{ML}}I_{\text{ML}}$ and $\alpha_{\text{L}}I_{\text{L}}$, where α_{ML} , I_{ML} , α_{L} and I_{L} are the molar ratio of metal-complexed organic ligand to total organic ligand, the limiting value of *I* at maximum

metal-ligand binding condition, the molar ratio of unbound organic ligand to the total organic ligand, and the fluorescence intensity of the initial free ligand in the absence of any added metal ions, respectively¹⁶. Therefore, the fraction of organic ligand bound with metal ion can be expressed as:

$$\frac{[\text{ML}]}{[\text{C}_\text{L}]} = \frac{\left\{1 - \frac{I}{I_\text{L}}\right\}}{\left\{1 - \frac{I_\text{ML}}{I_\text{L}}\right\}} \quad (1)$$

where $[\text{ML}]$ is the molar concentration of metal complexed organic ligand and $[\text{C}_\text{L}]$ is the total molar concentration of organic ligand. For simplicity, it is assumed a 1:1 stoichiometric relationship between metal and organic ligand for the complexation reaction¹⁶. Based on the mass balances on metal and organic ligand, the degree of complexation (Equation 1) can be written in terms of the overall stability constant (K), total metal concentration (C_M) as well as C_L :

$$\frac{I_\text{L} - I}{I_\text{L} - I_\text{ML}} = \frac{1}{2KC_\text{L}} \left\{ (KC_\text{L} + KC_\text{M} + 1) - \sqrt{(KC_\text{L} + KC_\text{M} + 1)^2 - 4K^2C_\text{L}C_\text{M}} \right\} \quad (2)$$

Using the measured parameters (I , I_L , and C_M from metal titration experiments), the overall stability constant, the total organic ligand concentration and the limiting fluorescence intensity at maximum metal-ligand binding condition (I_ML) are obtainable by fitting I and I_L as a function of C_M to Equation 2 using nonlinear regression techniques²³. The overall stability constant thus calculated was used to compute the reaction rate constants. As noted above, the reaction between metal and organic ligand was presumed to be one metal bound at one organic ligand, i.e., $\text{M} + \text{L} = \text{ML}$. Assuming elementary and reversible reaction, the differential rate equation that applies to this complexation reaction is

$$\frac{d[\text{ML}]}{dt} = k_1[\text{M}][\text{L}] - k_{-1}[\text{ML}] \quad (3)$$

Where k_1 and k_{-1} are the apparent forward and reverse rate constants. Replacing k_{-1} with k_1/K , the concentrations of metal-ligand complex over time are computed from the following

$$[\text{ML}]_{t+\Delta t} = [\text{ML}]_t + k_1 ([\text{M}]_t[\text{L}]_t - \frac{1}{K} [\text{ML}]_t) \Delta t \quad (4)$$

where $[\text{ML}]_t$, $[\text{M}]_t$, and $[\text{L}]_t$ are the concentrations of metal-complexed organic ligand, free metal, and unbound organic ligand at time t as the reaction proceeds. $[\text{ML}]_t$ is calculated from the observed fluorescence intensity such that $[\text{ML}]_t =$

$[ML]_e(I_L - I_t)/(I_L - I_e)$, where $[ML]_e$ is the concentration of metal-complexed organic ligand at equilibrium and I_t and I_e are the fluorescence intensity of the reaction system at time t and at equilibrium, respectively. From Equation 4 and the initial concentrations of metal and organic ligand, the rate of change of predicted $[ML]$ values can be constructed. By adjusting the value of k_1 to best fit the predicted $[ML]_t$ to the observed $[ML]_t$, the apparent forward and reverse reaction rate constants are optimized.

RESULTS AND DISCUSSION

The decrease in fluorescence intensity after quickly mixing SRFA (5 mg C/L) and Cu(II) (5×10^{-5} M) solution using the stopped-flow apparatus is illustrated in Figure 1. Two kinetic runs for each DOM and Cu system were conducted and the experimental results indicated a good reproducibility. The fluctuation of the fluorescence intensity in the studied system is probably due to data collection and integration process within a very short span (0.1–0.3 sec.). The original outputs from spectrofluorophotometer are simulated by curve-fitting technique using nonlinear regression technique. Similar kinetic results for WSSOM and Cu(II) reaction are presented in Figure 2. The fluorescence intensity at the beginning of the reaction for the WSSOM system was significantly higher than that of SRFA. The fluorescence intensities at the beginning of the reaction and at the end of observation period in the SRFA and WSSOM systems are 0.397 ($t = 0$ s)/0.344 ($t = 17$ s) and 1.266

Figure 1. Complexation kinetics at pH 6 in a system with 5×10^{-5} M of copper and 5 mg C/L of SRFA. The observed intensity readings were simulated by the solid curve.

Figure 2. Complexation kinetics at pH 6 in a system with 5×10^{-5} M of copper and 5 mg C/L of WSSOM. The observed intensity readings were simulated by the solid curve.

($t = 0$ s)/1.048 ($t = 45$ s), respectively. The difference in initial fluorescence intensity between SRFA and WSSOM system can be reasoned by two facts. WSSOM is water soluble soil organic matter with complex mixtures resulting in higher fluorescence intensity. Also, a filter lense was employed to enhance the detection sensibility in the kinetic run of the WSSOM system. Results have shown that the binding of metal to the DOM is fairly rapid and suggest that the reaction is virtually at equilibrium in about 10 seconds in the SRFA system and 30 seconds in the WSSOM system. It is important to note that the first fluorescence intensity readings (i.e., the fluorescence intensity data detected immediately after triggering) from kinetic runs have deviated substantially from those of metal titration experiments²³, with the fluorescence intensity of metal-free organic ligand being much larger than that observed at time “zero” in kinetic experiments. Therefore, “blank” experiments were also conducted for comparison. As one of the reactant reservoirs filled with Cu(II) solution was replaced with NaClO₄ solution, the fluorescence intensity in the SRFA system from kinetic run came out to be 0.82. Similar phenomenon was noticed in the WSSOM system where the intensity reading was 3.12. The results probably imply that a significant portion of the organic ligands have already associated with Cu(II) within few milliseconds after mixing.

Based on the assumption that the quenching of the fluorescence intensity of DOM is proportional to the degree of metal-organic ligand complexation¹⁶, the equilibrium ML concentrations derived from Equations 1 and 2 are 7.45×10^{-6} M in the SRFA system and 1.51×10^{-5} M in the WSSOM system (Appendix A). The Cu-organic ligand (SRFA) complex concentration calculated at time “zero” (it should be 0.3

second—the first fluorescence reading obtained) is 6.62×10^{-6} M (Appendix B). In other words, the complexation reaction for Cu and SRFA (and also WSSOM) is about 89% complete in less than 0.3 second. To our best knowledge, the results represented are performed within the millisecond range, as compared to those of other investigation conducted in 10–30 sec^{19–21}.

DOM is a complex mixture containing multireacting components^{19,28–29}. At present, it is not possible and not our intention to characterize the thermodynamic and kinetic nature of each metal reacting site of DOM. Instead, the apparent kinetic properties of organic ligands were investigated. A simple monomeric ligand model has been employed to simulate the reaction kinetically. Experimentally observed fluorescence readings shown in Figures 1 and 2 were translated into Cu-organic ligand concentrations over time ($[ML]_t$) as displayed in Figures 3 and 4, (Appendix B). Figures 3 and 4 also present the optimization results for the kinetic model. It seems that the model results fit the experimental data quite well. The forward and reverse rate constants are $5.15 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ and $3.48 \times 10^{-2} \text{ s}^{-1}$ for the Cu-SRFA system, respectively, and $2.2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ and $8.24 \times 10^{-3} \text{ s}^{-1}$ for the Cu-WSSOM system. These rate constants are within the same order of magnitude with different type of DOM. The rate constants defined represent the averages over the distribution of the reacting sites on SRFA and WSSOM. Past studies on the kinetics of Cu-DOM complexation are relatively scarce. Shuman²¹ studied the dissociation pathways of Al bound to aquatic fulvic acid (FA) and reported that the $[FA]$ -dependent “disjunctive” rate constant is $1.46 \times 10^{-5} \text{ sec}^{-1}$. If this “disjunctive” rate constant is treated as the reverse rate constant, our SRFA result ($3.48 \times 10^{-2} \text{ s}^{-1}$) is about

Figure 3. Best fit model results for the SRFA system. Forward rate constant, k_1 , was optimized for the predicted $[ML]_t$ to best fit the observed $[ML]_t$. The k_1 thus calculated is $5.15 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. The overall stability constant of Cu-SRFA reaction is 1.48×10^5 . The calculation of the observed $[ML]_t$ is explained in Appendix B. The predicted $[ML]_t$ is computed from Equation 4 with optimized rate constant.

Figure 4. Best fit model results for WSSOM system. Forward rate constant, k_1 , was optimized for the predicted $[ML]_t$ to best fit the observed $[ML]_t$. The k_1 thus calculated is $2.2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. The overall stability constant of Cu-WSSOM reaction is 2.67×10^5 . The calculation of the observed $[ML]_t$ is explained in Appendix B. The predicted $[ML]_t$ is computed from Equation 4 with optimized rate constant.

three orders of magnitude higher. No Al-FA forward rate constant was reported in his study. Plankey and Patterson¹⁹ reported that the forward rate constants for Al-FA complexation ranged from $3.39 \text{ M}^{-1} \text{ s}^{-1}$ to $3.5 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, depending on Al speciation and degree of FA protonation. They also noted that the upper limit for Al-salicylic acid rate constant was $3.9 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. It may not be justified to compare the kinetic results here because of the different metal and organic ligand used. Nevertheless, it does imply that the kinetic behavior of SRFA and WSSOM is somewhat comparable to that of salicylic acid.

It is interesting to examine the reaction rate of Cu(II) with organic ligand at the very beginning of the reaction period -0.3 second. We assumed an elementary second order reaction and ignored the reverse reaction such that

$$\frac{d[ML]}{dt} = k_1^0 [M][L] \quad (6)$$

Where k_1^0 designated for the forward rate constant during the first 0.3 seconds. By integrating the differential rate equation and substituting total Cu, total ligand, Cu-organic ligand complex concentration at time "zero" and 0.3 second into the

integrated equation, k_1^0 is calculated to be $1 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ for the SRFA system and $1.34 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for the WSSOM system. The 2nd-order rate constants are two orders of magnitude higher than those forward rate constants calculated previously (Figures 3 and 4). Hence, based on the measured kinetic data, we propose two classes of reacting components of DOM: fast- and slow-reacting ligands. The magnitude of the rate constants coupling with the fact that the complexation reaction does complete 89% within 0.3 sec indicate that the faster reacting ligands are in excess of that slower reacting sites. These fast-reacting ligands of SRFA might be modeled as nitrilotriacetic acid (NTA) whose rate constant for the complexation with Cu is estimated to be $9 \times 10^7 \text{ M}^{-1} \text{ s}^{-120}$. Our work provided direct measurement to calculate the fast and slow reaction rate constants for the complexation reaction of metal and DOM, and these results should be useful in interpreting the interactions of metal and DOM in natural environments.

CONCLUSIONS

Experiments were conducted using values of pH and organic ligand concentration that are similar to natural water conditions. Kinetic study using a stopped-flow method suggested two reaction rate constants for DOM complexation with Cu(II). Since it is not possible to define each of the different reacting ligands, for simplicity, the various ligands are generalized to two types based on the experimental observations. The two reaction rate constants may represent the averages over the distribution of the fast- and slow-reacting ligands on SRFA or WSSOM. The fast-reacting ligands of DOM in the presence of Cu(II) behave more like NTA kinetically. This work provided direct evidence that under natural conditions of pH and DOM concentrations the metal complexation by DOM is fairly rapid, i.e., half-lives in millisecond level. The results are useful for modeling copper transport in surface and ground waters.

APPENDIX A

From metal titration experiments and nonlinear regression analysis of Equation 2, the following parameters are defined.

SRFA system:

- 1) The relative fluorescence intensity at equilibrium, I_e/I_L , is 0.36.
- 2) $I_{ML}/I_L = 0.25$
- 3) $[C_L] = 8.67 \times 10^{-6} \text{ M}$
- 4) The metal-organic ligand concentration at equilibrium, $[ML]_e$, is $7.45 \times 10^{-6} \text{ M}$ (Eq. 1).

WSSOM system:

- 1) The relative fluorescence intensity at equilibrium, I_e/I_L , is 0.42.
- 2) $I_{ML}/I_L = 0.354$.
- 3) $[C_L] = 1.67 \times 10^{-5}$ M.
- 4) The metal-organic ligand concentration at equilibrium, $[ML]_e$, is 1.51×10^{-5} M (Eq. 1).

APPENDIX B

From kinetic runs, the following data are obtained. The following equation is used to calculate $[ML]_t$.

$$[ML]_t = [ML]_e \frac{\{I_b - I_t\}}{\{I_b - I_f\}} \quad (A-1)$$

SRFA system:

- 1) The fluorescence intensity at time zero, I_i , is 0.40.
- 2) The fluorescence intensity at time 17 seconds, I_f , is 0.34.
- 3) The fluorescence intensity of "blank" sample, i.e., SRFA solution without Cu, I_b is 0.82.
- 4) The metal-organic ligand complex concentration at time zero, $[ML]_i$, is 6.62×10^{-6} M (substitute $I_t = I_i = 0.40$ in Eq. A-1).

WSSOM system:

- 1) The fluorescence intensity at time zero, I_i , is 1.27.
- 2) The fluorescence intensity at time 45 seconds, I_f , is 1.05.
- 3) The fluorescence intensity of "blank" sample, i.e., SRFA solution without Cu, I_b is 3.12.
- 4) The metal-organic ligand complex concentration at time zero, $[ML]_i$, is 1.35×10^{-5} M (substitute $I_t = I_i = 1.27$ in Eq. A-1).

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References

1. W. Stumm, H. Hohl and F. Dalang. *Croatica Chemica Acta*, **49**, 491 (1976).
2. W. Stumm, R. Kummert and L. Sigg. *Croatica Chemica Acta*, **53**, 291 (1980).

3. W. Stumm, G. Furrer and B. Kunz. *Croatica Chemica Acta*, **56**, 593 (1983).
4. M. M. Benjamin and J. Leckie. *Environ. Sci. Technol.*, **15**, 1050 (1981).
5. J. A. Davis. *Geochim. Cosmochim. Acta*, **48**, 679 (1984).
6. B. D. Honeyman and P. H. Santschi. *Environ. Sci. Technol.*, **22**, 862 (1988).
7. L. J. Evan. *Environ. Sci. Technol.*, **23**, 1046 (1989).
8. R. A. Saar and J. H. Weber. *Canadian J. of Chem.*, **57**, 1264 (1979).
9. R. A. Saar and J. H. Weber. *Environ. Sci. Technol.*, **16**, 510A (1982).
10. J. H. Weber. "Binding and Transport of Metals by Humic Materials", in *Humic Substances and Their Role in the Environment*, F. H. Frimmel and R. F. Christman, eds., John Wiley, New York (1988).
11. W. G. Sunda, D. W. Engel and R. M. Thuotte. *Environ. Sci. Technol.*, **12**, 409 (1978).
12. H. E. Allen, R. H. Hall and T. D. Brisbin. *Environ. Sci. Technol.*, **4**, 441 (1980).
13. d. A. Dzombak, W. Fish and M. M. Morel. *Environ. Sci. Technol.*, **20**, 669 (1986).
14. F. J. Stevenson. *Soil Sci. Soc. Am. J.*, **40**, 665 (1976).
15. R. A. Saar and J. H. Weber. *Environ. Sci. Technol.*, **14**, 877 (1980).
16. D. K. Ryan and J. H. Weber. *Anal. Chem.*, **54**, 986 (1982).
17. F. H. Frimmel and R. F. Christman. "Polymerization of Humic Substances in Natural Environments", in *Humic Substances and Their Role in the Environment*, John Wiley, New York (1981).
18. D. L. Olson and M. S. Shuman. *Anal. Chem.*, **55**, 1103 (1983).
19. B. P. Plankey and H. H. Patterson. *Environ. Sci. Technol.*, **21**, 595 (1987).
20. J. G. Hering and M. M. Morel. *Environ. Sci. Technol.*, **24**, 242 (1990).
21. M. S. Shuman. *Environ. Sci. Technol.*, **26**, 593 (1992).
22. K. J. Laidler. *Chemical Kinetics*, 3rd ed., Harper & Row, New York (1987).
23. D. Y. Lee, C. F. Lin, L. M. Houg and K. S. Lo. *Chinese Insti. of Environ. Engr.*, **2**, 57 (1992).
24. E. M. Thurman and R. L. Malcolm. *Envir. Sci. Technol.*, **15**, 463 (1981).
25. E. M. Thurman and R. L. Malcolm. "Structural Study of Humic Substances: New Approaches and Methods", in *Aquatic and Terrestrial Humic Materials*, R. F. Christman and E. T. Gjessing eds., Ann Arbor Science, Ann Arbor, MI (1983).
26. S. E. Cabaniss. *A Potentiometric and Fluorometric Study of Copper Complexation by Dissolved Organic Matter*, Ph.D Thesis, University of North Carolina, Chapel Hill, NC (1986).
27. R. A. Saar and J. H. Weber. *Anal. Chem.*, **52**, 2095 (1980).
28. D. S. Gamble, A. W. Underdown and C. H. Langford. *Anal. Chem.*, **52**, 1901 (1980).
29. D. M. Grimm, L. V. Azarraga, L. A. Carreira and W. Susetyo. *Environ. Sci. Technol.*, **25**, 1427 (1991).