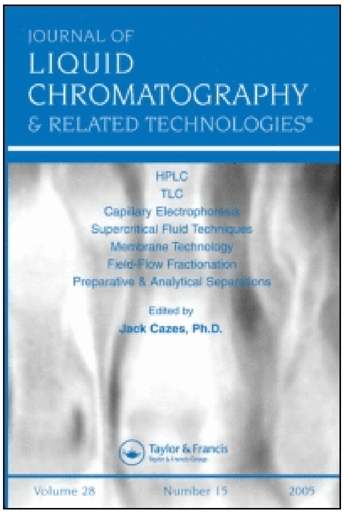

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CONDITIONAL STABILITY CONSTANT DETERMINATION OF METAL-FULVIC ACID COMPLEXES

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ABSTRACT

Conditional stability constants for metal complexes of a terrestrial fulvic acid were determined using an ion-exchange chromatography - atomic absorbance spectroscopy method. Employing the Scatchard model, conditional stability constants were determined for the metal(II) fulvic acid complexes of cadminum, copper, lead, nickel, manganese, and zinc. The order of metal binding by the fulvic acid was determined to be: Cu > Ni > Pb > Zn > Cd > Mn. Complexes of weakly bound metal ions were determined with an added metal ion concentration of 2×10^{-5} M to 1×10^{-4} M while complexes of strongly bound metal ions were determined with an added metal ion concentration of 1×10^{-4} M to 8×10^{-4} M. The fulvic acid concentration was kept constant at 4×10^{-4} M. The effect of pH and ionic strength on the copper-fulvic acid complex also was investigated.

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INTRODUCTION

Independent of their origin, humic substances can be divided into three classes: humic acid, fulvic acid and humin. Fulvic acid is soluble in both acid and base and can be derived from either vascular plant remains (terrestrial fulvic acid) or soil humus and aquatic or terrestrial plants (aquatic fulvic acid). Fulvic acid is complex mixture of degradation products which can bind metals primarily through its carboxylic acid and seconarily through its phenolic acid functional groups. Since fulvic acid is a naturally occuring metal complexer, the nature of these metal-fulvic acid complexes is necessary to understand the role of fulvic acid in the transport of metals in our environment.

The strength of the metal-fulvic acid complex is measured by the magnitude of its stability constant. Numerous techniques have been used to measure metal-fulvic acid complex parameters from which stability constants are determined. The parameters involved are the concentration of free metal, concentration of complexed metal and/or concentration of fulvic acid. The advantages and disadvantages of the methods were reviewed by Tuschall and Brezonik (1), and Saar and Weber (2). Comparisons of stability constants obtained by different methods cannot always be made because of the undefined ionic strength and pH conditions or because of the different manners by which the experimental data was modeled. The theoretical aspects of the mathematical models used to determine the stability constants were evaluated by Fitch and Stevenson (3) and Perdue and Lytle (4).

The techniques used to measure these parameters can be divided into two major types. The first type are techniques employing two-step processes in which metal determinations are made after the metal has been separated into its free and complexed forms. Gel permeation chromatography (5-7), equilibrium dialysis (8,9), and ultrafiltration (10,11) have been used to separate free metal from metal-fulvic acid complexes. Each separation technique has the potential problem of complex adsorption to the separation medium. In addition there is the possibililty of the separation process shifting the equilibrium causing dissociation of the complex. The advantage of separating the two metal species is that it enables atomic absorption spectroscopy(AAS) to be used in the metal determination. AAS allows a wide range of metals to be investigated.

The second type are techniques employing one-step processes in which the species measured (complexed metal, free metal, complexed fulvic acid, or free fulvic) is not separated from solution but measured in situ. Electrochemical methods of anodic stripping voltammetry (12-14) and ion-selective electrodes (12,14-16) are used to measure the free metal ion. Hydrogen-ion potentiometry (17) and UV-visible spectroscopy (18-19) are used to measure the amount of complexed fulvic acid while fluorescence spectroscopy (20-22) is used to measure the amount of uncomplexed fulvic acid.

The limiting factor in determining the stability constants for metal-fulvic acid complexes is the accuracy of the determination of the amount of free and complexed metal. The one-step processes offer a low limit of detection but are limited to the number of metals that can be studied. The electrochemical methods suffer from adsorption effects of the fulvic acid that make the accuracy of free metal measurement values questionable. Two-step processes are applicable to a wide range of metals due to the use of AAS for metal determinations. However, separation of free metal from metal-fulvic acid complexes has not been well-characterized for adsorption effects and possible equilibrium shifts.

This paper presents the results of an ion-exchange chromatography - atomic absorption spectroscopy system to measure the concentration of free metal and complexed metal-fulvic acid in order to calculate the metal-fulvic acid conditional stability constants. Previous work on our model system for the copper complex of citric acid has shown the method is valid for stability constant determinations (23). The work presented here was applied to a series of metals. Chromatographic parameters that influence the separation and determination of the free metal as well as the solution parameters that influence the metal standard used for calibration are investigated.

EXPERIMENTAL

Fulvic Acid Extraction and Characterization Using the accepted method of Schnitzer and Skinner (24), a soil sample obtained from a local garden center was air dried to reduce the moisture content, pulverized, and passed through a 40 mesh sieve to remove stones, plant material, and other debris. Two hundred and fifty g of sieved soil was added to 1.6 L of distilled water that contained 64 g of 50% NaOH and which had been nitrogen purged for 0.5 h. The mixture was stirred for 24 h under a nitrogen atmosphere. After stirring, the mixture was allowed to settle for 24 h and the dark brown solution decanted. The solution was centrifuged for 30 min at 10,000 rpm at a temperature of 5°C to remove suspended clay particulates. It was then passed through a strong cation exchange column loaded in the hydrogen form. Humic acid precipitated on the column, and, the fulvic acid remained in solution. The solution was rotary evaporated to a volume of 1 L and passed through the cation exchange column three more times. Any additional humic acid was removed by centrifuging the solution at 10,000 rpm and 5°C for 30 min. The solution was freeze dried to obtain the fulvic acid. Terrestrial fulvic acids are shiny, black compounds.

The elemental composition for carbon, hydrogen, nitrogen, oxygen, and sulfur was 48.02, 4.39, 1.86, 41.17, and 2.93 percent, respectively, with an ash content of 1 percent. The fulvic acid was found to contain 5.3 meq/g carboxylic and 1.8 meq/g phenolic content through titration.

The infrared spectrum of the fulvic acid was very similar to those obtained by Stevenson and Goh (25) which they classified as characteristic of Type II humic substances. The unique features are the strong absorption band at 1720 cm^{-1} (C=O stretching of carboxylic ketones) and the weaker band centered at 1640 cm^{-1} (C-O stretching of amide, quinone, conjugated ketones, or aromatic C=C). The intensity of the band at 1720 cm^{-1} is equivalent to the 3400 cm^{-1} band (O-H stretch). There also were bands at 1400 cm^{-1} (phenolic OH), 1200 cm^{-1} (C-O stretching and OH deformation of carboyxlic acid), and 1050 cm^{-1} (C-O stretching of polysaccharides or polysaccharide like structures).

UV-visible and the fluorescence spectra were obtained with a Perkin-Elmer 320 Spectrophotometer and a Perkin-Elmer LS-5 Fluorescence Spectrofluorometer (Norwalk, CT), respectively and they were typical for a terrestrial fulvic acid having carboxylic and phenolic chromophores (26,27).

The molecular weight of the sample was found to be 750 daltons as determined by gel permeation chromatography using a method and calibration standards similar to that of Plechanov (28). The method employs a mobile phase of dimethylformamide-acetic acid (99:1 v/v). Polystyrene, polyethylene glycol, and various organic dyes were used as standards. A SynChropak GPC 100 column was used.

The results obtained on the isolated terrestrial fulvic acid correlate well with these obtained on fulvic acids previously studied. It can be concluded from the above analyses that the sample possesses the same structural, chemical, and physical properties as fulvic acids described in the literature (25-28).

<u>Solution Preparation</u> Stock metal ion solutions were prepared from their respective high purity (99.99%) nitrate salts (Aldrich Chemical Co. Milwaukee, WI). The divalent metal ions used in this study were cadmium, copper, manganese, nickel, lead, and zinc. The stock solutions had approximate concentrations of 2.50 x 10^{-2} M with the values verified by atomic absorbance spectroscopy using primary standards.

Stock solutions of 1.0 x 10^{-3} M of the terrestrial fulvic acid were prepared. The pH of the solutions was adjusted to 3, 5, and 7 with sodium hydroxide.

The metal-fulvic acid solutions were prepared from the stock solutions. The concentration of the fulvic acid was kept constant at 4 x 10^{-4} M as the concentration of the added metal ion was varied. The concentration range of the added metal ion depended on the strength of the metal-fulvic acid complex. A weakly complexed metal such as manganese had an added concentration range of 2 x 10^{-5} M to 1 x 10^{-4} M while a strongly complexed metal such as copper had an added concentration range of 1 x 10^{-4} M to 8 x 10^{-4} M.

Instrumentation and Ion Exchange Column The liquid chromatograph consisted of a Tracor 995 isochromatographic pump having a Rheodyne 7125 injector. Mobile phase selection was obtained through a series of solenoids. A semi-micro column was constructed of Teflon tubing and 316 stainless steel Swagelok column end fittings. The end fittings contained 2 micron stainless steel frits. The column's dimensions were 40 mm x 1.6 mm I.D. The column was slurry packed with HC-Pellionex SCX (Reeve Angel, Clinton, NJ) a high capacity strong cation exchange resin. The packing particle size was 37-53 μm and consisted of a polystrene-divinylbenzene copolymer base to which sulfonic acid ($-SO_3H$) functional groups were attached. The exchange capacity of the resin was 60 μequivalents per g of dry material. The resin was stable between pH 2 to 10 and can be used with organic solvents.

The chromatographic system was interfaced to an atomic absorbance spectrometer by connecting the outlet tube of the UV detector cell (Tracor 960) to the aspirator inlet of the AAS premix burner (Instrumentation Laboratory 251 AA/AE). The atomic absorbance spectrophotometer was interfaced to a Rockwell International AIM 65 microcomputer, which was used for data accumulation and manipulation.

Chromatographic Parameters Injections were made using a 100 μL loop into a water mobile phase of flow rate 4.0 mL/min. After 30 s the mobile phase was switched to 0.10 M barium nitrate for 15 s and then to 0.10 M sodium nitrate for 15 s. The mobile phase was switched back to water for 3.5 min to remove all sodium ions not electrostatically adsorbed to the ion-exchange column. Additional injections were made at the end of the water rinse cycle and the mobile phase sequence repeated. The 100 μL injection loop was used except when the free metal ion in solution exceeded the linear dynamic range of the system. In this case, calibration standards of higher concentrations were prepared and a 20 μL injection loop was used.

The aspiration rate of the premix burner was set at 2.0 mL/min for a chromatographic mobile phase flow rate of 4.0 mL/min. The optimization of aspiration rate as a function of chromatographic flow rate had been previously determined in our laboratory.

Mobile Phase Time Sequence The time sequence was chosen to minimize the metal ion peak base width and analysis time while maximizing resolution. The initial 30 s water mobile phase determines the resolution between the metal complex and the free metal ion in the sample. The free metal ion adsorbs to the ion-exchange column while the metal complex is eluted in a narrow band at the column bed volume. A 15 s elution with the barium nitrate mobile phase is the minimum necessary to elute the metal ion from the column in a narrow band. Barium nitrate was chosen because of the barium ion´s high exchange constant. Times longer than 15 s did not decrease the band width while times shorter than 15 s would increase it. A sodium nitrate elution of 15 s was the minimum needed to replace the barium ions adsorbed to the column with sodium ions. Very little band spread ing occured when the counter ion is sodium due to sodium´s small exchange constant. The column was rinsed with water for 3.5 min to remove all non-adsorbed sodium ions. Sodium was not detected in the eluent at the end of the rinse.

Calibration Curves The injected metal ion was determined by monitoring the eluent by AAS. A calibration curve was constructed by plotting the peak height versus the concentration of the free metal ion injected. Peak height was chosen because of the narrowness of the peak basewidth. The concentration of bound metal was calculated by subtracting the concentration of free metal in solution from the total concentration of metal added to solution.

RESULTS AND DISCUSSION

Determination of Conditional Stability Constants The conditional stability constants (K´) for the metal-fulvic acid complexes were determined through the Scatchard model (29). The Scatchard parameters of ν, which is the concentration of metal bound divided by the total concentration of fulvic acid, and $\nu/[M]$, which is ν divided by the concentration of free metal in solution, were calculated. The parameters were plotted as $\nu/[M]$ versus ν as given in eq. 1

$$\frac{\nu}{[M]} = K'\frac{C_1}{C_L} - K'\nu \tag{1}$$

where C_1/C_L is the number of binding sites per fulvic acid molecule. The conditional stability constants were obtained from the negative slopes of the linear portion of the curves. The intercept of this line divided by its negative slope gives the ratio of the average number of binding sites per fulvic acid molecule. Most of the curves showed two linear portions: (1) a linear portion with a large slope at low free metal ion concentration, and (2) a linear portion exhibiting little or no slope at high free metal ion concentrations. The portion of the curve with the larger slope is due to the strong binding sites on the fulvic acid molecule while the latter portion of the curve is due to binding at weaker sites. Metal binding at the weaker sites occurs after saturation of the stronger binding sites.

Metal binding by the terrestrial fulvic acid was studied at a pH of 6.5 and an ionic strength of 0.10 M using the divalent metal ions of cadmium, copper, lead, manganese, nickel, and zinc. The Scatchard parameters determined for the metal-fulvic acid complexes are plotted as $\nu/[M]$ versus ν in Figures 1 and 2.

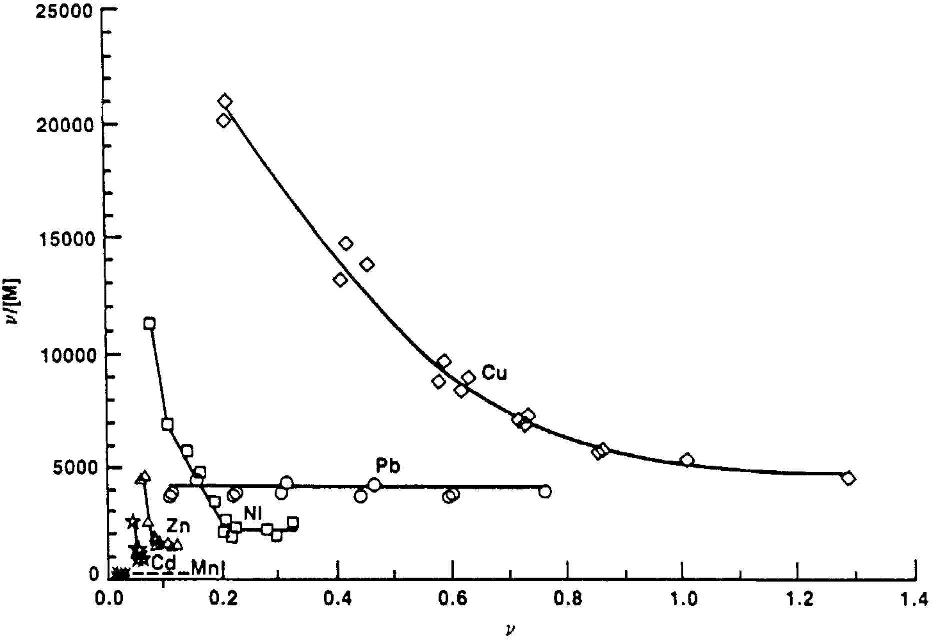

FIGURE 1. Scatchard plots for the binding of Cd(II), Cu(II), Mn(II) Ni(II), Pb(II), and Zn(II) by a terrestrial fulvic acid.

The plots for the lead-fulvic acid complex and the manganese-fulvic acid complex do not exhibit a large linear slope at low free metal ion concentrations. The reason the lead complex fails to show this line is due to the high limit of detection (LOD) of the atomic absorbance detector to lead determinations. The LOD for lead is at least a factor of four times higher than the LOD for the other metals studied. In addition, the sensitivity is less for lead than other metals studied. These two factors combined result in the inability of the pre-

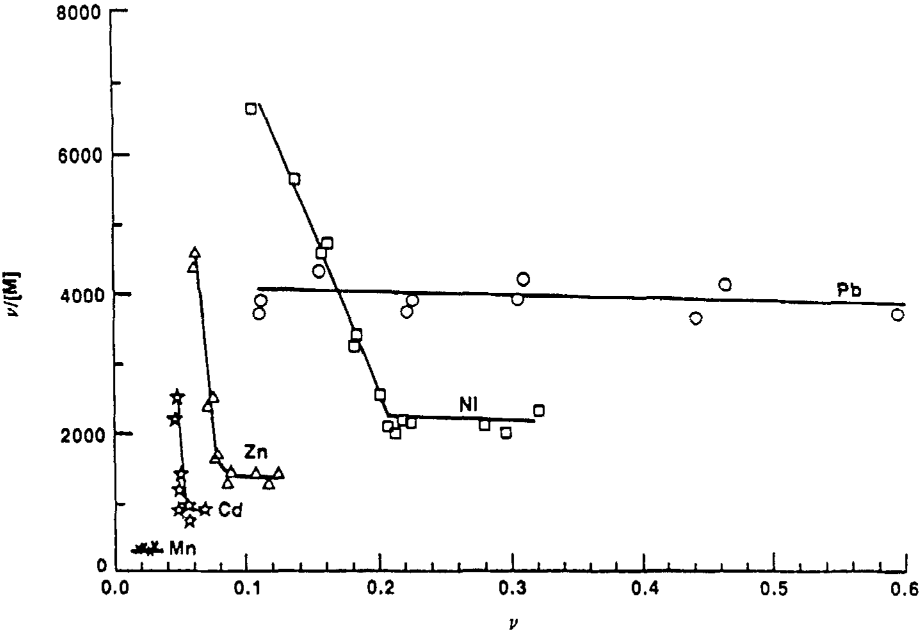

FIGURE 2. Scatchard plots for the binding of Cd(II), Mn(II), Ni(II), Pb(II), and Zn(II) by a terrestrial fulvic acid.

sent system to sufficiently measure the low free lead concentrations necessary to determine the strong binding sites for the concentration of fulvic acid employed in this study. A similar problem exists for making the manganese-fulvic acid complex determinations. The manganese complex is weak as compared to the other metals. Investigation of the strong binding sites reqiries a very low manganese concentration be added to the initial manganese-fulvic acid solution. The resulting free metal concentration at equilibrium is below the limit of detection for this system and the Scatchard parameters for the strong binding sites are not obtainable.

The conditional stability constants and the number of metal binding sites per fulvic acid molecule, C_1/C_L, were determined from the Scatchard plots. The log of the conditional stability constants and the values of C_1/C_L for the strong binding site complexes are given in Table 1. The conditional stability constant and the number of binding sites must be considered when evaluating the metal-fulvic acid complexes. While the strength of the metal-fulvic acid complex is important in considering the

Table 1. Conditional Stability Constants and Number of Binding Sites per Fulvic Acid Molecule for Cd(II), Cu(II), Ni(II), Pb(II), and Zn(II) Binding by a Terrestrial Fulvic Acid

Metal	Log K´	C_1/C_L
Cd	4.90 ± 0.10	0.07 ± 0.01
Cu	4.51 ± 0.03	0.85 ± 0.02
Ni	4.66 ± 0.03	0.25 ± 0.01
Pb[a]	2.20 ± 0.40	---
Zn	5.29 ± 0.05	0.09 ± 0.01

[a] Value for weak binding site.

transport of the metal through the environment, the number of metals bound to the fulvic acid must also be considered. The effectiveness of metal binding by fulvic acid as determined in this work was Cu > Ni > Pb > Zn > Cd > Mn. This ranking of metal complexation agrees with the order determined by Schnitzer and Hansen (30).

Literature values for the conditional stability constants copper-fulvic acid complexes determined by other researchers are listed in Table 2. The values determined in this work agree with those conditional stability constant values listed and

Table 2. Conditional Stability Constant Values for Cu(II) Binding by a Terrestrial Fulvic Acid[a]

Origin of Fulvic Acid	pH	Ionic Strength	Log K′
Soil	3.0	0.1 M $NaClO_4$	3.22
Soil	3.0	0.01 M KNO_3	3.30
Soil	5.0	0.01 M KNO_3	4.00
Soil	5.0	0.1 M $NaClO_4$	4.35
Soil[b]	5.0	0.1 M $NaNO_3$	4.44

[a] Taken from reference 3 and references cited therein.
[b] This work.

these data indicate that the terestrial fulvic acid possesses the same metal binding characteristics as other fulvic acids studied.

Effect of pH and Ionic Strength on the Stability Constant The two main factors that affect the stability constant are pH and ionic strength, therefore, these constants are referred to as conditional stability constants, K′, in this paper. The ionic strength was adjusted to 0.10 M with sodium nitrate and to a pH of 3, 5, and 7 for solutions containing 4 x 10^{-4} M fulvic acid and 5 x 10^{-4} M to 4 x 10^{-3} M copper nitrate. The Scatchard parameter values calculated for these solutions are plotted in Figure 3. The conditional stability constants, K′, and the ratio of number of metal binding sites per fulvic acid molecule, C_1/C_L, determined from the Scatchard plots are presented in Table 3.

The K′ values for the copper-fulvic acid complex are not significantly affected by pH changes. This result agrees with those of Stevenson (17). However, the number of binding sites per fulvic acid molecule decreases as the pH of solution decreases. This result was not observed in previous studies. The results show that the number of binding sites are a function of solution pH. As the pH decreases, the fulvic acid molecular structure changes from a flexible linear colloid to a rigid spherocolloid (31). This change in structure decreases the

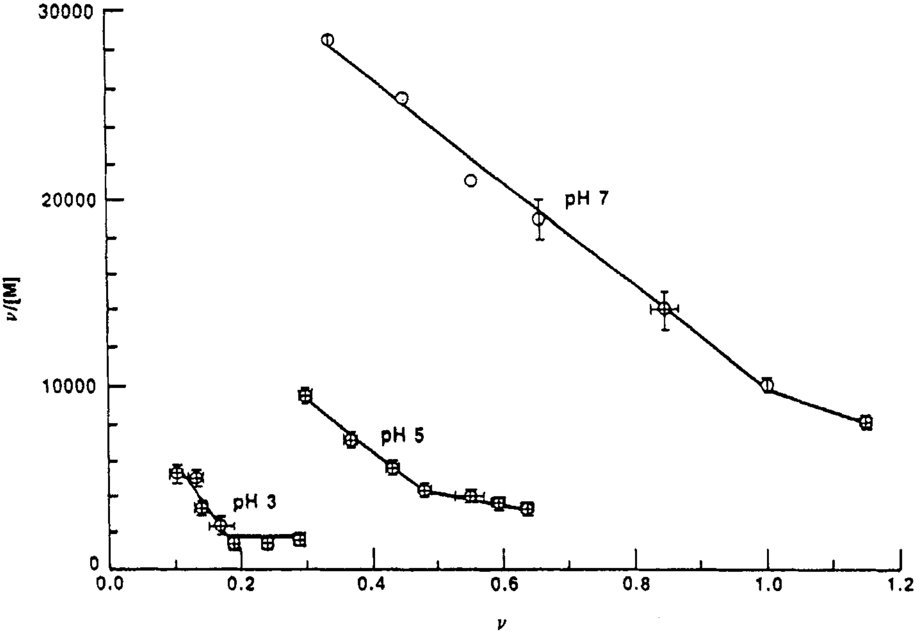

FIGURE 3. Scatchard plots for the binding of Cu(II) by a terrestrial fulvic acid at pH 3.0, 5.0, and 7.0.

Table 3. Conditional Stability Constants and Number of Binding Sites per Fulvic Acid Molecule for Cu(II) Binding by a Terrestrial Fulvic Acid at pH 3.0, 5.0, and 7.0 and Ionic Strength of 0.10 M

pH	Log K′	C_1/C_L
3.0	4.44 ± 0.04	1.35 ± 0.03
5.0	4.44 ± 0.04	0.63 ± 0.02
7.0	4.67 ± 0.04	0.02 ± 0.02

number of sites available for metal ion binding; however, the strength of the binding site is essentially pH independent.

The effect of the solution's ionic strength on the conditional stability constant of the copper-fulvic acid complex also was investigated. The metal-fulvic acid solutions contained 4 x 10^{-4} M fulvic acid and 1.5 x 10^{-4} M to 3.0 x 10^{-4} M copper nitrate. These solutions had a pH of 5.0 and ionic strengths of 0.01 M, 0.05 M, and 0.10 M. The conditional stability constants and C_1/C_L values determined through the Scatchard plots are listed in Table 4. K′ increases with increasing ionic strength due to the decrease in the species activity. This result is contradictory to the results obtained by Stevenson (17). A plausible explanation for this discrepancy is given below. The

Table 4. Conditional Stabililty Constants and Number of Binding Sites per Fulvic Acid Molecule for Cu(II) Binding by a Terrestrial Fulvic Acid at Ionic Strength of 0.01 M, 0.05 M, and 0.10 M and pH 5.0

Ionic Strength	Log K′	C_1/C_L
0.01 M	4.1 ± 0.1	0.9 ± 0.1
0.05 M	4.2 ± 0.1	0.8 ± 0.1
0.10 M	4.5 ± 0.1	0.6 ± 0.1

data in Table 4 shows that the number of binding sites per fulvic acid molecule increased with decreasing ionic strength. This increase in the number of binding sites can explain the increase in the conditional stability constant values with increasing ionic strength.

The conditional stability constants were determined for copper-fulvic acid solutions in which the copper binding was due to strong and weak sites. If the strong sites are much more selective to copper binding than the weak sites, the strong sites will be less influenced by the ionic strength of solution as compared to the weak sites. At high ionic strength the metal binding will be dominated by strong site binding. As the ionic strength decreases, the metal will begin to bind to the weak sites in addition to the strong sites. The conditional stability constant will decrease and there should be a corresponding increase in the number of binding sites per fulvic acid molecule as there are more weaker binding sites than stronger binding sites. This is what was experimentally observed. Stevenson's values may have been obtained in which the metal binding occured only at the strong sites.

Separation Effects The amount of complex dissociation within a given time period is dependent on both the dissociation rate constant and the formation rate constant. Dissociation of the metal-fulvic acid complex during the time it is in contact with the ion-exchange column will result in adsorption of the liberated metal ion to the ion-exchange resin. The metal ion from dissociation will be eluted with the free metal ion adsorbed from the injected solution. Therefore the conditional stability constant determined for the solution will have a lower value than the actual value because of the dissociation.

A comparison of peak parameters (basewidth, skewness) for metal-fulvic acid solutions and standard metal solutions show that the peak parameters for the metal ion are equivalent. These equivalent peak parameters indicate that the extent of dissociation of the metal-fulvic complex during the separation process is negligible. The metal-fulvic acid contact time with the ion-exchange column was calculated to be 0.71 s.

The extent of complex dissociation was also determined by an alternative method. Solutions containing 8 x 10^{-4} M copper nitrate and 4 x 10^{-4} M fulvic acid prepared at pH 7.0 and ionic strength of 0.10 M were allowed to equilibrate for 24 h. One of the solutions was passed through a 55 mm x 5 mm I.D. polystyrene syringe packed with a HC-Pellionex SCX ion-exchange resin to remove any free copper ion in solution. After 30 s the solution was analyzed on the ion-exchange chromatography-atomic absorbance system. The peak height of the metal-fulvic acid complex was compared to that obtained by the solution not subjected to the ion-exchange procedure. The two peak heights were equivalent indicating the dissociation of the copper-fulvic acid would not be signficant within the time frame of the experiment.

CONCLUSIONS

A novel method using ion-exchange chromatography interfaced to atomic adsorption spectroscopy was developed to separate and determine the amount of free metal ion in solution. The amount of metal was determined from a calibration curve that plots the metal concentration in a matrix matched standard versus AAS peak height. Parameters that influence the separation, ionic strength and pH, and hence the AAS detection of the metal ion were evaluated. This method was applied to the determination of the conditional stability constants for a series of metal-fulvic acid complexes. The metal-terrestrial fulvic acid complexes had conditional stability constants and/or a number of metal binding sites per fulvic acid molecule sufficient to make the determinations within the linear dynamic range of the method. The accuracy and reproducibility of the conditional stability constant determinations are directly dependent on the accuracy and reproducibility of the free metal ion determinations. Separation effects on the metal-fulvic acid complex were shown to be negligible.

REFERENCES

1. Tuschall, Jr., J.R. and Brezonik, P.L., Aquatic and Terrestrial Humic Materials, Christman, F. and Gjessing, E.T., eds., Ann Arbor Sci., Ann Arbor, MI, 1983, Chapter 13.

2. Saar R.A. and Weber, J.H., Environ. Sci. Technol.,16, 510A, 1982.

3. Fitch, A. and Stevenson, F.J., Signif. Trace Elem. Solving Petrog. Probl. Controversies 1983, p.645-669.

4. Perdue, E.M.and Lytle, C.R., Aquatic and Terrestrial Humic Materials, Christman, F. and Gjessing, E.T., eds., Ann Arbor Sci., Ann Arbor, MI, 1983, Chapter 14.

5. Chen, Y., Senesi, N. and Schnitzer, M., Soil Sci. Am. J., 41, 352, 1977.

6. Mantoura, R.F.C. and Riley, J.P., Anal. Chim. Acta, 78, 193, 1975.

7. Adamic, M.L. and Bartak, D.E., Anal. Chim. Acta, 158, 43, 1984.

8. Guy, R.D. and Chakrabarti, C.L., Can. J. Chem., 54, 2600, 1976.

9. Truitt, R.E. and Weber, J.H., Anal. Chem., 53, 337, 1981.

10. Buffle, J. and Stauab, C., Anal. Chem., 56, 2837, 1984.

11. Laxen, D.P.H. and Harrison, R.M., Water Res., 15, 1053, 1981.

12. Sterritt, R.M. and Lester, J.N., Water Res., 18, 1149, 1984.

13. Bhat, G.A. and Weber, J.H., Anal. Chim. Acta, 141, 95, 1982.

14. Bhat, G.A., Saar, R.A., Smart, R.B. and Weber, J.H., Anal. Chem., 53, 2275, 1981.

15. Saar R.A. and Weber, J.H., Anal. Chem., 52, 2095, 1980.

16. Takamatsu, T. and Yoshica, T., Soil Sci., 125, 377, 1978.

17. Stevenson, F.J., Soil Sci., 123, 10, 1977.

18. Langford, C.H., Gamble, D.S., Underdown, A.W. and Lee, S., Aquatiac and Terrestrial Humic Materials, Christman, F. and Gjessing, E.T., eds., Ann Arbor Sci., Ann Arbor, MI, 1983, Chapter 11.

19. Blaser, P., Fluhler, H. and Polomski, J., Soil Sci. Soc. Amer. J., 44, 709, 1980.

20. Waite, T.D. and Morel, F.M.M., Anal. Chim. Acta, 162, 263, 1984.

21. Ryan, D.K., Thompson, C.P. and Weber, J.H., Can. J. Chem., 61, 105, 1983.

22. Ryan, D.K. and Weber, J.H., Anal. Chem., 54, 986, 1982.

23. Pitluck, M.R., Pollard, B.D. and Haworth, D.T., Anal. Chim. Acta, accepted for publication.

24. Schnitzer, M. and Skinner, S., Soil Sci., 105, 392, 1968.

25. Stevenson, F.J. and Goh, K.M., Geochim. Cosmochim. Acta., 35, 471, 1971.

26. Plechanov, N., Josefsson, B., Dyrssen, D. and Lundquist, K., Aquatic and Terrestrial Humic Materials, Christman, R.F. and Gjessing, E.T., eds., Ann Arbor Sci., Ann Arbor, MI, 1983, Chapter 19.

27. Ewald, M., Belin, C. and Berger, P., Environ. Sci. Technol., 17, 501, 1983.

28. Plechanov, N., Org. Geochem., 5, 143, 1983.

29. Scatchard, G., Ann. N.Y. Acad. Sci., 51, 660, 1949.

30. Schnitzer, M. and Hansen, E.H., Soil Sci., 109, 333, 1970.

31. Stevenson, I.L. and Schnitzer, M., Soil Sci., 133, 179, 1982.