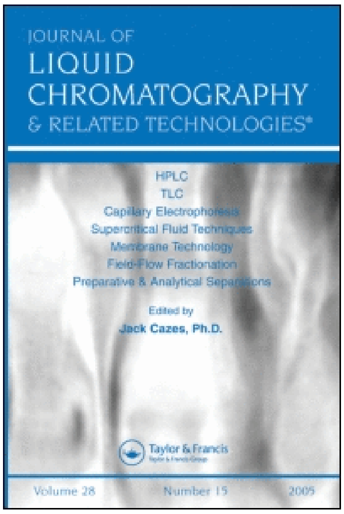

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CONDITIONAL STABILITY CONSTANT DETERMINATION OF METAL AQUATIC FULVIC ACID COMPLEXES

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ABSTRACT

An ion-exchange chromatography/atomic absorbance spectroscopy method for the determination of the conditional stability constants for metal complexes of an aquatic fulvic acid is described. Employing the Scatchard model, conditional stability constants were determined for metal(II) fulvic acid complexes of cadmium, copper, nickel and zinc at pH 7. The effect of pH on the stability constant of the copper-fulvic acid complex is also described.

INTRODUCTION

Fulvic acid is the class of humic substances which is soluble in both acid and base. Terrestrial fulvic acid is

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derived from either vascular plant remains or soil humus and aquatic fulvic acid is obtained from either aquatic or terrestrial plants. Fulvic acid is a complex mixture of degradation products which can blind to metals primarily through its carboxylic acid functional groups and also through its phenolic acid functional groups (1,2). As a naturally occuring chelate, the nature of metal-fulvic acid ineteractions is necessary to understand the role of fulvic acid in the transport of metals in our environment.

Recently we have reported on the determination of conditional stability constants for metal complexes of a terrestrial fulvic acid using an ion-exchange chromatography/atomic absorbance spectroscopy method (3). The advantages and disadvantages of other methods of stability constant determination have been reviewed by Tuschell and Brezonik (4) and by Saar and Weber (5) and we have summarized these techniques (3). Using the copper complex of critic acid as our model system, we have shown that the above method is valid for stability constant determinations (6).

The aim of the present work is to show that the same methodology can also be used to determine conditional stability constants of metal complexes of Cd, Cu, Ni and Zn with an aquatic fulvic acid ligand. The effect of pH on the stability constant of the copper-fulvic acid complex has also been investigated.

EXPERIMENTAL

Aquatic Fulvic Acid Aquatic fulvic acid (100 mg) was obtained from the International Humic Substances Society and is designated as the Suwanee Stream Standard Fulvic Acid.

Reagents. Stock solutions containing the divalent metal ions of Cd, Cu, Ni and Zn were prepred with concentrations of approximately 2.5×10^{-1} M with the values verified by AAS using primary standards. Solutions of 4×10^{-4} M aquatic fulvic acid were adjusted to a pH of 7.0 and ionic strength of 0.1 M with 0.2 M $NaNO_3$.

Instrumentation and Ion Exchange Column The liquid chromatograph consisted of a Tracor 995 isochromatographic pump, Rheodyne 7125 injector equipped with a 100-µl loop, and a Tracor 960 ultralviolet (UV) detector. Solvent was selected with a series of solenoid valves. An ion-exchange column (40 mm long, 1.6 mm i.d.) was constructed of teflon tubing and 316 stainless steel Swagelok column end-fittings with 2-µm stainless steel frits. The column was slurry-packed with HC-Pellionex SCX (37-53 µm particle size; Reeve Angel, Clinton, NJ) a strong cation-exchange resin consisting of a polystyrene/divinylbenzene copolymer base with sulfonic acid functional groups. The resin has an exchange capacity of 60 µeq g^{-1} (dry weight) and is stable between pH 2 and 10.

The outlet of the UV detector was connected to the premix burner of the atomic absorption spectrometer

(Instrumentation Laboratory 251 AA/AE) with 1/16 in. teflon tubing and appropriate Swagelok fittings. The spectrometer was interfaced to a Rockwell International Aim 65 microcomputer.

Chromatographic Parameters Solutions were injected onto the ion-exchange column using a 100-µl loop and a water mobile phase at 4.0 ml min^{-1}. The complex eluted from the column at the bed volume while the free metal ions were retained. After 30 s, the mobile phase was switched to 0.10 M barium nitrate for 15 s to elute the adsorbed metal. Barium nitrate was chosen because the high exchange constant for Ba^{2+} produces minimal band spreading of the metal ions being eluted. The mobile phase was switched to 0.10 M sodium nitrate for 15 s to remove barium ions from the resin and then back to water for 3.5 min to remove excess of sodium ions. Additional injections were made at the end of the water rinse cycle and the mobile phase sequence was repeated. A 20-µl loop was substituted for the 100-µl loop when the free metal concentration of the metal/fulvic acid solutions exceeded the linear dynamic range of the system; metal calibration standards of higher concentrations were then used.

Spectrometric Parameters The parameters for the spectrometer were: 324.7 nm wavelength, 640 µm slitwidth, 0.2 mA per count, intensity at 4 V, 620 V on the photon multiplier, 2 mA lamp current, and 22 mA deuterium lamp current. The aspiration rate of the premix burner was set at 2.0 ml min^{-1}. The aspiration rate as a function of chromatographic flow rate was optimized for signal-to-noise.

Calibration 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

The method of Scatchard (7) was used to determine the conditional stability constant K′, for the metal-aquatic fulvic acid complexes. The Scatchard parameter, ν, is equal to the concentration of metal bound to the fulvic acid, [MFA], divided by the total concentration of complexing ligand (fulvic acid) in solution [FA].

$$\nu = \frac{[MFA]}{[FA]} \qquad (1)$$

Taking $[M^{2+}]$ as the concentration of free metal ion in solution, and n as the number of binding sites per molecule as given in equation 2,

$$\frac{v}{[M^{2+}]} = K'n - K'v \qquad (2)$$

a plot of $v/[M^{2+}]$ versus v yields a straight line with a negative slope equal to the conditional stability constant (K') for the metal ion - fulvic acid complex. Dividing the intercept (K'n) by the negative slope gave a value equal to the number of binding sites per fulvic acid molecule, n. The concentration of the free metal ion was determined from a standard calibration curve. The concentration of metal ion - fulvic acid complex formed was calculated by subtracting the free-metal ion concentration in solution from the concentration of metal ion added to the solution (equation 3).

$$[MFA] = [M_T]-[M^{2+}] \qquad (3)$$

From the values for free and complexed metal ion and from the initial concentration of fulvic acid, the Scatchard parameters of v and $v/[M^{2+}]$ are calculated. The calculated Scatchard parameters are the average for four separate solution preparations and determinations and a typical Scatchard plot is shown in Fig. 1 for Cu^{2+} at pH 7.0 and ionic strength 0.10 M.

The Scatchard plots 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 attributed

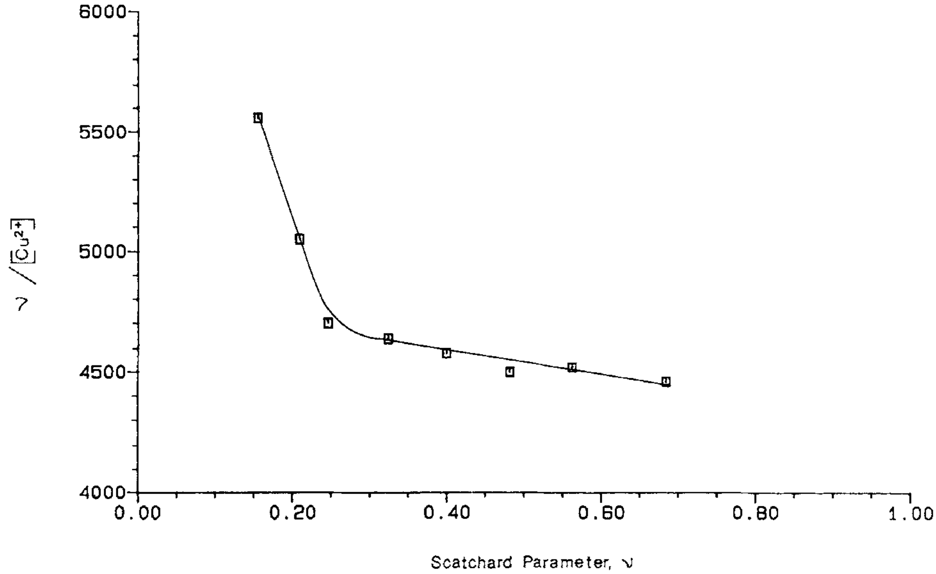

Figure 1. Scatchard plot for the binding of Cu(II) by an aquatic fulvic acid.

to the strong binding sites on the fulvic acid molecule while the latter portion of the curve is attributed to binding at weaker sites. Metal binding at the weaker sites occurs after saturation of the stronger binding sites. The log of the conditional stability constant was obtained from the negative slopes of the plots. The log K′ at the stronger binding sites and the corresponding number of binding sites per fulvic acid molecule, n, are presented in Table 1 for the metal ions of Cd, Cu, Ni, and Zn. The complexing ability of the aquatic fulvic acid for these metal ions is ranked: Cu > Ni > Cd > Zn which

Table 1

Conditonal stability constants and number of binding sites per fulvic acid molecule for metal binding by an aquatic fulvic acid

Metal	Log K′	n
Cd	4.80 ± 0.20 (4.90)*	0.05 ± 0.02 (0.07)*
Cu	3.98 ± 0.02 (4.51)	0.74 ± 0.02 (0.85)
Ni	4.63 ± 0.02 (4.66)	0.08 ± 0.03 (0.25)
Zn	4.98 ± 0.05 (5.29)	0.033± 0.003 (0.09)

*Values taken from reference 3 for a terrestrial fulvic acid.

agrees with the ranking determined by Schnitzer and Hansen (8). This was also the order of stability for metal complexes, Cu > Pb > Cd > Zn, of humic acids as determined by potentiometric titration(9). Sposito has also obtained a stability constant of log K of 3.9 for the Cu-fulvic acid complex for fulvic acid derived from sewage sludge-soil mixtures (10). The ranking of Cd and Zn are predicted to be reversed using linear correlation analysis from data also taken by potentiometric titrations (11). For comparison purposes the log K′ and n values for our terrestrial fulvic acid determinations are also included in Table 1. While the order of binding is the same, it is noted that this sample of aquatic fulvic acid is a weaker complexer than terrestrial fulvic acid. This is due to the smaller number of binding sites per fulvic acid molecule as seen by the n values listed in Table 1. Scatchard plots only distinguish between different complexes according to their stability constants and not according to their stoichiometries or their molecular structures (10).

The smaller number of binding sites results in the use of very low concentrations of added metal ion. The Scatchard parameters, which are dependent on the determination of free metal in solution, were determined at the limit of detection for the ion-exchange chromatography/atomic absorbance spectroscopy system. An increase in the accuracy and reproducibility of the measurements would require a greater concentration of fulvic acid be used in the solutions or much larger sample injections.

The effect of the solution's pH on the copper-fulvic acid complex was also investigated. The solutions had an ionic strength of 0.10 M and contained 4 x 10^{-1} M fulvic acid and 1.0 x 10^{-4} M to 4 x 10^{-4} M copper nitrate. The Scatchard parameter values determined for the solutions are plotted as $\nu/[Cu^{2+}]$ versus ν in Fig. 2. The Scatchard plot for the pH 3 solution is given as an error bar because of the inconsistency of the determined values.

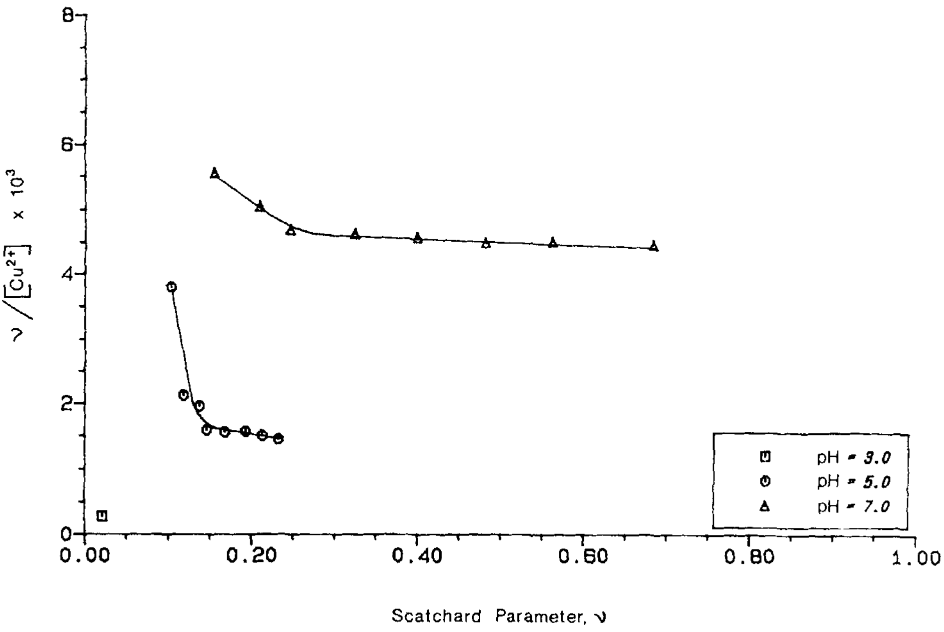

Figure 2. Scatchard plots for the binding of Cu(II) by an aquatic fulvic acid at pH 3.0, 5.0 and 7.0.

The pH 7 solution had a log K′ value of 3.9 ± 0.1 and a n value of 0.81 ± 0.04. The pH 5 solution had a log K′ value of 4.6 ± 0.3 and a n value of 0.18 ± 0.04. The lower stability constant for pH 7 could possible be due to the inability of the method to determine the copper binding to strong sites as seen by the Scatchard plot in Fig. 2 having only a small part of the curve with a large slope. The stability constant determined for pH 7 may also be a combination of string and weak binding sites. Again no assumption on stoichiometry can be implied from the Scatchard plots nor can the plots distinguish between the existence in a mixture of several 1:1 complexes with different stability constants (12). The decrease in the number of binding sites per fulvic acid molecule with decreasing pH is consistent with the results obtained for the terrestrial fulvic acid. (3) Bresnahan using an ion-selective electrode method has shown that the number of binding sites per molecule of fulvic acid increased as the pH increased. This increase is probably due to a structural change in the fulvic acid macromolecule molecule which brings more functional groups to the surface where they can act as ligands (13).

We have already shown that dissocation of the complex on the ion-exchange column was not expected to be significant as the contact time of the complex with the ion-exchange column is only 0.71 s (6). Furthermore, the fact that peak parameters of peak width, tailing and asymmetry of the metal ion peak separated from the complex were equivalent to peaks of the metal standard solutions tends to support this expectation.

CONCLUSIONS

An ion-exchange chromatography/atomic abosrbance spectroscopy method was developed to determine the conditional stability constants for a series of metal-fulvic acid complexes. These metal-aquatic fulvic acid complexes had conditional stability constants which were smaller than those reported for a terrestrial fulvic acid. The accuracy and reproducibility of the conditonal stability constants determination are directly dependent on the accuracy and reproducibility of the free metal determination. The developed method is applicable to a wide range of metals, has a low limit of detection and the ion strength of sample solution is limited to less than 0.05 M. The described novel chromatographic method provides a useful technique for stability constant determination for complexes with dissociation rates that are slow relative to the chromatographic process.

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