*Environ. Sci. Technol.* **1998**, *32,* 2410−2416

# Models of Metal Binding Structures in Fulvic Acid from the Suwannee River, Georgia

J. A. LEENHEER,*,† G. K. BROWN,† P. MACCARTHY,‡ AND S. E. CABANISS†,§

*U.S. Geological Survey, Denver, Colorado 80225, and Department of Chemistry, Colorado School of Mines, Golden, Colorado 80401*

Fulvic acid, isolated from the Suwannee River, Georgia, was assessed for its ability to bind $Ca^{2+}$, $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, and $Zn^{2+}$ ions at pH 6 before and after extensive fractionation that was designed to reveal the nature of metal binding functional groups. The binding constant for $Ca^{2+}$ ion had the greatest increase of all the ions in a metal binding fraction that was selected for intensive characterization for the purpose of building quantitative average model structures. The "metal binding" fraction was characterized by quantitative $^{13}C$ NMR, $^{1}H$ NMR, and FT−IR spectrometry and elemental, titrimetric, and molecular weight determinations. The characterization data revealed that carboxyl groups were clustered in short-chain aliphatic dibasic acid structures. The $Ca^{2+}$ binding data suggested that ether-substituted oxysuccinic acid structures are good models for the metal binding sites at pH 6. Structural models were derived based upon oxidation and photolytic rearrangements of cutin, lignin, and tannin precursors. These structural models rich in substituted dibasic acid structures revealed polydentate binding sites with the potential for both inner-sphere and outer-sphere type binding. The majority of the fulvic acid molecule was involved with metal binding rather than a small substructural unit.

## Introduction

Aquatic humic substances are frequently responsible for controlling the speciation, solubility, and toxicity of certain metal ions in water through various metal binding reactions (*1*); however, the mechanisms of these metal binding reactions are not well understood, and various models have been proposed. Metal binding with humic substances is a highly complex and variable phenomenom that depends on the pH and the ionic strength of the water medium, the concentration ratio of the metal ion to the humic ligand, competitive binding between certain metal ions, the intermolecular and intramolecular heterogeneity of of metal binding functional group distributions in humic molecules, and size, shape, and phase differences between different humic molecules.

The earliest models focused on specific functional group substructures in humic substances that are known to form metal−chelate complexes of moderate to high stability. Aromatic salicylate and phthalate models were first postulated (*2, 3*) followed by aliphatic citrate and malonate models (*4*) that better describe fulvic acid-metal complexes in acidic waters. Because of the chemical heterogeneity and complexity of humic substances, recent models have emphasized the mathematics and thermodynamics of metal binding (*5*). These models have widely differing assumptions, from a few discrete metal binding sites to a continuous Gaussian distribution of binding sites. Different electrostatic effects and molecular phases, sizes, and shapes have also been assumed in these models.

The modeling approach of this study is a return to molecular structural models under one specific set of conditions, but the entire molecule is modeled rather than substructures of functional groups that are postulated to bind metals. The set of conditions are for metal binding with a certain well-characterized fulvic acid at pH 6 at metal-to-ligand ratios where metal binding functional groups are sufficiently abundant to be detected by spectral methods. Although these specific conditions may limit environmental applications to metals found at trace concentrations, these conditions are relevant to metals such as calcium found in major concentrations.

The average structural model approach has been previously used to reveal major chemical structural differences in aquatic fulvic acid from different sources and environments (*6*) and has been applied to proton binding mechanisms in fulvic acid from the Suwannee River (*7, 8*). The modeling approach uses quantitative elemental, molecular weight, spectrometric, and titrimetric data to analytically constrain the molecular models. The models also incorporate subjective considerations of source structures (lignins, tannins, carbohydrates, lipids) and various degradative reaction pathways to fulvic acid.

Fulvic acid from the Suwannee River, Georgia, was chosen for this study because of its extensive characterization (*7*−*9*) and because its molecular weight is sufficiently low to act as an analytical constraint on the structural models. Intermolecular heterogeneity in this fulvic acid was minimized by extensive, large-scale fractionation (*10*). $Cu^{2+}$ binding by each fraction was examined by $Cu^{2+}$ ion titrations (*10*). The fraction with the highest conditional binding constant for $Cu^{2+}$ at pH 6 was used to determine conditional binding constants for $Ca^{2+}$, $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, and $Zn^{2+}$ by Schubert's ion exchange method (*10*). This fraction was used for molecular structural models presented in this study. The objective of this paper is to consider various analytically constrained structural models of fulvic acid−metal complexes that may reveal new structural relationships that are relevant to fulvic acid−metal binding under the specific conditions of this study.

## Experimental Section

**Fractionation Methods.** The fulvic acid fractionation methods are completely described by Brown (*10*). Briefly, 40 g of fulvic acid, isolated from the Suwannee River sampled at its source (*11*), was adsorbed onto a 9.4-L bed-volume column of Amberlite XAD-8 resin at pH 1 and was then fractionated by sequential eluent buffers that raised the pH in increments of 0.5−1.0 pH units up to pH 13. The fulvic acid fractions were re-isolated from each buffer eluent and were assayed for proton and $Cu^{2+}$ binding by titrimetry. The fraction that eluted at pH 5.0 had the strongest binding characteristics, and this fraction was subfractioned by normal-phase chromatography on a 1.9-L bed-volume column of silica gel. This

* Corresponding author phone: (303)467-8290; fax: (303)467-9598; e-mail: leenheer@usgs.gov.

† U.S. Geological Survey.

‡ Colorado School of Mines.

§ Present address: Department of Chemistry, Kent State University, Kent, OH 44242.



S0013-936X(97)00897-3 CCC: $15.00 
Published on Web 07/10/1998

column was eluted with eight different eluents of increasing polarity with the first eluent chloroform being the most nonpolar and 75% acetonitrile/25% water containing 0.25 M oxalic acid being the most polar. In this fractionation, the fractions that eluted with 75% acetonitrile/25% 2-propanol and with the solvent mixture containing oxalic acid had the strongest $Cu^{2+}$ binding. The acetonitrile/2-propanol fraction contained the majority of the mass of fulvic acid, and this was the fraction selected for intensive characterization and molecular modeling. For this paper, this fraction will be referred to as the "metal binding" fraction.

**Metal Binding Methods.** $Cu^{2+}$ ion selective electrode potentiometry was used to screen the fractions for metal binding ability, and the detailed experimental protocols and calculation of conditional stability constants from the titration data are given by Brown (*10*). Briefly, all $Cu^{2+}$ titrations were performed at pH 6.0 in 0.001 M $KClO_4$. The useful range of the $Cu^{2+}$ titration ranged between $10^{-6}$ and $10^{-3}$ M total Cu(II). Fulvic acid concentrations were $7.86 \times 10^{-5}$ M assuming a number average molecular weight of 700 Da for the fulvic acid (*9*).

Conditional stability constants for $Ca^{2+}$, $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, and $Zn^{2+}$ were also determined for the metal binding fraction at high ligand-to-metal ratios by Schubert's ion exchange method (*12*); the detailed procedures and results are given by Brown (*10*). Briefly, the metal ions at a concentrations of $1.0-8.0 \times 10^{-6}$ M were equilibrated with fulvic acid at concentrations of 0.25−1.0 M in the presence of sodium-form AG 50W−X8 cation-exchange resin. All metals were as nitrate salts in a background electrolyte of 0.10 M $NaNO_3$. After equilibration, the total metal in solution was determined by inductively coupled plasma−atomic emission spectrometry (ICP−AES), and the metal on the resin was eluted with nitric acid and determined by ICP−AES. From the knowledge of total metal added to the system and the free metal bound to the resin, conditional stability constants were calculated for metal bound to fulvic acid. The method was calibrated with citric acid at pH 3.5 and pH 6.0. Conditional stability constants were determined at pH 6.0 for both the unfractionated fulvic acid and for the metal binding fraction of fulvic acid.

**Fulvic Acid Characterizations.** Elemental analyses for carbon, hydrogen, oxygen, nitrogen, sulfur, moisture, and ash were performed by Huffman Laboratories, Wheat Ridge, CO, with methods optimized for humic substances (*13*). Number-average molecular weight was determined by Huffman Laboratories by four-point vapor pressure osmometry in tetrahydrofuran. An evaluation of vapor pressure osmometry for determination of molecular weight of fulvic acid is given by Aiken and Gillam (*14*).

$^{13}C$ and $^{1}H$ nuclear magnetic resonance (NMR) spectra were obtained on a Varian XL-300 NMR spectrometer. The pulse sequences and operating conditions to obtain quantitative spectra have been reported previously (*15*). Approximately 200-mg samples of fulvic acid were dissolved as potassium salts in 2 mL of $D_2O$ at pH 8. For $^{1}H$ NMR determinations, the fulvic acid was repeatedly dried from $D_2O$ to minimize the exchangeable proton peak.

Carboxylic acid groups in fulvic acid are frequently measured by titrimetry (*7*); however, overlap of phenolic hydroxyl p$K_a$ values with carboxyl group p$K_a$ values and hydrolysis of labile ester linkages during titration (*16*) are sources of error for the carboxyl group determination. A quantitative infrared spectrometric method was developed for the carboxyl group that measured the integral of a broad NH stretching band of fulvic acid dissolved in pyridine-$d_5$ between 1815 and 2150 $cm^{-1}$ that was unique to the carboxyl group (*17*). Strongly acidic enols such as ascorbic acid did not interfere, and esters and ketone groups could also be determined after spectral subtraction of the carboxyl C=O stretch near 1720 $cm^{-1}$. Binary mixtures of aliphatic and aromatic carboxylic acids served as standards for the carboxyl group subtraction procedure that approximated the relative proportions of carboxyl groups in fulvic acid from the Suwannee River (*7*). A Fourier tranform infrared spectrometer (Perkin-Elmer System 2000) was used with a 0.037 path length potassium bromide cell. Approximately 3 mg of fulvic acid was dissolved in 0.25−0.5 mL of pyridine-$d_5$.

**Structural Modeling.** Several approaches have been used to derive chemical structural models of complex heterogeneous natural organic matter such as humic substances (*18*) and coal (*19*). The coal chemists in particular (*19*) have emphasized the importance of independent, multidimensional fractionation and analytical approaches and then building models that meet the analytical constraints of the data. The first quantitative structural model of an aquatic fulvic acid that was built upon similar approaches used by coal chemists was for the unfractionated fulvic acid from the Suwannee River (*20*). This modeling approach has been extended to aquatic fulvic acid from diverse environments (*6*) and to proton binding structures in fulvic acid from the Suwannee River (*7, 8*).

The first step in deriving a structural model is to compute an average molecular formula by determining the empirical formula from the elemental analyses data and then converting to the molecular formula from the average molecular weight. The index of hydrogen deficiency $\Phi$ (number of rings and double bonds) is then computed from the equation, $\Phi = \{(2C + 2) - H\}/2$, where C and H are the number of these atoms in the molecular formula. Carbons in the molecular formula are then assigned to various structural units based upon quantitative $^{13}C$ NMR data (*15*), and then oxygen is similarly assigned from quantitative $^{13}C$ NMR and FT-IR data. The number of exchangeable hydrogen atoms is estimated from the oxygen functional group assignments, and exchangeable hydrogen is subtracted from total hydrogen to determine nonexchangeable hydrogen. The nonexchangeable hydrogen atoms are then assigned to various structural units based on the quantitative $^{1}H$ NMR data (*21*).

The assembly of structural data into a chemical structure is a subjective, speculative activity that takes the following considerations into account:

(1) The source of fulvic acid in the Suwannee River is primarily allochthonous plant organic matter(from the Okefenokee Swamp), i.e., a mixture of degraded tannins, lignins, carbohydrates, and lipids.

(2) Both biological and abiotic photolytic degradative processes are important. Phenolic rings in tannins and lignins are likely degraded to substituted aliphatic glutaric and succinic acid structures by the $\beta$-keto adipate pathway (*22*). Straight-chain aliphatic structures are readily degraded, but highly branched and aliphatic alicyclic structures are resistant to degradation (*23*). Olefins are highly reactive to oxidation by biotic and abiotic processes, and oxidative linkages such as aliphatic ethers result when precursors such as unsaturated lipids react with oxygen (*24*). Photolytic rearrangements of phenolic esters to benzophenone linkages are likely important with hydrolyzable tannins. The photo-Fries rearrangement may produce ketone linkages from phenolic ester linkages (*25, 26*).

The ChemWindow 3 graphics program from SoftShell International was used to draw the structural models. This graphics program is not as sophisticated as three-dimensional space-filling graphics programs, but it presents recognizable structures to the majority of readers.

## Results

The metal binding fraction that is the focus of the modeling effort constituted 7.1% of the mass of the fulvic acid. The pH 5.0 fraction from the pH gradient fractionation constituted

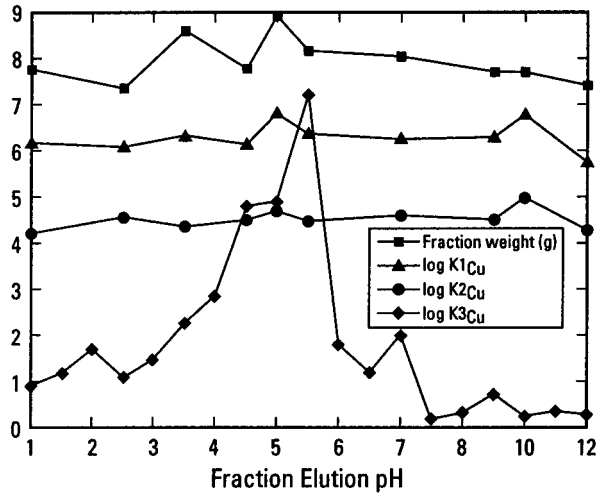


**FIGURE 1. Conditional stability constants of $Cu^{2+}$ ion at pH 6 and mass fractionation of pH gradient fractions of fulvic acid from the Suwannee River.**

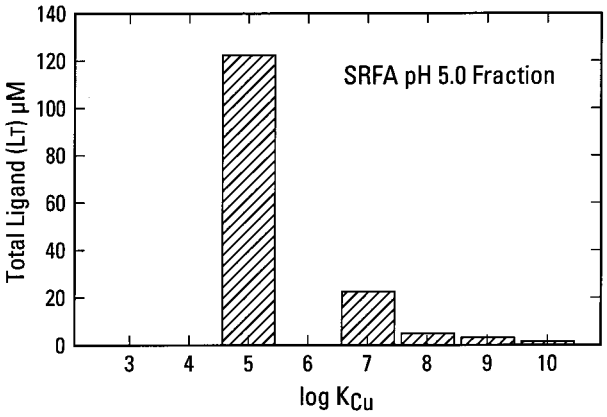


**FIGURE 2. Fulvic acid ligand abundance from fraction eluting at pH 5 as a function of Cu(II) conditional stability constants.**

12.5% of the fulvic acid, and the metal binding subfraction was 57% of the mass of the pH 5.0 fraction. The mass distribution of the pH gradient fractionation and the conditional $Cu^{2+}$ stability constants (three-site model) determined by ion selective electrode potentiometry (*10*) of this fractionation are presented in Figure 1. All the fractions in Figure 1 have significant $Cu^{2+}$ stability constants such that $Cu^{2+}$ binding is more of a bulk property of the fulvic acid as a whole than a trace property of a minor fraction with large metal binding constants. The calculated ligand abundance for a five-site $Cu^{2+}$ binding model for the pH 5.0 fraction is shown in Figure 2. Eighty percent of the $Cu^{2+}$ binding ligands were calculated to have log *K* values near 5.0, and it is this population of ligands that will be modeled because ligands with greater binding constants are not sufficiently abundant to be detected by the analytical techniques used in this study.

Conditional stability constants for binding of the five metals with the metal binding fraction determined by Schubert's method are presented in Table 1. The slope values of the Schubert's plots are near 1.0, which indicates 1:1 metal ion−humic ligand complex stoichiometry.

The only metal that has a large increase in its conditional stability constant value between the unfractionated fulvic acid and the metal binding fraction is calcium. Organic ligands that are rich in carboxyl groups with ether cross links, such as oxydisuccinic acid (*27*) and ditartronic acid (*28*), are known to have exceptionally large stability constants for $Ca^{2+}$. Thus, the fractionation procedure enriched the fulvic acid

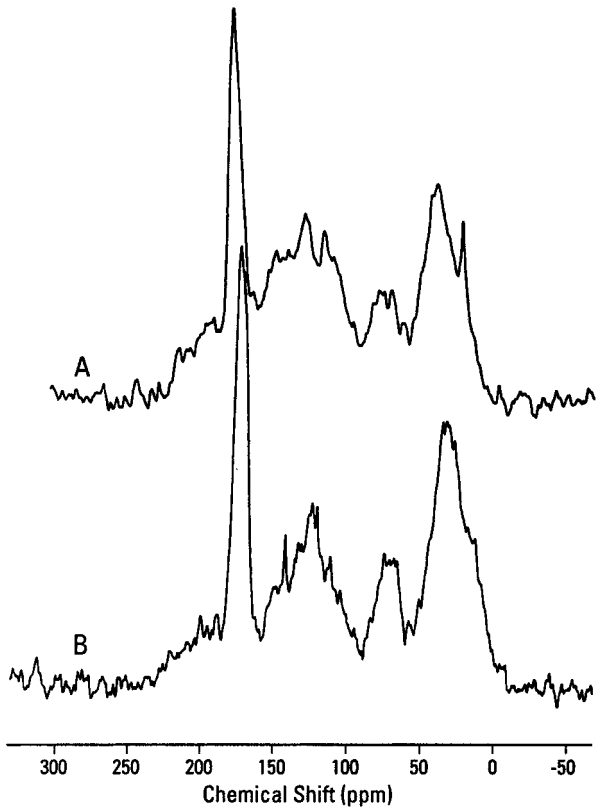


**FIGURE 3. Quantitative solution state $^{13}C$ NMR spectra of (A) metal binding fraction and (B) unfractionated (*15*) fulvic acid from the Suwannee River.**

**TABLE 1. Conditional Stability Constants (log *K*) at pH 6 for Unfractionated Fulvic Acid and for the Metal Binding Fraction of Fulvic Acid**

| metal | unfractionated fulvic acid (log *K*) | metal binding fraction (log *K*) |
|---|---|---|
| $Ca^{2+}$ | 3.0 | 4.1 |
| $Cd^{2+}$ | 4.4 | 4.5 |
| $Cu^{2+}$ | 5.2 | 5.5 |
| $Ni^{2+}$ | 3.8 | 4.1 |
| $Zn^{2+}$ | 3.8 | 4.1 |

with calcium binding ligands that may have structural similarities to oxy-polycarboxylic acids.

A comparison of the $^{13}C$ NMR spectra of the unfractionated fulvic acid (*15*), and the metal binding fraction is shown in Figure 3. In terms of intensity differences, the aliphatic C−O peak from 60 to 90 ppm is smaller in the metal binding fraction, and the carboxylate carbon peak for the metal binding fraction appears to reside upon a larger background (in the same region of chemical shifts) that includes ester and aromatic ketonic carbonyl groups. In terms of chemical shift difference, several peaks in the metal binding fraction are shifted downfield (greater chemical shifts), with the aliphatic carbon peak shifted from 36 to 40 ppm, the aliphatic C−O peak shifted from 74 to 80 ppm, and the carboxylate peak shifted from 177 to 180 ppm. On the basis of the $^{13}C$ NMR spectra of model polycarboxylic acids, these chemical shift changes indicate that electron-withdrawing oxygen functional groups are likely clustered closer together in molecular structures of the metal binding fraction than in the unfractionated fulvic acid. The 180 ppm chemical shift of the carboxylate group of the metal binding fraction also indicates that aromatic carboxylate groups with chemical shifts near 172 ppm are minimal.

**TABLE 2. Molecular Data Used for Formulating Average Structural Model of Metal Binding Fraction of Fulvic Acid from the Suwannee River**

| parameter | method | value |
|---|---|---|
| number-average molecular weight | vapor pressure osmometry in tetrahydrofuran (four point) | 956 Da |
| elemental analyses corrected for moisture and ash contents | | |
| carbon (C) | combustion, measurement of $CO_2$ | 53.1% |
| hydrogen (H) | combustion, measurement of $H_2O$ | 4.8% |
| oxygen (O) | reductive pyrolysis, measurement of CO | 41.0% |
| nitrogen (N) | Dumas method | 0.7% |
| sulfur (S) | combustion, measurement of $SO_4$ | 0.4% |
| average molecular formula | calculated from number-average molecular weight and elemental data | $C_{42}H_{46}O_{25}$ |
| index of hydrogen deficiency | $\phi = [(2C + 2) - H]/2$ | 20 |
| carbon distribution by type of carbon | quantitative $^{13}C$ NMR spectrometry for indicated integration ranges | 42 C atoms per molecule |
| aliphatic | 0−60 ppM | 10.8 C |
| H−C−O (alcohol, ether, ester) | 60−95 ppM | 4.7 C |
| aromatic | 95−165 ppM | 15.1 C |
| aromatic C−O | 140−165 ppM | 4.7 C |
| carboxyl, ester, quinone, flavone | 165−190 ppM | 8.3 C |
| ketone | 190−220 ppM | 3.1 C |
| oxygen distribution by type of oxygen | | 25 oxygen atoms per molecule |
| carboxyl | titrimetry | 10.8 O |
| carboxyl | FT-IR in pyridine | 10.0 O |
| ester | FT-IR in pyridine | 3.6 O |
| quinone and flavone | difference between carboxyl, ester, quinone, flavone ($^{13}C$ NMR) and carboxyl, ester (FTIR) | 1.3 O |
| ketone | $^{13}C$ NMR | 3.1 O |
| phenol + ether (aromatic) | $^{13}C$ NMR | 4.7 O |
| aliphatic alcohol, ether, ester | $^{13}C$ NMR | 4.7 O |
| ester plus ether | excess oxygen from $^{13}C$ NMR accounting | 2.6 O |
| ether | subtraction of ester C−O | 0.8 O |
| phenol | subtraction of ether | 3.9 O |
| aliphatic alcohol | subtraction of ester plus ether | 2.1 O |
| hydrogen distribution by type of hydrogen | | 46 hydrogen atoms per molecule |
| exchangeable hydrogens | sum of alcohol, carboxyl, and phenol groups | 11.2 H |
| nonexchangeable hydrogens | quantitative $^1H$ NMR spectrometry for indicated integration range | 34.8 H |
| isolated aliphatic | 0−1.9 ppM | 8.1 H |
| H−C−C=O, H−C-$\phi$ | 1.9−3.5 ppM | 13.3 H |
| H−C−O, $\phi$-CH−C=O | 3.5−6.2 ppM | 9.6 H |
| H-$\phi$ | 6.2−8.6 ppM | 3.8 H |

A comparison of the $^1H$ NMR spectra of the unfractionated fulvic acid (*21*) and the metal binding fraction is shown in Figure 4. The most significant difference is that the peak near 2.2 ppm in the unfractionated spectrum is shifted downfield to 2.7 ppm in the metal binding fraction. Protons in this spectral region are attached to aliphatic carbons adjacent to carbonyl groups or aromatic rings (*21*). The most abundant of the carbonyl groups in fulvic acid are carboxylate groups, and clustering of carboxylates into substituted phenyl and phenoxy succinic acid structures would cause the observed downfield shift. The aromatic proton distribution is similar for the two spectra, so a significant change in the aromatic ring substitution that might affect the shift of the peak from 2.2 to 2.7 ppm is unlikely; thus, this shift is related to carboxyl group clustering.

The aromatic proton distribution indicated by $^1H$ NMR spectra show that aromatic rings are primarily substituted with singly bonded oxygen functional groups that cause upfield shifts of protons from unsubstituted benzene (7.23 ppm) to near 6.8 ppm for many phenol structures. Carboxyl and aromatic ketone group substitution causes downfield shifts to near 8.0 ppm; however, a combination of phenol and carbonyl group substitution on the same ring can nullify significant shifts of aromatic protons.

The elemental and spectral data used to derive structural models for the metal binding fraction are listed in Table 2.

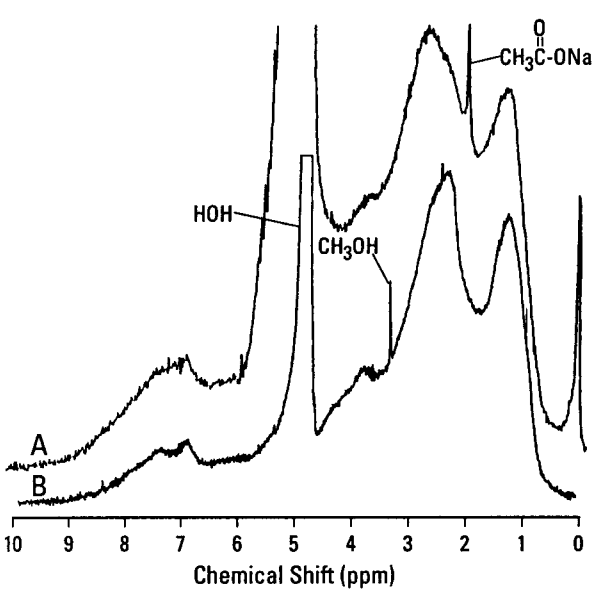


**FIGURE 4. Quantitative solution state $^1H$ NMR spectra of (A) metal binding fraction and (B) unfractionated (*21*) fulvic acid from the Suwannee River.**

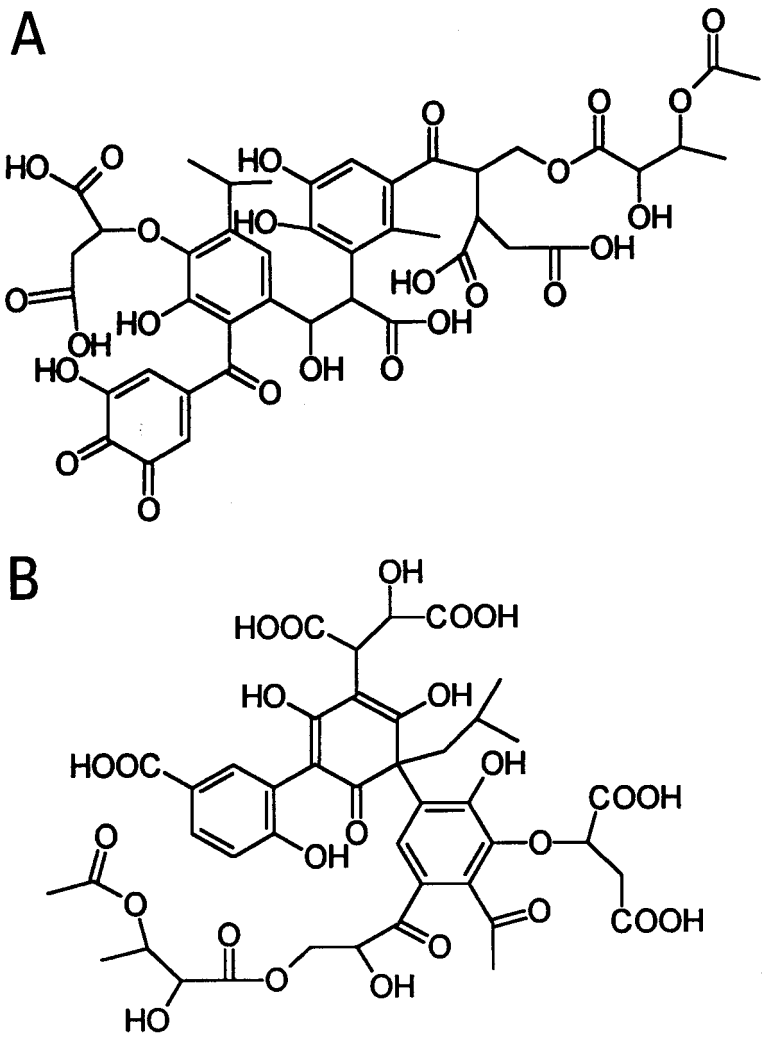


**FIGURE 5. Structural models of the hydrogen form of the metal binding fraction derived from a cutin−lignin−tannin complex (A) and derived from a condensed flavonoid tannin (B).**

Nitrogen and sulfur were not included in the average molecular formula (and in the molecular models) because there is less than one atom of these elements in each molecule of fulvic acid.

**Molecular Models of Metal Binding.** Two hydrogen-form average models of the metal binding fraction based on the data in Table 2 are presented in Figure 5. The model of Figure 5A is based upon a cutin−lignin−tannin complex for the plant precursor as suggested by Tegelaar et al. (*29*). The ester structures contain acetate esters known to be abundant in cutin coatings on plant vegetation. Evidence for acetate comes from the peak at 22 ppM in the $^{13}C$ NMR spectrum of Figure 3 and from the peak near 2.0 ppM in the $^{1}H$ NMR spectrum of Figure 4. Acetate is known to be released in significant quantities from the fulvic acid from the Suwannee River by base hydrolysis (*30*). Aromatic rings from the lignin structures are interconnected by aliphatic propane side chains, and the substituted succinic acid and oxysuccinic acid are remnants of aromatic rings that have be oxidized and opened by the $\beta$-keto adipate pathway (*22*). The ortho-quinone ring attached by the benzophenone linkage may be derived from a gallotannin that was originally attached to the adjacent phenol as a ester linkage and was photolytically rearranged through the photo-Fries rearrangement (*25, 26*). Oxidation of the phenol groups in the gallic acid ring is the basis for the ortho-quinone structure. The methyl and isopropyl groups on the aromatic rings do not have any basis in plant precursors or diagenetic processes; they are artifacts of modeling and the mixture properties of the metal binding fraction.

The model of Figure 5B was derived from initial oxidative degradation of a flavonol tannin structure to a phloroglucinol ring (*31*) that condensed with the phenolic acid fragment derived from other tannins and lignins to form a completely substituted phloroglucinol ring that partially tautomerized to the keto structure (*32*). The electron-rich phloroglucinol ring in flavonoid tannins is very reactive to condensation reactions that result in clustering of phenol, carboxyl, and ketone structures. Even though the plant precursors are significantly different for models in Figure 5A,B, the oxidative degradation and aromatic ring condensation processes result in carboxyl groups clustered together with ether, hydroxyl, and ketone groups that would make strong metal binding sites.

The Figure 5A model of a hypothetical inner-sphere calcium complex of the metal binding fraction is presented in Figure 6. Calcium is coordinated with four carboxyl groups from the two substituted succinate structures and one ether group that illustrates the importance of oxy-polycarboxylic acid structures. This model illustrates the folding conformational changes in fulvic acid that occur upon metal binding. Much of the molecular structure is involved with calcium binding; thus, binding is not confined to small substructural units. Metal binding is not by functional groups directly attached to aromatic rings such as salicylate and phthalate groups; these types of structures were not important

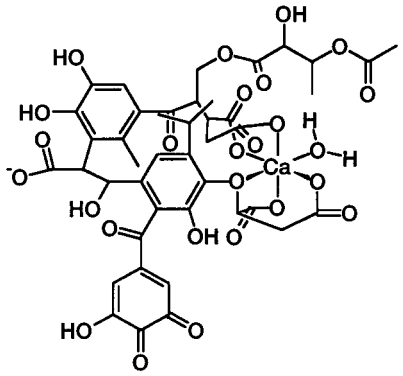


**FIGURE 6. Structural model of a calcium inner-sphere complex of the metal binding fraction.**

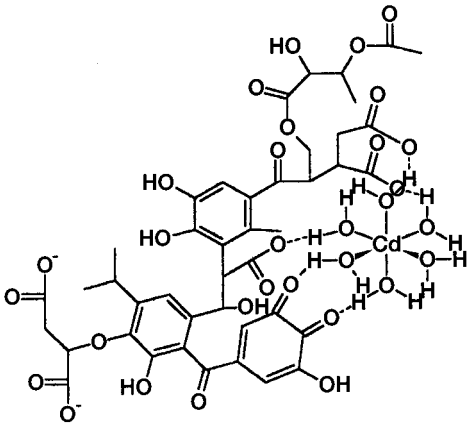


**FIGURE 7. Structural model of a cadmium outer-sphere complex of the metal binding fraction.**

at pH 6 because of proton competition with the metal for the phenolic hydroxyl group (*10*). Aromatic structures are indirectly important in that the substituted succinic acid structures involved with metal binding are probably derived from oxidation of aromatic rings, and these dibasic acids are attached in close proximity to phenol and ketone groups on aromatic structures that may contribute to metal binding. Phenols ortho to quinone groups are more acidic than normal phenols and are likely contributors to metal binding at pH 6.

The Figure 6A model of a hypothetical outer-sphere cadmium complex of the metal binding fraction is presented in Figure 7. The size of this complex is much larger because of the coordination of cadmium with water, and a different assemblage of functional groups that includes quinone carbonyl groups is associated with metal binding. Given the diversity and abundance of oxygen functional groups in the metal binding model structure, it is likely that multiple polydentate structures with many different conformations exist for metal complexes with just this one model structure.

The inner-sphere model of Figure 6 and the outer-sphere model of Figure 7 were presented to illustrate extreme cases for metal binding. Metal complexation with fulvic acid is usually a combination of both inner- and outer-sphere binding, and Figures 6 and 7 are not intended to imply that calcium only binds by the inner-sphere mechanism and cadmium binds by the other-sphere mechanism. A recent study of $Eu^{3+}$ binding with fulvic acid from the Suwannee River that used fluorescence lifetimes of the europium ion as a probe found that inner-sphere binding predominated (tetradentate complexes) at small metal-to-ligand ratios and that outer-sphere complexes predominated at high metal-to-ligand ratios (*33*).

The models of Figures 5−7 illustrate the considerable intramolecular heterogeneity for metal binding site possibilities. After considering the fact that the metal binding fraction is still a complex mixture despite efforts to maximize homogeneity by the two-stage fractionation, it is easy to understand why thermodynamic models that use continuous distributions of binding sites are conceptually popular because of intra- and intermolecular heterogeneity of binding sites.

Metal binding substructures that are considered important in the metal binding fraction are the short-chain substituted dibasic acids and, particularly, the oxy-succinic acid model for calcium binding. The NMR spectral data present compelling evidence for the abundance of substituted dibasic acid structures, and the metal binding data are consistent with the oxy-succinic acid model. However, structural models of this one fraction of one fulvic acid are not intended to be representative of all fulvic acids. The authors recognize the importance of nitrogen and sulfur as metal binding sites in other fractions and other humic substances. Other environments with different plant precursors will produce different fulvic acid with different metal binding properties. However, the results of this study hopefully present good approximations of certain fulvic acid structures that are important for metal binding under the conditions of this study.

## Acknowledgments

The authors wish to thank Robert L. Wershaw of the U.S. Geological Survey for his assistance in obtaining the $^{13}C$ and $^{1}H$ NMR spectra data presented in this report. The use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

## Literature Cited

(1) Buffle, J. *Complexation reactions in aquatic systems: An Analytical Approach*, Ellis Horwood Ltd.: Chichester, U.K., 1988; p 692.
(2) Schnitzer, M.; Skinner, S. I. M. *Soil Sci.* **1965**, *99*, 278−284.
(3) Schnitzer, M. *Soil Sci. Soc Am. Proc.* **1969**, *33*, 75−81.
(4) Gregor, J. E.; Powell, H. K. J.; Town, R. M. *Sci. Total Environ.* **1989**, *81/82*, 597−606.
(5) Westall, J. C.; Jones, J. D.; Turner, G. D.; Zachara, J. M. *Environ. Sci. Technol.* **1995**, *29*, 951−959.
(6) Leenheer, J. A. In *Environmental Chemistry of Lakes and Reservoirs*; Baker, L. A., Ed.; Advances in Chemistry Series 237; American Chemical Society: Washington, DC, 1994; pp 195−222.
(7) Leenheer, J. A.; Wershaw, R. L.; Reddy, M. M. *Environ. Sci. Technol.* **1995**, *29*, 393−398.
(8) Leenheer, J. A.; Wershaw, R. L.; Reddy, M. M. *Environ. Sci. Technol.* **1995**, *29*, 399−405.
(9) Averett, R. C.; Leenheer, J. A.; McKnight, D. M.; Thorn, K. A. *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*; U.S. Geological Survey Water-Supply Paper 2373; U.S. Government Printing Office: Washington, DC, 1994; p 224.
(10) Brown, G. K. Structural characterization of the metal complexation sites of Suwannee River fulvic acid. M.S. Thesis, Colorado School of Mines, Golden, CO, 1996.
(11) Leenheer, J. A.; Brown, P. A.; Noyes, T. I. In *Aquatic Humic Substances, Influence on Fate and Treatment of Pollutants*; Suffet, I. H., MacCarthy, P., Eds.; Advances in Chemistry Series 219; American Chemical Society: Washington, DC, 1989; pp 25−40.
(12) Schubert, J. *J. Phys. Colloid. Chem.* **1948**, *52*, 340−350.
(13) Huffman, E. W. D., Jr.; Stuber, H. A. In *Humic Substances in Soil, Sediment, and Water*; Aiken, G. R., McKnight, D. M., Wershaw, R. L., MacCarthy, P., Eds., John Wiley & Sons: New York, 1985; pp 433−456.
(14) Aiken, G. R.; Gillam, A. H. In *Humic Substances II, In Search of Structure*; Hayes, M. H. B, MacCarthy, P., Malcolm, R. L., Swift, R. S., Eds.; John Wiley & Sons: Chichester, U.K., 1989; pp 515−544.
(15) Thorn, K. A.; Folen, D. W.; MacCarthy, P. *Characterization of the International Humic Substances Society Standard and Reference Fulvic and Humic Acids by Solution State Carbon ($^{13}C$)*

*and Hydrogen-1 ($^1$H) Nuclear Magnetic Resonance Spectrometry*; U.S. Geological Survey Water-Resources Investigations Report 89-4196; U.S. Geological Survey: Denver, CO, 1989; p 93.
(16) Antweiler, R. C. In *Organic Substances and Sediments in Water Vol 1. Humics and Soils*; Baker, R. A., Ed.; Lewis Publishers: Chelsea, MI, 1991; pp 163−177.
(17) Wershaw, R. L.; Leenheer, J. A.; Kennedy, K. R.; Noyes, T. I. *Soil Sci.* **1996**, *161*, 667−679.
(18) Larson, R. A.; Weber, E. J. *Reaction Mechanisms in Environmental Organic Chemistry*; Lewis Publishers: Ann Arbor, MI, 1994; pp 64−83.
(19) Smith, K. L.; Smoot, L. D.; Fletcher, T. H.; Pugmire, R. J. *The Structure and Reaction Processes of Coal*; Plenum Press: New York, 1994; pp 69−76.
(20) Leenheer, J. A. In *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*; Averett, R. C., Leenheer, J. A., McKnight, D. M., Thorn, K. A, Eds.; U.S. Geological Survey Open-File Report 87-557; U.S. Geological Survey: Denver, CO, 1989; pp 340−344.
(21) Leenheer, J. A.; Noyes, T. I.; Wershaw, R. L. In *NMR Spectroscopy in Environmental Science and Technology*; Nanny, M. A., Minear, R. A., Leenheer, J. A., Eds.; Oxford University Press: New York, 1997; pp 295−303.
(22) Crawford, R. L. *Lignin Biodegradation and Transformation*; John Wiley and Sons: New York, 1981.
(23) Gibson, D. T., Ed. *Microbial Degradation of Organic Compounds*; Microbiology Series, Vol. 13; Marcel Dekker: New York, 1984.
(24) Harvey, G. R.; Boran, D. A.; Chesal, L. A.; Tokar, J. M. *Mar. Chem.* **1983**, *12*, 119−132.
(25) Anderson, J. C.; Reese, C. B. *Proc. Chem. Soc.* **1960**, *June*, 217.
(26) Leenheer, J. A.; Wilson, M. A.; Malcolm, R. L. *Org. Geochem.* **1987**, *11*, 273−280.
(27) Nieuwenhuizen, M. S.; Kieboom, A. P. G.; van Bekkum, H. *Tenside Deterg.* **1985**, *22*, 247−251.
(28) Motekaitis, R. J.; Martell, A. E. *J. Coord. Chem.* **1984**, *13*, 265−271.
(29) Tegelaar, E. W.; de Leeuw, J. W.; Saiz-Jimenez, C. *Sci. Total Environ.* **1989**, *81/82*, 1−17.
(30) Wilson, M. A.; Collin, P. J.; Malcolm, R. L.; Perdue, E. M.; Cresswell, P. *Org. Geochem.* **1988**, *12*, 7−12.
(31) Nordstrom, C. G. *Soumen Kemistilehti*, **1968**, *B-41*, 351−353.
(32) Robinson, T. *The Organic Constituents of Higher Plants*, 4th ed.; Cordus Press: North Amherst, MA, 1980; pp 114−117.
(33) Thomason, J. W.; Susetyo, W.; Carreira, L. A. *Appl. Spectrosc.* **1996**, *50*, 401−408.

*Received for review October 13, 1997. Revised manuscript received May 12, 1998. Accepted May 26, 1998.*

ES9708979