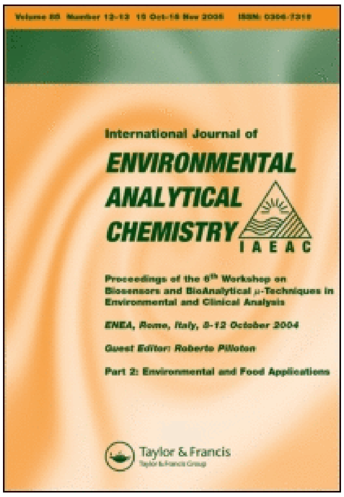

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A NOVEL APPROACH TO METAL-HUMIC COMPLEXATION STUDIES BY LANTHANIDE ION PROBE SPECTROSCOPY*

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Naturally occurring humic substances are known to be potentially strong binders of metals in the environment. A sensitive spectroscopic technique, based on the unique luminescence properties of the tripositive lanthanide metal ions, has been developed to selectively probe metal binding sites in humic substances. A continuous, multiligand pH dependent ligand model is proposed to describe complexation of metals with humic materials in terms of mean binding strengths, distributions, and concentrations.

KEY WORDS: Humic substance, lanthanide ion, complexation, hypersensitive, Gaussian distribution.

INTRODUCTION

Humic materials are present in all terrestrial and aquatic environments. These materials are complex in nature and vary in composition depending on their geographical location. It is generally assumed that humic substances contain at least two important classes of acidic binding sites, carboxylic and phenolic functional groups. The carboxylic type ligands represent an abundant source of metal binding sites in the natural environment.

The biological and physicochemical properties of metals are often changed dramatically as a result of complexation with humic materials.[1] Complexation of metal ions can often alter the toxicity of metallic elements. Metal ion complexation also can provide a source of rapid and efficient geochemical transport of metals throughout the environment. To develop a site-dependent model for metal complexation with humic materials, a detailed knowledge of the material is

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required and a method for directly probing these acidic binding sites is necessary. This probe should not only assess the total metal bound but also should be sensitive to the bound ion environment, i.e. the distribution, geometry, and the binding strengths of the different sites. Both discrete multiligand models and continuous multiligand models have been used to describe the binding of protons and metals by humic substances. An excellent critical review of many of these models has been provided by Perdue and Lytle.[2]

In this paper, we describe the development of a sensitive spectroscopic technique to selectively probe the metal binding sites in humic substances. This technique is based on the unique luminescence properties of the tripositive lanthanide metal ions, Ln(III). The use of lanthanide metals as fluorescent probes has been well documented.[3–13] The europium metal ion, Eu(III), has been the lanthanide of choice in most of these applications and its spectral features are widely documented.

We also describe an application of the Gaussian distribution multiligand model (independent of pH) to characterize the metal binding sites of humic materials. In addition, we have also developed a pH-dependent Gaussian distribution model which accounts for the competitive binding of protons and the metal ions. The incorporation of pH into this present modeling scheme represents a significant advancement in the current state of mathematical modeling of metal-humic interactions.

EXPERIMENTAL

Lanthanide Ion Probes

Lanthanide tripositive ions (Ln(III)) and their complexes are of little biological importance; however, the unique spectral chracteristics of Ln(III) ions render them useful as spectroscopic probes. The biologically more interesting d-metal complexes and alkaline earth metals lack the necessary spectroscopic properties to serve as useful probes. The fluoresence emission profile of the Eu(III) ion contains information about the binding environment of the complexing ligand.

The lowest lying excited states of the Ln(III) are comprised of various $4f^n$ configurations. The 4f orbitals are largely shielded from the surrounding environment by the outer lying d-electron orbtals and are minimally involved in bonding. The total amount of ligand-field splitting of an f-electron term is small, rarely exceeding a few hundred wavenumbers. Consequently, radiationless deexcitation processes in Ln(III) ions are relatively inefficient and the emission of radiation as luminescence is able to compete with various radiationless pathways (unlike many other transition metals).

Excited state transitions between the $4f^n$ configurations of the Ln(III) metal ions are formally forbidden by selection rules. Consequently, the absorption and emission of energy between these states is weak. A very small molar extinction coefficient, approximately $10\,M^{-1}\,cm^{-1}$, results in low transition probabilities and long luminescence lifetimes.

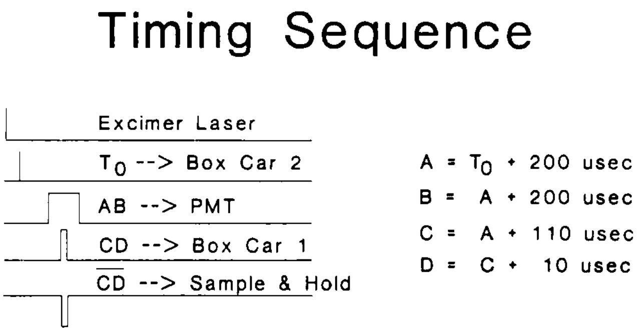

Figure 1 Timing sequences set by the delayed pulse generator.

Lifetimes of several milliseconds are experimentally observable. In contrast, the humic organic materials have molar extinction coefficients reaching the tens of thousands and fluoresence lifetimes that are typically in the nanosecond.[14] The disparity in fluorescence lifetimes means that the relatively weak lanthanide ion luminescence can be temporally resolved from the strong background fluorescence of the humic material by means of a fairly simple, time-gated detector response.

Instrumentation

A Lambda Physik EMG 102 XeCl excimer laser and a FL3002 tunable dye laser were used in tandem to supply a high intensity tunable source. The averaged output of the XeCl excimer laser was 1.6 W (10 Hz repetition rate) at 308 nm. A tunable ultraviolet emitting dye was used to provide an excitation source of 394 nm, which corresponds to a resonant absorption transition of the Eu(III) metal ion.

The Eu(III) fluorescence was collected and frequency analyzed with Jarrell Ash, one-meter, double focusing-monochromator. A Products For Research gated socket and a RCA C134A photomultiplier tube (PMT) were used to reject the prompt background fluorescence of the humic material and detect the long lived fluorescence of the lanthanide metal ion. The gated socket was designed to reverse bias the second and fourth dynode with respect to the first and third with a slight negative potential. A 5 volt TTL pulse was applied to the gate to change the reversed biased state to a normally biased state. This had the effect of turning the tube on and off.

A Stanford Research Systems model 535 digital delay pulse generator provided the necessary timing control for the experiment. The timing sequence set by the digital delay pulse generator is illustrated in Figure 1. Each pulse from the excimer laser triggered the pulse generator, which subsequently set trigger T_0 (85 nanoseconds after the laser pulse) to establish time zero for the experiment. A 200-microsecond delayed pulse, AB, altered the reverse biased state of the PMT, and the tube was turned "on" for 200 microseconds (width of AB). The 200-microsecond delay allowed for complete rejection of the short lived background fluorescence. One hundred and ten microseconds into pulse AB, a 10-microsecond pulse, CD, set the trigger for a sample-and-hold device that sampled the resulting voltage from a current to voltage convertor directly off of the PMT. The conditioned signal then was sampled by boxcar No. 1 (model 510 Stanford Research Systems) with an 80-microsecond delay to allow time for the signal to settle. The output of the boxcar was directed into the IEEE interface (model SR 245 of Stanford Research Systems) equippped with a 12-bit analog to digital (A/D) converter. A small portion of the dye laser output was directed to the photodiode detector. This reference signal monitored changes in power of the dye laser as a function of time and wavelength. The output of the photodiode was directed into boxcar No. 2. The results of each conversion (from boxcar No. 1 and boxcar No. 2) were stored in the memory of the interface. At the completion of the scan, the interface was polled and the entire set of data points was read into a file and stored on hard disk. All measurements were taken as the ratio of the fluorescence signal and the reference signal.

Reagents and Sample Preparation

The humic substances selected for this study were aquatic fulvic acid standards of the International Humic Substances Society. These standards were isolated from the Suwannee River near Fargo, Georgia, from November 1982 to February 1983, by Leenheer *et al.*[15]

Stock solutions of the fulvic acids and the Eu(III) metal ion were prepared. In the preparation step 6.5 mg of the dry fulvic material was weighed and dissolved in 50 ml of distilled water, to give 130 ppm of the fulvic acid. The pH of this solution was adjusted to 3.5. This fulvic stock solution was diluted by 1/7 to give an 18.5 ppm solution. A 100 ml solution of 2×10^{-2} M $EuCl_3$ was prepared. Eleven serial dilutions of the Eu(III) solution were made to give a range of concentrations extending over five orders of magnitude. The pH of each solution was adjusted to 3.5.

Twelve samples were prepared by mixing 1:1 (volume/volume) solutions of the diluted fulvic acids and the Eu(III) dilutions. All final solutions were adjusted to pH 3.5. Table 1 summarizes the initial and final concentrations of each sample. Complexation of the Eu(III) metal ion occurs very rapidly, and spectroscopic measurements could be taken immediately following sample preparation.

RESULTS AND DISCUSSION

The most fundamental and necessary requirement of any probe in a study of this kind is the possibility to determine quantitatively the concentration of the free and the total bound metal. The lanthanide ion probe can measure the concentration of both the complexed metal and the aquo or free metal ion simultaneously. This information is uniquely encoded in the experimental Eu(III) emission profiles. Figures 2a, 2b and 2c illustrate the Eu(III) emission profiles of three solutions containing equal amounts of fulvic acid, but different concentrations of total

Table 1 Experimental concentrations, pH conditions and observed ratio value (I_{592}/I_{616}) for each sample.

Initial Eu(III)	*Initial FA*	*Final Eu(III)*	*Final FA*	*Ratio*
$2.00\mathrm{X}^{-2}$ M	20 ppm	$1.00\mathrm{X}^{-2}$ M	10 ppm	2.65
$1.00\mathrm{X}^{-2}$ M	20 ppm	$5.00\mathrm{X}^{-3}$ M	10 ppm	2.63
$4.00\mathrm{X}^{-3}$ M	20 ppm	$2.00\mathrm{X}^{-3}$ M	10 ppm	2.47
$2.00\mathrm{X}^{-3}$ M	20 ppm	$1.00\mathrm{X}^{-3}$ M	10 ppm	2.31
$1.00\mathrm{X}^{-3}$ M	20 ppm	$5.00\mathrm{X}^{-4}$ M	10 ppm	2.02
$4.00\mathrm{X}^{-4}$ M	20 ppm	$2.00\mathrm{X}^{-4}$ M	10 ppm	1.70
$2.00\mathrm{X}^{-4}$ M	20 ppm	$1.00\mathrm{X}^{-4}$ M	10 ppm	1.50
$1.00\mathrm{X}^{-4}$ M	20 ppm	$5.00\mathrm{X}^{-5}$ M	10 ppm	1.21
$4.00\mathrm{X}^{-5}$ M	20 ppm	$2.00\mathrm{X}^{-5}$ M	10 ppm	0.96
$2.00\mathrm{X}^{-5}$ M	20 ppm	$1.00\mathrm{X}^{-5}$ M	10 ppm	0.77
$1.00\mathrm{X}^{-5}$ M	20 ppm	$5.00\mathrm{X}^{-6}$ M	10 ppm	0.58
$5.00\mathrm{X}^{-6}$ M	20 ppm	$2.00\mathrm{X}^{-6}$ M	10 ppm	0.55

Eu(III): 10^{-2} M, 10^{-3} M, 10^{-4} M, respectively. We have developed a method of data analysis to interpret these spectra. This technique is based on the ratio of the integrated intensity of two emission peaks, 592 nm and 616 nm, of each spectrum. The changes in the relative ratios of the two emission bands in these spectra are due to the hypersensitive nature of one of these transitions.

Hypersensitive Transitions

The basis of the experimental determination of the binding of lanthanide ions with humics lies in the existence of the hypersensitive emissive transition of the Eu(III) ion, $^5D_0 \rightarrow {}^7F_2$ (616 nm). Hypersensitive transitions are specific absorption or emission transitions of the lanthanide metal ions that are extremely sensitive to ligation. The intensity of a hypersensitive transition is enhanced in the metal-ligand complex relative to that transition in the aquo ion. Experimentally, this is observed by making the concentration of the Eu(III) small relative to the amount of ligand present such that the majority of the metal ion is complexed with the ligand.

The hypersenitive effect has been treated theoretically by several researchers[16] and is generally thought to be the result of a quadrupolar interaction of the ligand with the metal. For known hypersensitive transitions, most ligands are expected to produce the hypersensitive effect with respect to the aquo ions; however, there may be some exceptions. The degree of signal enhancement in the hypersensitive transitions depends on the specific system. Intensity enhancements as large as 100 fold have been reported for the $^5D_0 \rightarrow {}^7F_2$ hypersensitive transition of the Eu(III) ion.[17]

The effect of the hypersensitive transition is most obvious from the observation of several experimental emission spectra. This effect is illustrated in Figures 2a–2c. These three spectra represent the emission spectrum of the Eu(III) ion in the

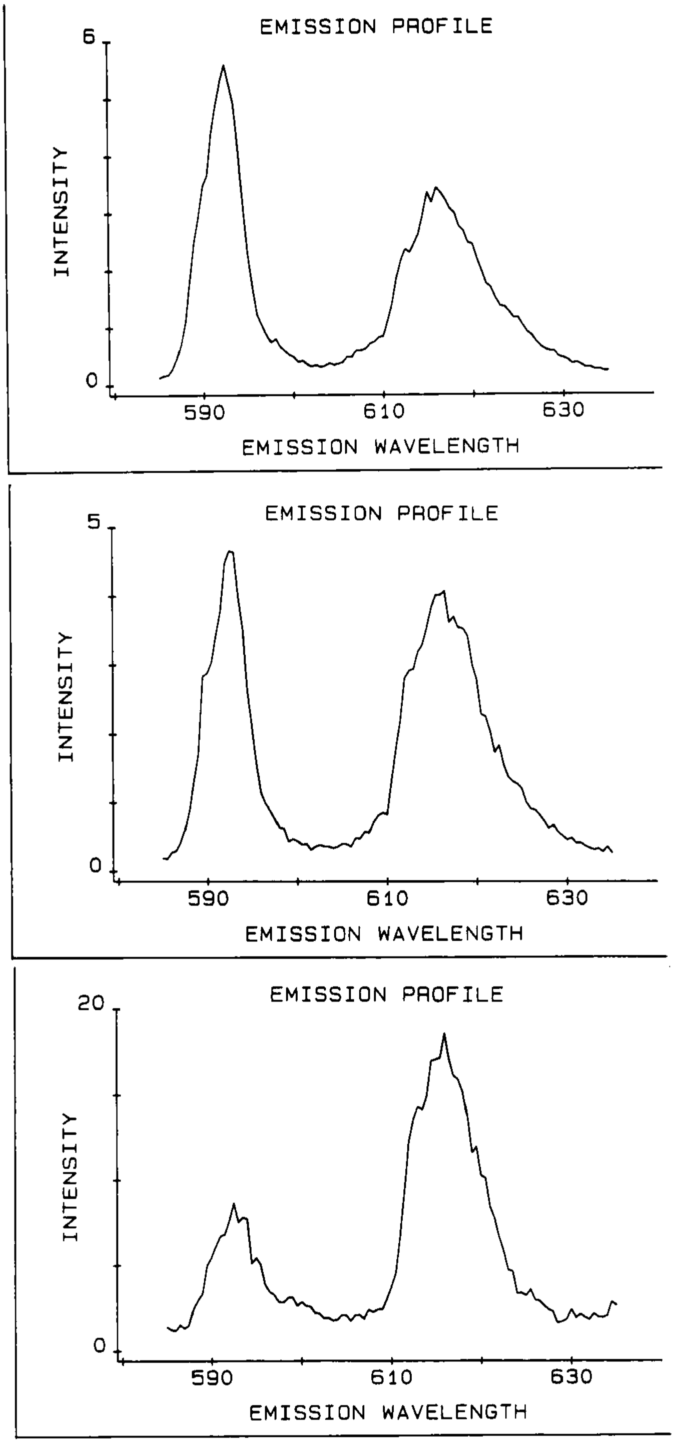

Figure 2 Emission spectra of Eu(III) bound with fulvic acid material: (a) 1×10^{-2} M Eu(III), 100 ppm fulvic acid; (b) 1×10^{-3} M Eu(III), 100 ppm fulvic acid; (c) 1×10^{-5} M Eu(III), 100 ppm fulvic acid.

presence of ligating fulvic acid. The concentration of Eu(III) was different in each sample while the concentration of fulvic acid was held constant. For each spectrum, the monochromator was scanned over the same spectral wavelengths, and included both the non-hypersensitive band at 592 nm and the hypersensitive band at 616 nm. Both of these transitions are always present in each spectrum; however, their relative intensities are different. In Figure 2a, the concentration of Eu(III) was 5×10^{-2} M, which was large relative to the total concentration of fulvic acid, 100 ppm. Because of the large excess of free metal ion (aquo ion) in solution, the intensities of the two transitions in this spectrum are characteristic of the Eu(III) free metal ion. The intensity of the band at 592 nm is much larger than the intensity of the band at 616 nm. Enhancement of the hypersensitive transition is not readily observed for the case of the aquo ion. When the concentration of the Eu(III) ion is small relative to the concentration of the fulvic acid, the majority of the probe metal ion is bound and intensity of the hypersensitive band (616 nm) is dramatically increased relative to the non-hypersensitive band. This effect is demonstrated in Figure 2c, where the concentration of Eu(III) is 5×10^{-5} M and the concentration of fulvic acid is 100 ppm. For intermediate cases, where both metal species (aquo and complexed) contribute significantly to the total observed fluorescence, it is expected that the relative intensities of the hypersensitive and non-hypersensitive band should also be intermediate. This is illustrated in the spectrum shown in Figure 2b, where the concentration of the Eu(III) was 5×10^{-4} M, respectively.

Qualitatively, one can easily see that the ratio of the intensities of these bands (592 nm and 616 nm) in these spectra is related to the overall extent of complexation of the Eu(III) ion. However, a detailed quantitative description of the metal complexation requires further mathematical analysis of these spectra.

Titration Curves

The result of the ratio of the integrated intensity of the non-hypersensitive transition at 592 nm to the hypersensitive transition at 616 nm as a function of log C_M, the total concentration of the metal ions, yields a sigmoidal shaped curve. This sigmoidal curve can be treated as a spectral titration curve. A representation of this curve is depicted in Figure 3. This curve asymptotically approaches two extreme limiting values. These conditions represent the maximum and minimum ratio values, X_b and X_s. In order for these conditions to be experimentally observed, certain criteria involving the relative concentration of the total metal, C_M, and the concentration of the total carboxylic ligand, C_L, are required. When the value of C_M/C_L, where C_M is the total metal concentration and C_L is the total ligand concentration, is large (greater than 100), the majority of the metal ions in solution are free. This state represents limiting condition X_b. At high C_L, when the value of C_M/C_L is small, the majority of the metal ions are bound with the ligands. This particular case is representative of the limiting condition X_s. Both of these limiting conditions can be obtained experimentally. The value for X_b was taken to be the ratio of the two peaks in the absence of any humic material. The value for

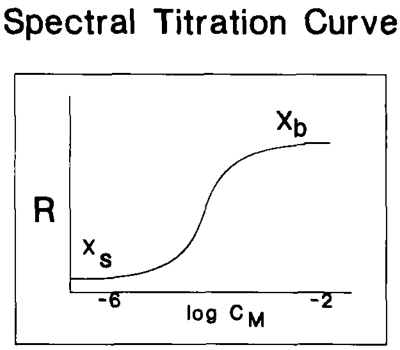

Figure 3 Representation of spectral titration curve. The ratio values asymptotically approach X_s when the majority of metal added is bound to the humic ligand.

X_s was taken to be the ratio of the two peaks when the Eu(III) was small (1×10^{-5} M) and the humic concentration was large (200 ppm). In this study, the values of X_b and X_s were measured to 2.71 and 0.35, respectively. The following mathematical derivation of the intensity ratio, R, will show how the concentration of the bound and free metal species are quantitatively determined from the experimentally observed parameters. In this mathematical derivation, the concentrations of the two metal species, bound and free, as represented by $\Sigma[ML_i]$ and $[M]$.

Let the relative ratio of the integrated peak intensity of these two emissive peaks, R, be defined as

$$R = \frac{I_{592}}{I_{616}}. \qquad (1)$$

The integrated intensities of the emissive transitions at 592 nm and 616 nm are proportional to the concentrations of both the bound and free metal species, and their respective contribution factors.

$$I_{592} \quad [M]*X+\Sigma[ML_i]*X \qquad (2)$$

$$I_{616} \quad [M]*Y+\Sigma[ML_i]*Z. \qquad (3)$$

Let X be the fluorescence contribution factor for the non-hypersensitive band occurring at 592 nm. This factor is the same for both of the metal terms because the intensity of this band is not influenced by the metal-ligand complexation. However, the emissive transition occurring at 616 nm is a hypersensitive band. The corresponding intensity factors, Y and Z, are not equal because the complexed metal term, $\Sigma[ML_i]$, is influenced by the hypersensitive effect. It is assumed in this study that the hypersensitive effect is constant for all ligands; therefore, the intensity factor Z is the same for each ML_i complex. This should be a valid assumption because the majority of the active binding sites are of the same class (i.e. carboxylic).

From Eqs. (1)–(3), the intensity ratio, R, can be expressed as:

$$R=\frac{[M]*X+\Sigma[ML_i]*X}{[M]*Y+\Sigma[ML_i]*Z} \tag{4}$$

where $Z=Y*T$ and T is another constant greater than one. For the limiting condition when C_M/C_L is large, i.e. greater than 100, $\Sigma[ML_i]\ll[M]$ and $\Sigma[ML_i]$ can be neglected in Eq. (4). At this limiting value, R approaches X_b, and Eq. (4) can be simplified to

$$X=X_b*Y \tag{5}$$

Substitution of this expression for X into Eq. (4) and simplification by use of the mass balance equation for the total metal, C_M, yields the following expression for R

$$R=\frac{C_M*X_b}{[M]+\Sigma[ML_i]*T} \tag{6}$$

In order to obtain a final expression for the free metal term, $[M]$, it is necessary to further simplify Eq. (6). The expression for the total bound metal, $\Sigma[ML_i]$, is related to $[M]$ through the mass balance equation of the total metal C_M and is given as

$$\Sigma[ML_i]=C_M-[M] \tag{7}$$

This expression for the bound metal can be used to simplify Eq. (6). Following simplification and rearrangement, Eq. (6) can be expressed in terms of the free metal, $[M]$, and is given as

$$[M]=\frac{C_M*(X_b-R*T)}{R*(1-T)}. \tag{8}$$

The concentration of the total bound metal is known from the mass balance equation of the total metal, C_M (Eq. 7). Equation (8) provides an expression for the concentration of the free metal species at some given C_M value. However, it contains the variable T which has not yet been defined. The value of T is actually a known constant. At the extreme limiting condition, where the majority of the metal ions are complexed with the humic material, the ratio value, R, asymptotically approaches X_s. At this limiting condition, the concentration of the free metal, $[M]$, is negligible to the concentration of the bound metal, $\Sigma[ML_i]$, and therefore can be ignored. It can also be assumed that $\Sigma[ML_i]$ approaches C_M and Eq. (6) can be simplified such that T can be expressed in terms of two experimental parameters, X_b and X_s.

$$T=\frac{X_b}{X_s}. \tag{9}$$

The significance of this mathematical derivation is summarized in Eqs. (7) and (8). From these expressions, the analytical concentrations of both the bound and free probe metal species can be readily determined from the experimentally observed parameters R, C_M, X_b and X_s.

METAL-MULTILIGAND COMPLEXATION MODEL

Mathematical modeling of proton-humic and metal-humic interactions have been approached with many different points of view. Both discrete multiligand models and continuous multiligand models have been applied to these type interactions. To describe the binding characteristics of europium with humic substances, we have used a model based on a Gaussian distribution. The Gaussian distribution model has been proposed by several authors to describe the binding of protons by humic substances. Initially, the Gaussian distribution model was proposed by Posner[18,19] and later developed by Perdue.[2,20] The Gaussian distribution model arises from the assumption that humic substances contain a large number of non-identical ligand sites that span a wide range of binding energies. Application of the Gaussian distribution model to the binding site energies of humic substances implies that the most probable distribution of acidic functional groups of these substances is normal with respect to the mean binding energy of humic ligands. This is of course an *a priori* assumption. However, because of the complex nature of humic substances and the combined contributions of statistical, electrostatic, and delocalization effects, this model should serve as a good first approximation of the actual distribution. Although the Gaussian distribution model was initially developed to describe the binding of protons by humic substances, it can be easily adapted to describe the binding of metals by humic substances.

The actual distribution of the humic ligands is given by the Gaussian error function

$$\frac{C_i}{C_L}=\frac{1}{\sigma\sqrt{\pi}}\exp\left[-\frac{1}{2}\left[\frac{\mu-\mathrm{Log}\,K_i}{\sigma}\right]^2\right]d\log K \tag{10}$$

where C_i/C_L is the mole fraction of ligands in the interval $d\log K$ whose metal binding constant is expressed as $\log K_i$, and σ is the standard deviation for the distribution of $\log K_i$ values about the mean $\log K_i$ value (μ) for the mixture of the ligands. This ligand distribution is illustrated in Figure 4. The probability of occurrence of any site is dependent on the Gibbs free energy of binding of that site, where the most probable site has a $\log K_i$ value equivalent to μ. By definition, stronger binding sites have $\log K_i$ values greater than μ and weaker binding sites have $\log K_i$ values less than μ. The height at any position along the Gaussian shaped curve is equal to C_i, the concentration of the ith ligand site. Hence, the total area under the curve is defined as the ΣC_i which is equivalent to C_L, the total carboxylic ligand concentration.

A typical approach which is often taken when a new model is developed to

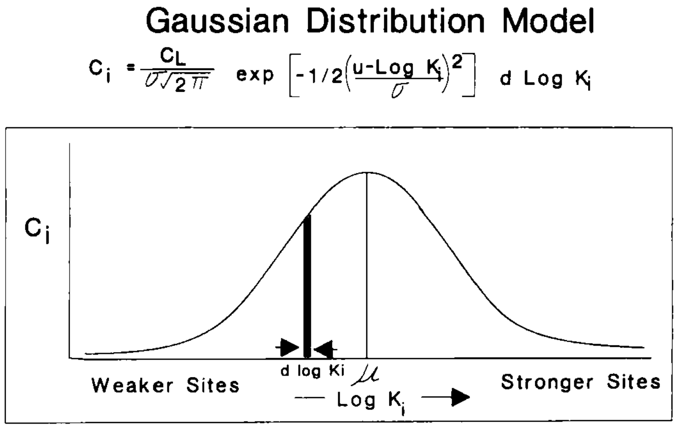

Figure 4 Graphic representation of the Gaussian distribution model.

describe an observed event is to make some initial assumptions which will simplify and limit the degrees of freedom inherent to the model. Such an approach was taken in this initial application of the Gaussian distribution model. This initial prototype model did not include the effect of pH. Consequently, the ionization of the carboxylic functional groups was neglected which resulted in the disregard of the inherent competition between hydrogen and the probe metal ions for the ligand binding sites. This initial application to the Gaussian distribution model has been referred to as a pH-independent model.

The derivation of the following continuous multiligand pH-independent equilibrium model includes only the single binding species $[M]$. This single binding species should be recognized as the experimental fluorescent probe metal ion. For the single type of binding site, i, at constant pH, the complexation reaction for a 1:1 stoichiometric concentration of metal (M) and ligand (L) is

$$M + L_i \leftrightarrow ML_i. \tag{11}$$

The binding constant K_i for the reaction is

$$K_i = \frac{[ML_i]}{[M] * [L_i]}. \tag{12}$$

where $[ML_i]$ is the concentration of the complexed metal at site i, $[M]$ is the concentration of free metal, and $[L_i]$ is the concentration of the free ligand at site i.

The expression for the free ligand concentration, $[L_i]$, in Eq. (14) was difficult to measure quantitatively. For this reason, it was expressed in some other terms which could be evaluated more easily. This was accomplished given the mass balance form of the equation for $[L_i]$:

$$[L_i] = C_i - [ML_i] \tag{13}$$

where C_i is equal to total concentration at site *i*. This expression for $[L_i]$ was substituted into Eq. (13) and solved for $[ML_i]$. Upon rearrangement and simplification, $[ML_i]$ was expressed as

$$[ML_i]=\frac{K_i*[M]*C_i}{1+K_i*[M]}. \tag{14}$$

This expression represents a generalized chemical equilibrium equation for a single bindng species (M) with a ligand (L_i). This general expression can be made more explicit as needed. For the Gaussian distribution model, the distribution function of C_i, which has been explicitly defined and given in Eq. (10), is incorporated into Eq. (14). Substitution of this distribution function into Eq. (14) provides an expression for concentration of the complexed metal at the single ligand site *i*, in terms of the modeling parameters μ and σ, where μ is the $\log K_i$ value of the most probable ligand site in the C_i distribution, and σ is one standard deviation of the mean value.

In order to apply the resulting single species model (Eq. 14) to the titration data, it is necessary to realize that the resulting expression in Eq. (14) provides quantitative solutions for only the bound metal species at a single ligand site *i*. As previously stated, the objective was to derive an expression for the total bound metal (over all *i* sites) in terms of the Gaussian distribution model. Therefore, it is necessary to sum Eq. (14) over all *i* sites to obtain an expression for the total bound metal, $\Sigma[ML_i]$. The resulting solutions to this expression can be directly compared to the experimentally observed values for the total bound metal which were obtained from the Ratio Plot analysis of the Eu(III) emission spectra. Following the substitution of the Gaussian distribution function for C_i (Eq. 10), into Eq. (14), the summation of Eq. (14) over all *i* sites Eq. (14), the summation of Eq. (14) over all *i* sites can be expressed as the following integral.

$$\Sigma[ML_i]=\frac{C_L}{\sigma\sqrt{2\pi}}\int_{-\infty}^{+\infty}\frac{K_i[M]}{1+K_i[M]}\exp\left[-\frac{1}{2}\left[\frac{\mu-\mathrm{Log}\,K_i}{\sigma}\right]^2\right]d\,\mathrm{Log}\,K \tag{15}$$

The solutions to this integral provided the calculated values for the total bound metal, in terms of the modeling parameters μ and σ. It was not feasible to solve the integral in Eq. 15 analytically, therefore, it was evaluated numerically. This integral contains a number of variables K_i, $[M]$, C_L, μ, and σ. In order to reduce the number of actual curve fitting parameters to only two, μ and σ, the values of $[M]$ and C_L were measured from the experimental data and taken as known values. The individual values for the free metal term, $[M]$, were taken directly from the results of the titration data (see Eq. 8) and the value of C_L as taken to be the height of the $\Sigma(ML_i]$ versus $\log[M]$ plot at the saturation point. Initial guesses were provided for the modeling parameters μ and σ. The integration was performed over one hundred individual $\log K_i$ values with integral limits set at $\mu\pm 4\sigma$, which accounted for more than 99.99% of the sites. The calculated $\log K_i$ values were dependent on the initial guess of μ. These calculated values for

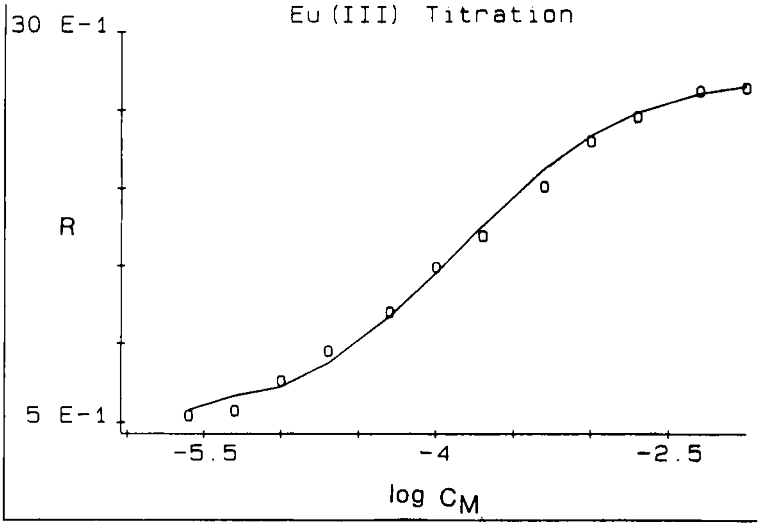

Figure 5 Experimentally observed titration curve (*) and calculated titration curve (—) at pH of 3.5. The modeling parameters μ, σ, and C_L were found to be 3.5, 1.1, and 3.5×10^{-56} M, respectively.

$\Sigma[ML_i]$ were fitted in terms of modeling parameters to the observed titration data obtained from the results of the Ratio Plot analysis. A nonlinear regression technique was utilized to minimize the residual sum of the squares (RSS) and determine the best fit for the modeling parameters μ and σ.

SUWANNEE RIVER FULVICS

Least Squares Fit

Twelve europium-humic samples were prepared as previously described. The pH of each sample was adjusted to 3.5. The emission spectrum of each sample was taken. In each spectrum, the ratio of the integrated peak intensity of the 592 nm band and the 616 nm band was determined. The resulting twelve measured ratio values were plotted as a function of the concentration of the total metal in each sample to yield the corresponding titration curve, where each ratio value, $R = I_{592}/I_{616}$, is mathematically related to the concentrations of the bound and free metal species.

Figure 5 shows the experimentally observed titration curve, R versus $\log C_M$, and the calculated fit according to the single metal pH-independent-Gaussian distribution model. The modeling parameters μ and σ were fitted to the experimental data by means of a non-linear regression technique. The best fit for μ and σ was found to be 3.5 and 1.1, respectively. The value of C_L was determined to 3.6×10^{-5} M. According to the Gaussian distribution model, this value of μ is representative of the average binding constant of europium, with a complex mixture of non-identical carboxylic binding sites. It is extremely important note that μ and σ are conditional constants that are constant only at constant pH.

pH DEPENDENT MODEL

In the previouslyb described model, we have shown how the metal binding sites of humic material can be characterized with respect to their binding strength, distribution, and concentration, according to Gaussian statistics. In order to simplify the development of the model, we initially chose to ignore the effects of pH in the previous discussion. The results of the pH-independent model provided conditional binding constants which are functions of pH. A pH-dependent model should account for the competitive binding of protons and metal ions for the humic ligands. The result of this model should remove the pH dependence from the calculated constants μ and σ.

In order to incorporate the effects of humic acid ionization into the present model, it was assumed that the distribution (in C_i) of the sites available for binding by the two species, protons and metal ions, are only different with respect to their μ values, μ_M and μ_H. The width of the distribution (σ) is equivalent for both protons and metal ions. This implies that the ratio of K_{Mi}/K_{Hi} remains constant, where K_{Mi} is the binding constant for europium and K_{Hi} is the humic acid protonation constant. The value for this ratio was taken to be $10^{\mu_M}/10^{\mu_H}$. The value of μ_H was taken to be 4.02.[21] The objective of the following derivation is to develop an expression for $[ML_i]$ in terms of known variables, including $[H^+]$ and K_{Hi}. A similar reaction to that previously shown for the single metal-humic interaction, Eq. (12), can be written for the protonation of the ith binding site:

$$H^+ + L_i \leftrightarrow HL_i \tag{16}$$

The formation constant K_{Hi} is

$$K_{Hi} = \frac{[HL_i]}{[H^+][L_i]} \tag{17}$$

where $[HL_i]$ is the concentration of the protonated ith binding site and $[H^+]$ is the concentration of hydrogen ion.

The ratio of K_is (Eqs (12) and (17)) can be expressed as

$$\frac{K_{Mi}}{K_{Hi}} = \frac{[ML_i][H^+]}{[M][HL_i]} \tag{18}$$

As previously stated, the objective in this derivation is to solve Eq. (18) in terms of $[ML_i]$. However, in doing so, it is necessary to realize that some of the terms in Eq. (18) are difficult to determine quantitatively; therefore, they should be expressed in other terms. The mass balance equation of the ith binding site, $[HL_i]$, can be expressed as

$$[HL_i] = C_i - [ML_i] - [L_i]. \tag{19}$$

The term $[L_i]$ can be expressed in other terms according to Eq. (12).

$$[L_i] = \frac{[ML_i]}{K_{Mi} * [M]} \tag{20}$$

where subscript M is added for clarity. Following the substitution and rearrangement of these terms, $[ML_i]$ (in Eq. (18)) can be expressed as:

$$[ML_i] = \frac{K_{Mi} * [M] * C_i}{1 + K_{Mi}[M] + K_{Hi}[H^+]} \tag{21}$$

The only difference between the final expression of $[ML_i]$ shown above and the expression for $[ML_i]$ for a single metal system, Eq. (14) is the additional term, $(K_{Hi} * [H^+])$, found in the denominator. Again, this generalized expression can be made more specific as needed. To apply it to the Gaussian distribution model, C_i is replaced by the distribution function given in Eq. (10). Following the replacement with this distribution function, it is necessary to sum equation (21) over all i sites. The summation of this expression is expressed as the following integral.

$$\Sigma[ML_i] = \frac{C_L}{\sigma\sqrt{2\pi}} \int_{-\infty}^{+\infty} \frac{K_{Mi}[M]}{1 + K_{Mi}[M] + K_{Hi}[H^+]} \left[-\frac{1}{2} \left[\frac{\mu_M - \mathrm{Log}\, K_{Mi}}{\sigma} \right]^2 \right] d\, Log\, K_M \tag{22}$$

The solutions to this integral provide calculated concentrations of the bound and free metal ions. This integral is numerically solved exactly as it was done in the previous pH-independent model. The K_{Hi} values are constrained to the K_{Mi} values by means of the initial assumption that K_{Mi}/K_{Hi} remains constant over all i values. The value of $[H^+]$ is estimated by the pH. The final solutions are in terms of the modeling parameters μ_M and σ. These two modeling parameters are fitted by means of the least squares analysis technique.

Prior to performing the metal titrations at various pHs, we were able to predict the effect of pH on the experimental titration curves, R versus $\log C_M$, by means of a computer simulation study. In this study, a series of R values (I_{592}/I_{616}) were computed for a given C_M, X_b, X_s, μ_M, μ_H, σ, C_L and pH. All parameters were held constant with the exception of pH and the values of R were plotted as a function of C_M. The resulting titration curves are shown in Figure 6. As pH is decreased, more protons are available to compete with the metal ions for the binding sites of the humic material, consequently, for a given C_M value, less Eu(III) can bind with the humic material. Hence, more of the total Eu(III) is present as the aquo ion and the contributions of the hypersensitive transition at 616 nm are much less effective. The relative intensity of these two emissive bands become more representative of the pure aquo ion which results in very small changes in R over the range of titrant concentrations.

The metal-humic titration were performed at three different pH values, 2.5, 3.0 and 3.5. The experimental titration curves and the corresponding calcuulated curves as predicted from the pH-dependent model are shown in Figure 7. The pH-dependent model predicts the mean binding constant (μ_M) for Eu(III) to be 4.4 and

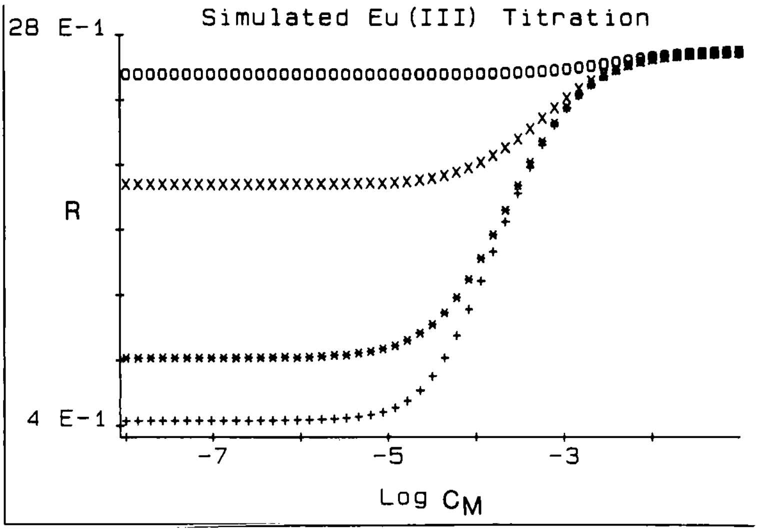

Figure 6 Simulation titration curves as a function of pH: pH = 2.0 (O), pH = 3.0 (×), pH = 4.0 (*) and pH = 5.0 (+).

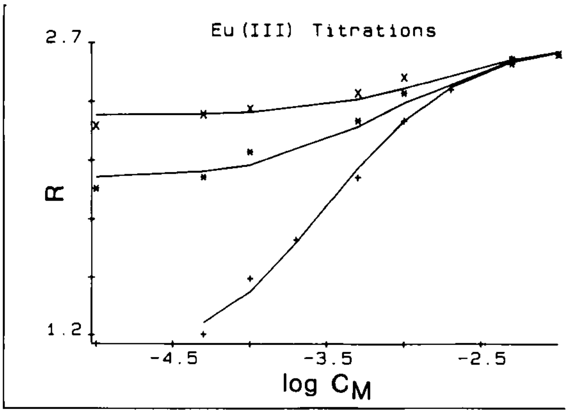

Figure 7 Calculated and observed titration curves at pH 2.5 (×), 3.0 (*) and 3.5 (+).

breadth of the distribution (σ) to be 2.17. These values are significantly larger than the previously predicted values of 3.5 (μ) and 1.1 (σ). This discrepancy can be explained as follows.

It is expected that protons and metal ions will preferentially bind to the stronger binding sites of the humic ligand. Before saturation of the sites is reached, at equilibrium, some of the stronger sites are occupied by the protons as well as the metal ions. However, those sites that are occupied by the protons are not accounted for in the pH-independent model. This has the effect of decreasing the population of the stronger sites which results in an effectively narrower and weaker distribution. Hence the lower μ_M and σ values are observed if the pH-independent model is used to interpret the experimental data.

CONCLUSIONS

The lanthanide ion probe can be used as a sensitive analytical tool to selectively probe the metal binding sites of humic substances. We have shown that metal-humic complexation studies can be made even at natural environmental concentrations of humic acids. Such experimental concentrations should best represent the true metal-humic interactions occurring in the environment.

The results of the continuous-multiligand model characterize the metal binding sites of complex humic substances as to their mean binding strength, distribution, and concentration. The pH-dependent model represents a more realistic model of metal-humic complexations because it accounts for the competitive interactions between protons and Eu(III) metal ions. The significance of this model is demonstrated by its ability to represent the binding strengths of many ligand sites in humic substances by two constants, i.e. μ and σ, where these constants are not dependent on the pH of the system.

Note—Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

References

1. J.H. Weber, *Aquatic and Terrestrial Humic Materials* (R. S. Christian and E. T. Gjessing, eds.) (Ann Arbor Science, Michigan, 1983), Ch. 15.
2. E. M. Perdue and C. R. Lytle, *Environ. Sci. Technol.* **17,** 654 (1983).
3. C. K. Luk, *Biochem.* **10,** 2838 (1971).
4. M. S. Kayne and M. Cohn, *Biochem.* **13,** 4159 (1974).
5. M. P. Miller, D. R. Tallant, F. J. Gustafson and J. C. Wright, *Anal. Chem.* **49,** 1479 (1977).
6. J. C. Wright, *Anal. Chem.* **49,** 1690 (1977).
7. W. DeW. Horrocks and D. R. Sudnick, *J. Am. Chem. Soc.* **101,** 334 (1979).
8. W. DeW. Horrocks and D. R. Sudnick, *Science* **206,** 1194 (1979).
9. W. DeW. Horrocks and D. R. Sudnick, *Acc. Chem. Res.* **14,** 384 (1981).
10. P. B. O'Hara and R. Bersohn, *Biochem.* **21,** 5269 (1982).
11. L. C. Porter, J. R. Aske, M. V. Johnston and J. C. Wright, *Appl. Spectrosc.* **37,** 360 (1983).
12. M. Albin, G. K. Farber and W. DeW. Horrocks, *Inorg. Chem.* **23,** 1648 (1984).
13. G. J. M. van Scharrenburg, A. J. Slotboom, G. H. de Haas, P. Mulqueen, P. J. Breen and W. DeW. Horrocks, *Biochem.* **24,** 334 (1985).
14. W. DeW. Horrocks and M. Ailbin, *Progress in Inorganic Chemistry*, Vol. 31, New York, 1984.
15. J. A. Leenheer, M. A. Wilson and R. L. Malcolm, *Org. Geochem.* **11,** No. 4, 273 (1987).
16. S. F. Mason, R. D. Peacock and B. Stewart, *Chem. Phys. Lett.* **29,** 149 (1974).
17. W. T. Carnall, *et al.*, *Systematics and the Properties of the Lanthanides* (S. P. Sinha, ed.) (D. Reidel Publishing Co., Boston, 1983).
18. A. M. Posner, *8th Intern. Congress Soil Sci.* Buccharest, Romania 161 (1964).
19. A. M. Posner, *J. Soil Sci.* **17,** 65 (1966).
20. E. M. Perdue, J. H. Reuter and R. S. Parrish, *Geochim. Cosmochim. Acta* **48,** 1257 (1984).
21. E. M. Perdue, Private Communications, June, 1988.