MODELING THE INTERACTIONS OF TRACE METALS AND AQUATIC HUMIC MATERIALS

by

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MODELING THE INTERACTIONS OF TRACE METALS AND AQUATIC HUMIC SUBSTANCES

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ABSTRACT

The modeling of interactions between humic materials and trace metals has been hindered by inappropriate applications of experimental and mathematical procedures. Furthermore, little is known of the competitive interactions among trace metals binding to humic molecules. This work clarifies the correct interpretation of metal-humic binding data and applies the improved methodology to a study of competition among cations binding to an aquatic fulvic acid.

The first section considers the magnitudes of errors in three commonly used metal titration methods and demonstrates that the propagation of error into metal-binding data sets is non-uniform through the course of a titration. The error propagation pattern is characteristic of each titration method. Optimally precise metal-binding data can be obtained over a wide range of species concentrations by appropriate combinations of experimental methods.

The second section is an analysis of the mathematical models used to interpret metal-humic interactions. Discrete ligand representations are contrasted with continuous distributions of ligands. The discrete ligand model is advanced as the most useful representation of binding data.

In the final section, a combination of ion-selective electrode and fluorescence quenching titration data are applied in a study of the competition between Cu(II) and Cd(II) ions for binding by an aquatic fulvic acid at various pH values. A new empirical model is developed as an alternative representation of the experimental data.

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CHAPTER ONE: INTRODUCTION

The term "humic materials" defines a group of randomly polymerized organic molecules that result from the degradation of lignin, cellulose and other biomolecules. In the past twenty years, scientists have recognized the importance of the interactions between trace elements and humic materials. A heightened interest in the behavior of toxic and nutritional trace metals in natural waters has led to investigations of the roles that humic materials can play in the geochemical cycles of metals.

This thesis focuses on the ways in which the interactions of trace metals with humic materials can be mathematically represented for predictive purposes. Developing a predictive model involves acquiring experimental data, formulating a mathematical theory to represent the data, and then testing the model under novel conditions. The goal of this thesis was not to create a particular model of metal-humic interactions, although a new model is developed in the fourth chapter. Instead, I have chosen to examine the general problem of developing models of chemical systems like humic materials that are thermodynamically pootly defined. The following three chapters entail a broad exposition of each of the three stages mentioned above.

Chapter Two addresses the issues of data acquisition and they way in which the precision and accuracy of datais affected by the analytical methods chosen. The most useful methods of analysis are organized into classes based on the metal species that are measured. The characteristic errors found in each group are determined and the effects of error propagation in data transformations are demonstrated. Recommendations are made as to the best ways of obtaining precise titration data.

Chapter Three focuses on the problem of mathematically representing metal-humic binding behavior. The theory underlying discrete and continuous models of metal binding is examined in detail and then applied in a critical evaluation of three models that have been advocated in the literature. The models were applied to experimental and synthetic titration data to test the effects of the precision and range of data on model results. The theoretical and practical shortcomings of these models are discussed as well as the useful features of each.

Chapter Four presents an experimental study of an aquatic fulvic acid. (Fulvic acid is the operationally defined, acid soluble fraction of humic matter and is usually the principal component of aquatic humics.) A series of Cu(II) titrations of fulvic acid are performed over a range of pH values and with several concentrations of Cd(II) present. The analytical procedures recommended in Chapter Two are employed, namely, a combination of ion-selective electrodes and fluorescence quenching techniques. The results demonstrate the competitive interactions among Cu^{2+}, Cd^{2+}, and H^{+} ions for binding on the fulvic acid. A discrete ligand model is shown to be a potentially useful means of representing these interactions. Application of the discrete ligand model required adjustments of ligand parameters to properly reflect competitive ion behavior and the limitations of the model are discussed. A new, empirical model fo metal-humic interactions is developed that is based on a power law relationship between the ratio of bound to free metal species. This empirical model is shown to be useful for representing many simple systems of humic materials.

Five appendices have been included. The first is a study of the release of Cu(II) binding agents released by the zooplankter, Daphnia

magna. The methodologies used in this study are nearly identical to those used for humic materials and the results suggest that zooplankton contribute metal binding agents to natural waters that are comparable in strength to fulvic acids.

The second appendix is a study of the effects of natural (fulvic) and synthetic chelating agents on the transport of Cu(II) through a porous, silica medium. The effects of synthetic materials on transport are analytically represented. The behavior of humic materials, which adsorb to the silica medium as well as bind metal, is approximated with a one-dimensional numerical model.

The third appendix describes the use of DEAE-cellulose in combination with XAD-8 resin procedures for the purpose of fractionating dissolved organic carbon into humic and non-humic metal-chelating agents. A significant portion of the metal binding properties of natural waters may be due to non-humic materials not isolated by the standard XAD-8 procedure.

The fourth appendix discusses the use of DEAE-cellulose as a rapid and convenient method for concentrating organic complexing agents from ocean water.

The last appendix is a compendium of spectrophotometric studies of the fulvic acid used throughout this work. Excitation and emission spectra are presented along with a discussion of the effects of pH and trace metals on the shapes of these spectra.

Mr. David A. Dzombak is a co-author on the research paper that constitutes Chapter Three. He is responsible for much of the development of the section on the theory of continuous distribution models, in particular, the normal distribution model.

CHAPTER TWO:

PROPAGATION OF ERROR IN FULVIC ACID TITRTATION DATA:

A COMPARISON OF THREE ANALYTICAL METHODS

ABSTRACT

Many methods have been employed for measuring the interactions of trace metals and humic materials but little consideration has been given to the magnitude of error in the resulting titration data. Most commonly used metal titration methods can be placed into three categories based on the chemical species that is most directly measured: free ionic metal, total labile metal, or ligand-bound metal. The propagation of random instrument error into a titration data set is not uniform throughout a titration and the pattern of error propagation is characteristic of the category of method used. Standard deviations are determined for measurements by ion-selective electrodes, fluorescence quenching, and fixed potential amperometry (each representative of a category of methods). Error propagation in resulting titration data is quantified. No single titration method yields uniformly precise data in all concentration ranges and a combination of complementary methods yields the best characterization of metal binding to a humic material.

INTRODUCTION

In recent years researchers have amassed a substantial body of information about the interactions of humic materials with trace metals. In gathering these data, many analytical methods have been used, each of which measures a single chemical quantity such as free or ligand-bound metal. The additional quantities necessary for mathematically modeling the system have been indirectly obtained by data transformations.

Valid comparisons among results of different methods are possible if each method yields, either directly or indirectly, the same concentration of a metal species for a particular system. Tuschall and Brezonik (1983) applied this test to four commonly used analytical methods: ion-selective electrodes, anodic stripping voltammetry, fluorescence quenching and continuous flow ultrafiltration. Saar and Weber (1980) compared and intercalibrated ion selective electrode and fluorescence quenching data and Waite and Morel (1983) compared fulvic acid titrations using ion-selective electrodes and fixed-potential amperometry. The results of these comparisons suggest that, over certain concentration ranges and with certain exceptions, the measurements of each chemical quantity by several different methods are in approximate agreement.

With the variety of apparently comparable techniques available, criteria are needed to guide the choice of methodologies appropriate to a particular application. In a review of the titration methods currently available, Saar and Weber (1982) emphasized the special merits and problems associated with each technique, such as sensitivity, interferences and the metals that can be measured. In addition to matching the experimental capabilities of the method to the system under study, it is

important to recognize the differences in the precision of data obtained from each method as a result of the different chemical species that are measured. The distinctions are a consequence of the arithmetic manipulations needed to calculate indirectly a species concentration. Errors propagate, resulting in different degrees of uncertainty for the directly and indirectly measured quantities.

Previous comparisons of techniques have not fully considered the implications of errors in transformed data. Parameters used to characterize humic materials, such as stability constants and equivalent ligand concentrations, vary widely in accuracy depending on the titration method and the metal concentrations used. Frequently, limitations of time and materials preclude repetitive titrations and error intervals are determined for stability constants and ligand concentrations. As an alternative to numerous replicate titrations, the precision of the analytical methods can be measured and the propagation of error quantified for each transformation of the data. The latter approach is employed in this study.

We examined the most useful analytical methods and grouped them into three classes based on the quantities they measure. We selected a representative method from each class and showed that this method, in addition to its particular practical limitations, is subject to predictable errors characteristic of its class and that these errors propagate through the transformations necessary for modeling the experimental results. As a consequence, no single technique can yield a complete and optimally precise set of metal-binding data. Results from different methods were overlapped to yield data that posses minimal error over a wide titration range. We applied these principles to series of Cu(II)

titrations of fulvic acid obtained from each representative method. The standard deviations of conditional stability constants obtained from each titration method were compared to demonstrate the predicted regions of minimal error.

THEORY

Classes of Methods

Commonly used titration techniques fall into three general classes, each defined by the quantity that is measured as a function of total metal added during the titration: free ionic metal, labile metal species or bound metal species.

Free-ionic metal activity may be measured with a solid-state, ion-selective electrode (ISE). These Nernstian devices generate a potential proportional to the logarithm of the free-metal activity in solution. Because stability constants are conventionally written in terms of the free metal species, the output of an ISE titration is especially convenient to use in speciation models. Furthermore, the logarithmic scaling allows data to be collected over many orders of magnitude. This feature is useful for characterizing humic materials which typically have a wide range of apparent metal-binding strengths.

Ion-selective electrodes have several well-known practical limitations. While very low free-metal activity can be measured, the lowest <u>total</u> metal concentrations possible are at or above the average concentrations typically found in unpolluted natural waters. Cupric ISEs, which are the most commonly used means of studying metal-humic interactions, are also subject to a large, uncorrectable interference

from chloride ions (Westall et al. 1979). To overcome these problems, ion exchange methods have been proposed for determining ionic activities of metals in titrations (Van den Berg and Kramer 1979). Such procedures fall into this class because the metal that partitions onto the exchanger surface is proportional to free-metal activity. Unfortunately, these methods still require relatively high ligand concentrations and are difficult to calibrate and interpret (Van den Berg et al. 1979). Consequently, the ion-selective electrode, which is very reliable within its optimal operating range (in the absence of interferences), was chosen for this study as the representative method of its class.

The second class comprises those methods that yield arithmetically scaled labile-metal concentrations. The labile-metal fraction is operationally defined for each method but is ideally taken to be the sum of all metal species except the organo-metallic complexes of interest. Anodic stripping voltammetry and fixed-potential amperometry (Waite and Morel 1983) fall into this category as well as all titration techniques involving separations such as dialysis (Truitt and Weber 1981), gel-permeation chromatography (Mantoura and Riley 1975) and ultrafiltration (Tuschall and Brezonik 1983). The separation techniques offer great flexibility in the choice of reactant metals and background electolytes, but uncertain molecular-size cutoffs make unclear the composition of the defined labile-metal fraction.

Because of its sensitivity and convenience, anodic stripping voltammetry (ASV) has been the most widely used technique in this class. Yet it is not clear which metal species are electrochemically stable and which are decomposed as "labile" at the negative potentials employed in the plating step (Tuschall and Brezonik 1981; Brezonik and Tuschall

1982). Much debate has centered on the accuracy of ASV techniques and there is substantial reason to believe that characterizations of metal binding agents by ASV methods are ambiguous or subject to artifact.

Waite and Morel (1983) describe fixed-potential amperometry (FPA) as a method of labile-copper analysis that appears to have a sensitivity comparable to ASV methods yet is free of the major artifacts associated with mercury electrodes operating in the negative potential region. The FPA method has been intercalibrated with cupric ISE data and is used in this study as the representative method for this class.

The third class of methods are those that directly measure the formation of organo-metallic complexes during the titration. Spectrophotometry is the classical technique in this category (Schnitzer and Hansen 1970) but has been little used for humic substances because of the weak absorptivity of metal-humate complex. Methods that extract organo-metallic complexes onto a hydrophobic column such as the C-18 Sep-Pak column procedure described by Mills and Quinn (1981) also fall into this class. Such methods are useful in estimating the extent of organic metal-binding in natural samples but are poorly suited to detailed titrimetric procedures because the kinetics of humic complex formation and dissociation in the column are not well defined. Quenching of natural humic fluorescence by certain paramagnetic metals (such as Cu^{2+}) has been recently applied to metal binding studies with considerable success (Ryan and Weber 1982; Tuschall and Brezonik 1983). Fluorescence quenching (FQ) is relatively sensitive and provides a direct, arithmetically scaled measure of metal-complex concentration. It is employed here as the third representative method.

Error Propagation in Data Transforms

Developing a thermodynamic equilibrium model of metal interactions with humic materials requires knowledge of the free ionic activity of the metal, {M}, the ligand-bound metal concentration, ML, and the unbound-ligand concentration, L_f, over a range of values. In practice, all three quantities are not measured directly or independently. One quantity is measured as a function of the total metal added to the system and the two remaining quantities are determined indirectly from conservation of mass considerations. Several transformations of the original data are necessary to obtain the three quantities used in an equilibrium model.

For example, in ISE titrations the electrode potential is converted to the logarithm of the metal activity by multiplying by a calibration factor (A). The log of activity is exponentiated to obtain the arithmetic metal activity, which in turn is multiplied by a proportionality constant (K_0) to generate the concentration of inorganic metal complexes, M_i. This value is subtracted from the total concentration of metal in solution to determine, indirectly, the concentration of ligand-bound metal. The value of L_f must be obtained by subtracting ML from the total ligand concentration, L_T. In each of these transformations the uncertainty in the quantity obtained increases as errors propagate through the data set. We can quantify this propagation by simple statistical methods, summarized in Table 1.

Examination of the error propagation equations leads to the observation that the least error in each quantity results from methods that most directly measure that quantity. Note also that many of the error terms are based on relative errors and that the absolute error in a derived

TABLE 1.

Standard deviations and error propagation equations for chemical quantities measured by three exemplary methods. Subscript "r" denotes relative standard deviation. Chemical species are in units of mol/L. Propagation formulas after Skoog and West (1981).

Quantity	Symbol	ISE	FQ	FPA
ISE potential (mV)	$V \pm S_v$	$S_v = 0.5$ V	-	-
ISE calibration	$A \pm S_a$	$S_a = 0.4$ mV/unit pCu	-	-
FQ calibration	$B \pm S_b$	-	$S_{br} = 0.05$	-
FPA calibration	$C \pm S_c$	-	-	$S_{cr} = 0.01$
-log (Cu^{2+})	$pCu \pm S_p$	$S_{pr} = [S_{ar}^2 + S_{vr}^2]^{\frac{1}{2}}$	-	-
Percent quenching	$\%Q \pm S_q$	-	$S_q = 0.001(RFU_0)$	-
Charge transferred	$U \pm S_u$	-	-	$S_u = 0.4 + 0.01(U)$
(Cu^{2+})	$Cu^{2+} \pm S_m$	$S_{mr} = S_p/0.434$	$S_{mr} = [S_{ir}^2 + S_{0r}^2]^{\frac{1}{2}}$	$S_{mr} = [S_{ir}^2 + S_{0r}^2]^{\frac{1}{2}}$
Inorganic constant	$K_0 \pm S_0$	(a)	(a)	(a)
Inorganic Cu(II)	$Cu_i \pm S_i$	$S_{ir} = [S_{0r}^2 + S_{mr}^2]^{\frac{1}{2}}$	$S_i = [S_1^2 + S_t^2]^{\frac{1}{2}}$	$S_{ir} = [S_{ur}^2 + S_{cr}^2]^{\frac{1}{2}}$
Total Cu(II) added	$Cu_T \pm S_t$	$S_{tr} = 0.02$	$S_{tr} = 0.02$	$S_{tr} = 0.02$
Humic-bound Cu(II)	$CuL \pm S_1$	$S_1 = [S_t^2 + S_i^2]^{\frac{1}{2}}$	$S_{1r} = [S_{qr}^2 + S_{br}^2]^{\frac{1}{2}}$	$S_1 = [S_t^2 + S_i^2]^{\frac{1}{2}}$
Free ligand	$L_f \pm S_f$	$S_f = S_1$	$S_f = S_1$	$S_f = S_1$

(a) $S_0 = 0.02$ (pH 6.0) or $S_0 = 19$ (pH 8.5) for all methods.

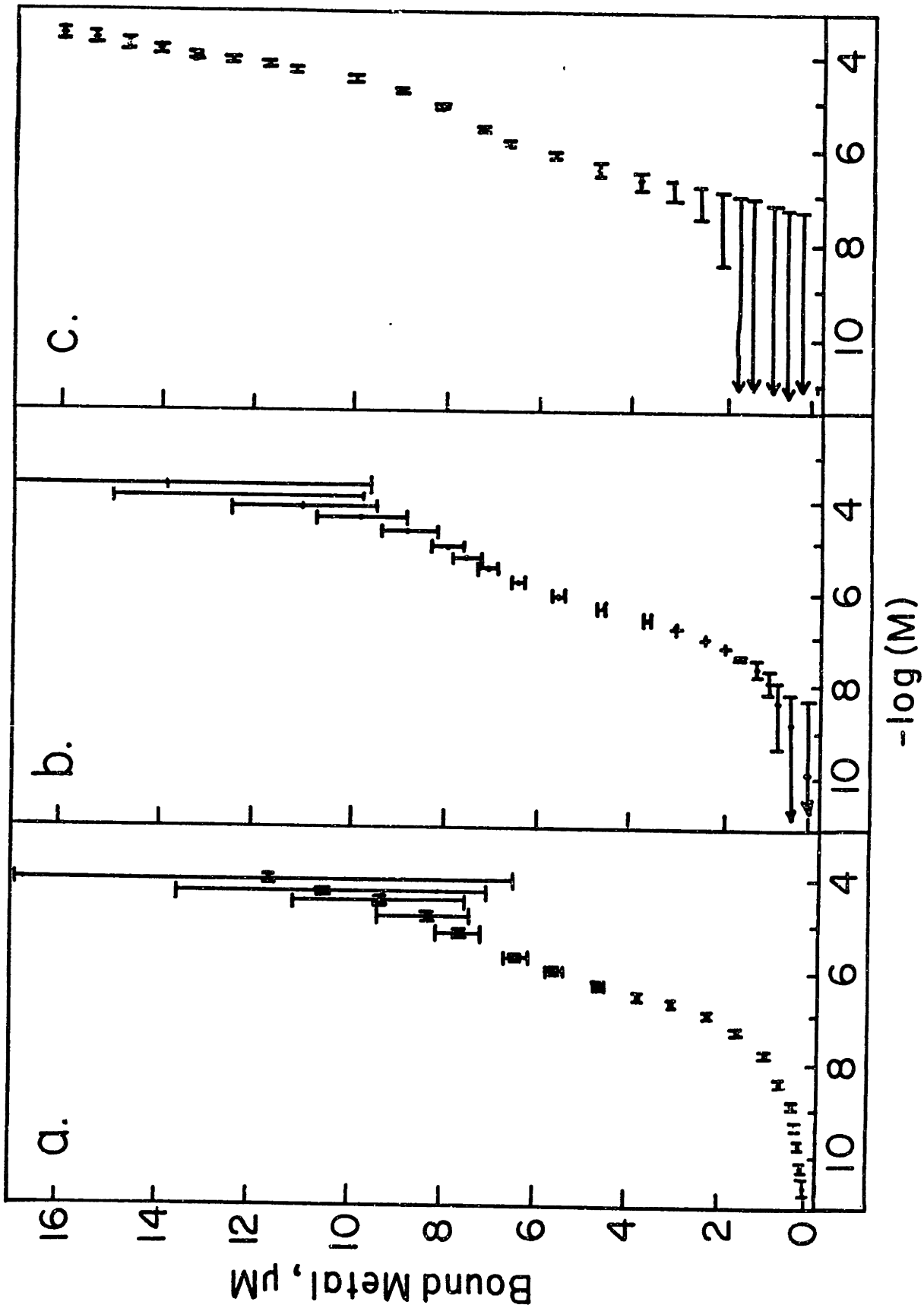
quantity may be dependent on the magnitude of the terms used in the derivation. Uncertainty in the indirectly measured quantities may increase dramatically through the course of a titration as concentrations of total metal and the directly measured species increase. This effect can be demonstrated graphically by drawing calculated error bars on plots of titration data.

The plots in Figure 1 are the so-called formation functions which depict $\bar{\nu}$ = (ML) / L_T as a function of the negative log of the free metal activity (pM). The underlying formation function is identical for the three plots and represents a simulated metal titration of a hypothetical three-ligand mixture calculated with the computer-based equilibrium program MINEQL (Westall et al. 1976). The three ligands yield titration curves essentially the same as those of humic acid but can be used to generate titration data to an arbitrarily high degree of accuracy (Fish et al. 1984).

The standard deviations in the quantities measured by ISE, FPA and FQ were calculated from experimental data obtained in this laboratory (as discussed below) and were translated through the propagation equations for each method. Error intervals of one standard deviation from the computer-calculated mean values for both pM and ML (the 68% confidence intervals) were drawn about each point in the calculated formation functions.

As noted earlier, the error intervals for each metal species are smallest for the methods that most directly measure that species: free metal for Classes I and II, bound metal for Class III. The wide error bars on the low pM values for the Class II (FPA-type) measurements (Fig. 1b) reflect the detection limit of this method for a solution in which

Fig. 1. A comparison of error propagation in hypothetical titration data: Bound metal vs. -log free metal activity (formation functions). Error bars represent ± one standard deviation for: a. ion-selective electrode type data; b. fixed potential amperometry type data; c. fluorescence quenching type data.


a.
b.
c.
Bound Metal, μM
0
2
4
6
8
10
12
14
16
4
6
8
10
-log (M)

the labile metal approximately equals the free-metal activity; a similar error region would also be observed in the Class I measurements if the titration were conducted below the detection limit of the electrode.

Above the detection limits, the greatest error is found for the indirectly measured species and the magnitude of this error varies widely across the titration range. For example, with methods that measure unbound metal, bound-metal errors increase greatly near the end of the titration. This reflects the propagation of relative errors as well as the fact that errors become most severe when we attempt to calculate a species concentration by subtracting two relatively large numbers.

A mathematical model of humic binding is of greatest predictive value if it is fitted to the most precise data in any concentration range. For each class of methods there is a titration region of minimal error in determinations of both free and bound metal. Figure 1 suggests that the best approach to modeling entails fitting Class I titration data in the low to moderate pM range and Class III titration data in the moderate to high pM range. Class II titrations might also be desirable, for reasons such as the high sensitivity of FPA, of for calibrating a model to data from a high-chloride medium.

Before combining the titration results of different techniques, the methods should be intercalibrated so there is reasonable congruence among the data. Intercalibration can be achieved in the central region of the titrations where all three titration classes yield data of approximately equal precision. From this central region, the data set can be extended for both free- and bound-metal species in either concentration direction with data from the appropriate methods. If only a single method is used, careful consideration should be given to the poor precision of data in

certain regions of the titration and the resulting errors in the modeling parameters derived for that region.

MATERIALS AND METHODS

Fulvic acid was obtained from 20 L of Grassy Pond water (Bedford, MA) by adsorption onto XAD-8 resin according to the method of Thurman and Malcolm (1981). The freeze-dried extract was 49% carbon by weight and this factor was used to prepare an aqueous stock solution of 50 mg-C/L of Grassy Pond fulvic acid (GP-FA). The stock solution was stored in a foil-covered flask (to exclude light) at 5°C and at pH 8. After preparation stock solutions were allowed 48 h to dissolve and hydrolyze completely before any dilutions for titrations were made.

Solutions of fulvic acid for ion selective electrode and fluorescence quenching titrations at 0.5, 1.0 and 5.0 mg-C/L were prepared with deionized water. Ionic strengths were adjusted using 1 N $NaClO_4$ to give an electrolyte concentration of 10^{-3}M. For fixed potential amperometry a background electrolyte of 0.5 M NaCl and 2 mM $NaHCO_3$ was used. The electrolyte was prepared from a 5 M NaCl, 20 mM $NaHCO_3$ stock solution that was passed through a Chelex resin column to remove contaminating trace metals according to the methods of Morel et al. (1979).

Ion selective electrode titrations were performed using a Radiometer F3000 Selectrode with a Cu(II) sensing membrane coupled with an Orion 90-02 double-junction reference electrode. The pH was monitored using a Fisher E5-A combination pH electrode and the pH was maintained by adjusting the alkalinity and bubbling with 1% CO_2/99% N_2 gas mixture.

Electrode potentials were measured by a pair of Orion 801 Ionanalyzer meters. The ISE was calibrated with a series of copper solutions the day before each titration and was allowed to equilibrate overnight in a 1 mM Na_2EDTA / 0.1 M TRIS buffer solution at pH 6.2. This procedure maximized electrode sensitivity in the low copper range. The fulvic acid solutions were titrated in 100 mL, water-jacketed beakers at 15°C with total copper concentrations ranging from 0.1 uM to 100 uM.

Fluorescence quenching titrations were performed using a Perkin-Elmer LS-5 Fluorescence Spectrophotometer equipped with a 1 cm flow cell coupled by Teflon tubing and a peristaltic pump to a 50 mL, water-jacketed titration vessel maintained at 15°C. The pH was maintained with a CO_2/N_2 mixture as in the ISE titrations. Fulvic acid solutions were deoxygenated by several hours of bubbling with the CO_2/N_2 gas mixture in order to eliminate the minor quenching of the fulvic acid fluorescence by O_2. For GP-FA titrations the excitation and emission wavelengths were set for maximum fluorescence at 325 nm and 405 nm respectively. Slit widths were set at 10 nm. Kinetic studies indicated that quenching equilibrium was reached within 10 min at copper concentrations less than 1 uM and in less than 5 min above 1 uM copper. In subsequent titrations equilibration times were allowed accordingly before measurements were taken. Fulvic acid solutions were titrated over the same total copper range as in the ISE experiments: 0.1 uM to 100 uM.

Fixed potential amperometry was employed according to the methods of Waite and Morel (1983). Measurements were made with an Environmental Science Associates (ESA) Model 3040 charge transfer analyzer equipped with a tubular pyrolytic graphite working electrode, a platinum wire counter electrode and an Ag/AgCl, saturated NaCl reference electrode.

The electrode potential was set at 90 mV and measurements were taken after a 10 min equilibration period following each copper addition. Titrations were performed with total copper ranging from 10 to 330 nM.

RESULTS AND DISCUSSION

Error Analyses

The millivolt output of the ISE system was recorded to within 0.1 mV and analysis of five pairs of replicate titrations indicates that the standard deviation in electrode potential is ± 0.5 mV. A slightly higher deviation is obtained at low Cu_T and pCu levels but, as an approximation, the standard deviation in potential was considered to be constant at ± 0.5 mV.

The calibration factors for the electrode were determined by measurements of standard Cu(II) solutions. The variation allowed in the calibration slope was ± 0.5 mV per decade pCu and the standard deviation was statistically derived from this spread to be ± 0.4 mV per decade pCu.

The hydroxo and carbonato complexes of copper were calculated with stability constants selected from the literature. Stability constants and standard deviations for the carbonato complexes are given by Smith and Martell (1976) as log β_1 = 6.75 ± 0.02 and and log β_2 = 9.92 ± 0.09. There is greater variability in the constants for the hydroxo complex. For example, Smith and Martell (1976) offer log β_1 = 6.3 while Sunda and Hanson (1979) reported log β_1 = 6.48. The reported stability constants for the dihydroxo complex are particularly variable but in two careful studies, values of log β_2 = 11.78 (Sunda and Hanson 1979) and 11.80 (Paulson 1978) were obtained. The mean of these is used in the present study. Standard deviations are not available for the hydroxo constants

but it is reasonable to assume that both log β values are known to within ± 0.10 log units. Because the concentrations of the hydroxide and the carbonate ions were held constant during the titrations, an aggregate proportionality factor, K_o, relating total inorganic and free ionic copper was calculated from the stability constants. The pH and $p(CO_3^{2-})$ were maintained within ± 0.05 log units and incorporating all of these error terms in the calculation yielded K_o = 1.12 ± 0.02 for pH 6.0 and 170 ± 19 for pH 8.5.

The relative error associated with the calculation of inorganic copper becomes very significant when the inorganic copper concentration equals or exceeds the the copper-fulvate concentration. This condition is dependent on both the metal/ligand ratio and the pH and is attained when either quantity is relatively high. Determinations of weak metal binding behavior by ion selective electrode are therefore most accurate at low pH. Because apparent binding strength also decreases with pH, an appropriate compromise is to perform titrations near pH 6 where inorganic copper-complex concentrations and proton competition for the ligands are both acceptably small.

The standard deviation in the fluorescence measurements of a fulvic acid solution was uniform throughout the titration and equal to approximately 0.1 % of the unquenched fluorescence. The relative fluorescence data are converted to bound-metal concentration by a calibration factor, B, linearly relating the percent-quenching (%Q) of fluorescence to the concentration of metal-fulvate complex. The simplest and most direct method for obtaining B is to plot percent quenching against the concentration of total metal added. If sufficiently small additions of Cu(II) (relative to the concentration of fulvic acid) are made, there is a

region at the beginning of the titration in which there is a linear relationship between the metal added and the percent quenching. This region corresponds to the strong metal-binding portion of the the titration in which essentially all of the metal added quantitatively binds to the fulvic acid. The slope of a least squares fit of this region provides a quenching calibration factor for a given concentration of fulvic acid. This factor is assumed to be constant throughout the titration. The appropriateness of this assumption is discussed below.

The variation in the relative fluorescence translates into a standard deviation for the bound metal of ± 0.1 x (calibration factor). For a typical titration of 5 mg-C/L GP-FA in which the calibration factor was 0.25 uM bound Cu(II) per unit pecent of quenching, the standard deviation on the bound copper measurements was ± 0.025 uM. The absolute error in bound metal is constant throughout so the relative error decreases as the titration advances. As a result, the relative error in the determination of free metal also decreases throughout the titration.

The labile copper measured by fixed potential amperometry includes all Cu(II) species that are reducible at the electrode potential, set in this system at + 90 mV. At this potential only the hydrated cupric ion or the inorganic complexes are reduced and the current transferred over a fixed time interval is proportional to the total inorganic copper in solution (Cu_i). The proportionality factor, C, is obtained from a set of standard solutions. After systematic errors were corrected (Waite and Morel 1983) we found the standard deviation in the current is described by the equation:

$$S = \pm [S_x + S_{cr}(U)]$$

in which S_x is a constant "background" error and $S_{cr}(U)$ represents the error that is proportional to the inorganic Cu(II) measured. For the experiments described here we found S_x = 0.4 nM and S_{cr} = 0.01; the latter value is the relative standard deviation in the proportionality factor. The minimum labile Cu(II) concentration accurately measurable was about 5 nM and the maximum copper concentration was about 350 nM.

The Davies equation was used to calculate the single-ion activity coefficients necessary for comparing the results of the FPA titrations to those of ISE titrations performed at lower ionic strength. Although there is significant uncertainty associated with these coefficients, the standard error could not be quantified and so is not incorporated in the error calculations.

Because total inorganic copper is directly measured by FPA, bound copper concentrations are more directly obtained with this method than by ISE. The inorganic copper concentration was converted to the cupric ion activity by K_o as defined earlier. For the solution conditions of the titration the minimum cupric ion activity measurable was about 0.01 nM or pCu 11.0.

A summary of the standard deviations for the data from the three methods is presented in Table 1.

Comparison of Titration Results

Data from the ISE and FQ titrations of 1.0 and 5.0 mg-C/L solutions of Grassy Pond fulvic acid at pH 6.0 were converted by the arithmetic transformations described into both the directly and indirectly measured

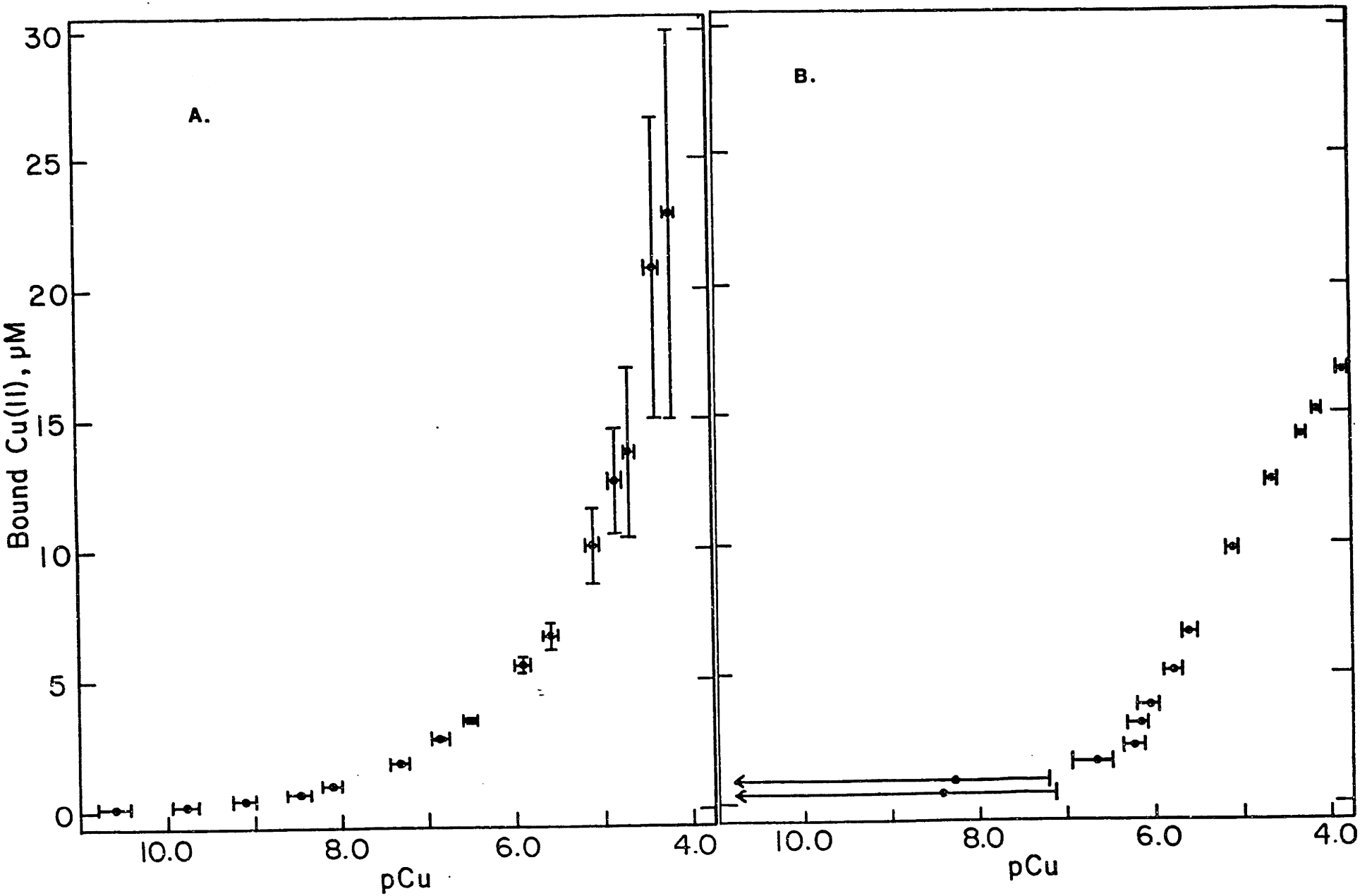
species concentrations. Plots of formation functions (bound metal vs. pCu) were prepared using the results of both techniques and are presented in Fig. 2 for comparison. The standard deviation for each data point was calculated with the error propagation equations and error bars representing one standard deviation were placed around the bound-metal values. The plots reveal systematic differences between the two methods as well as pronounced differences in the propagation of random error.

The formation functions demonstrate the limited sensitivity of fluorescence quenching for determining free-metal activity. Metal activity below 0.1 uM is measurable only by the ISE. The sensitivity of the ISE to low pCu allows the accurate calculation of stability constants or other model parameters for the strong binding region of the titration. Reliance on fluorescence quenching alone would preclude any quantification of this important property of humic materials.

The smallest amount of bound metal that can be detected in a fluorescence quenching titration is proportional to the smallest change in relative fluorescence that is distinguishable from noise. For the system described here that quantity is about 0.1 uM of bound copper and is independent of the total fulvic acid in solution. This amount is coincidentally the same as the smallest amount of strongly bound copper measurable with an ISE (equal to the minimum Cu_T).

Throughout the titration the ion-selective electrode provides relatively accurate measurements of pCu. Error propagation analysis reveals, however, that the accuracy of the ISE in measuring free metal does not translate into accurate bound-metal measurements over the entire titration range. The bound copper measured directly by fluorescence quenching exhibits higher accuracy than the indirect ISE data when (ML) >

Fig. 2. Formation functions and standard deviations for Grassy Pond fulvic acid at 5.0 mg-C/L and pH 6.0. A. ISE titration; B. FQ titration.


A.
B.
Bound Cu(II), μM
pCu
pCu
30
25
20
15
10
5
0
10.0
8.0
6.0
4.0
10.0
8.0
6.0
4.0

2 uM. Accuracy in the high-metal region of the titration is critical for estimating the total ligand concentration, L_T.

In order to compare directly the species concentrations measured by these two methods, plots of the formation functions for both the ISE and the FQ titrations were overlaid (without error bars, for clarity) in Fig. 3.

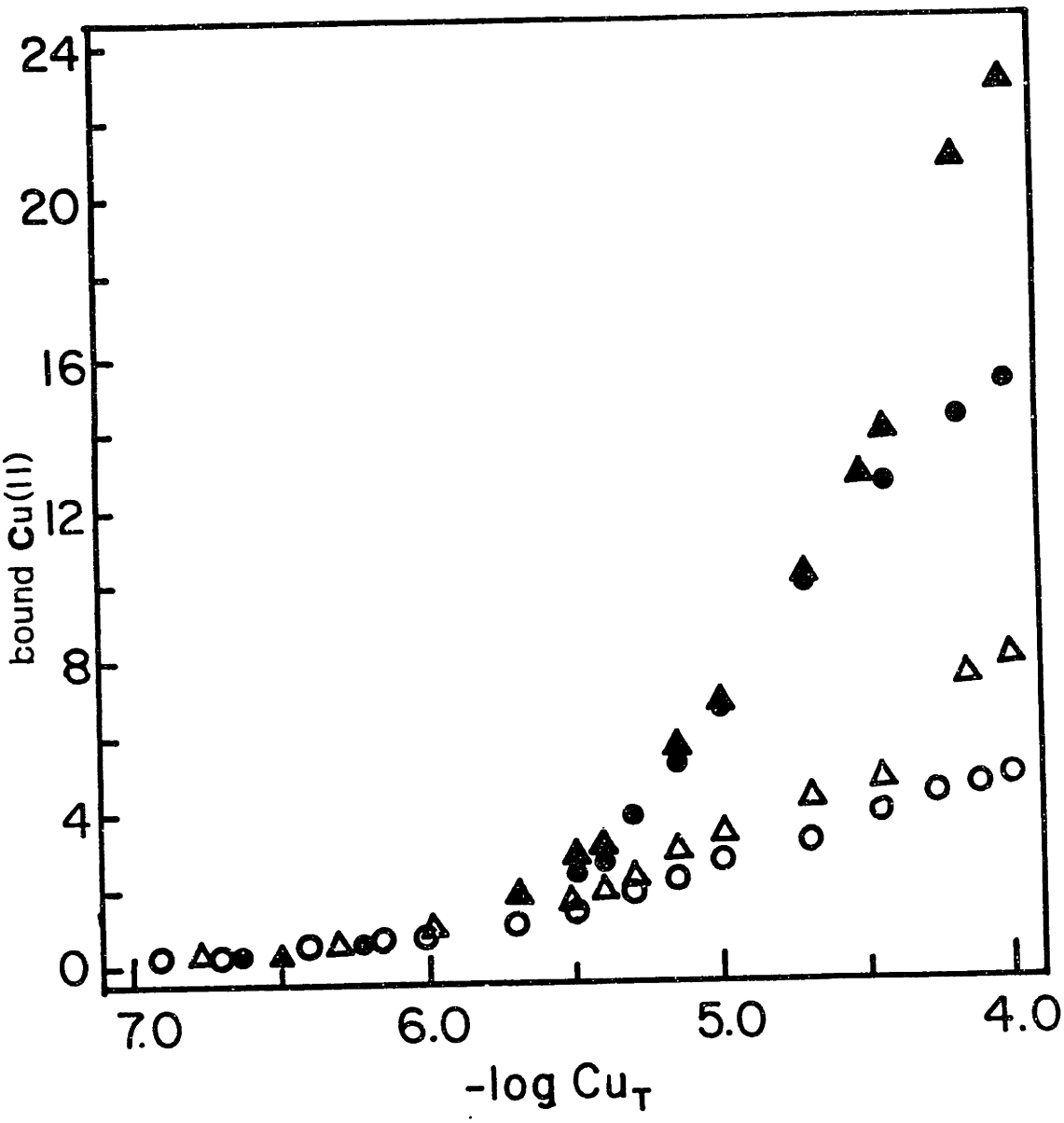
In the low total-metal regions of the titrations, both ISE and FQ yield nearly identical bound metal values. The free-metal values in this region differ because of the poor precision with which fluorescence quenching measures unbound metal at low M_T. In the mid-range the formation functions overlap but in the titration region corresponding to weak metal-binding, fluorescence quenching measures smaller bound-metal concentrations than does the ISE, a phenomenon observed in several other studies (Ryan and Weber 1982; Tuschall and Brezonik 1983). One explanation is that the weak binding sites have a different fluorescence efficiency than the stronger sites. Ryan and Weber (1982) suggested that some of the weak binding occurs on molecules that have already been quenched by strongly bound Cu(II). Another explanation is simply that a greater proportion of the weaker binding is due to either sites not associated with fluorophores or is due to a strictly electrostatic effect that does not involve the alteration of fluorescing electrons. No definitive explanation is currently possible and the discrepancy only adds greater uncertainty to the poorly defined end region of fulvic acid titrations.

The fulvic acid concentrations in these titrations are representative of many surface, freshwater systems but are perhaps an order of magnitude higher than in some deep groundwater or marine systems. The

Fig 3. Formation functions for Grassy Pond fulvic acid at pH 6.0.

5.0 mg-C/L: ISE (▲), FQ (●).

1.0 mg-C/L: ISE (△), FQ (○).


24
20
16
12
8
4
0
bound Cu(II)
7.0
6.0
5.0
4.0
-log Cu$_T$

relatively high total copper concentrations required by the ISE seriously limits the usefulness of the method for characterizing humic matter at concentrations below 1 mg-C/L.

A fixed-potential amperometry titration of a 0.5 mg-C/L solution of Grassy Pond fulvic acid at pH 8.5 in 0.5 M NaCl demonstrates that FPA can quantify binding behavior for Cu(II)-fulvate present in concentrations below the sensitivity of other methods. The pH and background electrolyte furnish information about fulvic acid behavior under marine solution conditions.

The results of the FPA and ISE titrations were converted, as in the previous section, into both the directly and indirectly measured quantities and the data from both methods presented in comparative plots (Fig. 4). All activities were corrected to the the lower ionic strength (I = 0.001) by coefficients calculated with the Davies equation. The formation functions demonstrate the relative merits of the two methods in the low-copper range. The ion-selective electrode is limited to measurements for which the total copper concentration is greater than about 0.1 uM although the cupric-ion activity can be measured at less than 10^{-10} M. Total Cu(II) concentrations can be two orders of magnitude lower for the FPA titration than with the ISE, but FPA has the same sensitivity for both total and labile Cu(II). Therefore, the minimum cupric-ion activity measurable by FPA in the presence of strong binding agents is higher than for the ISE.

A comparison of the plots indicates good agreement in the cupric ion activities determined by both methods for pCu $\leq$ 9.2. At lower copper concentrations the activities measured by the ISE fall below the FPA data (Fig. 5). At these low total-copper concentrations the ISE is

Fig. 4. Formation functions and standard deviations for Grassy Pond fulvic acid at 0.5 mg-C/L and pH 8.5. A. FPA titration; B. ISE titration.

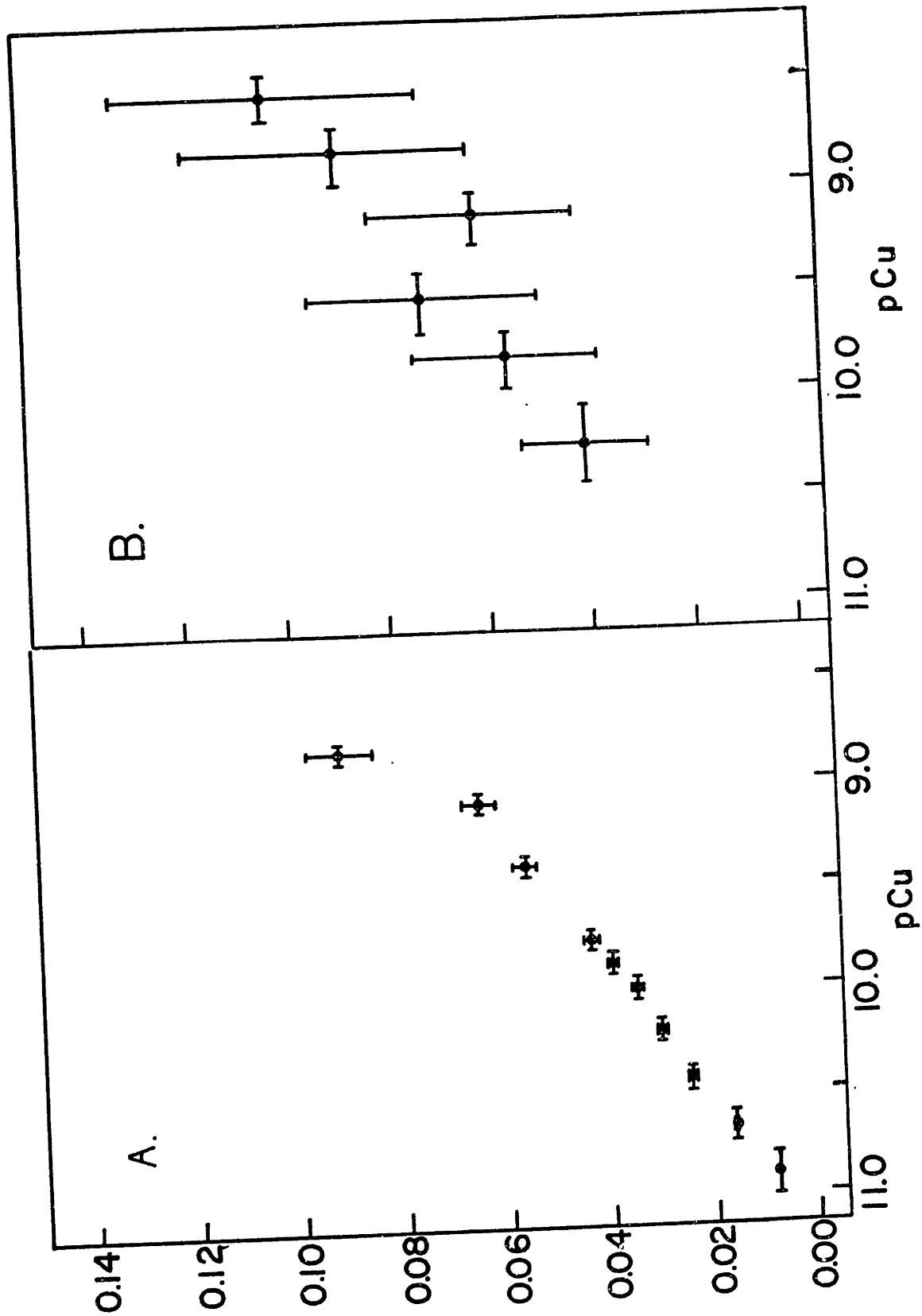
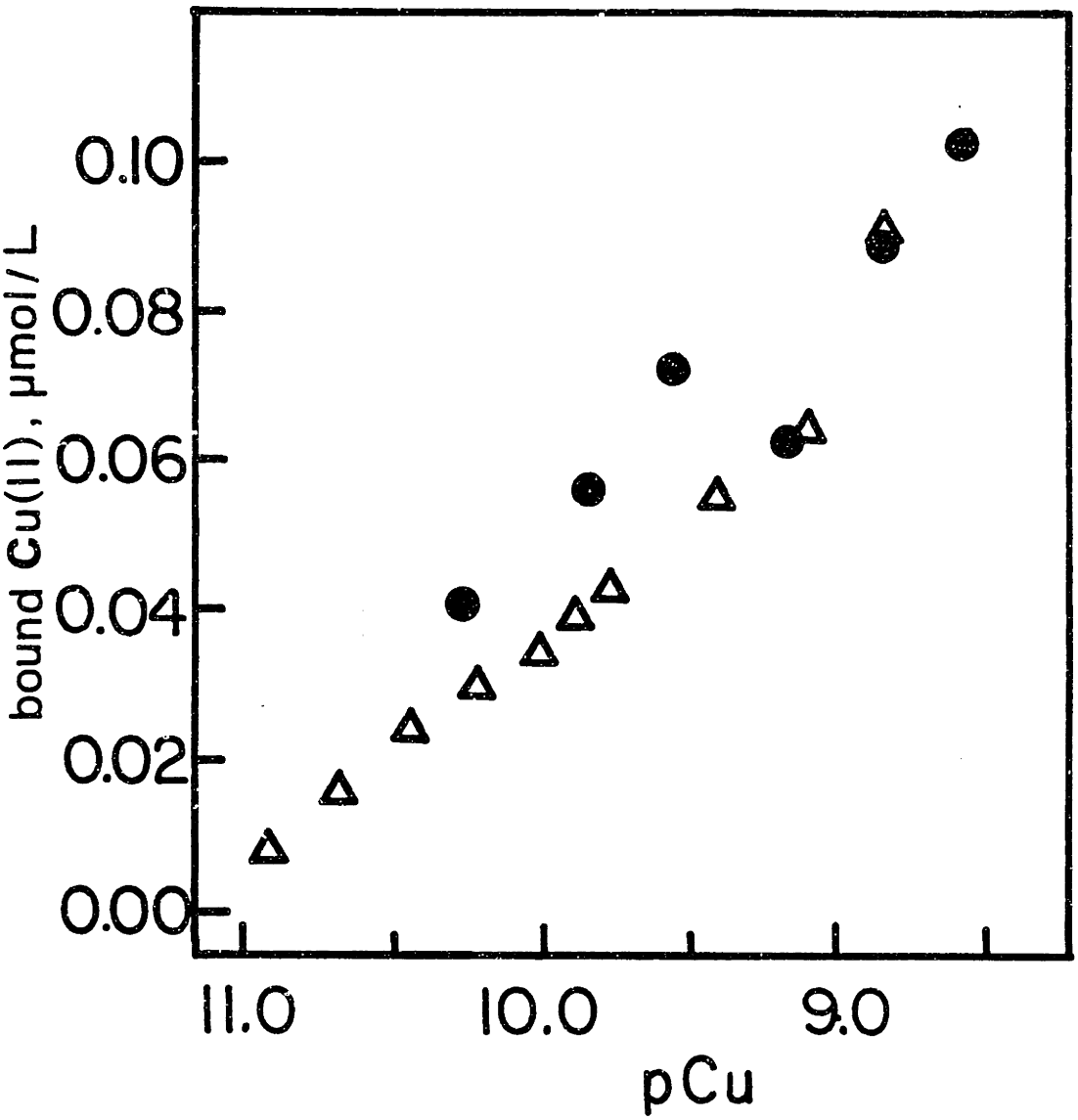

A.
B.
pCu
9.0
10.0
11.0
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00

Fig. 5. Formation functions for Grassy Pond fulvic acid at 0.5 mg-C/L and pH 8.5. FPA titration (△); ISE titration (●).


0.10
0.08
0.06
0.04
0.02
0.00
bound Cu(II), µmol/L
11.0
10.0
9.0
pCu

functioning below its optimal range and the equilibration time for the electrode is very slow, on the order of two to ten hours, depending on pH and pCu (low pCu and high pH have the slowest equilibration). Because the electrode potential drifts so slowly upward during this extended period it is likely that the apparent equilibrium reached and recorded for these data points was short of true equilibration. Another likely problem was the loss of copper through surface adsorption at high pH given the extended equilibration period. We estimated this quantity from atomic absorption spectrophotometry measurements of Cu_T in solution to be about 10% of total copper for Cu_T less than 1.0 uM.

In the same Cu(II) concentration range, fixed-potential amperometry operates under optimal solution conditions and we expect the FPA data to be more accurate than those of the ISE operating outside its appropriate range. Measurements at the low metal/ligand ratios confirm the binding behavior observed in more concentrated fulvic acid solutions with other methods and is of great use in evaluating the metal complexing properties of marine waters.

Note also that the bound-metal data obtained by the ISE are particularly imprecise at the higher pH of this titration compared to titrations at pH 6. The uncertainty in the inorganic complexation factor, K_o, is the primary cause. Recall that this constant is not needed in obtaining bound-copper values from FPA data because the total inorganic Cu(II) is measued directly by FPA.

The major limitations on FPA are the maximum permissible ligand concentration (about 0.1 uM), the maximum Cu(II) concentration (about 350 nM in the present system) and the need for relatively high chloride concentrations. These restrictions do not hinder the special usefulness

of FPA for marine studies but indicate that the method cannot fully characterize humic material over a wide range of species concentrations, especially in the weak metal-binding region. While these problems are specific to FPA, all methods in Class II (linear, labile-metal) are hindered by the difficulty of collecting metal binding data over many orders of magnitude with a linearly responding method. As a class, these methods appear to be most useful for measuring a limited titration range under solution conditions that preclude the use of methods such as ISE and FQ.

The results of the fixed-potential amperometry titrations were not compared directly to the the fluorescence quenching results because there is only a small region of overlap in the concentration ranges of the two methods. The overlap is in the region of least precision for free-metal measurements by fluorescence quenching. Hence, FPA and FQ, while complementary in the concentration ranges measured, cannot be directly intercalibrated.

Total Ligand Measurements

Determination of the total ligand concentration in solution is essential for defining conditional stability constants or parameters for other models of humic-metal interactions. If the bound-metal concentration in a plot of the complex-formation function levels off, the maximum concentration of bound metal is taken to equal L_T. Unfortunately, such unambiguous endpoints are rarely seen in Cu(II) titrations of humic materials. The large proportion of weak binding sites may not be fully saturated at the maximum metal activity in the titration. Formation of copper hydroxide solid at cupric ion activities greater than 10^{-4} M poses

an upper limit for Cu(II) titrations as does the flocculation of humic colloids (Tuschall and Brezonik 1983).

The absence of a clear endpoint is exacerbated in ISE titrations by the scattered bound-metal data in the end region. Low ligand concentrations favor the likelihood of reaching the endpoint before pCu 4 and a low pH (less than 5) would permit continuing the titration to higher cupric-ion activities. However, L_T determinations are not improved by these conditions because the bound-metal concentration at the end of the titration in either case is smaller while the error in the data remains about the same.

For Grassy Pond fulvic acid the best estimate of L_T, based on the ISE titration of 1.0 mg-C/L, is 6.0 ± 1.5 meq/mg-C (the standard deviation represents the variance in the bound-metal determination). For the fluorescence quenching titration of the same solution, L_T = 4.7 meq/mg-C. Although the uncertainty in the bound-metal measurements on which the latter figure is based is ± 0.025 uM, the error in L_T is presumably greater than that because of uncertainty as to the degree of saturation of binding sites, coagulation effects and uncertainty in the assumption that FQ calibration factor is constant. Because the definition of endpoint remains rather arbitrary for all methods, the uncertainty cannot be quantified by replicate titrations. The error in L_T may be the single largest error in the calculation of metal-humic interactions. Many models of humic-metal interactions normalize bound-metal concentrations to L_T (i.e., $\bar{\nu}$ = (ML) / L_T). Normalizing data to such a poorly defined quantity decreases the precision with which model parameters can be fitted (Fish et al. 1984). Eliminating the L_T term from empirical binding models would obviate this analytically intractable problem.

In general, L_T is a difficult parameter to measure by any method, or, in fact, to properly define. The poor resolution of L_T has been little recognized in studies of humic-metal interactions and an examination of the literature indicates that most L_T values or "complexation capacities" are actually operationally defined endpoint that do not reflect the true concentrations of humic binding sites. The errors in titration end-regions also suggest that "total ligand concentration" or "binding capacity" should not be used as a descriptive parameter for comparing humic materials. The concentration of humic material, the method used and the solution pH all have a dramatic effect on the maximum amount of metal binding observed.

Comparison of Individual and Combined Data Sets

Conditional stability constants for Cu(II)-fulvate compexes were derived from the results of the three exemplary methods by applying FITEQL, a computer-based, discrete-ligand optimization routine (Westall 1982). The concentrations and stability constants obtained are, of course, only fitting parameters but they are useful for both reproducing the titration data and for comparing different data sets.

In order to compare the effects of error propagation, the standard deviations calculated for the titration data from each method were used to estimate confidence intervals for the derived stability constants and ligand concentrations. The solution scheme in FITEQL has provisions only for fixed relative standard deviations in the titration data. Since relative errors are not constant over the entire titration, the fitting optimizations were repeated for each ligand with the appropriate relative standard deviations substituted. The values used each time were the mean

relative standard deviations for the titration region in which that ligand is predominant. The optimally fitted stability constants and equivalent ligand concentrations for all methods are presented in Table 2.

The results of applying a single analytical method to complicated mixtures such as fulvic acids are reflected in the diversity of standard deviations in the stability constants obtained. The regions of high error for each method translate predictably into poor accuracy for the conditional stability constants that predominate in those regions. This can be demonstrated by plotting the standard deviations associated with the ratios of bound metal to free metal for each titration (Fig. 6). Prediction of bound-metal and free-metal concentrations is the goal of metal-humic speciation models and the error in the ratio of the two quantities is a convenient way of summarizing the errors expected in model calculations based on these data. The ratio is also proportional to the apparent stability constant that would be calculated at any point in the titration. In addition, the plot is a useful summary of the concentration ranges over which each method is operational.

The region in which the bound-metal/free-metal ratio is high (the left-hand side of the plot) corresponds to the low Cu_T, strong-binding region of the titration. The ISE data here have much lower relative standard deviations than do the FQ data. Conversely, in the region of low bound-metal/free metal ratios (weak metal-binding), the FQ data are more precise. The entire range of the FPA titration lies in the high-ratio region and the method yields lower relative standard deviations in the strong-binding region than does the ISE.

Fig. 6. Relative standard deviations for the ratio of bound Cu(II) to free Cu(II) for all titrations, plotted against the log of the bound/free ratio.

ISE: 1 mg-C/L (●), 5 mg-C/L (○).

FQ: 1 mg-C/L (▲), 5 mg-C/L (△).

FPA: 0.5 mg-C/L,(□).

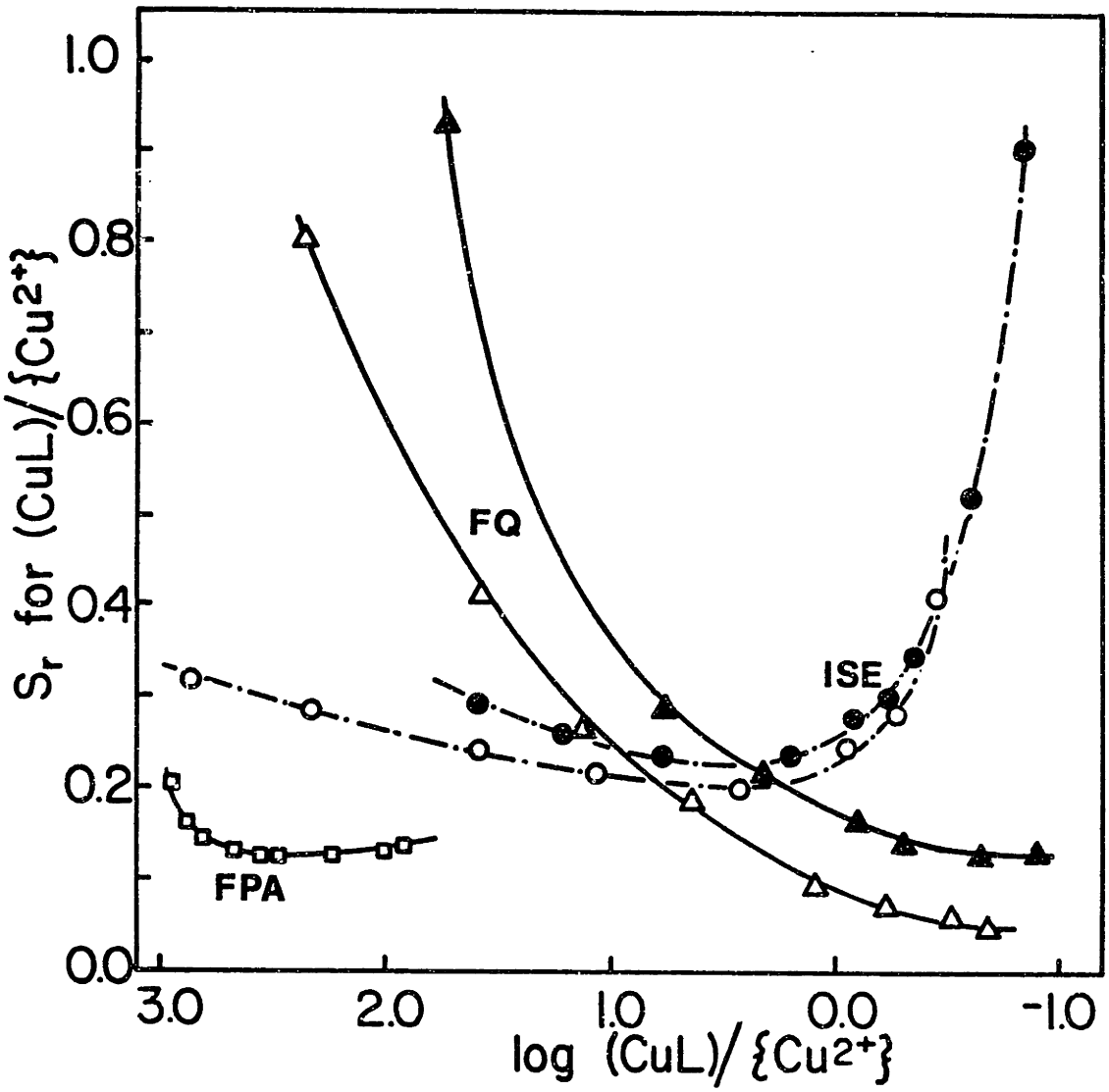

1.0
0.8
0.6
0.4
0.2
0.0
S_r for (CuL)/{Cu^{2+}}
FQ
ISE
FPA
3.0
2.0
1.0
0.0
-1.0
log (CuL)/{Cu^{2+}}

TABLE 2. Conditional stability constants, discrete ligand concentrations and associated standard deviations for Grassy Pond fulvic acid (GP-FA). Values calculated with FITEQL using errors defined in Table 1.

GP-FA (mg-C/L)	Method	Stability Constants			Ligand Concentrations, ueq/L		
		log K_1	log K_2	log K_3	L_1	L_2	L_3
0.5 [a]	ISE	-	9.02 ± 0.90	-	-	0.40 ± 0.25	-
	FPA	-	9.27 ± 0.04	-	-	0.33 ± 0.02	-
1.0 [b]	ISE	-	8.25 ± 0.29	6.27 ± 0.18	-	0.25 ± 0.10	1.98 ± 0.35
	FQ	-	8.10 ± 0.36	5.25 ± 0.10	-	0.53 ± 0.11	3.98 ± 0.28
5.0 [b]	ISE	10.52 ± 0.34	8.28 ± 0.20	5.63 ± 0.14	0.18 ± 0.06	1.34 ± 0.26	9.15 ± 1.20
	FQ	-	8.57 ± 0.25	5.14 ± 0.05	-	1.53 ± 0.25	8.80 ± 0.80

a : pH 8.5, 0.5 M NaCl

b : pH 6.0, 10^{-3} M $NaClO_4$

The results in Fig. 6 also indicate that each method is more accurate at higher fulvic acid concentrations. Examination of the error propagation equations shows that higher ligand concentrations result in smaller relative errors in the bound-metal data, hence less error in stability constants.

Only a combination of several methods yields the most precise set of ligand parameters. Within any broad region of a titration only two of the three methods operate within their concentration limits and the more precise data of the two sets available can be used to create a combined-methods data set.

Conclusions

Any comprehensive study of the interactions of a humic material with one or more metals is subject to unnecessary imprecision if only a single analytical method is employed or if inappropriate methods are selected. Precision at high bound-metal/free-metal ratios is important for quantifying the strongest metal binding behavior of a humic substance while accuracy at low bound-metal/free-metal ratios is critical for assessing the total number of sites available for binding. We have demonstrated that analytical methods can be grouped according to the quantity that they most directly measure and that the precision of metal binding data over a wide range of solution conditions can be enhanced if methods from at least two different classes are used. Examining the error propagation associated with the formation-function plots for each class is helpful in choosing complementary methods that yield the most precise data throughout a wide titration range. If only a single method can be utilized, the variance calculations and error propagation

equations listed in Table 1 can be used to estimate confidence intervals for species concentrations or model parameters.

An overall improvement in the precision of titration data leads to better predictive calculations of trace-metal speciation in natural waters. Quantification of the variance in the parameters used to describe metal-humic interactions allows for more useful and realistic comparisons among the results of different studies. Error analysis can also indicate which parameters (L_T for example) should not be incorporated in models or used for comparison.

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CHAPTER THREE:

ANALYSIS AND COMPARISON OF DISCRETE-LIGAND AND CONTINUOUS-DISTRIBUTION MODELS OF METAL-HUMIC INTERACTIONS

ABSTRACT

Discrete-ligand and continuous ligand distribution models of metal-humic interactions are compared and analyzed using both experimental and synthetic data. Discrete ligands are shown to be a simple and accurate predictive representation of experimental results if the parameters are correctly fitted by means of a non-linear regression. A continuous representation of binding requires knowledge of the distribution of ligand concentrations with respect to apparent stability constants. We demonstrate that a "stability function" proposed in the literature fails to correctly establish this relationship and that so-called afinity spectra should not be confused with binding-site distributions. Assumed ligand distributions can be rigorously applied to experimental results but cannot be used to model competitive interactions among several binding ions.

INTRODUCTION

The past decade has been one of expanding interest in the speciation of trace metals in natural waters. This interest stems from an increased awareness of the importance of metals in biological uptake and toxicity phenomena and in geochemical cycles. Detailed computation of metal speciation is now possible with computer-based solution schemes such as MINEQL (Westall et al. 1976) if all possible interactions can be represented mathematically via mass law equations. Humic materials are a major metal-binding constituent of many natural waters and a correct mathematical description of their interactions with metals is essential to any prediction of element speciation in those waters.

Inasmuch as the humic component of an aquatic system is an assemblage of molecules with similar but not identical structures, data obtained by titrating these compounds with metals cannot be adequately described by a single ligand with an associated stability constant. Consequently, several models have been proposed to account for the observed metal-binding behavior over the full range of titration data.

Most experimental results in the literature have been modeled by sets of discrete ligands (Buffle et al. 1977; Mantoura and Riley 1975; Sunda and Hanson 1979; Bresnahan et al. 1978; Tuschall and Brezonik 1983). In the discrete ligand model of humic-metal behavior humic molecules are represented by distinct classes of binding sites, each class having a characteristic stability constant. The actual distribution of sites is unknown, so the number of ligand classes as well as their concentrations and stability constants are constrained only by the goal of optimally fitting the titration data. The discrete ligands

obtained from these curve-fitting procedures may have little physical significance.

Because solutions of humic materials are thought to contain a spectrum of possible binding sites, models based on a continuous frequency distribution of stability constants have been advanced as physically more realistic. Two recently proposed continuous distribution models are the affinity spectrum model (Shuman et al. 1983) and the normal distribution model (Perdue and Lytle 1983a,b). Another approach is the continuous stability function theory advanced by Gamble and co-workers (Gamble 1972; Gamble et al. 1970, 1973, 1980, 1983).

Researchers using the approaches described above have all claimed success in modeling humic-metal interactions. For other investigators interested in the study of trace metal speciation, the apparently equal success of these disparate models makes the choice of a humic binding model difficult. The relative merits of the continuous and the discrete approaches are not easily ascertained from the literature. Are distribution models more accurate or detailed in their representation of humic behavior than the discrete model? How easily are the models applied to experimental data? To address these questions, we critically examined the theory underlying models of metal-humic interactions. We then compared the discrete ligand model to the affinity spectrum and the normal distribution models by applying them to carefully selected data sets. The stability function model was not included for comparison because our analysis revealed that the method does not correctly derive an actual stability function. The values of the function obtained by this procedure are conditional stability constants that are properly applied only to discrete ligands, as discussed below.

We applied the discrete ligand model and the two continuous distributions models using the methods as published in the literature. We examined the results for accuracy and consistency, considered the effects of data error or artifact on the results, and tested each model for the special advantages that have been attributed to it. Finally, we developed recommendations for the selection and proper use of the appropriate model. Our intent was not only to compare model results but also to address the general problem of modeling a chemical system for which only limited data are available and for which no simple thermodynamic description is possible.

THEORY

Discrete Ligand Model

A solution of humic material may be considered to be a mixture of non-identical metal-binding molecules characterized by a wide range of affinities for metals. Although our definition includes the possibility of several binding sites on each humic molecule, in the models discussed here each site is assumed to behave independently of all others. Furthermore, while each site may have a unique affinity for a metal, we define the term "ligand" to refer to a class of molecules that have molar free energies of metal binding that are within some defined interval. Note that a class is simply a numerical concept and need not refer to a chemically distinguishable group of binding sites (Perdue and Lytle 1983a). We then characterize this class by a concentration, L_i, and a single conditional stability constant

$$K_i = (ML_i) \; / \; [M](L_i^f) \qquad [1]$$

where [M] is the concentration of free metal, (ML_i) is the concentration of metal bound to ligands of the ith class and (L_i^f) is the unbound-ligand concentration. A titration of this ligand with metal, M, can be represented at each point by the expression

$$\nu_i \equiv \frac{(ML_i)}{L_i} = \frac{K_i[M]}{1 + K_i[M]} \qquad [2]$$

For a titration of a mixture of ligands the macroscopic binding parameter $\bar{\nu}$ can be expressed

$$\bar{\nu} \equiv \frac{\Sigma \nu_i L_i}{\Sigma L_i} = \Sigma \left[n_i \cdot \frac{K_i[M]}{1 + K_i[M]} \right] = \frac{M_T - [M]}{L_T} \qquad [3]$$

where L_T is the total concentration of all ligands in solution and $n_i = L_i/L_T$ is the mole fraction of ligands in the ith class. Note that the number of molecules included in each ligand class depends on how many ligands are used to represent the system. For a very large number of ligands, the stability constant of each will be the average of a narrow interval of individual binding strengths. For a small set of ligands, each stability constant will reflect the binding behavior of a wide range of molecules.

Ligands can be selected so that the metal titration curves of each will overlap and yield the smooth, relatively inflectionless curves typical of humic materials. Only a few ligands are generally required to achieve a good fit of experimental data. Techniques for selecting discrete ligands to fit the data are discussed below.

The minimum number of ligands required to fit titration data can be determined by examining the effect on the quality of fit of increasing numbers of ligands or by employing a statistical significance test such as the the F-test (Sunda and Hanson 1979). The results of such tests nearly always indicate that two to four ligands are needed to model metal titrations of humic materials and the number of ligands is correlated to the amount of metal binding observed. Based on these observations we found that the optimal number of ligands can be estimated as one ligand for each order of magnitude of bound metal concentrations observed in the titration data. For example, if a Cu(II) titration begun at 10^{-7}M Cu_T (all Cu(II) is ligand-bound) exhibits only inorganic binding above 10^{-4}M Cu_T, the concentration of bound metal ranges over three orders of magnitude and three discrete ligands will be required to model the whole range of data.

This convenient rule of thumb was developed by noting that a plot of pM vs. -log M_T (where pM = -log [M]) for a humic titration has a slope that is always greater than unity until the endpoint is approached. For a single ligand the slope of a plot of pM vs. -log M_T approaches unity when either the ligand is nearly saturated with metal ($(ML_i)/(L_i^f) \gg 1$) or when the free ligand predominates ($(ML_i)/(L_i^f) \ll 1$). The former condition will position the weakest ligand in relation to the observed endpoint of the titration. The latter condition, however, indicates that for a set of discrete ligands, (L_i^f) can never greatly exceed (ML_i) and still reproduce the slope of the humic titration curve. In the concentration ranges typical of such a titration, we found that this condition can be met if $(ML_i)/L_i^f \geq 0.1$, that is, if the amount of bound ligand is never less then about 10% of the strongest ligand in the system. Thus,

for every tenfold increase in the observed bound-ligand (or bound-metal) concentration, an additional ligand will be needed to reproduce the titration results.

Continuous Distribution Models

As discussed above, the complexity of humic materials suggests that humic-metal interactions are most correctly modeled by a large number of non-identical binding sites characterized by a continuous frequency distribution with respect to their apparent stability constants. Instead of defining a discrete set of stability constants and ligand concentrations, it can be assumed that K varies continuously and that ligand frequency distributions can be integrated over this variable. We define the variable of integration $\kappa = \log K$ in order to simplify notation and make a clear distinction between the discrete constants K_i and the differential variable κ. The discrete mole fraction n_i of Eq. 3 is replaced by the differential mole fraction $N(\kappa)d\kappa$ which is defined as the mole fraction of ligand molecules in the infinitesimal interval, $d\kappa$. Recalling that $K = 10^{\kappa}$, the macroscopic binding function (Eq. 3) can be transformed from a summation to an integral:

$$\bar{\nu} = \bar{\nu}([M]) = \int_{-\infty}^{\infty} N(\kappa) \frac{10^{\kappa}[M]}{1 + 10^{\kappa}[M]} d\kappa \qquad [4]$$

Evaluating the integral in Eq. 4 gives a continuous mathematical representation of the formation function, $\bar{\nu}$ vs pM. To evaluate this integral for any metal concentrations we must first know the distribution function $N(\kappa)$. Vice versa, we can solve this convolution integral for $N(\kappa)$ using experimental data which define $\bar{\nu}([M])$. In principle this can

be done by taking the Fourier transform and then the inverse Fourier transform of both sides of Eq.4 (Dzombak, in preparation).

Hunston (1975) determined $N(\kappa)$ by employing a second order approximation of the integral in Eq. 4 based on the work of Ninomiya and Ferry (1959). The approximation involves a change of variable in which the derivative of the formation function, $d\bar{\nu}$ / dpM, is evaluated assuming that κ = pM. As a result of this assumption, the density function obtained does not depict the mole fraction of ligands at each κ but is instead a measure of the proportion of metal binding that could be attributed to ligands with κ = pM. This is a fundamental distinction because the value of $\bar{\nu}$ at each pM value is determined not only by a ligand with κ = pM, but by all ligands with nearby values of κ.

Put simply, the integral approximation offered by Hunston imposes a κ at each pM and determines its apparent contribution to $\bar{\nu}$, even though all metal binding at that pM may be due to nearby κ values. Whether a ligand truly exists at a particular log K is not determined and a continuous spectrum is obtained even when the actual ligands are discrete. The result of the Hunston procedure is not necessarily the actual distribution of binding sites but is instead an "affinity spectrum." An affinity spectrum represents the relative contribution made by a hypothetical (and imposed) log K to the formation function at that pM value. In essence, $N(\kappa)$ is obtained by numerically differentiating the experimental formation function and the peaks in the spectrum correspond to inflections in the titration curve. Each peak in the spectrum indicates the most probable κ (log K) controlling metal binding in that titration region. The area under the peak determines the concentration of a single ligand which will reproduce that portion of the

formation function when assigned the peak log K. The affinity spectrum procedure for identifying discrete ligands was employed by Shuman et al. (1983) in a study of Cu(II) binding to humic materials.

Because the metal binding curves of individual ligands overlap and tend to smooth out inflections in the formation functions, only a few discrete ligands can be identified by the affinity spectrum. For a highly complex mixture of ligands the method yields only those stability constants and ligand concentrations that will reproduce the formation function. Accordingly, we would not expect the affinity spectrum to identify more than about three predominant ligands over the typical titration range. The results of Shuman et al. (1983) appear to confirm this expectation. In that work an affinity spectrum of Cu(II) binding to estuarine humic material revealed three predominant binding regions, although small sub-peaks could be distinguished in each of the main affinity peaks.

Thakur et al. (1980) have shown that the affinity spectrum method can resolve discrete ligands only when their affinities differ by about a factor of 100, and that ligands present in very low concentrations are difficult or impossible to detect. Thus, for metal-humic binding data in which the observable range of log K (= pM) is approximately 4 to 11, four ligands are the maximum that can be detected. However, because L_i values for humic ligands with log K > 8 are very small, any peak that occurs in the range log K = 8 to 11 will be difficult to detect.

As an alternative to solving Eq. 4 for the actual distribution of ligands, an analytical form for the distribution can be assumed. The distribution imposed can be any type that is convenient and that provides a fit of the titration data. A normal distribution has been employed for

this purpose in many studies and was first developed by Pauling et al. (1944) as a quantitative description of the distribution of affinities in antibodies. It was used in subsequent studies of antibody binding (as reviewed by Karush 1962) and also to describe the binding of organic anions by protein (Karush and Sonenberg 1949). More recently, the model has been applied to describe proton and metal binding by humic substances (Posner 1966; Perdue and Lytle 1983a,b).

Following the derivation of Perdue and Lytle (1983a,b), the normally distributed mole fractions of ligands can be described by

$$N(\kappa) = \frac{L_i}{L_T} = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{1}{2}\left(\frac{\mu - \kappa}{\sigma}\right)^2\right] \qquad [5]$$

where μ is the log of the mean binding constant, K_o. This expression can be substituted in Eq. 4 to yield

$$\bar{\nu}_{calc} = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{\infty} \frac{[M]\ 10^{\kappa}}{1 + [M]\ 10^{\kappa}} \cdot \exp\left[-\frac{1}{2}\left(\frac{\mu - \kappa}{\sigma}\right)^2\right] d\kappa \qquad [6]$$

The integral in Eq. 6 can be evaluated numerically for any value of [M], given values of μ and σ. Karush and Sonenberg (1949) showed that when $pM = \mu$ in Eq. 6, $\bar{\nu} = 0.5$ for all values of σ. Hence the mean binding constant K_o ($\mu \equiv \log K_o$) can be obtained directly from a formation function plot as the reciprocal of the value of [M] at which the ligand system is half-saturated. The value of σ that provides the best fit for a particular $\bar{\nu}$ vs. [M] data set can be determined by matching experimental results with a family of theoretical binding curves (Karush and Sonenberg 1949; Karush 1962). Alternatively, both μ and σ

can be treated as fitting parameters and the inverse problem (determining optimal μ and σ values for a set of data) can be solved by means of nonlinear regression techniques (Perdue and Lytle 1983b).

Perdue and Lytle (1983b) were able to model Cu(II) binding to aquatic humus with a single normal distribution but found that a description of proton binding required a bimodal distribution. Note that the single-mode normal distribution model is a three fitting- parameter model (μ, σ, L_T) while the bimodal model is a six parameter model.

The continuous stability function theory developed by Gamble et al. (1972, 1980) is also based on the assumption of a continuous distribution of binding strengths and purports to solve the fundamental convolution integral (in fact, Eq. 4 multiplied by 1/[M]) by differentiating with respect to the total unbound ligand concentration. This is of couse not possible in general and is achieved in the derivation of the theory by changing variables and considering the differential unbound ligand concentration to be a function only of the total unbound ligand concentration. For example, in our notation one would consider $N(\kappa)/(1 + 10^{\kappa}[M])$ to be a function of $\bar{\nu}$ only and not a function of κ (see Eq. 4). This reduction from two variables to one is similar to the affinity spectrum approach; it assumes implicitly that the differential ligands are titrated one at a time. Interestingly enough, the parameter extraction procedure recommended from the continuous stability function theory amounts to the extraction of <u>discrete</u> constants and is formally similar to the Scatchard method (compare Gamble et al. 1980, Fig. 5, with the discussion of the Scatchard method below).

Data Precision and Model Results

The precision with which model parameters can be determined is directly related to the precision of the data used to calibrate the model. Furthermore, the precision of analytical measurements is not constant throughout a titration and the errors propagated in the indirectly determined quantities may change dramatically as concentrations vary. The experimental data used in this study were obtained with an ion-selective electrode. The relative error in free metal concentrations measured by this device is approximately constant at all concentrations but the error in the indirectly determined bound-metal concentration increases sharply near the end of a titration. The results of a previous study of error in titration data (Fish and Morel 1984a) were used to quantify this effect. Synthetic data sets were also employed to study the effects of error on model behavior, as outlined below.

METHODS

Both experimental and synthetic metal-humic binding data were used for comparing the models. Experimental data were obtained from a Cu(II) titration of a fulvic acid while the synthetic data were generated using equilibrium calculations for a hypothetical system of six ligands at various total metal concentrations. By investigating the response of the models to data from a well-defined system we were able to infer how the models transform experimental humic titration data.

Data Set 1: Experimental Data

Experimental metal-humic binding data were obtained from a Cu(II) titration of a 5.0 mg-C/L solution of fulvic acid extracted according to the methods of Thurman and Malcolm (1981) from Grassy Pond in Bedford, Massachusetts. Grassy Pond fulvic acid has been used in several other studies of trace metal binding (Fish and Morel 1984a,b) and iron photochemistry (Waite and Morel 1984). The solution was maintained at pH 6.0 and copper was added in increments from an initial concentration of 1×10^{-7} M up to a final concentration of 1×10^{-4} M. The cupric ion activity was measured with a Radiometer Selectrode F-3000 cupric-ion selective electrode. Details of the methodology are discussed by Fish and Morel (1984a); results of the titration are presented in Fig. 1.

In Fig. 2 the experimental data are replotted in terms of bound Cu(II) vs. - log Cu_T for which bound Cu(II) was determined by subtracting the cupric ion activity and calculated hydroxo and carbonato Cu(II) complexes from the total Cu(II) concentration. The total ligand concentration, L_T, for the experimental data was estimated to be 20 μM (Fig. 2), corresponding to the maximum observed concentration of bound Cu(II). It is important to note that the estimate for L_T obtained with this commonly used procedure is very imprecise because of the large error associated with bound-metal concentrations determined by the ion-selective electrode at high M_T.

The experimental data were also transformed into a formation function by normalizing the bound-metal data to L_T and plotting $\bar{\nu} = (CuL)/L_T$ against pCu (Fig. 3).

Fig. 1. Discrete-ligand fitting of ion-selective electrode titration results; pCu vs. total Cu(II) added. Grassy Pond fulvic acid, 5.0 mg-C/L at pH 6.0 (●); three-discrete-ligand model (———); synthetic data(·····).

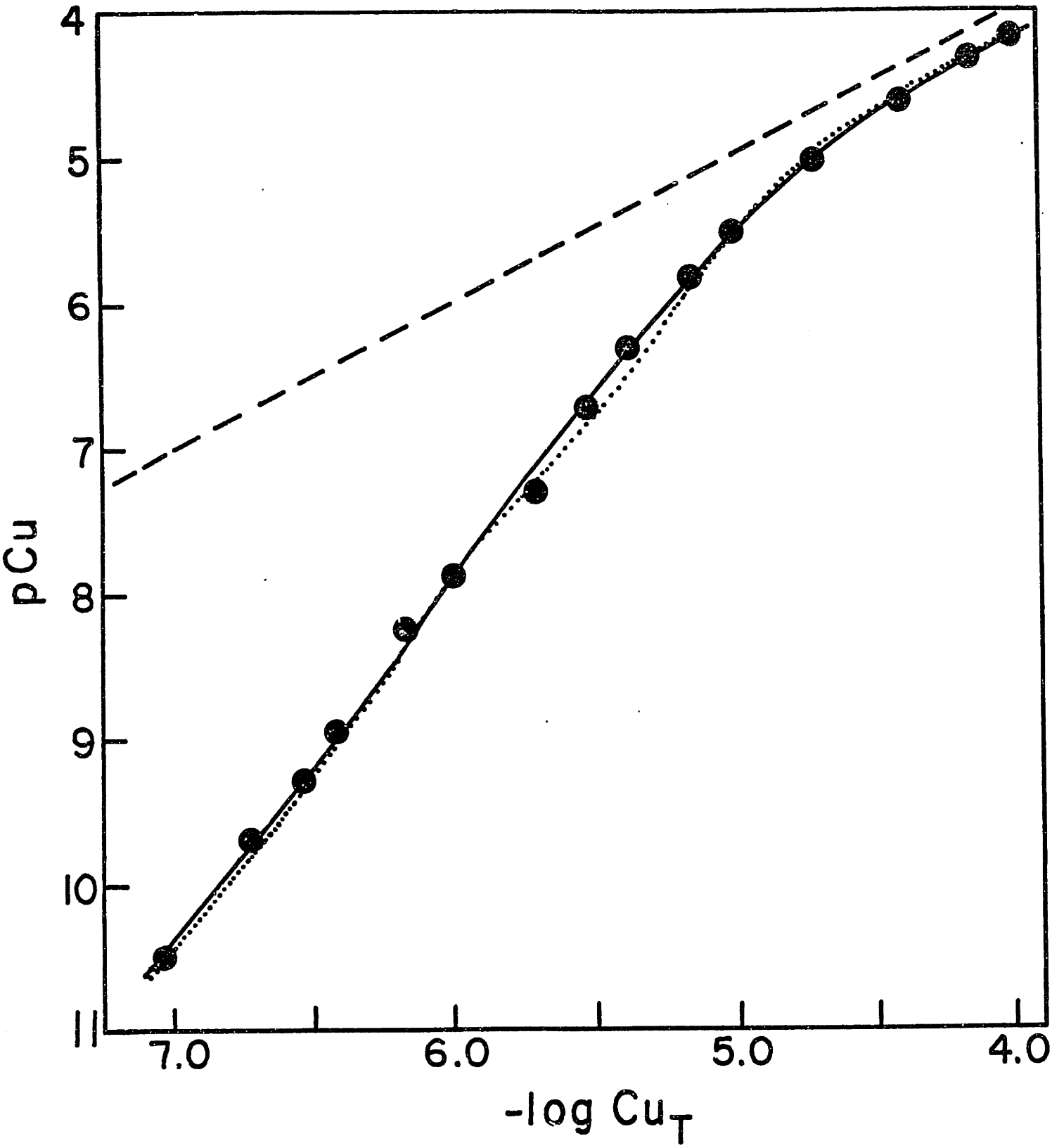

4
5
6
7
8
9
10
11
pCu
7.0
6.0
5.0
4.0
-log Cu$_T$

Fig. 2. Concentrations of bound metal vs. -log(total metal) for experimental data (○) and the truncated synthetic data (△).

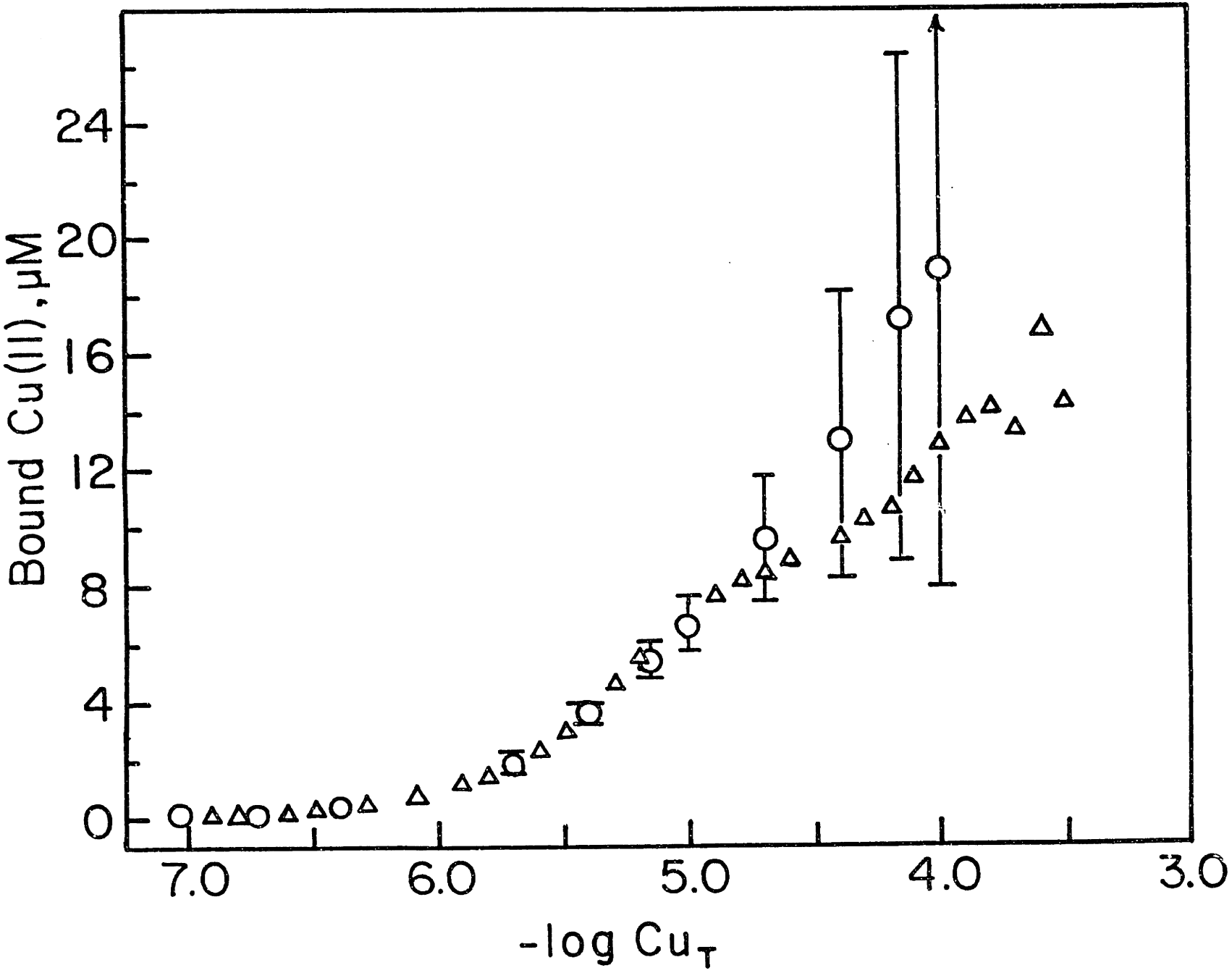
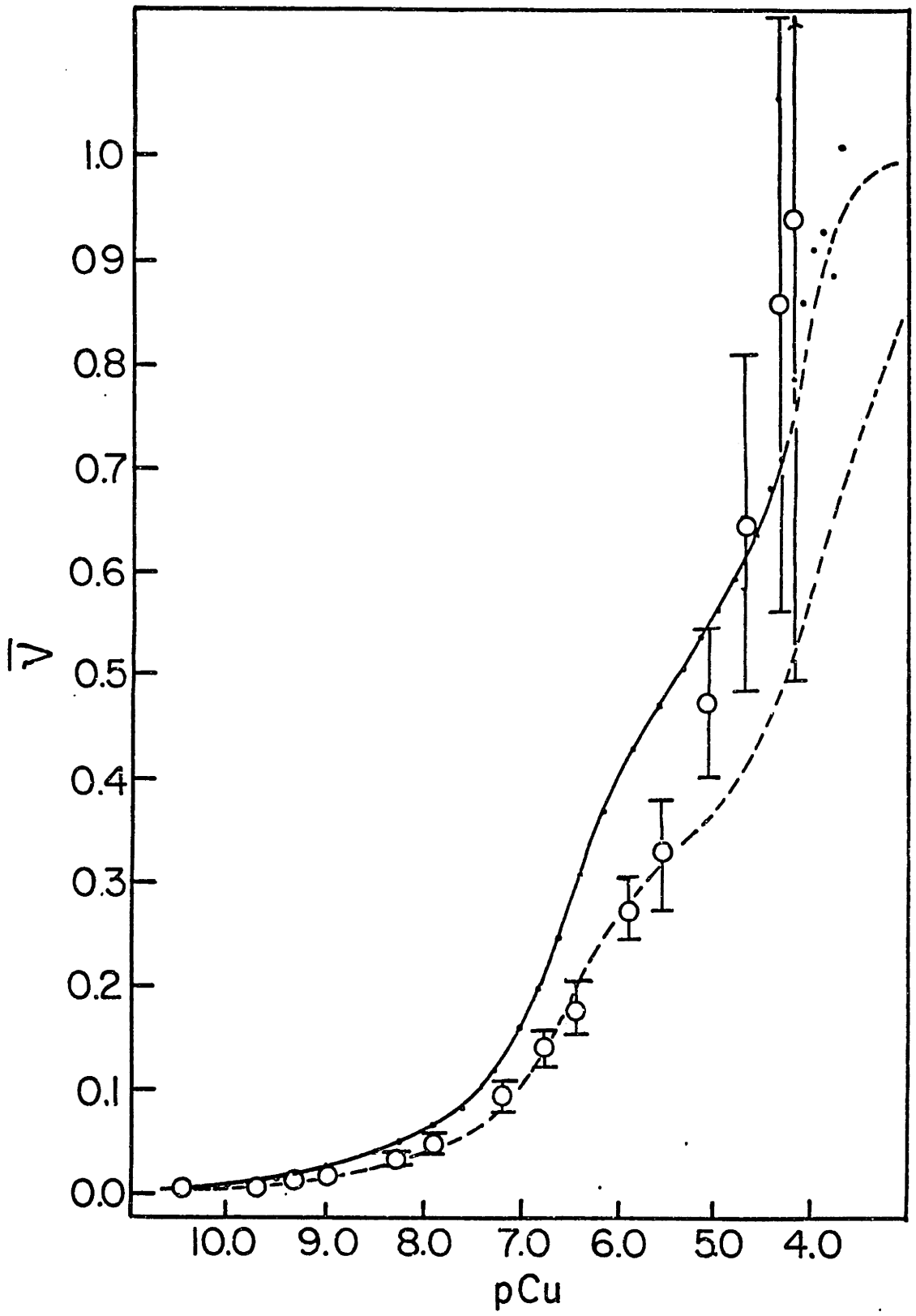

Bound Cu(II), μM
0
4
8
12
16
20
24
7.0
6.0
5.0
4.0
3.0
-log Cu$_T$

Fig. 3. Formation functions ($\bar{v}$ vs. $-\log[Cu^{2+}]$) for synthetic data (--), truncated synthetic data (——) and experimental data (○).


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
r
10.0
9.0
8.0
7.0
6.0
5.0
4.0
pCu

Data Set 2: Synthetic Data

A set of synthetic metal-humic binding data was generated by calculating the equilibrium speciation of a hypothetical six-ligand system (Table 1) at various total metal concentrations. The stability constants and concentrations of the ligands were selected so that the computed titration curve (see Fig. 1) was similar to the experimental Cu(II) titration of Grassy Pond fulvic acid. Calculations were performed with the chemical equilibrium program MINEQL. The advantage of a synthetic data set based on a well-defined multi-ligand system is that the chemical interactions responsible for the observed metal binding are completely understood. For real titration data nothing is known of the underlying chemical speciation.

Although the number of ligands in the hypothetical system was arbitrary, we selected the concentrations and stability constants listed in Table 1 for specific reasons. In order to have the synthetic titration plot resemble the experimental titration plot, we selected the parameters for three "backbone" ligands by means of a Scatchard fit of the experimental data (a method described below). These are ligands 2, 3, and 4 in Table 1. Three more ligands were added to the system to examine particular capabilities of the continuous models. A strong ligand at low concentration ($\log K_1 = 15.0$, $L_1 = 1 \times 10^{-10}$M) was added to test the sensitivity of the models to small amounts of strong binding sites suspected to exist in humic materials but not experimentally verified. A large quantity of weak ligand ($\log K_5 = 4.0$, $L_5 = 1 \times 10^{-5}$M) was added to reflect the observed weak binding region. Finally, an even weaker ligand was added ($\log K_6 = 3.0$) but at a somewhat smaller concentration ($L_6 = 5 \times 10^{-6}$M) than the next weakest ligand. This ligand was

TABLE 1. Stability constants and ligand concentrations for discrete-ligand data sets.

		1	2	3	4	5	6	L_T(μM)
Three-ligand set: Scatchard Method	log K_i	-	10.83	8.80	6.48	-	-	
	log L_i	-	-6.95	-6.17	-5.17	-	-	7.55
Six-ligand set: Synthetic Data	log K_i	15.0	10.8	8.8	6.5	4.0	3.0	
	log L_i	-10.0	-7.0	-6.2	-5.2	-5.0	-5.3	22.1
Three-ligand set: FITEQL	log K_i	-	10.72	8.29	5.94	-	-	
	log L_i	-	-6.84	-5.87	-5.04	-	-	10.6

added to test the detectability of smaller concentrations of ligands symetrically distributed around a "peak" log K.

The formation function for the synthetic data is plotted in Fig. 3. Computed bound-metal concentrations were normalized with respect to the known L_T for the hypothetical six-ligand system. Note that at pM = 4, L_T is only slightly over half saturated for the synthetic data, whereas the formation function for the experimental data indicates complete saturation. This is because the two data sets are normalized to different values of L_T. For the experimental data, complete saturation at the upper limit of the titration (pM = 4) was assumed in determining L_T.

Data Set 3: Truncated Synthetic Data

To evaluate the sensitivity of the models to measurement error, we imposed limits on the degree of accuracy of the synthetic data by extracting only the total metal concentrations (to three significant digits) and the logarithms of the free metal activities (to two significant digits in the mantissa). These are the data and the accuracy obtainable from a metal-humic titration, as monitored by an ion-selective electrode, and we treated these extracted data as we did the experimental data. Bound metal concentrations were computed by subtracting the free metal activities from the corresponding total metal concentrations, and L_T was estimated to be 15 μM from the plot shown in Fig. 2. The bound-metal concentrations were normalized to L_T and the formation function plotted (Fig. 3). Note that the variability evident in Figs. 2 and 3 for the truncated synthetic data is due only to roundoff error in the free metal activities and the resulting propagation of error in the bound-metal concentrations.

RESULTS AND DISCUSSION

Discrete Ligand Model

A number of methods exist for selecting discrete ligands to reproduce titration data. There are graphical linearization methods, and linear or non-linear numerical optimization schemes. Linear graphical techniques are easier to implement than parameter optimization procedures which require computerized solution methods. However, graphical methods, particularly linear ones, can yield erroneous ligand parameters when applied to non-linear, multi-ligand systems.

The most commonly applied graphical method is a linearization of titration data introduced by Scatchard (1948) for use in studies of ion-binding by proteins. Rearrangement of Eqs. 2 and 3 leads to the formula

$$\frac{\bar{\nu}}{[M]} = \Sigma(n_i K_i) - \sum_i (K_i \bar{\nu}) \qquad [7]$$

so that a plot of $\bar{\nu}/[M]$ vs. $\bar{\nu}$ for one ligand has slope K_i, a vertical intercept equal to $\Sigma(n_i K_i)$ and a horizontal intercept Σn_i. For more than one ligand the Scatchard plot assumes a hyperbolic shape that can be divided into two or more quasi-linear regions. For i = 2 the two slopes and the horizontal and vertical intercepts yield four known quantities, enabling an analytical solution of Eq. 7 for the four unknowns. In order to obtain the best fit of the data, more than two ligands are frequently required. For more than two ligands, the number of unknowns exceeds the number of measurable quantities (there are always only two intercepts) and the solution is indeterminate. In this case, values of K_i and n_i can be obtained by dividing the Scatchard curve into three or more

quasi-linear regions and determining a slope for each by least-squares linear regression. The values of n_i are then determined by trial and error such that the conditions

$$\Sigma\, n_i = \bar{\nu}_{max} \qquad \Sigma(n_i K_i) = (\bar{\nu}/[M])_{max} \qquad [8]$$

are met. Examples of this approach are given by Sunda and Hanson (1979) and Tuschall and Brezonik (1983).

Linearized graphical methods such as the Scatchard plot are subject to two major sources of errors. The first is the error inherent in fitting a line to non-linear data, a problem that results in standard errors for the slope and intercept (see for example Bevington 1969). The second and greater problem is that of equally weighting all data points, regardless of the fact that the relative error associated with the data varies throughout the titration. We demonstrated these problems by applying the Scatchard method to the experimental data set.

Three orders of magnitude of bound metal concentration were observed in the titration data so we estimated that three ligands would best fit the data. Values of K_i and n_i were selected as described above, yielding the set of constants in Table 1.

A calculated Cu(II) titration of these three ligands reproduces closely the experimental data in a plot of pCu vs. $-\log Cu_T$ (Fig. 1). However, the apparently small discrepancies in the pCu values at high Cu_T translate into large errors in the concentration of bound metal predicted in this range (Fig. 2). The deviation between model and experimental results can be explained by examining the amount of error associated with the points used to evalulate the parameters of the weakest ligand.

The Scatchard regression line is a best fit of all the data, including data points of very poor precision near the end of the titration. The scatter in the last few data points means that the slope of the regression line will be controlled by the more precise (hence more collinear) stonger-binding data points. The weakest binding has little influence on the regression line and is not reflected in the constants obtained. The uncertainty in the last few bound-metal data points translates directly into uncertainty in the parameters fitted to the weakest ligand.

Several other graphical techniques involving linear transformations have been applied for fitting discrete ligands to ion-binding data for macromolecules (Klotz and Hunston 1971). The problems outlined above are applicable to all such procedures although the difficulties in defining L_T may not be as severe for well-defined biological macromolecules as they are for humic substances.

Choosing discrete ligand parameters by optimization offers several improvements over graphical methods. A routine that optimizes all parameters eliminates the arbitrariness of dividing a non-linear curve into quasi-linear subsections. The parameters obtained by optimization yield the set of minimum residuals between experimental and model data and not simply a "reasonable" fit. The parameters so obtained are more useful than Scatchard parameters for comparing the results of different titrations because they are reproducible; i.e., a set of data always yields the same parameter values as long as the same optimization algorithm is employed.

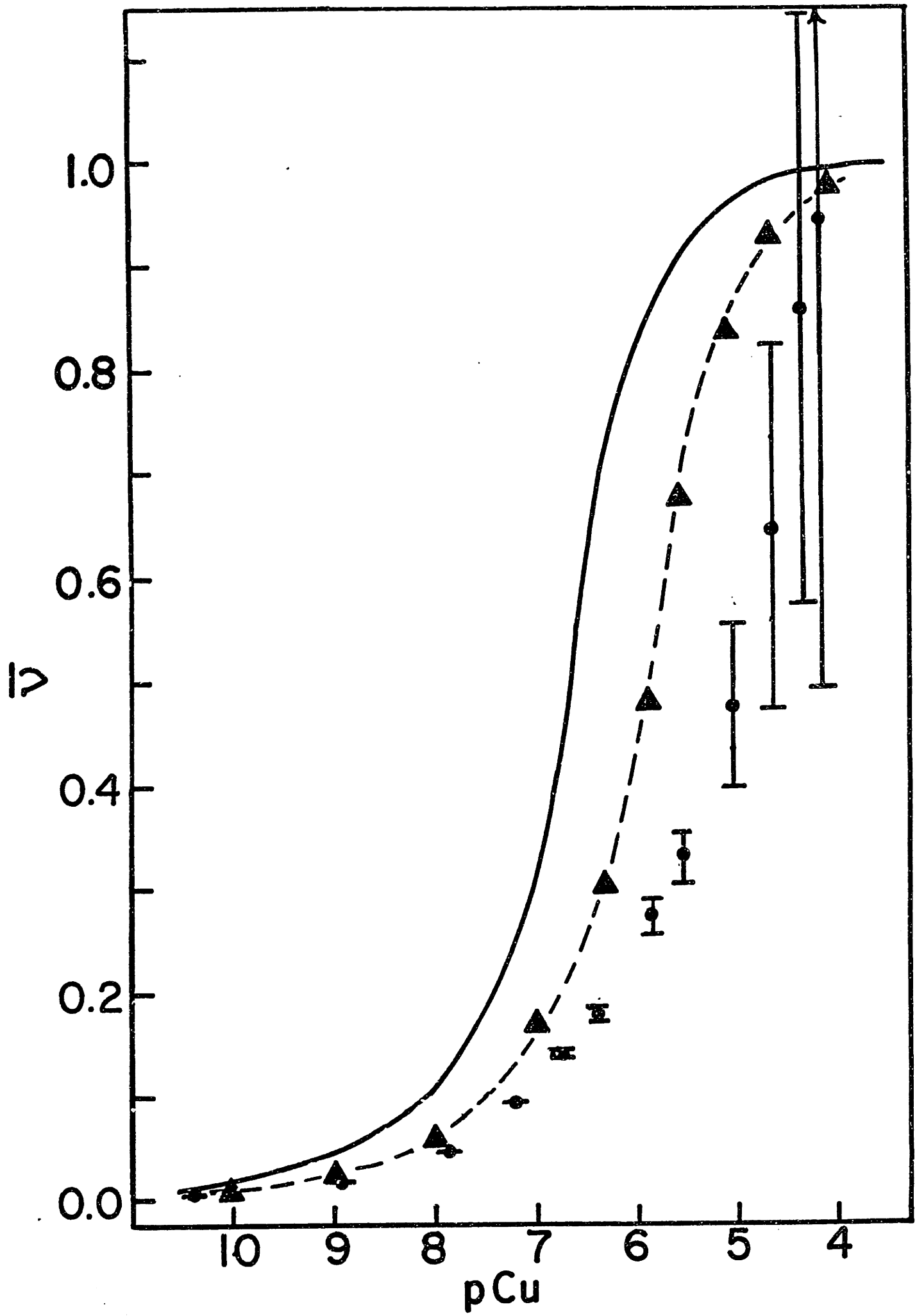
A second advantage of parameter optimization over graphical methods is the relative ease with which statistical aspects of the data can be

incorporated. An optimization program can automatically calculate error propagation, apply weighting factors to the data and calculate the standard error associated with each optimized parameter. Such data treatment is essential for correctly comparing the results of different titrations. Differences among results can be tested for statistical significance. The arbitrariness of graphical fitting methods precludes a quantitative evaluation of parameter error. Note however, that very weak binding may be ignored by an optimization procedure if the data are of low precision and are weighted accordingly.

We applied the discrete-ligand optimization program FITEQL (Westall 1982) to the experimental data and obtained an optimal set of stability constants and concentrations for three ligands (Table 1). The parameters for the first two ligands are very close to those obtained by the Scatchard method, a reflection of the precision of the data in the stronger metal-binding region. The weakest ligand is assigned a conditional stability constant by FITEQL that is about an order of magnitude smaller than the value we obtained from the Scatchard plot. The concentration of weak ligand is somewhat higher when determined by FITEQL than by the Scatchard plot.

The difference in fit between the two procedures is barely apparent in the pCu vs. -log Cu_T plot but is clearly distinguished in the formation functions in which the FITEQL-fitted data are closer to the experimental data (Fig. 4). The difference between the experimental, the FITEQL-based and the Scatchard-based formation functions is mostly attributable to a difference in L_T and not to differences in the bound-metal concentrations.

Fig. 4. Formation functions of $\bar{\nu}$ vs. pCu. Grassy Pond fulvic acid, 5.0 mg-C/L at pH 6.0 (●), error bars indicate ± one standard deviation; Scatchard, three-ligand fit of data (———); FITEQL three-ligand fit (– – –).


1.0
0.8
0.6
0.4
0.2
0.0
I>
10
9
8
7
6
5
4
pCu

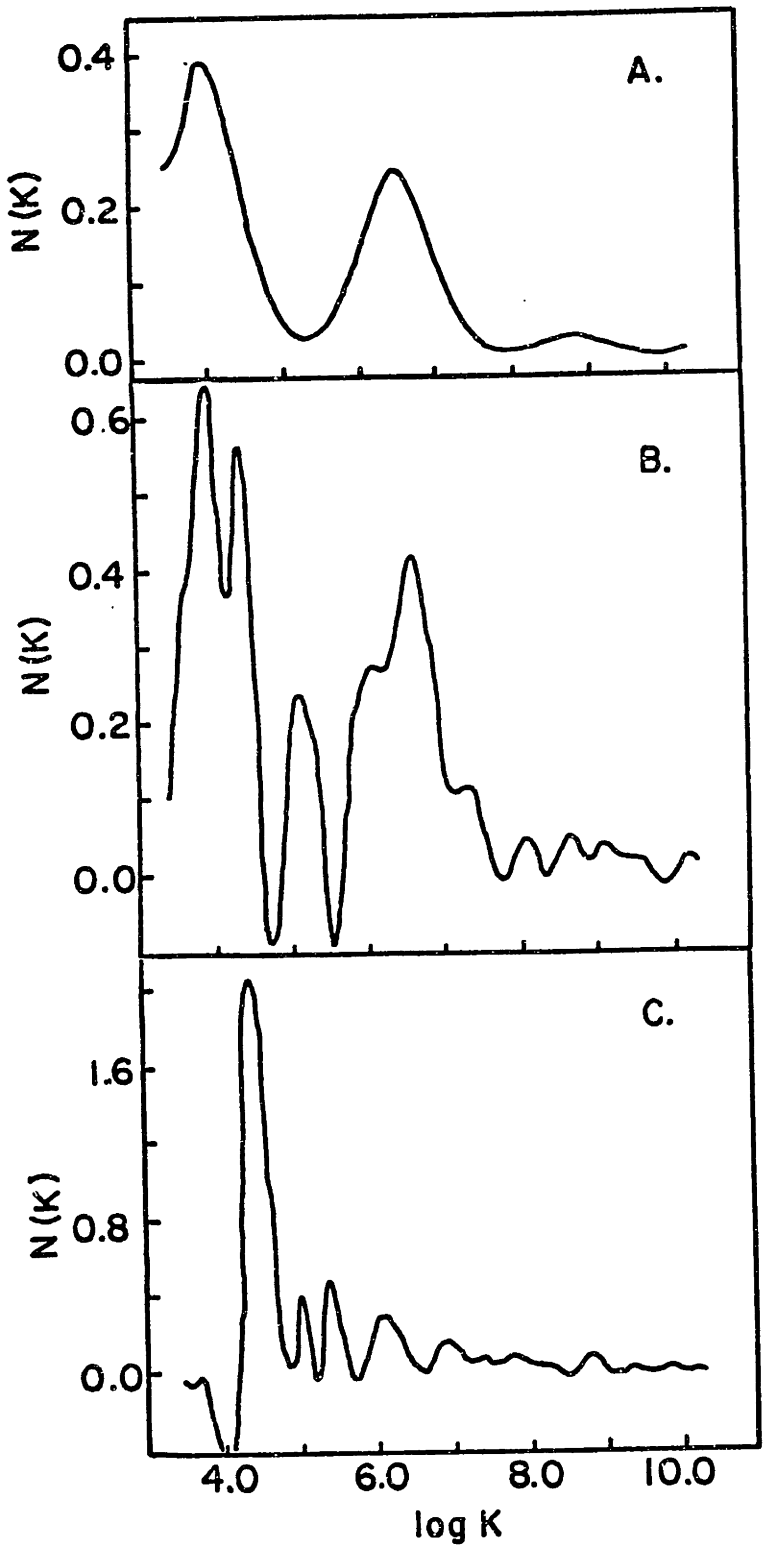
Note that all of the formation functions are normalized to L_T and that L_T is only operationally defined, either as the summation of ligand concentrations fitted to the data (for calculated data) or as the maximum amount of bound metal detectable in the titration data (for experimental data). Consequently, the three formation functions in Fig. 4 are normalized to different L_T values. The normalization that is intended to facilitate comparison among results actually makes comparisons difficult because L_T is so poorly defined. The only solution to this dilemma is to obtain the most precise data available for the weak-binding region of the titration so that L_T is determined with greater precision. Unfortunately the absence of a clear endpoint in most titations of humic material precludes an accurate value of L_T.

Affinity Spectrum Model

The affinity spectrum for each of our three data sets was obtained by smoothing the formation function data (from the plots in Fig. 3) with a cubic spline and using the smoothed data in a second-order solution of Eq. 4 after the methods of Ninomiya and Ferry (1959) as applied by Hunston (1975), Thakur et al.(1980) and Shuman et al. (1983).

The affinity spectrum for the synthetic data set exhibits three smooth and clearly defined peaks at log K = 8.75, 6.50 and 3.95 (Fig. 5a) corresponding to ligands 3, 4 and 5 (log K = 8.80, 6.48 and 4.00). Only three of the six ligands are identified because only these ligands affect the shape of the formation function (i.e., the metal speciation) between the realistically imposed activity measurement limits of pCu 10.40 to pCu 2.50. Stability constants are identified at pCu = log K, hence it was

Fig. 5. Affinity spectra for the synthetic data set (A.), the truncated synthetic data set (B.) and for experimental data (C.).


A.
B.
C.
N(K)
0.4
0.2
0.0
0.6
1.6
0.8
4.0
6.0
8.0
10.0
log K

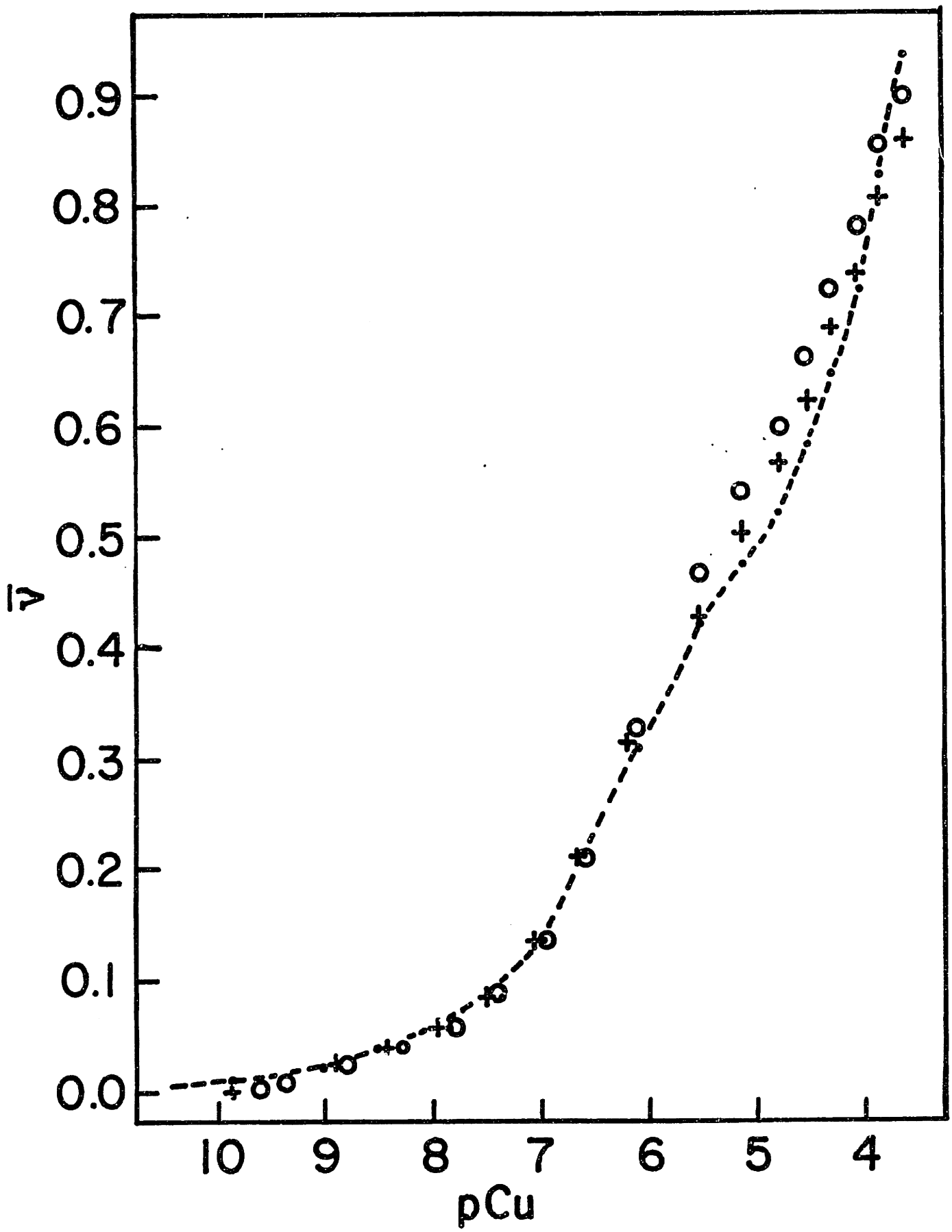
not possible to detect the strongest ligands (log K_1 = 15.0, log K_2 = 10.83) or the weakest ligand (log K_6 = 3.00).

The affinity spectrum for the truncated synthetic data shows a dramatic increase in the number of peaks compared to the six-ligand set on which it is based. At least twelve peaks or shoulders can be distinguished (Fig. 5b). Recall that the truncated synthetic data differ from the synthetic data only in the inclusion of the roundoff error typical of bound-metal data from ion-selective electrode titrations. This small error produces enough scatter to generate small inflections in the formation function and causes numerous spurious peaks in the affinity spectrum. Adjustments of the parameter that controls the sharpness of the peaks in the numerical integration method (Ninomiya and Ferry 1959) had negligible effect on the noise in the spectrum and adjusting the smoothing was of no value because we had no a priori knowledge of which inflections were artifacts and which were authentic.

Our knowledge of the components underlying the truncated synthetic data assisted us in sorting the numerous peaks into three groups corresponding to the predominant ligands. The mean log K and L_i values for each group are in reasonable agreement with the actual component ligands and yield a good fit of the formation function (Fig. 6). An improved fit of the data may be expected if a large number of ligands are assigned from the spectrum, thereby approximating a continuous ligand distribution. Dividing the spectrum into seventeen ligands greatly magnifies the computation necessary to calculate the speciation but improves the fit of the formation function only slightly (Fig. 6).

The impact of random error is especially problematic when analyzing experimental data, as seen in the affinity spectrum for the Cu(II)

Fig. 6. Formation functions for the truncated synthetic data set (---), the three-ligand, affinity spectrum fit (○) and the 17-ligand, affinity spectrum fit (+). Note: symbols represent continuous data; discrete points are used only for the purpose of clarity.


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
ν
10
9
8
7
6
5
4
pCu

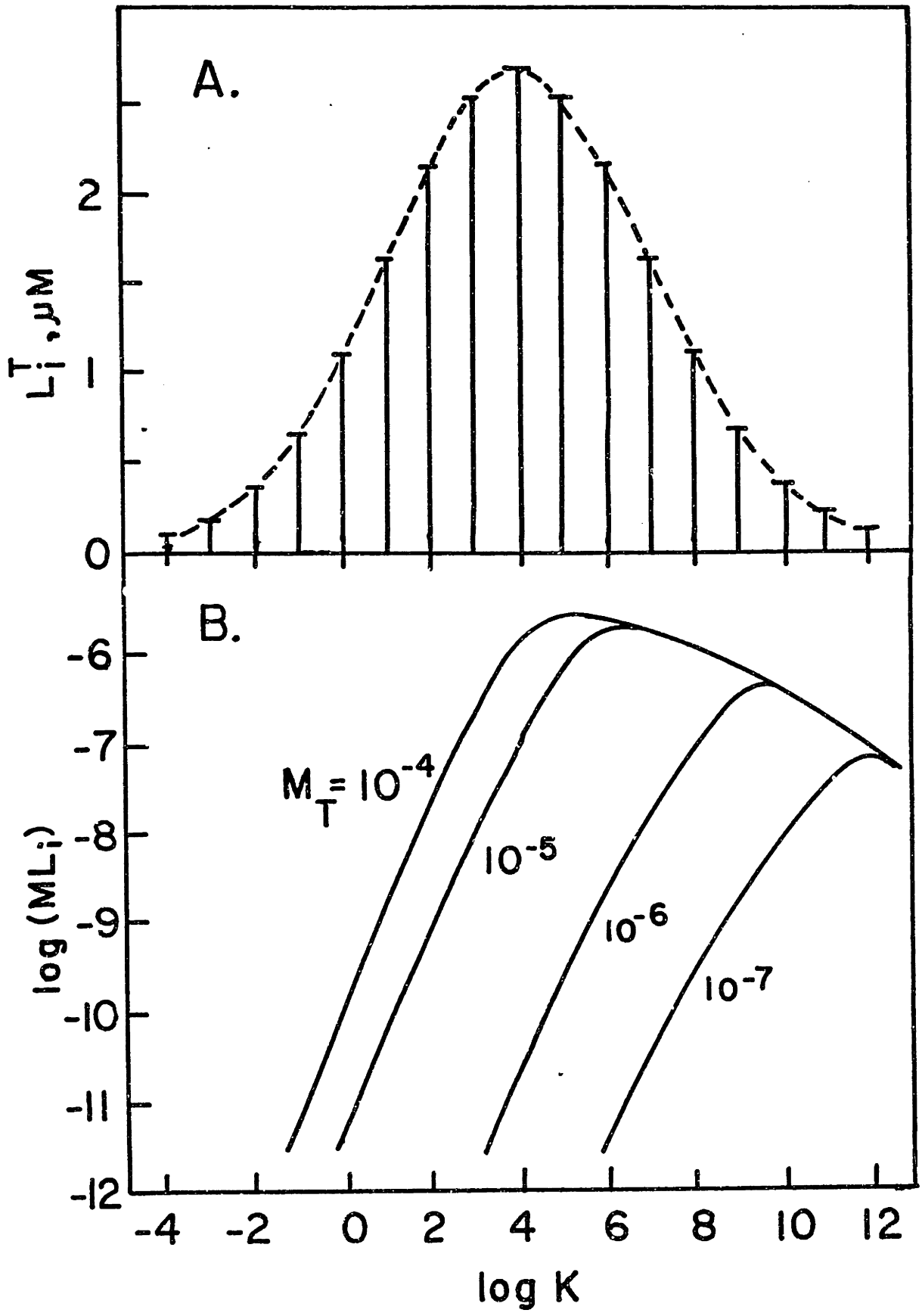
titration of Grassy Pond fulvic acid (Fig. 5c). Numerous small peaks appear but we cannot distinguish between authentic binding sites and the sort of artifacts observed in the truncated synthetic data spectrum. Again we are faced with a choice between a few ligands representing arbitrary groups of peaks or a large number of discrete ligands that are computationally unwieldly.

In principle, an affinity spectrum is a useful numerical method for selecting discrete ligands to fit titration data. A few ligands will suffice for most data sets and under ideal conditions the affinity spectrum will reveal them. Under experimental conditions, however, small, random errors cause fragmentation of the spectral peaks and the choice of discrete ligands becomes arbitrary.

The Normal Distribution Model

We applied the single-mode normal distribution model to the three data sets. To determine the optimal values of μ and σ for each data set, we employed the curve-matching technique of Karush and Sonenberg (1949; see also Karush 1962). The mean log K values were obtained directly from the formation functions in which μ (= log K_o) is given by the value of pM when $\bar{\nu} = 0.5$, as explained earlier. These μ values were then substituted in Eq. 6 and families of theoretical binding curves were computed for various values of σ. For each data set, the optimum value of σ was determined by comparing the theoretical binding curves with the actual binding curve. The theoretical curve providing the closest fit to the most precise binding data ($\bar{\nu} < 0.3$, approx.) was selected as optimal. In The theoretical binding curves computed for the truncated synthetic data are presented in Fig. 7. The optimal values of μ and σ determined for

Fig. 7. A) Seventeen discrete ligands normally distributed with respect to log K. B) Log concentration of metal bound to each of the seventeen normally distributed ligands. Bound metal distributions are shown for $M_T = 10^{-7} - 10^{-4}$.


A.
L_i^T, μM
2
1
0
B.
log (ML_i)
-6
-7
-8
-9
-10
-11
-12
$M_T = 10^{-4}$
10^{-5}
10^{-6}
10^{-7}
-4
-2
0
2
4
6
8
10
12
log K

the three data sets with the curve matching technique are listed in Table 2.

The fit obtained for the truncated synthetic data is similar in quality to those obtained for the other data sets. In accord with the original fitting objective, the more precise low $\bar{\nu}$ data were fitted more closely than the less precise high $\bar{\nu}$ data. Note that in fixing $\mu = \log K_o$ the pivot point of the family of theoretical curves is also fixed. The location of this pivot point strongly influences the quality of fit achievable. Given the size of the error bars associated with the experimental binding data at $\bar{\nu} \cong 0.5$ (e.g., see Fig. 3), and hence the range of possible values for μ, improved fits might be possible by treating both μ and σ as adjustable parameters as did Perdue and Lytle (1983b). However, it is interesting to note that the optimum μ values obtained by Perdue and Lytle for two data sets are identical to the pM values for $\bar{\nu} = 0.5$ in their corresponding formation function plots.

The optimum normal distribution of log K values for the truncated synthetic data set has $\mu = 5.35$ and $\sigma = 1.5$. As expected, the distribution reflects the large concentrations of weak ligands in the hypothetical six ligand system near log K = 4 - 5 and the successively smaller concentrations of strong ligands. Since no ligand in the hypothetical system has a stability constant less than 10^3, a question arises as to the meaning of the left hand (low log K) side of the distribution. Comparing the known, six-ligand distribution used to create the truncated synthetic data with the shape of the normal distribution of log K's that enables a fit of these data, it seems that only the high log K (right hand) side of the distribution influences metal binding.

TABLE 2. Optimal parameters for the single-mode normal distribution fitted to three data sets.

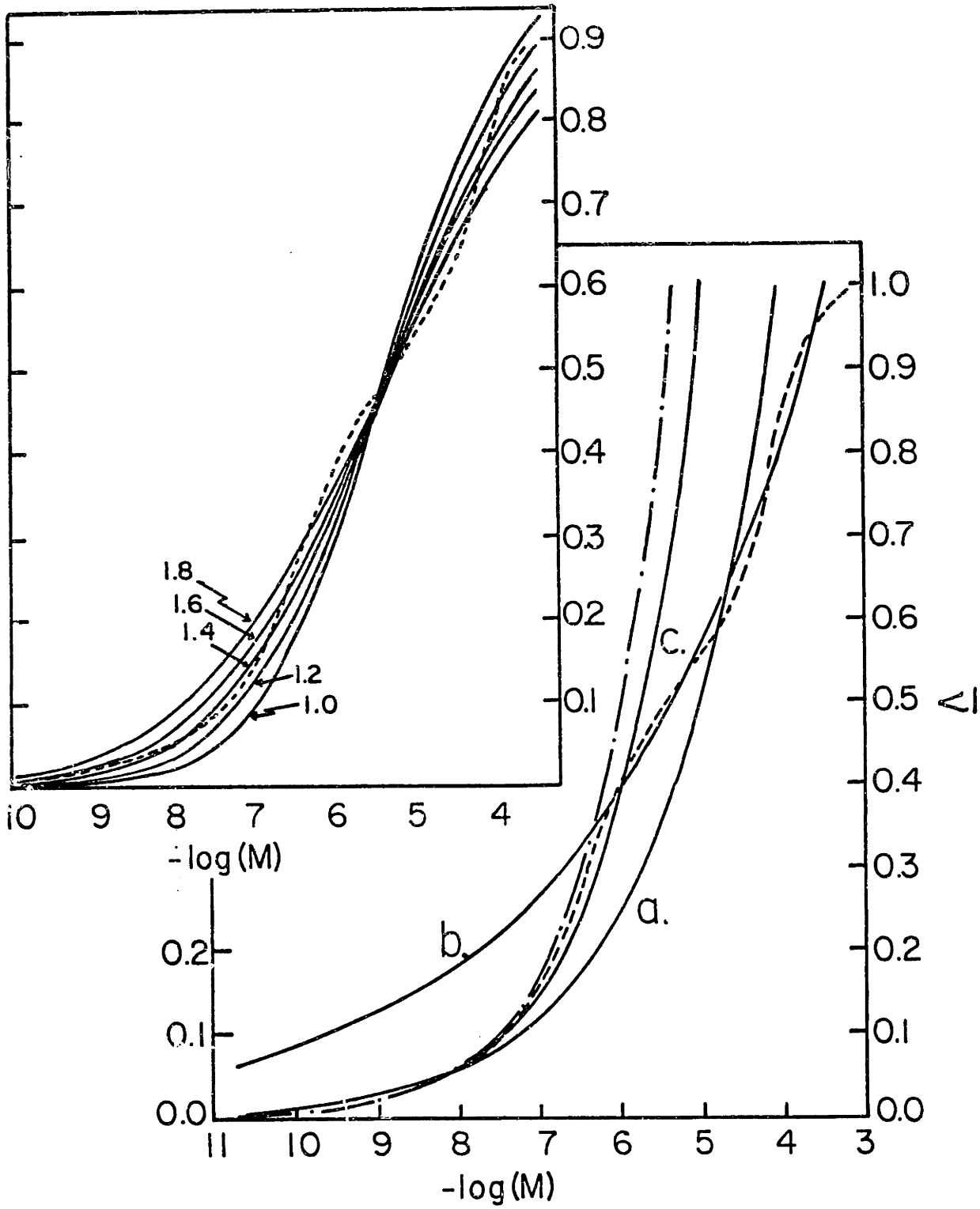
	Experimental	Synthetic	Truncated Synthetic
$\mu = \log K_o$	5.0	4.25	5.35
σ	1.6	2.2	1.5

To demonstrate the dominance of the high log K side of a normal distribution on the predicted metal speciation, we considered a hypothetical system of 17 ligands normally distributed in log K. The concentrations were selected assuming $\mu = 4$, $\sigma = 3$ and $L_T = 2 \times 10^{-5}$ M; the resulting normal distribution is depicted in Fig. 8a. We calculated the metal speciation in this system for several concentrations of M_T, using MINEQL. When the concentration of metal bound to each ligand is plotted as a function of their stability constants, we observe that most of the metal is bound only to the strong ligands; i.e., those with log K > μ (Fig. 8b). The maximum amount of metal binding occurs with ligands in the high log K tail of the distribution and this maximum shifts to stronger ligands as M_T decreases. The latter effect is due to the increasing influence of the strongest ligands as M_T approaches their (small) concentrations. We confirmed the dominance of the strong side of the normal distribution by reproducing <u>exactly</u> the results shown in Fig. 8b using only the 9 ligands to the right of and including the mean. We concluded that only the high log K side of a normal distibution of binding sites has any effect on metal speciation for the range of M_T considered here.

These results can be understood by noting that ligands are titrated in order of decreasing values of the product K_iL_i. At any point in a titration, additional metal will always bind to the available ligand that has the largest value of K_iL_i. The ligand with the next largest K_iL_i will not pick up significant metal until the previous ligand is nearly saturated. For the 17-ligand distribution depicted in Fig 8a, the order of the product K_iL_i is the same as the order of log K_i, hence the strongest ligands bind most of the metal at all values of M_T. At pM = 4,

Fig. 8. Upper left: Family of curves (solid lines) used to find normal distribution parameters by matching with the formation function of the truncated synthetic data set (– – –). Value of μ is fixed as described in the text. Trial values of σ are 1.0, 1.2, 1.4, 1.6 and 1.8.

Lower: Comparison of formation functions obtained with the Sips method. Truncated synthetic data (– – –); optimal fit from family of curves (— - —). Fits obtained from linearization method: regression of all data (a); regression of weak binding data (b); regression of strong binding data (c).


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1.8
1.6
1.4
1.2
1.0
10 9 8 7 6 5 4
-log (M)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a.
b.
c.
11 10 9 8 7 6 5 4 3
-log (M)
<1

the ligand at the apex of the titration (log K = 4) is still only half saturated and weaker ligands are almost completely unbound. The weaker ligands can only start to bind metal when the free metal is in great excess of the apex ligand concentration. Since the weaker ligands have smaller concentrations than the apex, the free metal will be in great excess of the amount that they can bind. Thus, the weak ligands beyond the apex can have no measurable effect on the amount of free metal in solution.

In summary, our analysis of the normal distribution model shows that a single, relatively narrow mode centered at relatively low log K is adequate to describe metal-humic binding. If the strong-binding (low M_T) portion of a titration curve can be fit, a reasonable fit of the weak-binding (high M_T) data will follow. The success of using a single mode means that the normal distribution model is an accurate three-fitting-parameter model for humic-metal binding. We have also demonstrated that only the high log K side of a normal distribution of binding sites has any influence on humic-metal binding. A normal distribution that enables a fit of metal binding data does not necessarily represent the actual distribution of ligands that produced the observed binding.

We reduced the difficulty of extracting constants for the normal distribution from experimental data by employing a modified version of the model. The approximation, first introduced by Nisonoff and Pressman (1958) to describe the heterogeneity of antibodies, evolved from the use of the normal distribution model by Pauling, Karush and others. Instead of describing the spectrum of antibody affinities with a normal distribution, Nisonoff and Pressman used a distribution function derived by Sips

(1948) which closely resembles a normal distribution but has the advantage that it can be integrated analytically.

Sips' original work was applied to the adsorption of gases on solids, but his derivation is also applicable to association equilibria in solution. Sips began with a modified Freundlich isotherm expression for binding. In metal-humic binding terms, this expression is

$$\bar{\nu} = \frac{K_s[M]^a}{1 + K_s[M]^a} \qquad [9]$$

where K_s and a are constants.

By means of statistical-mechanical arguments, Sips (1948) showed that the binding expression in Eq. 9 corresponds to a distribution "astonishingly similar to a Gaussian distribution." The Sips distribution in terms of humic-metal binding is given by

$$N(K) = \frac{1}{\pi} \frac{(K_s/K^a)\sin(\pi a)}{1 + 2\cos(\pi a)(K_s/K^a) + (K_s/K^a)^2} \qquad [10]$$

The distribution represented by Eq. 10 has a quasi-Gaussian shape with an apex at $K_o = K_s^{1/a}$ or $\log K_o = \frac{1}{a} \log K_s$ (from solving $N'(K) = 0$). K_o is thus an average equilibrium constant and analogous to the mean of a normal distribution. The index of heterogeneity, a, ($0 \leq a \leq 1$), is analogous to the standard deviation. When a = 1, corresponding to identical sites, Eq. 9 reduces to a simple mass law expression.

It can be shown from Eq. 9 that for $\bar{\nu} = 0.5$, $\log K_s = a(pM)$; and since $\log K_o = pM$ when $\bar{\nu} = 0.5$, $\log K_s = a(\log K_o)$. The parameters K_s

and a can be determined by changing Eq. 9 into logarithmic form and substituting for log K_s to obtain

$$\log \bar{\nu} = a\,[\log [M] + \log K_o] \qquad [17]$$

Eq. 11 contains only one fitting parameter (a) since log K_o can be obtained directly from a formation function plot (as the value of pM for which $\bar{\nu} = 0.5$). A family of theoretical binding curves can be hand calculated for various values of a, and the optimum value ascertained by matching the curve with experimental results. The optimal fit obtained in this way for the truncated synthetic data is shown in Fig. 9.

Alternatively, both K_s and a can be treated as adjustable parameters and a graphical technique can be used to determine their values. For a linear plot of log $\bar{\nu}$ against log [M], the index a is given by the slope and K_s by the intercept. However, log $\bar{\nu}$ vs. log [M] plots for metal-humic binding typically comprise two linear regions, one in the high M_T (weak binding) region and one in the low M_T (strong binding) region. The regression for the low M_T data provides the best fit of the most precise portion of the formation function, i.e., the low $\bar{\nu}$ data. As shown in Fig. 9, this fit is slightly better than the fit obtained with the curve matching technique. Both methods result in a single mode centered at a relatively low log K.

The Sips Distribution model is convenient since it can be implemented without the aid of a computer. The three fitting parameters a, K_o and L_T can all be determined graphically and are used in a simple analytical binding expression (Eq. 11) that can adequately describe metal-humic binding phenomena.

CONCLUSIONS

The discrete ligand approach to modeling humic-metal interactions has been widely used because it is a simple and accurate means of reproducing titration data. Ligand parameters (K_i and L_i^T) can be fitted to the data by graphical techniques, such as the Scatchard plot, but such linearizations can lead to substantial errors in the parameters fitted. Non-uniform errors and error propagation are not easily accounted for in graphical techniques although these methods are adequate for quick estimates.

Computer-based optimization routines like FITEQL (Westall 1982) offer several major improvements in the fitting of discrete ligands to humic-metal data. By minimizing the residuals between the calculated and experimental data, a properly applied optimization yields the best fit possible with discrete ligands and the resulting parameters are reproducible if the same optimization algorithm is used. The influence of error can also be properly accounted for in an optimization procedure by incorporating error propagation calculations and weighting factors. For most humic solutions two or three ligands will be required, resulting in four or six adjustable parameters. Titrations can be compared on the basis of these parameters if the fitting procedures are standardized for all data sets.

It is worth emphasizing once again that discrete ligands fitted to a titration curve are properly used only to reproduce the solution conditions of that titration. The fact that these ligands do not correspond to physically definable binding sites means, for example, that the proton ionization constants obtained for a fulvic acid are difficult

to assign to the ligands determined from a Cu(II) titration. In general, modeling multi-metal interactions with humic materials by assigning stability constants for each metal to the same set of ligands yields poor results, although the approach may be a reasonable first aproximation (Fish and Morel 1984b). The structure of computer programs such as MINEQL can easily lead the unwary modeller into incorrectly posing a complicated natural equilibrium problem by using a set of discrete ligands that may not account for competitive interactions among ions.

A discrete ligand model is also only properly applied to systems in which the ratios of total metal to total fulvic acid are comparable to those for which the model was calibrated. The strongest ligand in the model is dictated by the lowest metal/ligand ratio in the calibrating titration and extrapolations below this ratio may underestimate the strength of metal binding.

The affinity-spectrum technique is a means of fitting discrete ligands to titration data that is based on a postulated continuum of metal binding strengths. As we have shown, with ideally precise data the procedure is an improvement over the graphical Scatchard method when selecting binding parameters in the range log $K < 8$. For ligands stronger than this, the affinity spectrum is difficult to interpret, even when applied to ideally precise data. When indeterminate error is present in the titration data, the resulting small, random fluctuations in the slope of the formation function introduce sufficient noise into the affinity spectrum to make an unambiguous choice of fitting parameters difficult at best. In addition, the method requires a computer-based solution scheme even though the final selection of ligands involves a

visual evaluation of the spectrum and trial and error adjustment of parameters.

If a normal frequency distribution of humic binding sites is assumed, a continuous representation of the formation function is possible. With this formulation only three fitting parameters are needed to model a wide range of data. Characterizing a titration by only three parameters provides a concise description of a particular data set and makes comparisons among results easier. A computer-based solution scheme is necessary for applying the model but we have demonstrated that the Sips approximation of the normal distribution function allows for a graphical method of extracting optimal parameters.

Although the normal distribution model accurately reproduces titration data, it possesses several drawbacks. The computation of metal speciation by this method is not easily incorporated into existing chemical equilibrium programs. In addition, no general theory currently exists for calculating the competition among ions for a continuous distribution of binding sites. Binding-site distributions can be defined for single metals or for protons, but classical chemical equilibrium theory is of no help in combining these distributions for calculation of multiple equilbria among competing ions. Unless this problem is resolved, continuous distribution models will be of only limited use in predicting the speciation of metals in natural waters.

In contrast to the discrete ligand model, the normal distribution model postulates the presence of ligands with stronger stability constants than are experimentally verifiable. In fact, the model includes small but finite concentrations of ligands with nearly infinite stability conetants. Perdue and Lytle (1983a) showed that when the

normal distribution model is extrapolated beyond its calibration range, it predicts stronger binding than does a set of discrete ligands, but that this binding may not reflect the correct metal speciation. This follows from the fact that the assumed normal distribution of ligands has no intrinsic physical meaning and is simply a useful means of representing data.

Researchers interested only in concisely describing the results of a single-metal titration of a humic material should consider using the three-parameter, normal distribution model of metal binding. Incorporating humic titration results in a general equilibrium model of chemical speciation is most easily accomplished by discrete ligands. With either approach, care must be taken to insure that these empirically based models are not used to calculate ion speciation in systems for which they have not been calibrated.

No single model of metal-humic interactions has yet emerged that is clearly superior for all purposes. Continuous distribution models are at an early stage of development and currently offer few advantages over discrete ligand representations. The choice of a model should be guided by a careful consideration of the uses for which the model is intended, the quality and quantity of titration data at hand, and the computational resources that are available.

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CHAPTER FOUR:

POWER-FUNCTION AND DISCRETE-LIGAND REPRESENTATIONS OF THE INTERACTIONS OF CU^{2+}, CD^{2+}, and H^{+} WITH AN AQUATIC FULVIC ACID

ABSTRACT

Competition among cations for fulvic acid binding sites is quantified by a series of acidimetric, Cu(II), Cd(II), and mixed-ion titrations. The mixed-ion titrations measure the binding behavior of one ionic species in the presence of a second, competing ionic species. These direct competition measurements are essential for modeling the behavior of humic materials under natural water conditions in which different ions are present. Multiple-ion competition can be modeled by a set of discrete ligands that are optimized to fit simultaneously the single-ion titrations as well as the competing-ion titrations. The titration data can also be conveniently modeled by a newly developed power-function representation in which a single titration can be specified by only two adjustable parameters. Adding two more parameters allows an empirical representation of competition between two metals for the fulvic acid binding sites.

INTRODUCTION

The role of humic materials in the chemical speciation of natural waters has been the subject of considerable interest and research. The chemical complexity of humic matter has given rise to a number of models for representing the results of proton and metal binding studies. The speciation of elements in the presence of humic materials has been calculated with discrete-ligand models (Mantoura and Riley 1975; Buffle et al. 1977; Bresnahan et al. 1978; Sunda and Hanson 1979; Tuschall and Brezonik 1983), with stability function models (Gamble 1970; Gamble et al. 1972, 1983), and with a Gaussian, continuous ligand-distribution model (Perdue and Lytle 1983a,b).

Detailed evaluation of these representations has shown that all existing speciation models have little or no thermodynamic significance when applied to humic materials (Fish et al. 1984; Perdue and Lytle 1983a). Because of the complicated and variable structure of humic substances such as fulvic acid, and the limitations of the analytical procedures available, no classical thermodynamic representation of humic behavior may be possible.

Chemical speciation models are of greatest value if they predict the equilibrium composition of a system under conditions that are different from the experimental conditions. Ideally, a complexation model for fulvic acid could be calibrated in the laboratory at analytically convenient concentrations and then used to predict metal speciation at environmental concentrations. If an exact thermodynamic description of fulvic acid were possible, such predictions could be made as easily for fulvic acid as they are for well-defined compounds such as nitrilotriacetic acid.

In lieu of such an exact description, how well can existing humic models be applied to practical problems outside of the laboratory? At the minimum, a model should account for variable concentrations of fulvic acid and should predict the effects of competitive interactions among ions involved in fulvate binding. Although existing discrete and continuous models have been tested on limited experimental data, there has been no assessment of how well these models predict metal-humic interactions in natural systems. Sposito (1981), for example, developed a discrete ligand model for multiple ions binding to a fulvic acid but did not compare the results to titrations in which several metals were present and competing for binding sites. Laboratory verification of the competitive interactions predicted by a model is an essential step toward applying the model to natural waters.

Extrapolating titration results to fulvic acid concentrations other than those titrated is an essential, and on the face of it, a simple requirement for a model. Both discrete and Gaussian distribution models have ligand concentration terms that should be proportional to the fulvic acid concentration, [FA]. Problems arise, not in the variation of [FA] itself, but in predicting metal speciation when the ratio of total metal, M_T, to [FA] is outside the range used in the calibrating titrations. As will be discusssed below, neither continuous nor discrete models can accurately predict metal binding outside the M_T/[FA] ratios observed experimentally.

There is currently no theoretical framework for using continuous distribution models to predict competition among binding ions. A new distribution can be defined for each solution condition and empirical correlations drawn between ion concentrations and the values of distri-

bution parameters, thus defining conditional parameters. But no means exists for combining the distributions fitted to two different metal titrations and then predicting the speciation when both metals are present. Because of this intrinsic difficulty the Gaussian distribution model is not used in this study.

The discrete ligands fitted to fulvic acid titrations are not physically identifiable entities but are a representation of fulvic acid that enable the use of mass law equations for describing concentrations of free and bound metal in a simple metal-fulvate system (Sposito 1981). Through the mass law formulation one can in principle describe also the competition among ions for fulvic binding. Because these ligands do not physically exist, however, we cannot know a priori if the thermodynamic equilibrium calculated from these mass laws will quantitatively account for the competition among reacting ions.

A direct (and naive) approach consists of fitting ligands to single-ion titrations. The ligands are then assumed to be the same for all titrations and differ only in the stability constants for each ion. For this approach to work, the set of ligand concentrations, L_i, fitted to each metal must be equal. For example, the strongest Cu(II)-binding ligand must have the same concentration as the strongest Cd(II)-binding ligand so that they may be considered to be the same chemical component. This approach is termed Model I.

If the ligand concentrations are not equal, the first alternative, termed Model II, is to adjust the concentrations in each class of ligands so that L_i are the same for all metals. The stability constants are adjusted iteratively so that the ligands still reproduce the single-ion titrations. A ligand set that can predict the speciation for mixtures of ions is created from single-ion titrations.

The adjustment of parameters described for Model II is possible because a set of discrete ligands fitted to titration data does not have unique concentrations and stability constants. These two parameters are interdependent and reasonably small changes in stability constants can be balanced by changes in ligand concentrations. If each ligand parameter is thought of as an axis in n-space, the degree of "uniqueness" is related to the size of the region in which any combination of parameter values yields the minimum residuals between the model and the data. This region of minimal residuals (or optimal fit) becomes smaller as the precision of the data increases and as the system is more constrained.

For example, when ligands are fitted to single-ion titrations, the stability constants and concentrations are constrained only by a fit of the speciation of one metal and the parameters are are thus fairly flexible. If we require these ligands to also fit data from competing-ion titrations, then we are constraining the system more tightly. Only a limited range of constants and concentrations for each ligand can reproduce all of the data.

The third possibility, then, for discrete ligand fitting (Model III) entails the use of all experimental data, both single-ion and multiple-ion titrations to constrain the choice of ligand parameters. While this procedure is empirical, it does retain the conciseness of the thermodynamic mass law representation in that both single- and multiple-ion interactions are described by the same set of stability constants. This keeps the number of adjustable parameters relatively small when many chemical components are present.

If such a set of ligands cannot be created or if it is very difficult to optimize all parameters for all the data, the only recourse is to use direct measurements of competitive interactions to determine empirical correlations between single-ion stability constants and the concentrations of competing ions. With a sufficient number of adjustable parameters, these conditional stability constants can always be fitted to experimental data. However, there may be little reason to retain the mass law formulation if the necessary parameters (equilibrium constants and ligand concentrations) are not constant and must be determined from empirical correlations. The number of necessary parameters becomes very large if the stability constants for each component are dependent on the concentrations of all other components. Simpler and more direct empirical means of fitting the data may then be developed.

In this study we obtained experimental data for the binding of Cu(II), Cd(II) and hydrogen ions to an extracted fulvic acid. The competitive interactions among these ions were measured by titrating the fulvic acid with Cu(II) solutions at several pH values and Cd(II) concentrations. A combination of ion-selective electrodes and fluorescence quenching techniques were used to obtain the most precise binding data over a wide range of concentrations (Fish and Morel 1984).

Discrete ligands were fitted to the single-ion titrations using the data-fitting program FITEQL (Westall 1982). Within limits we were able to model variable fulvic acid concentrations with discrete ligands. We tested the possibility of directly combining ligands for ion-competition

studies. The parameters were then modified in an attempt to develop a more consistent set of ligands with stability constants for each ion. The complicated calibration procedures necessary to obtain discrete ligands to model ion competition suggested that a simpler model would be more appropriate for systems with only a few competing metals. As a result, we developed and tested a new, empirical model of metal-humic interactions defined by a simple power-law relationship between the ratio of bound metal to free metal and the total metal in solution.

MATERIALS AND METHODS

Fulvic acid was obtained from 20 L of Grassy Pond water (Bedford, MA) by adsorption onto XAD-8 resin according to the method of Thurman and Malcolm (1981). The freeze-dried extract was 49% carbon by weight and this factor was used to prepare an aqueous stock solution of 50 mg-C/L of Grassy Pond fulvic acid (GP-FA). The stock solution was stored in a foil-covered flask (to exclude light) at 5°C and at pH 8. After preparation stock solutions were allowed 48 h to dissolve and hydrolyze completely before any dilutions for titrations were made. The freeze-dried Suwannee River fulvic acid standard was treated in the same manner and was provided by the U.S. Geologic Survey, Denver. Fulvic acid from the Shawsheen River, Massachusetts, was provided by D.M. McKnight, USGS, Denver.

Fulvic acid solutions for ion-selective electrode and fluorescence quenching titrations at 1.0 and 5.0 mg-C/L were prepared with deionized water. Ionic strengths were adjusted using 1 M $NaClO_4$ to give an electrolyte concentration of 10^{-3}M. Fluorescence data were calibrated by estimating a linear proportionality factor between percent quenching

and bound-metal concentrations in the strong metal-binding portion of the titration (Fish and Morel, 1984).

Ion selective electrode (ISE) titrations were performed using a Radiometer F3000 Selectrode with a Cu(II) or Cd(II) sensing membrane coupled with an Orion 90-02 double-junction reference electrode. The pH was monitored using a Fisher E5-A combination pH electrode. Electrode potentials were measured by a pair of Orion 801 Ionanalyzer meters. The ISE was calibrated with a series of copper solutions the day before each titration and was allowed to equilibrate overnight in a 1 mM Na_2EDTA / 0.1 M TRIS buffer solution at pH 6.2. This procedure maximized electrode sensitivity in the low copper range. For the Cu(II) and Cd(II) titrations, the pH was maintained by adjusting the alkalinity and bubbling with 1% CO_2/99% N_2 gas mixture. The fulvic acid solutions were titrated in 100 mL, water-jacketed beakers at 15°C with total Cu(II) concentrations ranging from 0.1 uM to 100 uM.

For the alkalimetric titration, a 50 mg-C/L fulvic acid solution was acidified to pH 3.0, purged of carbonate with purified N_2 gas and titrated with standardized solutions of carbonate-free NaOH.

Fluorescence quenching (FQ) titrations were performed using a Perkin-Elmer LS-5 Fluorescence Spectrophotometer equipped with a 1 cm flow cell coupled by Teflon tubing and a peristaltic pump to a 50 mL, water-jacketed titration vessel maintained at 15°C. The pH was maintained with a CO_2/N_2 mixture as in the ISE titrations. Fulvic acid solutions were deoxygenated by several hours of bubbling with the CO_2/N_2 gas mixture in order to eliminate the minor quenching of the fulvic acid fluorescence by O_2. For GP-FA titrations the excitation and emission wavelengths were set for maximum fluorescence at 325 nm and 405 nm

respectively. Slit widths were set at 10 nm. Kinetic studies indicated that quenching equilibrium was reached within 10 min at copper concentrations less than 1 uM and in less than 5 min above 1 uM copper. In subsequent titrations equilibration times were allowed accordingly before measurements were taken. Fulvic acid solutions were titrated over the same total copper range as in the ISE experiments: 0.1 uM to 100 uM.

For studies of competition between Cd(II) and Cu(II), both the cupric-ISE and fluorescence quenching procedures were employed. After standardizing the cupric-ISE with Cu(II) solutions, $Cd(NO_3)_2$ was added to the Cu(II) standards to yield total Cd(II) concentrations of 10^{-4}M. The electrode response to cupric ion activity was found to be unaffected by the presence of Cd(II). The extent of fluorescence quenching induced by Cd(II) was measured by titrating a 1.0 mg-C/L solution of GP-FA with Cd(II) up to a total Cd(II) concentration of 10^{-5}M. There was no measurable effect of cadmium ions on the fluorescence of the fulvic acid. The absence of a Cd(II) interference with either ISE or fluorescence procedures meant that the effect of Cd(II) on Cu(II)-fulvate speciation could be measured directly by performing copper titrations in the presence of cadmium ions.

In the Cu(II)/Cd(II) competition studies 2, 10 or 100 uM of Cd(II) was added to either 1.0 or 5.0 mg-C/L solutions of GP-FA and allowed to equilibrate for 30 min. The solutions were titrated with Cu(II) as before and electrode potentials or relative fluorescence measurements recorded after equilibration periods of at least 30 min for the ISE titrations and 10 min for the FQ titrations. Rayleigh scatter measurements were made with both excitation and emission monochromators set at 410nm.

pH Titration

The alkalimetric titration of 50 mg-C/L GP-FA is presented in the usual form of "excess acidity" vs. pH where excess acidity is defined as $A = acid - base + [H^+] - [OH^-]$ (Fig. 1). The titration has the "smeared", weakly inflected shape that is typical of such curves for solutions of polyprotic fulvic acid molecules. The total "excess acidity" of Grassy Pond fulvic acid for the pH range titrated, A_T, is 11.5 meq/g-C, a number that corresponds to the number of ionizable protons in the fulvic acid. The exact average molecular weight of Grassy Pond FA is unknown, but most aquatic fulvic acids have number average molecular weights close to 1000 daltons (Thurman and Malcolm, 1983; Plechanov et al., 1983). Assuming GP-FA has a molecular weight of 1000 daltons, there are approximately 10-12 ionizable protons per fulvic acid molecule.

Cu(II) Titrations

Titrations of Grassy Pond fulvic acid with Cu(II) indicate that the strength of copper binding is strongly dependent on solution pH, as evidenced by shifts in the titration curves obtained at pH 5.0, 6.0, 7.0 and 8.0. Titration data for 1 mg-C/L and 5 mg-C/L solutions of GP-FA are presented in the form of pCu ($= -\log \{Cu^{2+}\}$) as a function of total Cu(II) added (Cu_T) (Fig. 2 a,b).

Fluorescence quenching data yield bound Cu(II) as a function of Cu_T (Fig. 3). The general increase in the strength of Cu(II) binding with increased pH is evidenced by the sharper rise in the complex formation as a function of M_T. Note also that the amount of bound Cu(II) at the

Fig. 1. Alkalimetric titration of 50 mg-C/L Grassy Pond fulvic acid (GP-FA). Excess acidity (defined in text), meq/g-C vs. pH (O). Results of Model I (— —) and Model IV (•—•).

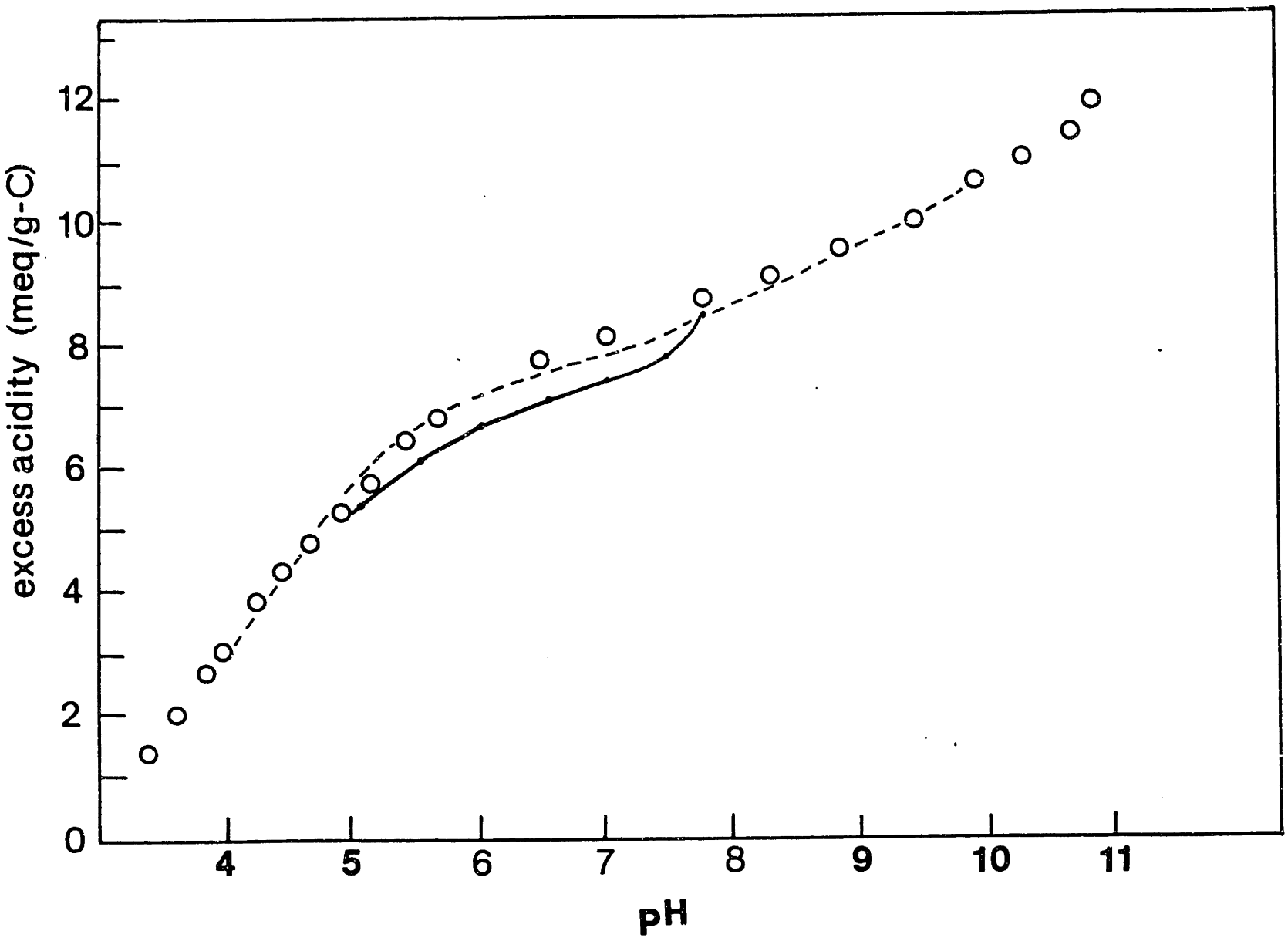

excess acidity (meq/g-C)
0
2
4
6
8
10
12
4
5
6
7
8
9
10
11
pH

Fig. 2. (a) Cu(II) titrations of 1 mg-C/L solutions of GP-FA at pH 5.0 (○), 6.0 (□), 7.0(△), 8.0 (▽). Data obtained with ion-selective electrode (ISE). Dotted lines are results of Model III using parameters in Table 5. Solid lines are results of Model VI using the parameters in Table 8. Upper dashed line is calibration line ($pCu = -\log Cu_T$).

(b) Same as (a) except for 5 mg-C/L GP-FA.

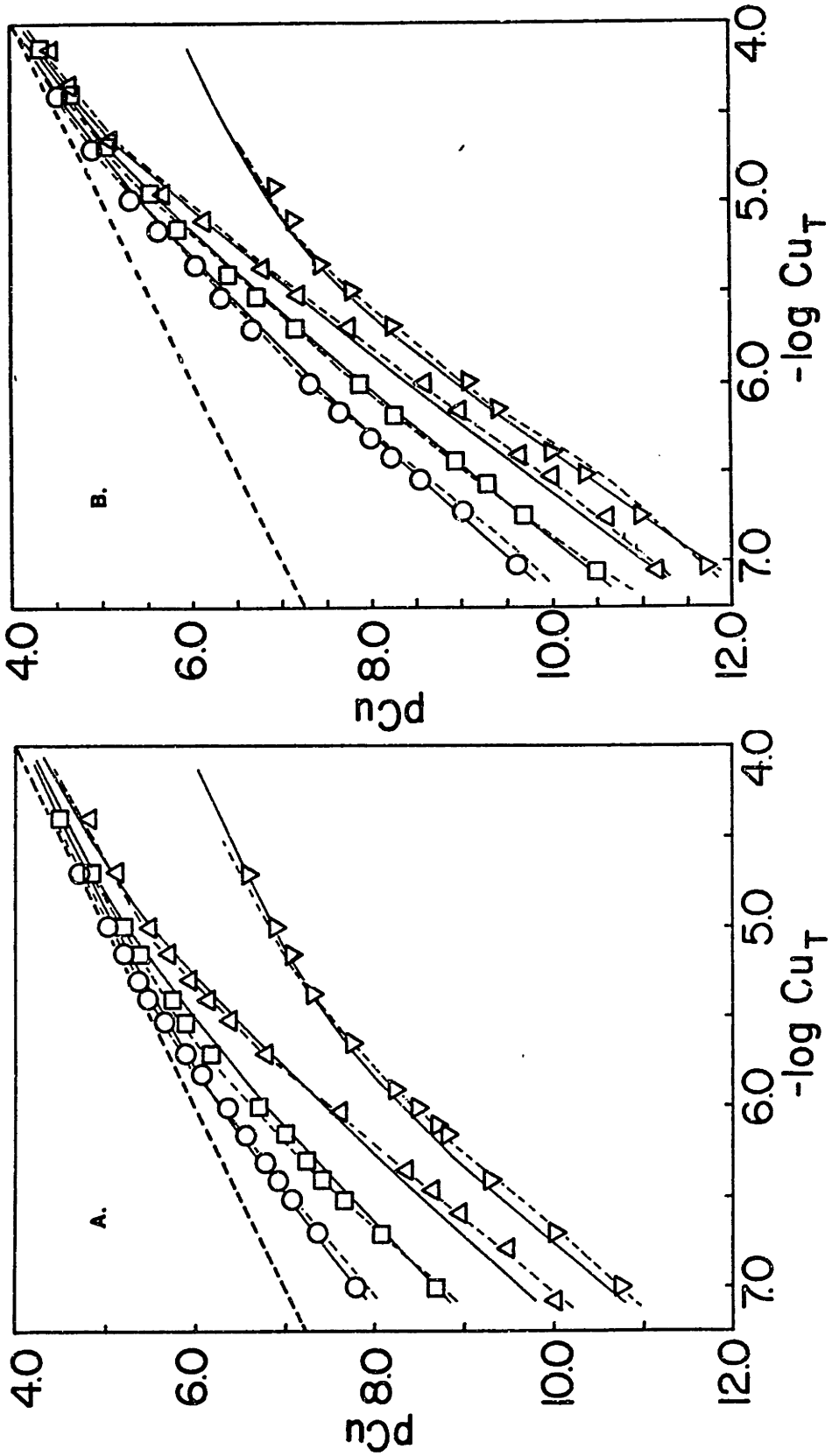

B.
A.
pCu
-log Cu$_T$
4.0
5.0
6.0
7.0
8.0
10.0
12.0

Fig. 3. Fluorescence quenching (FQ) Cu(II) titrations of 5 mg-C/L GP-FA at pH 5.0 (■), 6.0 (●), 7.0 (▲), 8.0 (▼). Dashed lines are results of Model I using parameters in Table 1. Rayleigh scatter ratios (F_R^{Cu} / F_R^{o}) for the same titrations: pH 5.0 (□), 6.0 (○), 7.0 (△), 8.0 (▽).

Rayleigh Scatter ratio, F/Fo
4.0
3.0
2.0
1.0
90
70
50
30
10
CuT, μM
20
15
10
5
0
Bound Cu(II), μM

end of each titration increases with pH. Rayleigh-scatter ratios are plotted to indicate when the formation of colloidal aggregates may become important (Fig.3). The Rayleigh-scatter ratio is defined as the 90°-angle light scattering at any point in the titration divided by the scattering at the start of the titration.

The estimates of error for free- and bound-metal concentrations indicated that ISE data were more precise for the titration region for which $[CuL]/\{Cu^{2+}\} > 3$ and that the FQ data were more precise for $[CuL]/\{Cu^{2+}\} < 3$. Accordingly, results from the more precise portions of the two titrations were combined to produce a data set for which the relative error is nearly uniform throughout the titration. The combined-method data with associated standard deviations are presented in the form [CuL] vs. pCu in Fig. 4. This format conveniently depicts bound and free metal data on a single plot but the variation of pCu within a titration and from one titration to another is not as easy to discern in this format as in the pCu vs. Cu_T plot, particularly in the low $\{Cu^{2+}\}$ region.

Cd(II) Titration

A titration of 5 mg-C/L of GP-FA, as monitored by the Cd-ISE, indicates that the affinity of the fulvic acid for Cd(II) is much weaker than the affinity for Cu(II) (Fig. 5). Note that the Cd-ISE is limited to higher initial total-metal concentrations than is the Cu-ISE (10^{-6}M for Cd_T vs. 10^{-7} for Cu_T). Thus, the Cd-ISE cannot measure as wide a range of Cd-fulvate stability constants as can the Cu-ISE for Cu-fulvate complexes.

Fig. 4. Bound Cu(II) vs. pCu for 5 mg-C/L GP-FA at pH 6.0. No Cd(II) added (●), 100 uM Cd(II) added (▲). Data are the more precise of either ISE or FQ titrations. Error bars are ± one standard deviation. Dashed lines are results of Model III using parameters in Table 3.

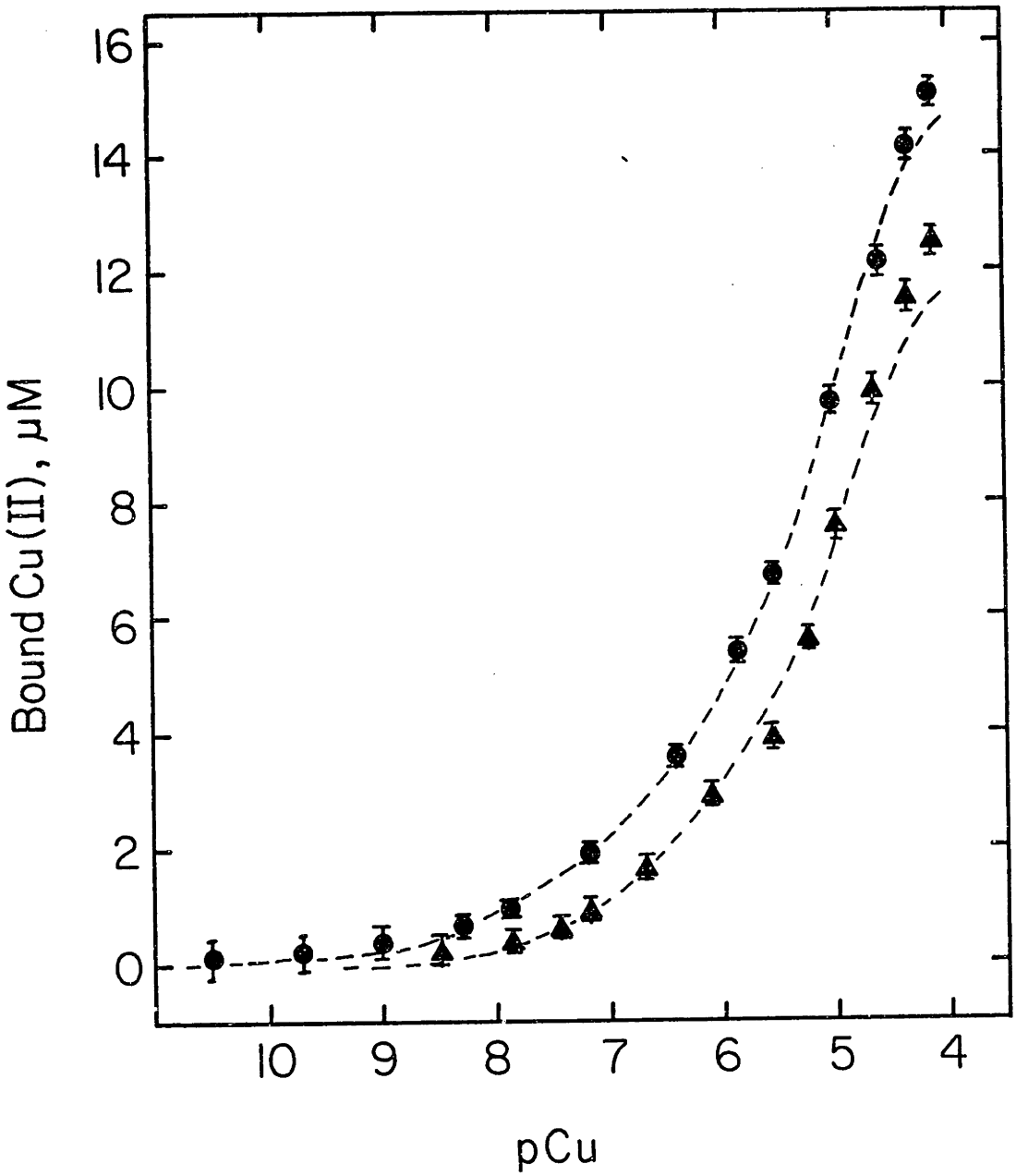
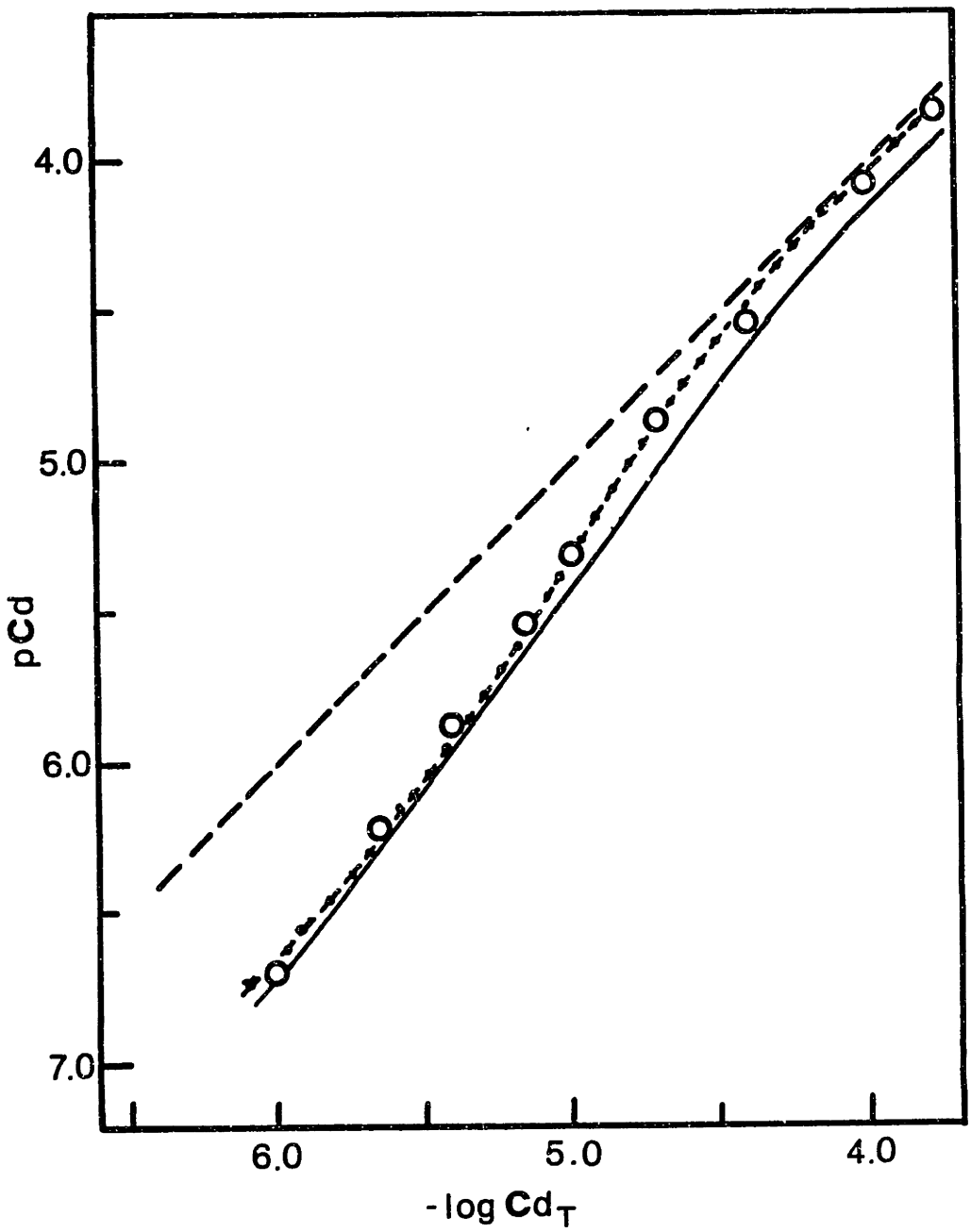

Bound Cu(II), μM
16
14
12
10
8
6
4
2
0
10
9
8
7
6
5
4
pCu

Fig. 5. Cd(II) titration of 5 mg-C/L GP-FA, pH 6.0, ISE data (O); Dotted line is the result of Model I, solid line is the result of Model III. Upper dashed line is the calibration line ($pCd = -\log Cd_T$).


4.0
5.0
6.0
7.0
pCd
6.0
5.0
4.0
- log Cd$_T$

Cu/Cd Competition Titrations

Cu(II) titrations of 1.0 mg-C/L GP-FA with Cd_T = 2 uM and 10 uM and 5.0 mg-C/L GP-FA with Cd_T = 10 uM and 100 uM indicate relatively small effects of Cd(II) on free and bound Cu(II) concentrations as measured by ISE (Fig. 6). The advantages of the combined-methods approach become particularly apparent in these studies. The low and relatively uniform standard deviation associated with data from a combination of ISE and FQ methods enables small differences in titration curves to be distinguished with greater confidence throughout the titration range (Fig. 4)

MODELING RESULTS

Discrete Ligand Models

Through the application of FITEQL, we obtained sets of discrete ligands that closely reproduce the Cu(II) and the Cd(II) titration data sets independently of each other. The minimum numbers of ligands needed to fit the titration data (two for 1 mg-C/L, three for 5 mg-C/L) are related to the concentration range of the titration data and were determined using the methods described by Fish et al. (1984). The log K_i values and concentrations of the discrete ligands constitute Model I and are presented in Table 1. Example of the quality of fit obtained with Model I (calculated using the equilibrium program MINEQL (Westall et al. 1976)) are shown in Fig. 7 for the Cu(II) titration of 5 mg-C/L GP-FA.

We tested Model I for its ability to represent fulvic acid concentrations outside the range of calibration. Two considerations are the concentrations of metal in the system relative to the fulvic acid concentration and the effect of curve fitting routines on the ligand concentrations that are extracted from titration data.

Fig. 6. (A) Cu (II) titration of 1 mg-C/L GP-FA at pH 6.0 in the presence of: No added Cd(II) (△), 2 uM Cd(II) (□), and 10 uM Cd(II) (○). Broken lines are the results of Model III. Upper dashed line is the calibration line.

(B) Cu(II) titration of 5 mg-C/L GP-FA at pH 6.0 in the presence of: No added Cd(II) (▲), 10 uM Cd(II) (■), and 100 uM Cd(II) (●). Solid lines are the results of Model III with the parameters listed in Table 3.

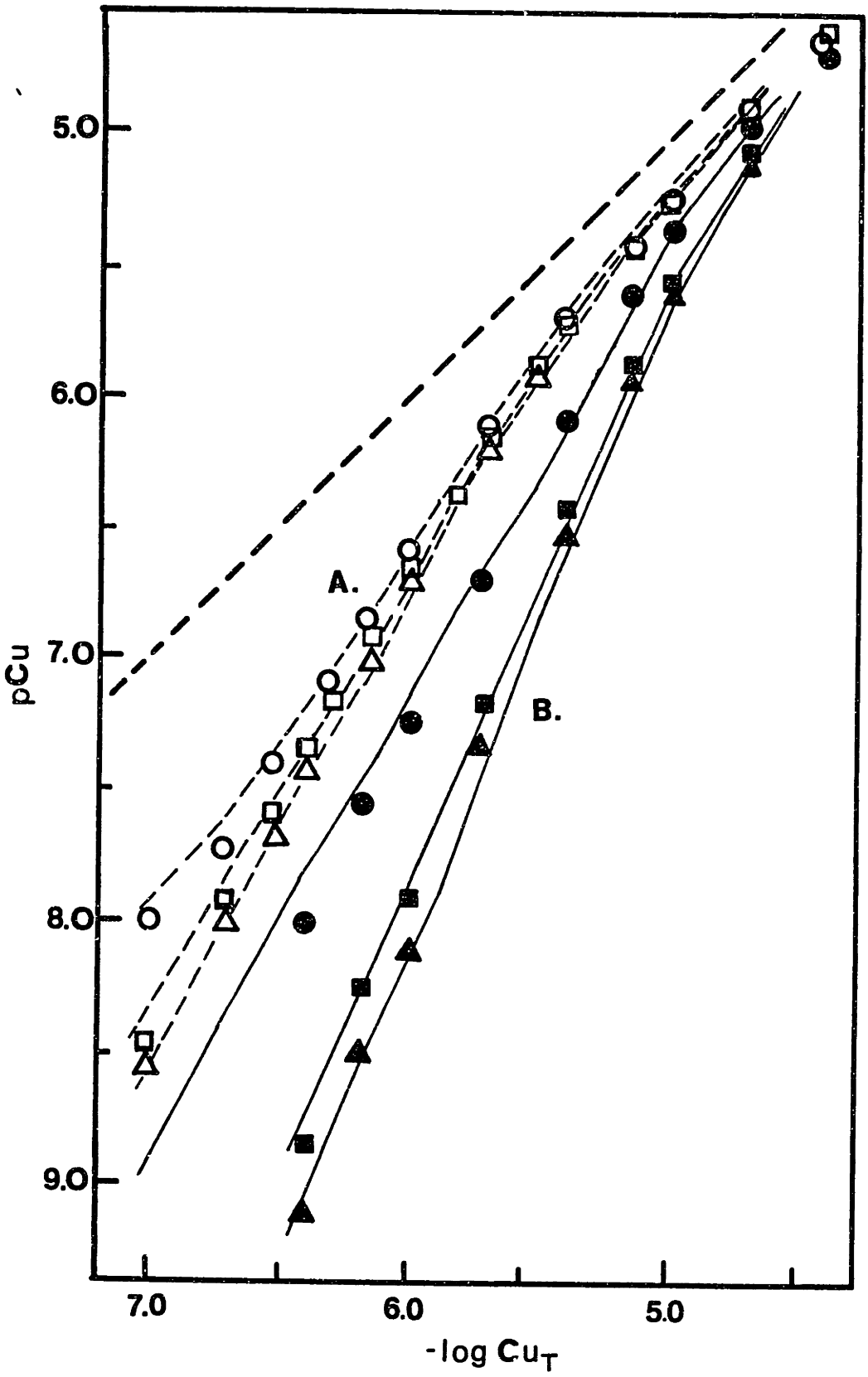

5.0
6.0
7.0
8.0
9.0
pCu
A.
B.
7.0
6.0
5.0
-log Cu$_T$

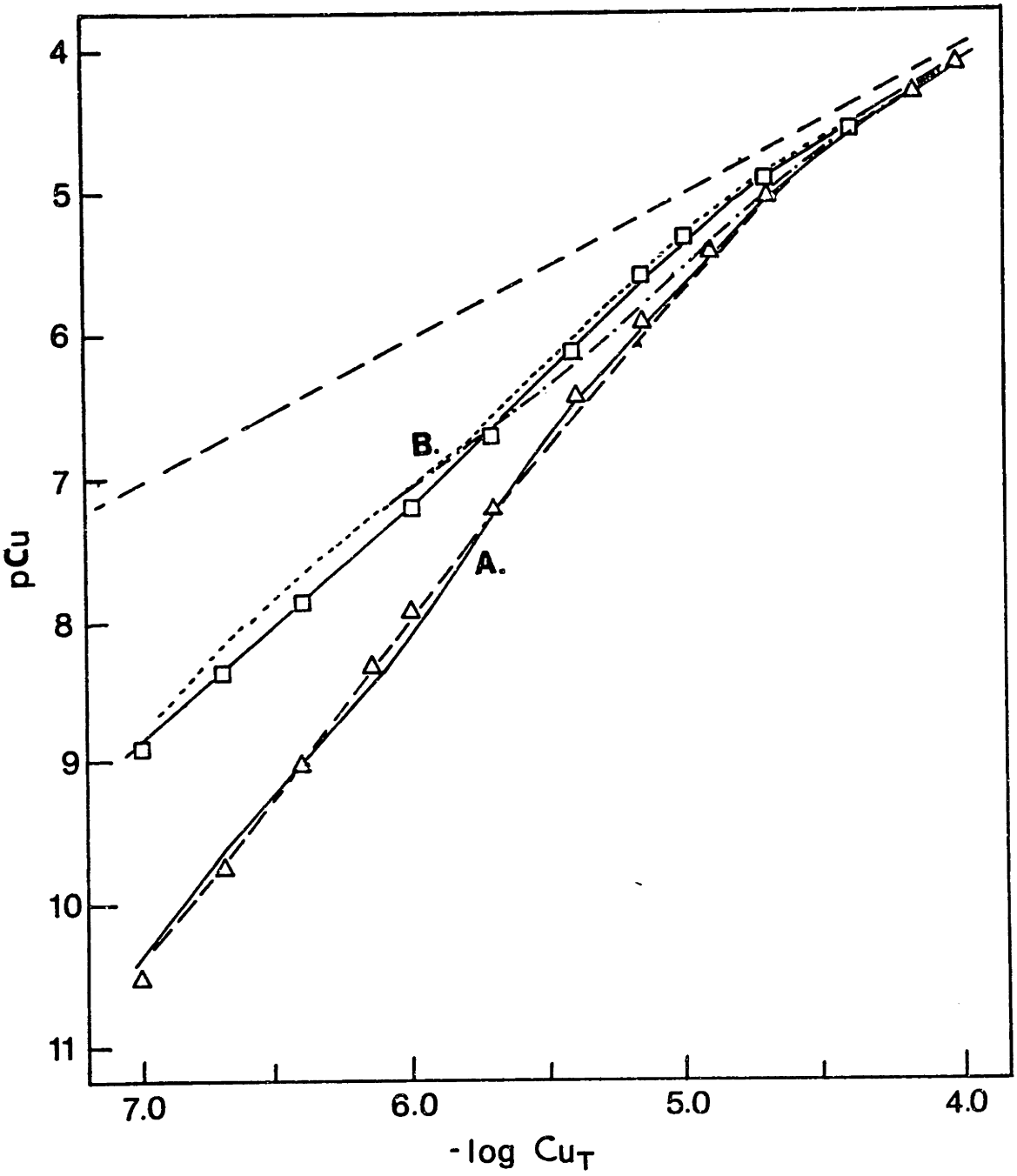
TABLE 1: Model I: Discrete ligands fitted to Grassy Pond fulvic acid (GP-FA) experimental data with FITEQL.

GP-FA Conc.	Metal	pH	log K_i			L_i, uM		
			1	2	3	1	2	3
1 mg-C/L	Cu(II)	5	5.61	7.37	-	1.12	0.17	-
		6	6.46	8.60	-	1.77	0.21	-
		7	6.47	9.07	-	4.70	0.48	-
		8	6.63	10.19	-	2.30	0.36	-
5 mg-C/L	Cu(II)	5	5.74	7.58	9.40	6.46	0.85	0.20
		6	5.95	8.28	10.52	9.15	1.34	0.18
		7	6.18	8.58	10.80	10.0	1.41	0.28
		8	6.41	9.15	12.04	11.1	1.47	0.29
5 mg-C/L	Cd(II)	6	3.83	5.65	-	11.2	4.58	-

Fig. 7. Comparison of experimental and model results.

(a) 5 mg-C/L GP-FA, pH 6.0, No Cd(II) added (Δ); results of Model I (——); results of Model V (– – –).

(b) 5 mg-C/L GP-FA, pH 6.0 with 100 uM Cd(II) added (_); results of Model II (—·—); results of Model III (----); results of Model VI.(——)


4
5
6
7
8
9
10
11
pCu
B.
A.
7.0
6.0
5.0
4.0
-log Cu$_T$

An empirical model of metal ions binding to a mixture of ligands can predict speciation over a range that is dictated by the experimental values of the ratio M_T/[FA], and not by either M_T or [FA] alone. For M_T/[FA] values less than the experimental (calibration) range, a model may fail to predict the effects of small quantities of strong binding sites. At M_T/[FA] values above the calibration range, weak binding may be incorrectly represented. Because a ratio of concentrations is the parameter that defines the range of applicability, it is possible to extend a model to lower M_T values than are experimentally obtainable by increasing the concentration of fulvic acid in the calibrating titration. Conversely, very low fulvic acid concentrations may be modeled from a titration of concentrated fulvic acid solution by calibrating the model to proportionally higher total metal concentrations than found in the low-[FA] system.

Titrations of fulvic acid indicate that the strongest metal binding that is detectable is function of the lowest value of the ratio M_T/[FA]. Within the attainable values of M_T/[FA], there is no clearly defined maximum metal-binding strength for fulvic acid. Thus, the strongest discrete ligand fitted to titration data is likely to underestimate binding strengths at lower values of M_T/[FA]. Yet we cannot impose stronger ligands without experimental justification. A demonstration of this problem is presented below. This limitation applies to all models of metal-humic interactions as long as there is no quantification of the strongest metal-binding sites.

If we limit Model I to the experimental range of M_T/[FA] we should be able to account for various values of [FA] by adjusting the ligand concentrations in proportion to [FA]. Comparison of L_i values for 1 and

5 mg-C/L GP-FA (Table 1) seems to indicate that this is not the case. Only L_1 and L_2 at pH 5.0 are very close to increasing five-fold as [FA] is increased by that amount. However, considering the error intervals associated with these ligand concentrations, only the data for pH 7.0 are significantly out of proportion.

Much of the discrepancy is an artifact of the procedure of optimally fitting ligands to titration data. Because binding strengths do not level off at the lowest M_T values, the strongest ligand is identified by the free metal activity at the minimum M_T. In order to reproduce the slope of the pM vs. -log M_T curve at that point (slope > 1.0) the strongest ligand must be about half saturated with metal. Hence, the strongest ligand fitted to a fulvic acid titration is expected to have a concentration that is about two or three times larger than the minimum M_T. In Table 1, we observe that the strongest ligands fitted to Cu(II) titrations (for which the minimum M_T = 0.1 uM) in fact vary in concentration from 0.17 uM to 0.36 uM (with the exception of 1 mg-C/L at pH 7 for which L_2 = 0.48).

At the weak-binding end of the titration, the ligand concentration that is fitted must account for the maximum amount of bound metal observed. A third, intermediate ligand is required if the strongest and weakest ligands differ in concentration by more than about a factor of ten (Fish et al. 1984). Thus, the concentrations of two successive ligands usually differ by a factor of five to ten. This means that the ligand concentrations fitted to a titration of a particular fulvic acid concentration depend on the range of titration data and not directly on [FA]. Any agreement between the relative values of L_i and [FA] is largely fortuitous.

Calculations made with discrete ligands demonstrate, however, that the concentrations of a set of ligands can be varied according to the value of [FA] and used to correctly predict metal speciation, as long as the restriction on M_T/[FA] is observed. For example, the optimal ligand concentrations fitted to titrations of 1 and 5 mg-C/L GP-FA at pH 7.0 do not differ by a factor of five. Yet simply decreasing fivefold the ligand concentrations for 5 mg-C/L enables a good fit of the titration of 1 mg-C/L. The differences between the optimal ligand concentrations and the extrapolated values are small on the logarithmic scale used in speciation calculations and the resulting difference in goodness of fit is also small (Fig. 8).

Having shown the ability of Model I to predict speciation for variable [FA], we tested the extent to which it correctly predicts multiple ion interactions. Recall that the simplest approach (defined as Model I) is to combine directly the ligand parameters fitted to single-ion titrations. The prerequisite for Model I is to have agreement between the ligand concentrations resulting from the single-ion titrations.

The concentrations of ligands fitted to the Cu(II) and Cd(II) titrations of 5 mg-C/L GP-FA are not in good agreement. This is predictable because the metal concentration ranges are not identical for the two titrations. If the Cu(II) titration data are limited to the range of M_T that is feasible for the Cd(II) titration (10^{-6}M to 10^{-4}M), two new ligands can be fitted that have concentrations in better agreement with the Cd(II) ligands, a modifcation that defines Model II (Table 2). Note that the predictive range of the ligands is now limited to the concentration range of Cd(II) data. Since the ligand

Fig. 8. Experimental results for Cu(II) titrations of 1 mg-C/L GP-FA at pH 5.0 (●), 6.0 (■), 7.0 (▲), 8.0 (▼) and MINEQL-calculated results using Model IV with the conditional stability constants in Table 6 (solid lines). Ligand concentrations were fixed at the values fitted to the titration at pH 6.0. Dotted lines are the results of Model I for 1 mg-C/L GP-FA as calibrated to 5 mg-C/L.

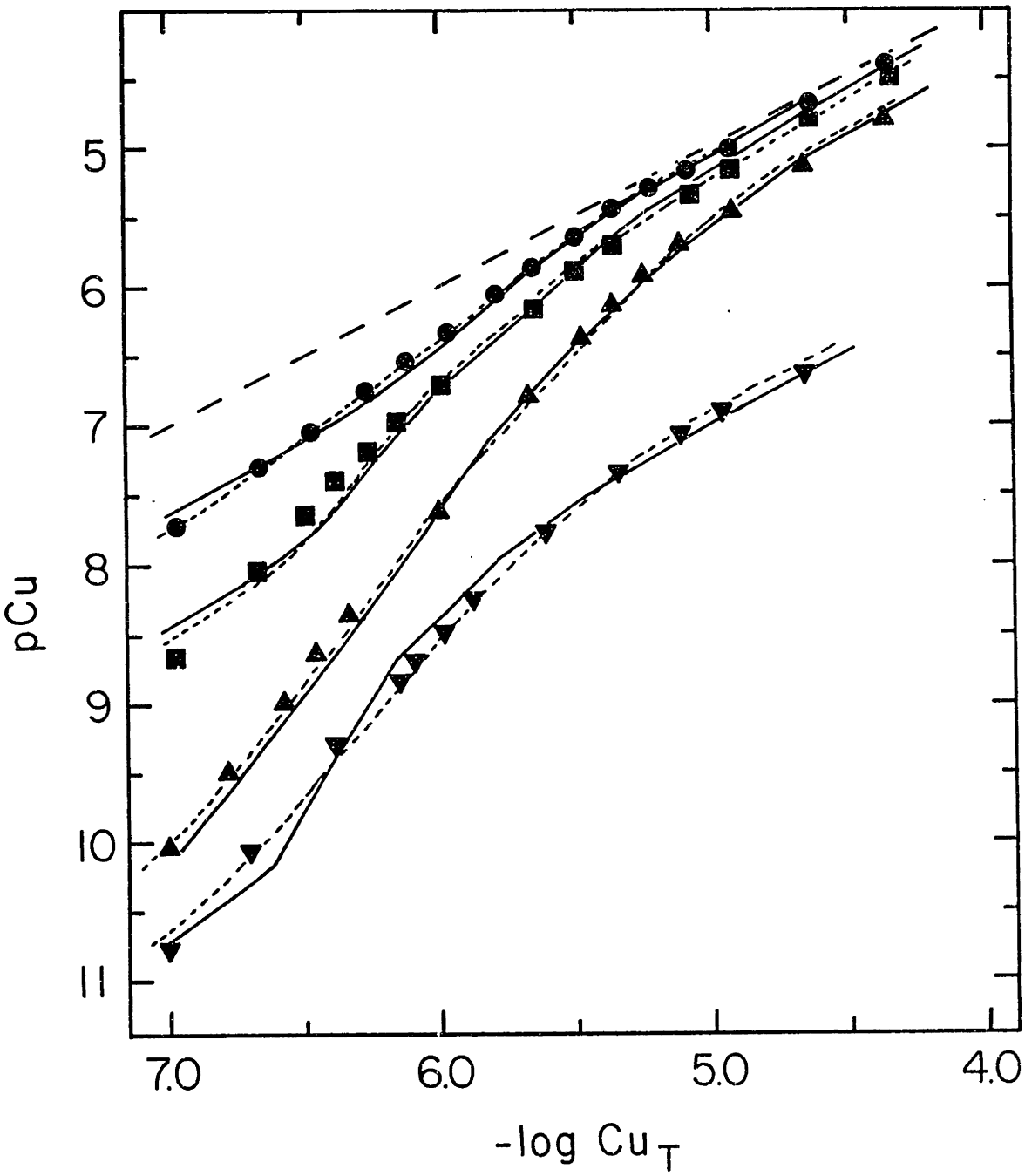

5
6
7
8
9
10
11
pCu
7.0
6.0
5.0
4.0
-log Cu T

TABLE 2: Model II: Modified discrete ligands fitted to Cu(II) and Cd(II) titrations of Grassy Pond fulvic acid (GP-FA). L_i values are the mean values defined in the text. K_i are fitted to experimental data with FITEQL imposing the mean L_i values.

GP-FA Conc.	Metal	pH	log K_i		L_i, uM	
			1	2	1	2
5 mg-C/L	Cu(II)	6.0	5.93	7.96	10.1	3.72
	Cd(II)	6.0	3.83	5.69	10.1	3.72

concentrations still are not equal, we computed mean values for L_1 and L_2 and used FITEQL to find the optimal stability constants for Cu(II) and Cd(II) assuming these new, uniform values of L_i (Table 2). The effect on the fits of the single-ion titrations is negligible because L_i values are changed only slightly and the K_i values are changed by a small amount to compensate.

The solution conditions for the Cd/Cu competition titrations were duplicated in a simulated titrations of Model II using MINEQL (Fig. 7). Model II closely reproduces the experimental results for Cu_T = 1 uM to 4 uM (-log Cu_T = 6.0 to 5.4). Close examination of the data at higher Cu_T values, however, indicates that Cd(II) has a greater effect on Cu(II) binding than is predicted by Model II. While the difference in predicted pCu is small, the resulting difference in bound Cu(II) is significant. At $Cu_T = 7 \times 10^{-6}$ M, Model II predicts 30% more bound Cu(II) than is observed experimentally.

Model II is a reasonable first approximation of competitive effects of Cd(II) on the cupric ion activity. However, the only way to fully account for competition between Cu(II) and Cd(II) in the weaker binding region with discrete ligands is to fit the ligands directly to both the single-metal and the competing-metal titrations (Model III). Unfortunately, optimizing ligands simultaneously for two different metals is beyond the capability of FITEQL. The alternative is a trial and error adjustment of parameters. The K_i and L_i values resulting from this iterative process are shown in Table 3 and the fit of the single- and dual-metal titrations are shown in Figs. 5, 6 and 7.

The problem of combining the results of separate titrations is more severe when modeling the competition between protons and trace metals.

TABLE 3: Model III: Discrete ligand parameters fitted to Cu(II), Cd(II) and Cu(II)/Cd(II) competition titrations of 5 mg-C/L at pH 6.0.

Metal	log K_i			L_i, uM		
	1	2	3	1	2	3
Cu(II)	5.83	8.25	10.52	9.0	2.0	0.20
Cd(II)	4.73	5.65	5.80	9.0	2.0	0.20

There is no chemically obvious way, for example, to assign proton ionization constants to the three ligands fitted to the Cu(II) titration. Application of FITEQL indicated that at least four ligands are required to yield a good fit of the alkalimetric titration data (Table 4).

Using the Model III approach, the proton binding sites can be arbitrarily partitioned among the metal binding sites by trial and error followed by adjustments of the ionization constants to achieve a fit of the experimental results. However, optimization of ligand parameters for both metal and proton binding is more difficult than optimization for the binding of two divalent metals. The binding of univalent protons and divalent transition metals are dissimilar processes because of differences in coordination numbers and in the effects of molecular charge on the binding ions.

The simplest expression of the difficulty of associating proton and metal binding constants can be seen in the variation of metal binding constants with pH, as given in Table 1. The log K values of Ligand 1 increase consistently by about 0.2 log units per unit increase in pH. Examination of the mass laws indicates that this change means an average of 0.2 protons are displaced when a metal ion binds to the ligand. A simple set of proton ionization constants cannot be devised for this ligand that will predict a relatively constant 20% protonation of binding sites from pH 5 to pH 8.

For the stronger ligands the approximation is somewhat easier. For Ligand 3, the change in log K per pH unit is about 1.2. This variation can be approximated by a bidentate binding site with one relatively

TABLE 4. Model IV: Proton binding parameters for discrete ligands fitted to alkalimetric titration of 50 mg-C/L GP-FA.

	1	2	3	4
log K_i	3.82	5.01	7.38	9.03
L_i, mM	0.215	0.145	0.083	0.010

basic proton and a second proton site that is partially ionized at pH 5. This does not account for the variation in log K above about pH 6.

Trial and error analysis of the Cu(II) titrations at various pH's (with the aid of MINEQL) indicates that a rough approximation of the data is possible with three bidentate ligands (Table 5, Fig. 2). A better approximation may be possible with an extended set of ligands but the number of variable parameters rapidly becomes too large for manual trial and error adjustment. The inclusion of a third competing ion makes the problem intractable by simple curve-fitting methods. A numerical curve-fitting routine that can optimize parameters for a number of components simultaneously is necessary for the proper application of Model III to proton metal interactions.

An alternative to Model III is to use stability constants that are conditional on pH (Model IV). Apparent stability constants can be defined as:

$$K_i^* = (ML_i)(H^+)^x/(M)(H_xL_i) \qquad [1]$$

The value of K_i^* will be constant as pH is varied over a limited range and $\underline{x}$ is a fitting parameter that accounts for the variation in K_i at different pH values ($K_i = K_i^*/(H^+)^x$). This approach has been used in several studies of Cu(II) binding to humic materials (Buffle et al., 1977; Sunda and Hanson, 1979). Using Model IV we modeled the Cu(II) titrations at pH 5 to pH 8 with nine parameters, as each of the three ligands has a different value for $\underline{x}$ (Table 6).

Note that Model IV does not account for the variation observed in the ligand concentrations. If the concentrations are required to be constant over all pH values, the small differences can be accounted for

TABLE 5: Model III: Proton binding parameters for discrete bidentate ligands fitted to Cu(II) titrations of 5 mg-C/L GP-FA at pH 5 to pH 8.

Binding Ion	log K_i			L_i, uM		
	1	2	3	1	2	3
Cu(II)	6.0	8.6	12.5	9.0	2.0	0.2
1st Proton	5.0	4.0	4.0	9.0	2.0	0.2
2nd Proton	10.0	5.0	6.0	9.0	2.0	0.2

TABLE 6: Model IV: Conditional stability constants fitted to Cu(II) titration data at pH 5 to pH 8. Values of K_i^* and $\underline{x}_i$.

GP-FA Conc. mg-C/L	log K_i^* 1	2	3	$\underline{x}_i$ 1	2	3
1	4.28	3.00	-	0.31	0.89	-
5	4.64	5.15	5.24	0.22	0.50	0.85

by adjusting the K_i values. The effect on the quality of fit to the cupric ion data is negligible, as seen in the results of a MINEQL simulation (Fig. 8) using the parameters listed in Table 6.

While such empirical procedures will reproduce the conditions found in the experiments, the predictive value of the mass law formulation has been lost. The use of stability constants and ligand concentrations is an unnecessarily complicated representation of metal binding by fulvic acid if the parameters (ligand concentrations and stability constants) are dependent on the composition of the system.

Empirical Power Function Model

In an effort to develop a simple empirical model of metal-humic speciation, we tested a number of data transformations. The most convenient format resulted when titration data were transformed into a plot of $-\log([ML]/(M)$ vs. $-\log M_T$. The data in such a plot are closely approximated throughout the titration by a straight line (Fig. 9).

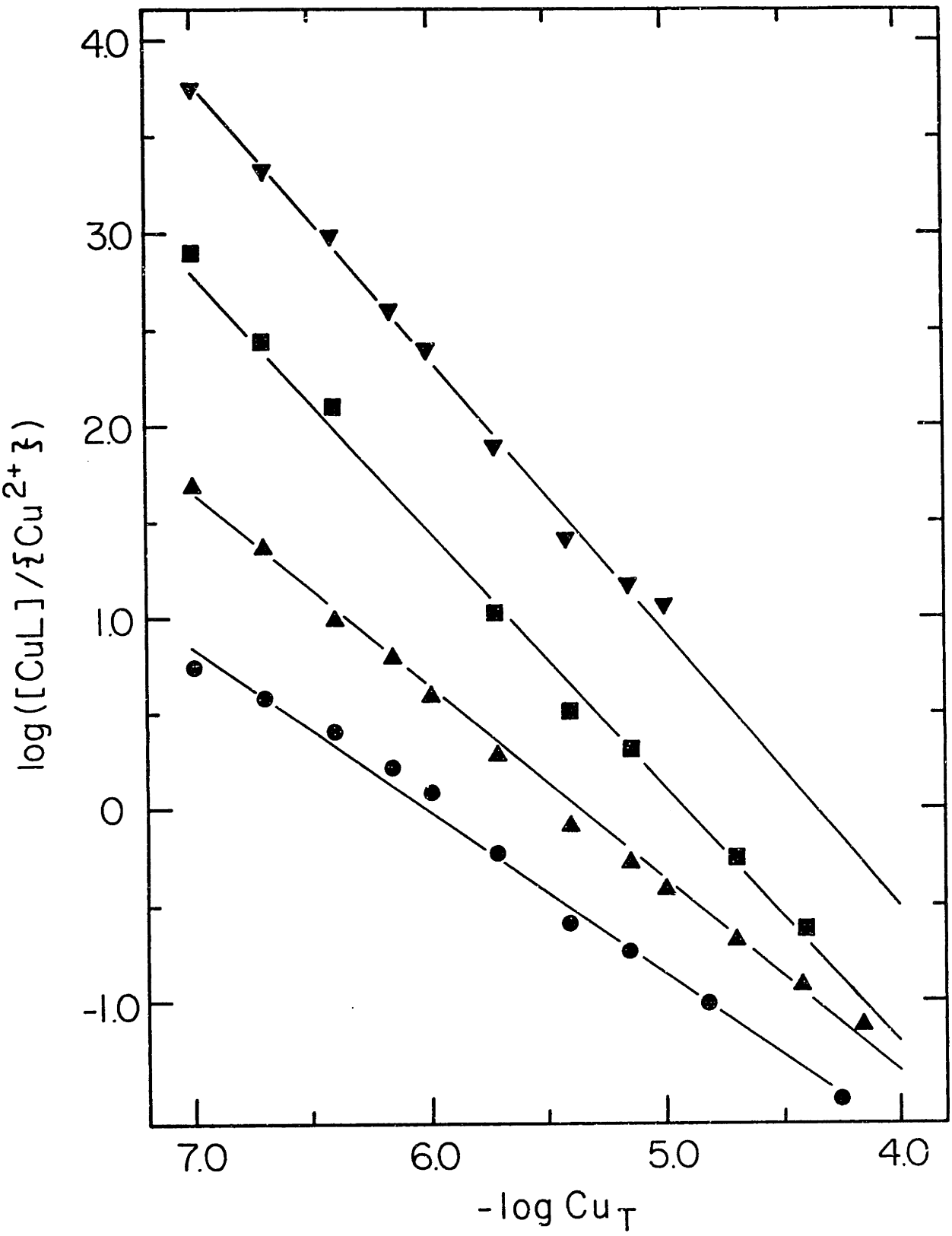
The formula that describes these data is the power function

$$Q_M \equiv [ML]/(M) = Q_o \, (M_T)^k \qquad [2]$$

for which k is slope of the log-log plot and Q_o is the intercept of log $(M_T) = 0$. Q_o has units consistent with that of M_T and the exponent k.

The parameters in the power function model probably do not have a specific physical meaning, as is also the case for other models of humic-metal interactions. However, the simplicity and ease of application of the power function suggested that it could be highly useful for

Fig. 9. Power function calibration plots for Cu(II) titrations (Model V). $\log Q_M = \log ([CuL]/[Cu^{2+}])$ vs. $-\log Cu_T$. 1 mg-C/L GP-FA at pH 5.0 (●), 6.0 (▲), 7.0 (■), 8.0 (▼). Data are the more precise results of either ISE or FQ titrations.


4.0
3.0
2.0
1.0
0
-1.0
7.0
6.0
5.0
4.0
log([CuL]/ξCu2+ξ)
-log CuT

calculating the partitioning of metal ions in systems approximated by experimental conditions. In addition, the power function has only two adjustable parameters that are easily obtained from a linear, least-squares fit of log Q_M vs. -log M_T. The power function model can also represent ion-competition studies (the parameters become conditional on solution composition) and the model may be useful for extrapolating experimental results to lower concentrations of M_T.

It is significant that the power function formulation does not require an estimate of a total ligand concentration, L_T (sometimes termed the "binding capacity"). Several studies of metal-humic interactions have noted the difficulty of obtaining accurate values of L_T for humic materials (Ryan and Weber 1983; Fish and Morel 1984). Both discrete and continuous ligand distribution models require estimates for L_T and Fish et al.(1984) demonstrated the problems associated with the use of this uncertain quantity. The power function representation obviates the need for L_T estimates and the model parameters are especially well suited for concise comparisons of titration data. Any humic titration curve can be represented by two parameters (and a statement of the pH and major ion composition) that are not subject to the uncertainties in the definitions of L_T used by different researchers.

After the metal titration data were plotted in the form log Q_M vs. -log M_T, lines were fitted through each set of data by least-squares linear regression (Fig. 9). The direct fit of the power function in this fashion was termed Model V. Correlation coefficients for all data sets were greater than 0.995. Copper-binding data from a combination of the ISE and FQ titrations yielded data sets in which the relative errors

did not vary significantly across the titration range. As a result no weighting factors were applied to data points in the regression. The slope and intercept of each regression line corresponds to k and log Q_o, respectively (Table 7).

Note that values of both k and Q_o are dependent on the fulvic acid concentration at a particular pH. The change in Q_o (the vertical intercept) is expected. For any system of ligands, a change in total concentrations will shift the ratios of bound metal to free metal by a fixed amount at all points in the titration. The reasons for the change in slope are far less obvious.

The slopes of these plots should be constant for any fulvic acid concentration if all binding sites were independent. The systematic increase in k values at higher fulvic acid concentrations implies that the ratio of bound metal to free metal is not only a function of the ratio M_T/[FA] (as assumed earlier) but is also dependent on the absolute concentration of metal in solution. This effect may be due to a conformational change in the fulvic molecules that is dependent on M_T rather than M_T/[FA]. In the previous discussion of discrete ligands such effects were not observed because the small impact on the calculation of either the free or bound metal concentrations is negligible. Only in the ratio of the two is the effect clearly observed.

Model V was applied to the Cd(II) titration data in the same fashion except that each point in the titration was weighted by the inverse of the standard deviation estimated for that point. The weighting balances the non-uniformity of precision in the ISE titration data. The values of k and log Q_o for the Cd(II) titration are presented in Table 7.

TABLE 7: Model V: Summary of power function parameters fitted to Cu(II) and Cd(II) titrations of GP-FA.

Conc. GP-FA mg-C/L	Metal	pH	-k	-log Q_o
1.0	Cu(II)	5	0.848	5.09
		6	0.983	5.27
		7	1.34	6.61
		8	1.41	6.68
5.0	Cu(II)	5	1.17	5.67
		6	1.40	6.43
		7	1.64	7.28
		8	1.82	8.10
5.0	Cd(II)	6	0.696	3.49
50.0	H^+	5-8	10.44	34.7

The power function given in Eq. 2 reproduces the metal activity data within ± 0.1 log units when the parameters are calibrated to each titration curve. Examples of the quality of fits obtained by regressions on the data from several titrations are shown in Fig. 8 where discrete ligand fits are shown for comparison. When calibrated to each data set (as were the discrete ligand parameters), the power-function Model V achieves a fit with two adjustable parameters that is comparable in quality to the six-parameter fit of Model I.

The alkalimetric titration data are plotted as $\log([A_T - A]/[H^+])$ vs. $-\log(\underline{TOTH})$, where $\underline{TOTH} = [H^+] - [OH^-] + (A_T - A)$, to be in a form that is analogous to that used for the metal titrations (Fig. 10). Model V is not a good representation of the entire range of alkalimetric titration data (Fig. 10). The relationship between $\log Q_H$ and $-\log \underline{TOTH}$ is non-linear at high and low pH but can be approximated by a straight line in the range pH 5 to pH 8.

The power function model can be generalized for Cu(II)-binding over the pH range of 5 to 8 by establishing relationships between pH and the values of k and $\log Q_M$. For the titrations of the 5 mg-C/L solutions the values of both k and Q_o as functions of pH were closely approximated by linear equations. The parameters from the 1 mg-C/L titrations were more scattered with respect to pH but still followed a reasonably linear trend. The results of the regressions are given in Table 8 for use in the formulas.

$$\log Q_o = A_o + B_o\,(pH) \qquad [3]$$

$$k = A_k + B_k\,(pH) \qquad [4]$$

Such a simplified approach (Model VI) appears to be justified by the good fits of the experimental data that are obtained over the ranges

Fig. 10. Power function calibration plots for alkalimetric titration, 50 mg-C/L GP-FA. Log Q_H = log $(A_T - A)/[H^+]$ vs. -log TOTH (terms defined in text).

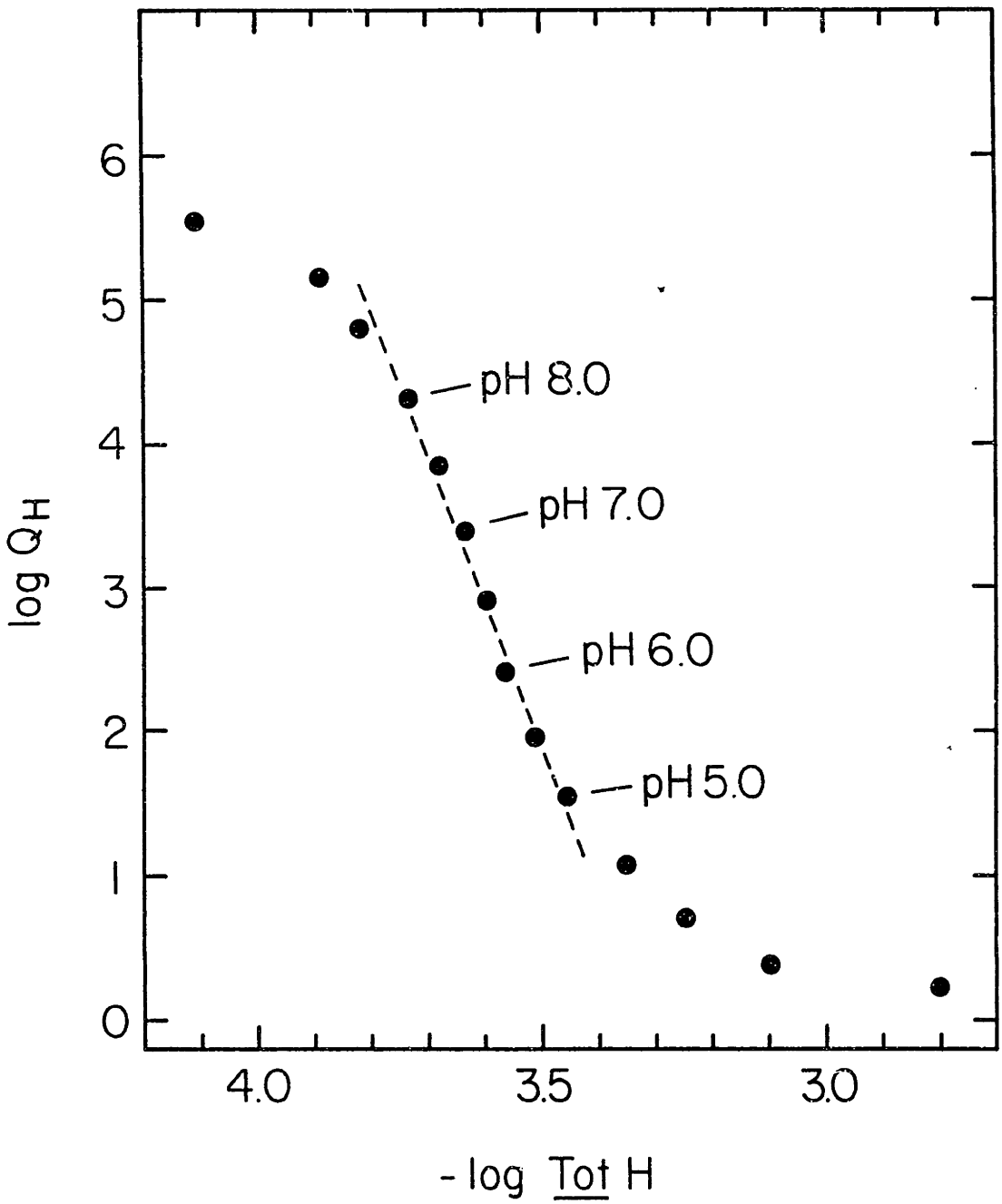

6
5
4
3
2
1
0
log Q_H
pH 8.0
pH 7.0
pH 6.0
pH 5.0
4.0
3.5
3.0
- log Tot H

TABLE 8: Model VI: Regression parameters relating log Q_o and k to pH for GP-FA.

	1.0 mg-C/L	5.0 mg-C/L
A_o	1.92	1.58
B_o	0.61	0.81
A_k	-0.32	0.23
B_k	0.08	0.22

$M_T = 10^{-7}M$ to $10^{-4}M$ and pH 5 to pH 8 (Fig. 2 a,b). As with Model IV (conditional stability constants), the generalized pH formulation of Modle VI reproduces each curve less exactly than the individually fitted the parameters of Model V. Yet the predictions of pCu are still within ± 0.2 log units of the experimental data. This degree of accuracy is reasonable considering the natural variability in humic materials in aquatic systems and the small number of parameters.

In comparison with the nine-parameter, conditional stability constant model, the Model VI power function predicts Cu(II) speciation in the calibrated concentration ranges with only four adjustable parameters, the values of which can be easily obtained by a series of linear regression. If data of approximately uniform precision are used, these regressions can be made directly on the entire range of titration data. This sets the method apart from other commonly used graphical ligand-fitting procedures, such as the Scatchard plot, which requires an estimation of the groups of data points to be linearized.

The Cd/Cu competition titrations can be modelled in the same way as the Cu/(H^+) data. In either system, the competing ions have the effect of reducing the slope k of the logarithmic plots and shifting the vertical intercepts (Fig. 11). There appear to be small difference in the nature of these shifts. Notice, for example, that a change in pH shifts the ratio of bound Cu(II) to free Cu(II) more uniformly across the titration range than does an increase in Cd_T which affects the strong Cu-binding more than the weak binding.

Slopes and intercepts for linear correlations between Cd_T and the values of k and Q are presented in Table 9 for 1 mg-C/L and 5 mg-C/L competition titrations. As with the variable-pH data, the Model VI

Fig. 11. Power function calibration plots for Cd/Cu competition titrations (Model VI). 1 mg-C/L GP-FA, pH 6.0 with: no added Cd(II) (▲), 2 uM Cd(II) (■), 10 uM Cd(II) (●). 5 mg-C/L GP-FA, pH 6.0 with no added Cd(II) (△), 10 uM Cd(II) (□), 100 uM Cd(II) (○).

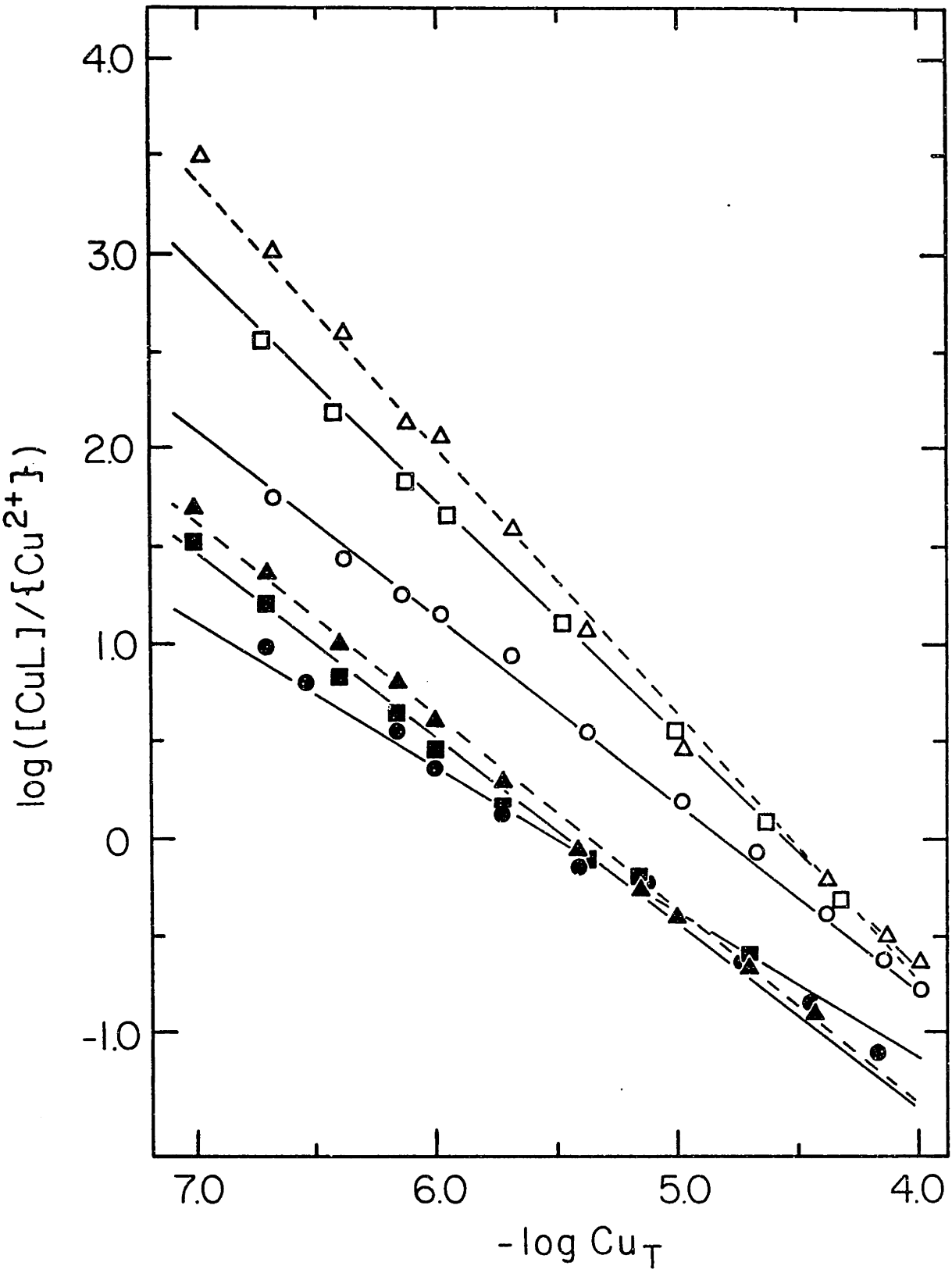

4.0
3.0
2.0
1.0
0
-1.0
7.0
6.0
5.0
4.0
log([CuL]/{Cu2+})
-log CuT

TABLE 9: Model VI: Summary of power function parameters fitted to Cu/Cd competition titrations of GP-FA.

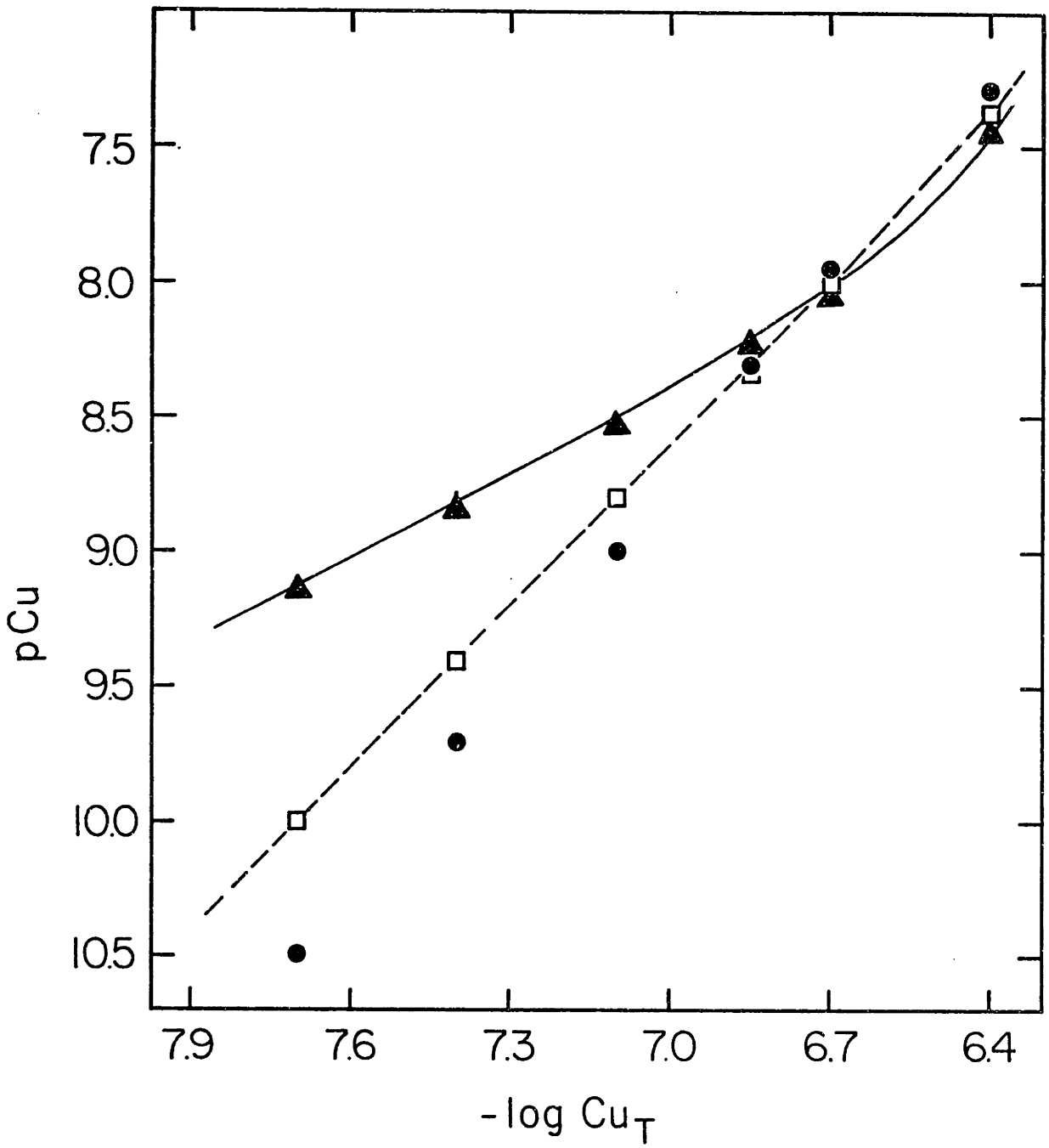
Conc. GP-FA mg-C/L	Cd(II) added	k	$-\log Q_o$
1.0	0	0.983	5.27
	2 uM	0.959	5.26
	10 uM	0.757	4.17
5.0	0	1.40	6.43
	10 uM	1.16	5.33
	100 uM	0.944	4.85

power function reproduces the titration data with only four adjustable parameters as compared to at least nine for a discrete ligand representation of comparable accuracy.

We tested the ability of the power function model to predict the ratio [ML]/[M] below the total metal concentration range of calibration by taking advantage of the fact that, in the strong-binding region, the value of pCu is approximately constant for a constant ratio of M_T/[FA]. That is, the pCu values of titrations at two different fulvic acid concentrations should overlap if M_T values are normalized to the fulvic acid concentrations. This is observed in the GP-FA data in which the strong-binding regions for 1 and 5 mg-C/L approximately overlap if the values of log M_T for the 1 mg-C/L titration are shifted down by 0.7 log units (a factor of five). In effect, the pCu values observed over the lowest 0.7 units of log M_T for the 5 mg-C/L titration are the same that we would observe if we could extrapolate the 1 mg-C/L titration to M_T values that are a factor of five lower than the experimental minimum. Thus we can use the pCu values from the 5 mg-C/L titration for $-\log M_T$ = 6.0 to 7.0 as the values we would observe in the 1 mg-C/L titration for $-\log M_T$ = 6.7 to 7.7.

We applied discrete ligand Model I and the Model V power function of 1 mg-C/L to predict pCu values for the range $-\log M_T$ = 6.4 to 7.7 for this fulvic acid concentration. The results of extrapolating the models below their calibrated concentration range are shown in Fig. 12, along with the pCu values expected in this range, as determined from the 5 mg-C/L titration. As discussed in the previous section, the discrete ligand model cannot predict stronger binding (i.e., lower cupric ion activity) than that of the strongest ligand calibrated. The cupric ion

Fig. 12. Extrapolated titration data for 1 mg-C/L GP-FA, pH 6.0. Data extrapolated from 5 mg-C/L GP-FA (●). Power function model calculations, calibrated for 1 mg-C/L GP-FA (-□-); Model I (discrete ligand) results for 1 mg-C/L GP-FA (-▲-).


7.5
8.0
8.5
9.0
9.5
10.0
10.5
pCu
7.9
7.6
7.3
7.0
6.7
6.4
-log Cu$_T$

activities predicted are well above the expected values. The power function model also fails to correctly predict the cupric ion activity but the values are much closer to those expected on the basis of the titration of the higher fulvic acid concentration.

While the power function model is known to be correct only at values of M_T/[FA] for which it has been calibrated, the model can be used to estimate metal speciation at M_T/[FA] values perhaps an order of magnitude lower than those measured in the laboratory.

Applications to Other Humic Materials

To demonstrate the general applicability of the power-function model to humic materials other than Grassy Pond fulvic acid, the linearalization and regression procedures were applied to Cu(II) titrations of several extracted fulvic acids and whole-water samples performed in this laboratory. The method was also applied to three Cu(II) titrations of natural waters published by Sunda and Hanson (1979) and Tuschall and Brezonik (1983). All titrations were monitored by ion-selective electrodes only, so data points in the high-Cu_T region are weighted according to the estimated precision. The plots of log Q_M vs. -log Cu_T are shown in Fig. 13. Regression parameters for all titrations are presented in Table 10.

The results demonstrate that the power function model can be applied to titrations of a variety of humic substances. Notice that the model can be used equally well for extracted fulvic acids and for titrations of whole water samples. The low Cu_T values of the Waldo Swamp water are of interest in that they deviate systematically from the regression line and appear to be levelling off near log $(CuL)/(Cu^{2+})$ =

Fig. 13. Power function (Model V) calibration plots for Cu(II) titrations of Shawsheen River fulvic acid, 10 mg-C/L, pH 6.45 (■); Suwannee River fulvic acid (I.H.S.S. standard), 5 mg-C/L, pH 6.0 (▽); Waldo Swamp water, 50 mg-C/L, pH 6.2 (△), data from Tuschall and Brezonik (1983); Newport River water, 15 mg-C/L, pH 8.0 (○) and pH 7.0 (●), data from Sunda and Hanson (1979).

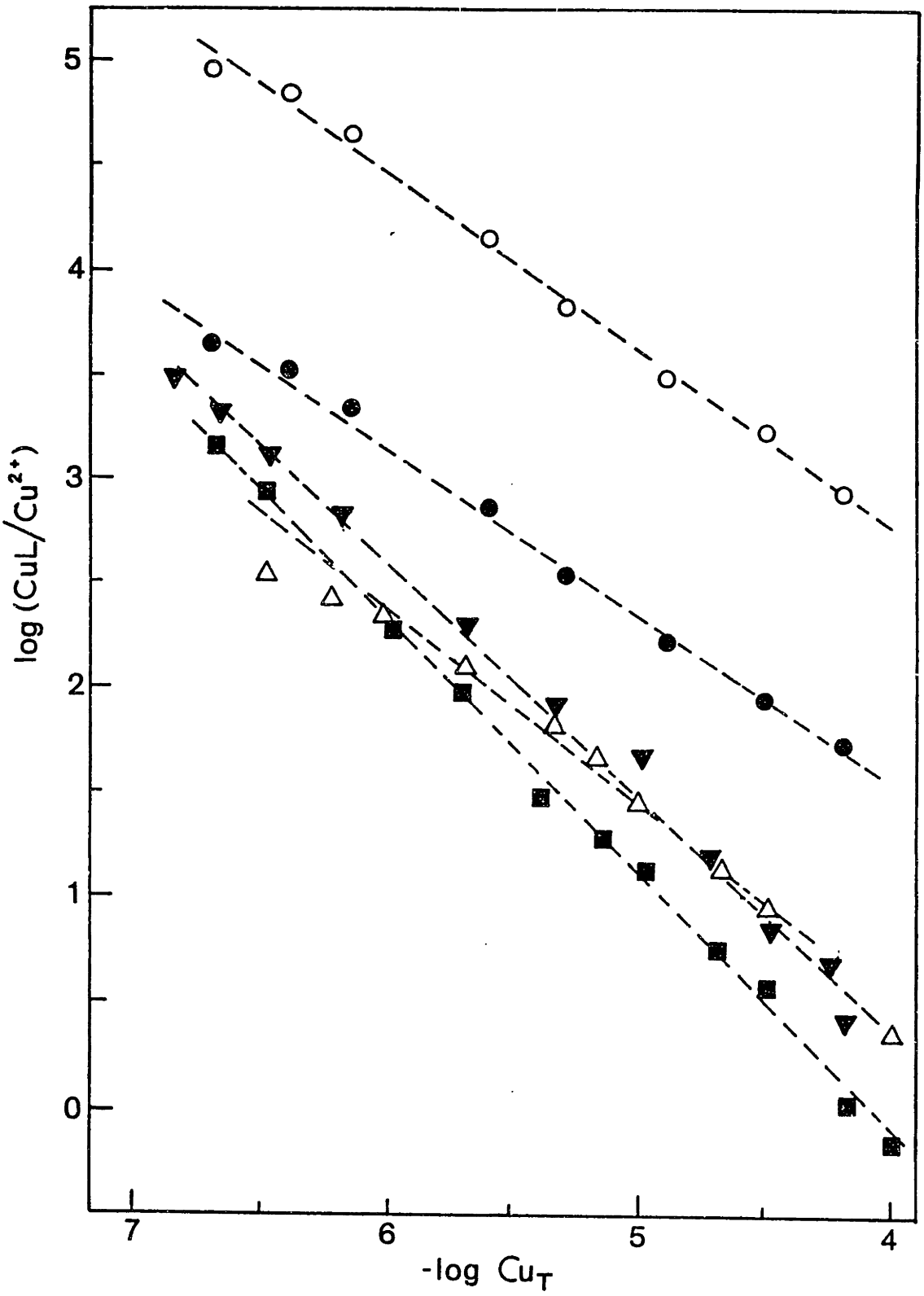

5
4
3
2
1
0
log (CuL/Cu²⁺)
7
6
5
4
-log Cu_T

TABLE 10: Values of $-\log Q_o$ and k for Cu(II) binding in humic-bearing waters and extracted fulvic acids (Model V).

Source	Ref.	pH	Conc. mg-C/L	$-\log Q_o$	k
Suwannee River fulvic acid	(1)	6.0	5	7.14	0.97
Shawsheen River fulvic acid	(1)	6.45	10	4.95	1.20
Newport River whole water	(2)	5.95	15	1.84	0.63
Newport River whole water	(2)	7.00	15	1.65	0.80
Waldo Swamp whole water	(3)	6.0	50	3.66	1.01

(1) This work.

(2) Sunda and Hanson (1979)

(3) Tuschall and Brezonik (1983)

2.5. This water contains much more dissolved organic matter (50 mg-C/L) than the other samples. The levelling of may be due to the attainment of maximum binding strengths or may be simply an artifact of poor electrode response for Cu_T below 1 uM in the presence of such high ligand concentrations.

CONCLUSIONS

As commonly used, the discrete ligand model of humic binding has been shown in several studies to have no intrinsic thermodynamic meaning. Yet discrete ligands can correctly predict the speciation of a single metal over the range of concentrations to which they have been calibrated. The success of using these non-thermodynamic parameters in a thermodynamic theory led us to investigate whether discrete ligands could be conveniently defined from single-ion titrations at a fixed fulvic acid concentration and then used in calculations of the equilibrium speciation of mixtures of ions and at other fulvate concentrations.

The discrete ligands fitted to a metal titration of a particular fulvic acid concentration can be used to predict metal speciation at other fulvic acid concentrations by adjusting the ligand concentrations in proportion to the change in [FA]. Ligand sets individually fitted to several values of [FA] will not appear to exactly follow this proportion, but this effect has been shown to be an artifact of the ligand fitting procedure. The discrete ligand model is never correctly applied at values of M_T/[FA] lower than the experimental range.

Competitive interactions between Cu(II) and Cd(II) were approximated using discrete ligands fitted to individual metal titrations. Ligand concentrations were defined as the mean values obtained for the

two metals and the stability constants were modified to fit the imposed concentrations. The range of metal concentrations that could be modeled by this approach were limited for both metals to the smaller titration range of the Cd(II) electrode. The calculation of the competitive effects of Cd(II) on Cu(II) binding to fulvic acid using Model II was least accurate in the weak binding region of the titration. Cd(II) ions have a greater effect on the weakly bound Cu(II) than is predicted from single-ion stability constants. Discrete ligand parameters that account for competition can be evaluated by extending the fitting procedure to the direct competition titration data (Model III). The requirement that additional data be fitted constrains more narrowly the domain of optimal ligand concentrations and stability constants. By decreasing the range of possible ligand parameter values, the parameters obtained are a more exact chemical description of the fulvic acid.

Assigning proton ionization constants to metal binding ligands was found to be difficult even when the procedure was constrained by proton/Cu(II) competition measurements. The number of possible combinations is unwieldy for manual trial and error adjustments and the data-fitting procedure employed (FITEQL) cannot optimize for more than a single component simultaneously. A more sophisticated curve-fitting routine is necessary to properly apply Model III to proton/Cu(II) competition but a reasonable fit of the data from pH 5 to pH 8 was obtained by trial and error adjustments.

Conditional metal binding constants that are empirically correlated to solution pH offer a simple alternative to the discrete ligand approach. However such conditional constants negate the intended predictive use of discrete ligands but are convenient in systems for

which only a single metal is the primary reactant and for which the pH varies over a few log units.

The power function representation of humic binding is introduced as an alternative to the more detailed, but equally empirical discrete ligand approach. Individual titration curves can be closely approximated by a power function that has only two adjustable parameters.

The power function model offers several important advantages over other empirical models. The model parameters can be fitted with a simple graphical technique that avoids the major ambiguities of commonly used graphical methods for discrete ligands. The appropriateness of the model for a particular data set and the goodness of fit are immediately obvious from the calibration plot. No estimate of the poorly defined "total ligand concentration" is necessary in the power function formulation. The competitive effects of protons on metal binding can be approximated over the range pH 5 to pH 8 by fitting only four parameters to the data. A similar correlation can be made to account for the effects of Cd(II) competition. The power function can be used (with caution) to estimate metal speciation to perhaps an order of magnitude lower metal concentrations than those to which the function was calibrated. Applications to a variety of humic titration data verified the generality of this approach. The model is particularly useful as a concise, unambiguous, two-parameter description of titration curves for comparisons of different materials and the results of different researcher.

Empirical models of metal-humic interactions offer a practical scientific tool for estimating the effects of humic substances on the

chemistry of aquatic systems. However, the shortcomings of all empirical models underscore the need for a thermodynamically well-defined model of metal-humic interactions.

ACKNOWLEDGMENT

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CHAPTER FIVE: SUMMARY

The prediction of interactions between metal ions (including protons) and aquatic humic materials has been shown to be a complex process that is greatly influenced by the type and quality of experimental data as well by the computational model chosen to represent the chemical system.

A comparison of analytical techniques showed that all methods of measuring metal-humic interactions can be usefully divided into three categories, based on the chemical species that are most directly measured. A study of the ways in which indeterminate error propagates through titration data sets indicates that the most precise data over the maximum titration range can be obtained by combining the results of complementary analytical methods for two or more categories.

This approach was demonstrated by applying ion-selective electrode, fluorescence quenching and fixed-potential amperometry methods to a series of Cu(II) titrations of an aquatic fulvic acid. The optimally precise data obtained by these methods and the error analyses developed in this study were used in the remaining sections of the thesis.

A number of models have been advanced for computing the speciation of trace metals in the presence of humic substances. A detailed analysis of the theory underlying these models as well as the applications of the models to experimental and synthetic data demonstrated the fundamentally empirical nature of existing models.

Discrete-ligand models were shown to be useful, in principle, for reproducing the experimentally observed speciation of metals, although

they are not predictive beyond the range of calibration values. Models based on the presumably more realistic continuous distribution of metal binding sites were shown to be dependent on the correct representation of the is model. Several techniques of data analysis appearing in the literature make fundamental errors in the mathematical treatment of this distribution with the results that they provide no new insights into the nature of this distribution.

The only successful continuous distribution models were shown to be those that impose a convenient and physically reasonable distribution, such as a Gaussian distribution of sites. While these models were effective in reproducing titration data, the imposition of a distribution and the subsequent fitting of parameters to the experimental or synthetic data revealed their empirical nature. Such models cannot be expected to be more accurate outside the calibrated concentration range than are discrete ligand models.

A detailed series of Cu(II), Cd(II) and H^+ titrations of an isolated fulvic acid were conducted employing the error analysis of the first section and the combined data of ion-selective electrodes (ISEs) and fluorescence quenching (FQ) methods. Conventional single-ion titrations were performed as well as competing-ion titrations that utilized innovative applications of ISE and FQ procedures. The latter titrations yielded direct measurements of the competitive interactions among ions for humic binding sites.

Application of the discrete ligand model was successful in reproducing the metal speciation observed in single-ion titrations. The discrete ligands fitted to these titrations could not, however, reproduce the binding behavior observed in the competing-ion titrations

unless the parameters were empirically adjusted. In general, six to nine ligand parameters were needed to approximate the experimental results.

By judicious use of "Occam's Razor", it was shown that titration results could be mathematically representd in a much simpler fashion by a power function. The ratio of bound metal to free metal was found to be proportional throughout the titration to the total metal added to the system raised to the power k. Such a model reproduced the titration data within approximately the same margins of error as the discrete ligand model but with only two adjustable parameters.

Generalization of the power function to competing-ion conditions was accomplished by linear regression. A four parameter model was found to reproduce titration data over the ranges $Cu_T = 10^{-7}M$ and pH 5 to 8. A similar regression procedure could account for the results of the Cu/Cd competing-ion experiments.

While the power function model is highly dependent on solution conditions, the analysis of the second section of this thesis revealed that complex mathematical representations of humic-metal interactions are no less dependent on calibration factors.

It is concluded that there are important differences in the analytical methods used to study humic materials and that these differences affect the quality of titration data and the corresponding accuracy of models fitted to these data. No exact representation of the microscopic reactions between humic binding sites and trace metal ions may be possible given only macroscopic binding data. Thus, the models discussed here are essentially empirical, fitting exercises. Because of these limitations, it is concluded that metal-humic interactions are

best represented by simple, easy calibrated models applied to the most precise data obtained over the widest range of conditions. This thesis offers improved methodologies in all of these areas.

APPENDIX ONE:

CHARACTERIZATION OF ORGANIC COPPER-COMPLEXING AGENTS RELEASED BY *DAPHNIA MAGNA*

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ABSTRACT

Zooplankton release a variety of organic and inorganic compounds into their aqueous surroundings. To test the effects of this on trace metal speciation, we raised Daphnia magna in a defined, synthetic medium at various population densities. A series of Cu(II) and pH titrations using an ion-selective electrode apparatus confirmed the excretion of moderately strong metal-binding organic compounds. Control experiments established that bacteria do not produce the complexing agents. The mean hourly excretion rate in the cultures was 40 ± 8 pmol/organism. The complexing material is characterized by two model ligands with conditional stability constants (at pH 6.30) log K_1 = 8.6 and log K_2 = 6.4. These stabilty constants are similar to those obtained for humic and fulvic acids and suggest a role for this material in the trace metal speciation of natural waters. The release of complexing agents may also affect the results of Daphnia toxicity tests by reducing metal activities during incubation.

Interactions between aquatic organisms and the chemistry of their surroundings have been a major focus of scientific attention in recent years. Most of this work addresses the effects of the medium chemistry on the organisms, but there is a growing interest in the ways in which the biota affect the chemistry of the aquatic milieu. The work presented here explores how a freshwater zooplankter, Daphnia magna, can alter the speciation of trace metals by releasing complexing agents in the water.

Chemical speciation is a key factor in the metal sensitivity of aquatic organisms. In general, the biological effects of trace metals are expected to be governed by the free metal ion activities. This has been demonstrated for several metals and many classes of aquatic microorganisms, most of the work focusing on phytoplankton (Sunda and Guillard 1977; Anderson and Morel 1978). In Daphnia magna the toxicity of copper has been shown to be a function of the free cupric ion activity (Andrew et al. 1977).

The major question concerning the speciation of trace metals in natural waters is the potential role of organic ligands. Whereas the most abundant organic ligands are the humic and fulvic acids (Mantoura et al. 1978; Reuter and Perdue 1977) other metal-complexing agents are released by aquatic organisms in culture studies. The strongest class of these ligands is that of the iron siderophores released by bacteria (Neilands 1967; 1981), macrophytes (Sueur et al. 1982) and cyanophytes (Simpson and Neilands 1976; McKnight and Morel 1980). Many bacteria

(Bitton and Freihofer 1978) and eukaryotic freshwater or euryhaline algae (McKnight and Morel 1979) have also been found to produce weaker ligands of undetermined chemical structure.

A logical extension of this work is to consider the release of ligands by zooplankton because these animals are known to excrete a variety of compounds. Banta and Brown (1929) attempted to relate the induction of male production in D. magna to the release of excretory products. Phosphate release by Daphnia is well documented (Rigler 1961; Peters and Lean 1973; Peters and Rigler 1973) as is ammonia production (Jacobsen and Comita 1976). Lampert (1978) reported the release of dissolved organic carbon by grazing zooplankton, some of which can be attributed to animal excretions. Recently, Gardner and Miller (1981) have demonstrated the release of amino acids by D. magna.

Whereas much is known about the release of nutrients by zooplankton, no work to date has centered on excretory products that function as metal-complexing agents. There is, however, indirect evidence for such a role. It has been observed that Daphnia in culture grow and reproduce more rapidly if their medium is only gradually replenished (TenBerge 1978). Daphnia kept in medium in which Daphnia were previously grown likewise show increased growth over organisms in fresh medium (J. Shapiro, Univ. Minn., personal comm.) Such observations are consistent with the notion of an excreted ligand that reduces the deleterious effects of metal ions in the growth medium. These ligands could be significant in both natural systems and in laboratory studies. Daphnia are used in many standardized metal-toxicity testing procedures that confine the organisms in metal solutions for periods of up to several days. Release of complexing ligands would change the speciation of

these metals and alter their uptake and toxicity. In natural systems, the release of ligands by zooplankton could make a contribution to the total pool of autochthonous complexing organic compounds that are probably important for metal speciation in many freshwater systems. This study documents the production of such organic complexing agents by D. magna.

Materials and Methods

In order to provide carefully defined, reproducible conditions, all experiments used parthenogenetically produced females that are genetically uniform. Most of the experiments described here used individuals of a single strain (all descended from a single parent) but some of the later experiments used genetically uniform animals of a different strain. Duplicate experiments with both strains revealed no differences in results.

The Daphnia were raised in large glass beakers or aquaria using dechlorinated tap water with a pH of approximately 8.0. They were fed daily with additions of a separately maintained culture of Scenedesmus sp. and with small additions of yeast extract after the methods of TenBerge (1978). All food additions to the stock cultures were buffered with phosphate buffer to minimize pH variations. The Daphnia cultures were maintained in a constant temperature room at 18°±1°C on a cycle of 14 h light and 10 h dark (cool-white fluorescent fixtures). These stock cultures provided organisms for the ligand collection experiments. All experiments were performed with organisms 20 to 30 d old. The average

body length was approximately 2.5 mm with an average dry weight of approximately 0.10 mg.

The collection of ligands was designed to minimize contamination with trace metals or complexing agents. All apparatus was acid-washed in 10% HCl and metal-clean techniques were used throughout. A defined experimental medium was developed to resemble closely the major ionic composition of the tap water in which they were cultured (Table 1). The major ion salts used in the medium were reagent grade. These salts were used at sufficiently low concentration such that they required no special cleaning procedure. Because most pH buffers are also metal complexing agents, no buffer could be used in the ligand collection experiments except the bicarbonate-atmospheric CO_2 system (alkalinity).

To obviate the problems of artifacts due to algal excretions, feces production and transfer shock, the Daphnia in most experiments were not fed during the course of the collections which ranged from 24 to 120 h. Such periods of time without food did not cause a perceptible increase in mortality or reduction in mobility, although undoubtedly the organisms were somewhat stressed by the end of the longer collections. Many standardized toxicity tests with Daphnia confine them without food for 48 h or more (LeBlanc 1980; Hall 1982).

The experimental Daphnia were transferred with a widebore (7 mm) glass pipet into a wash solution of the defined medium for a brief period. They were then placed in a second beaker of autoclaved, defined medium for 4 to 6 h to rinse away contaminating trace metals and organic compounds that could be carried over from the culture vessels. The second rinsing period also allowed time for the elimination of material from the digestive tracts of the animals. The organisms were subse-

TABLE 1. Composition of Defined Medium

Ion	Concentration (mmol/L)
Ca^{2+}	0.77
Na^{+}	0.75
Mg^{2+}	0.32
K^{+}	0.13
Cl^{-}	1.54
HCO_3^{-}	0.60
SO_4^{2-}	0.37
HSO_4^{-}	0.12
NO_3^{-}	0.04
SiO_3^{-}	0.03

quently transferred into the collection beakers of defined medium. This scheme was modified when an antibiotic treatment was utilized, as outlined below.

Ligands were detected in the excretions of D. magna and in the control experiments by the copper titration techniques described by Swallow, et al. (1978). One procedure is an alkalimetric titration at a fixed total copper concentration (Cu_T); in the other procedure increasing amounts of total copper are added to the solution at a fixed pH of 6.30. The pH was maintained by bubbling with a 1% CO_2/99% N_2 gas mixture and was monitored with a Fisher E5-A combination pH electrode. A Radiometer F3000 Selectrode was used to measure cupric ion activity in conjunction with an Orion 90-02 double-junction reference electrode. The electrodes were coupled to a pair of Orion 801 Ionalyzer meters. McKnight and Morel (1979) elaborate on these techniques in a similar application to that described here.

Several authors have discussed the presence of gut and carapace bacteria in zooplankters (Marshal and Orr 1958; Rigler 1961). These bacteria can be reduced or eliminated by adding antibiotics to the medium (Rigler 1961; Mayzaud 1973; Hall 1982). The Daphnia in control experiments were sterilized by a procedure based on that of Rigler (1961). The antibiotic chosen was Furan-2, a commercially available, broad spectrum (gram-positive and gram-negative) compound consisting of 69% nitrofurazone, 29% furazolidone and 2% methylene blue [Aquarium Pharmaceuticals]. A Furan-2 stock solution was prepared once a week with 15 g of antibiotic and 10 mL of deionized water and refrigerated. The stock was diluted 1:100 for use in the collection experiments. Preliminary tests insured that the Daphnia were not adversely affected

by exposure to this compound. The doses were based on the manufacturer's recommendations and tested with bacterial plate counts.

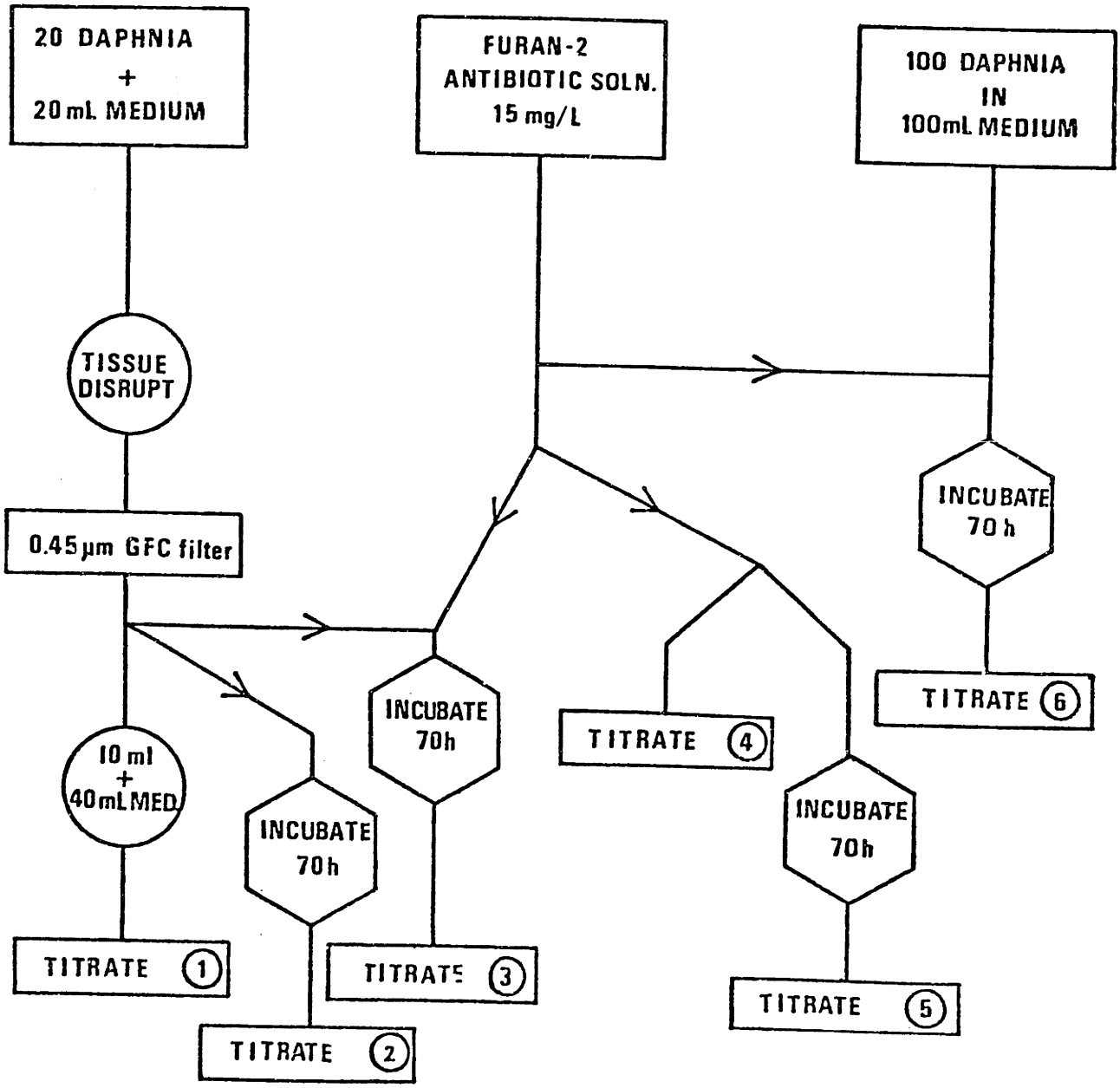
For the bacterial-action control experiments the animals were consecutively transferred for 0.5 h into autoclaved Daphnia medium (ADM) and antibiotic (20 $mg \cdot L^{-1}$), for 0.5 h into ADM, for 1.0 h into ADM and antibiotic (20 $mg \cdot L^{-1}$) and finally rinsed in ADM. They were then transferred to collection beakers containing ADM with an added dose of 15 $mg \cdot L^{-1}$ of antibiotic. After 48 h an additional 10 $mg \cdot L^{-1}$ was added. Sterile technique was used in all transfers.

Because of the confined conditions and several days of collection time, it was necessary to consider the possibility of artifacts due to bacterial production of ligands. Additional considerations were the metal-complexing properties of the antibiotic itself as well as the the breakdown products of the antibiotic. Finally, some measure of the effectiveness of the antibiotic in its intended capacity was needed. The control protocol systematically tests all of these possibilities (Figure 1).

Note that this protocol provides both positive and negative controls. One set of controls was designed to provide plentiful substrate for bacterial growth as a positive test of bacterial effects. Another set tests the bacterial-inhibition and metal-complexing effects of the antibiotics. These sets overlap so that all useful combinations were considered.

Twenty Daphnia were placed in 20 mL of defined medium and ground thoroughly in a glass and Teflon tissue grinder. The resulting suspension of tissue fluids and cytoplasm was filtered through a Whatman GFC glass-fiber filter to remove the solid material. Three aliquots of

Figure 1. Protocol for the bacterial activity and cytoplasmic content control experiments.


20 DAPHNIA
+
20 mL MEDIUM
FURAN-2
ANTIBIOTIC SOLN.
15 mg/L
100 DAPHNIA
IN
100mL MEDIUM
TISSUE
DISRUPT
0.45µm GFC filter
INCUBATE
70h
10 ml
+
40mL MED.
INCUBATE
70h
INCUBATE
70h
INCUBATE
70h
TITRATE 1
TITRATE 2
TITRATE 3
TITRATE 4
TITRATE 5
TITRATE 6

10 mL each were removed from the filtrate and each aliquot was mixed with 40 mL of defined medium. The first was titrated immediately. This tested the complexing ability of internal body fluids that might be released as cytoplasmic leakage. The second aliquot was inoculated with a small addition of Daphnia culture water (about 2 mL) and incubated for 72 h at 20°C to simulate collection conditions. The relatively high concentration of protoplasmic fluids provided substrate for bacterial growth. The third aliquot received the same treatment except that Furan-2 was added at the predetermined dosage to check the results of antibiotic breakdown in the presence of substrate. These aliquots were titrated with copper at the end of the incubations.

A sample of fresh antibiotic in medium was titrated to test its complexing ability. In addition, an antibiotic solution was incubated for 72 h and titrated to test for complexation by breakdown products. Finally, Daphnia excretions were collected in the presence of Furan-2 in order to inhibit epizoic or gut bacteria that survived the initial antibiotic treatment.

To demonstrate directly the absence of bacterial effects, a collection was made under axenic conditions in which the Daphnia were freed of gut and carapace bacteria by the antibiotic pretreatment. The collection was made with 70 Daphnia in 100 mL of medium over a 120 h period. After collection, a 0.5 mL sample of the medium was streaked on a Daphnia medium-peptone-agar plate and incubated for 72 h.

Subsequent to these controls, all experimental collections were made using autoclaved medium and glassware, organisms pretreated with autoclaved medium and sterile transfer procedures. Antibiotics were not

added to the collection medium so as to avoid any minor complexation effects.

Experimental collections were terminated at 24-h intervals up to a maximum of 120 h. Preliminary tests with various population densities indicated that rather high densities of organisms were necessary to produce accurately detectable concentrations of ligand in the 1- to 5-d collection times. The effect of population density on the rate of excretion was examined by collecting excretions in beakers with 50, 75 and 100 organisms per 100 mL of medium.

In order to determine the production rate of ligands by D. magna a time-course collection experiment was performed. Five beakers were prepared, each containing 100 mL of autoclaved medium. Fifty axenically pretreated Daphnia were placed in each of four of the beakers and incubated. The fifth beaker was titrated as a blank. The remaining beakers were collected after 24, 48, 72 and 120 h and titrated with copper.

To determine the effects of a feeding regime compared to a non-feeding regime on ligand production, a collection was set up that included several transfers of the organisms to a separate feeding vessel. Fifty Daphnia were placed in 150 mL of medium and incubated for a total collection period of 84 h. The organisms were transferred to the feeding vessel for a 2 h period after 24 h of collection and again after 72 h.

Membrane ultrafiltration was used to test for the approximate molecular size range of the complexing agents released by D. magna. A sample was collected using 200 Daphnia in 400 ml of medium for 96 hr and was divided into two aliquots, each prefiltered through a Whatman GFC

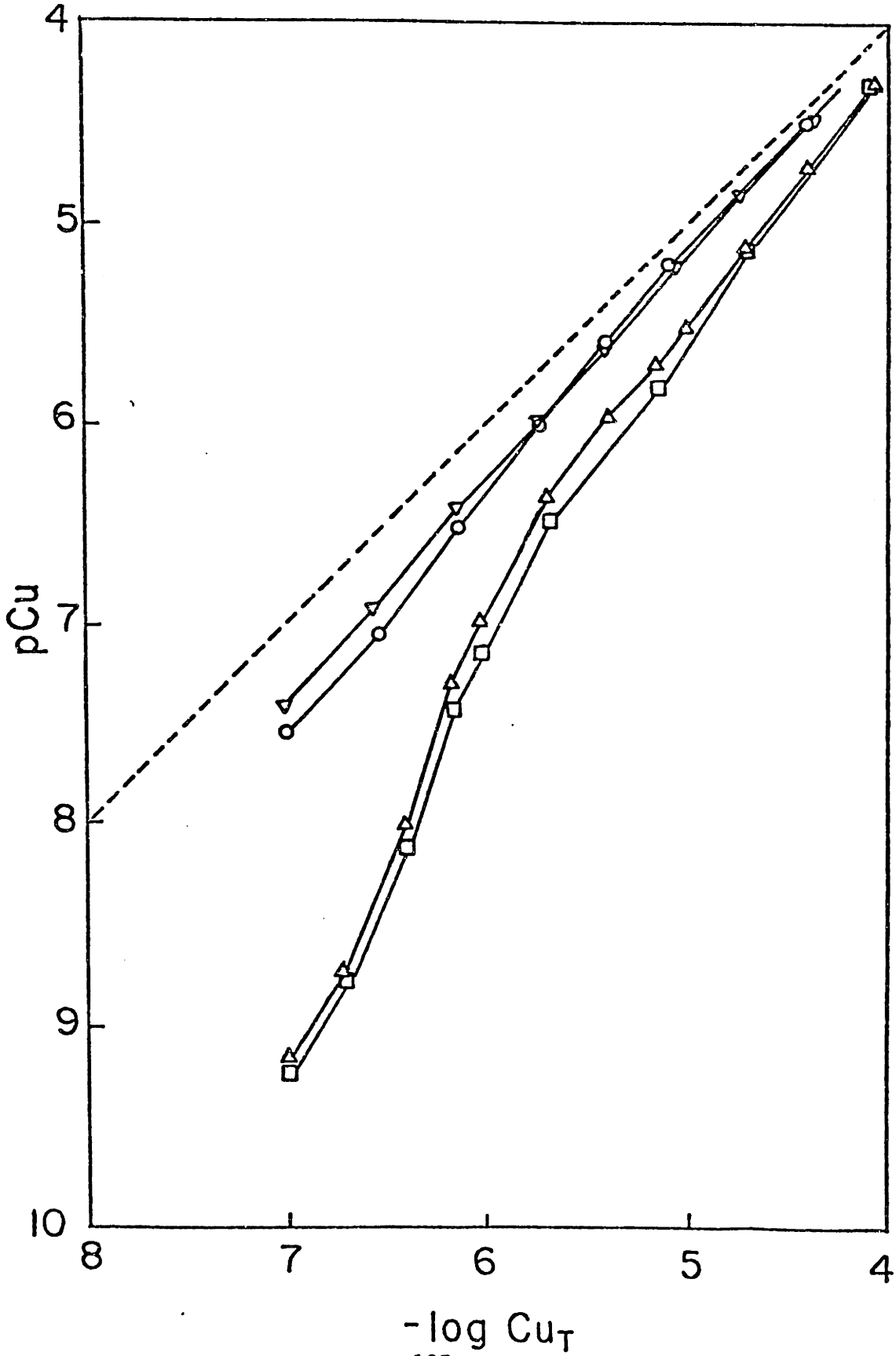
filter. One aliquot was titrated with copper immediately and the other was passed through an Amicon UM05 membrane filter. The UM05 filter, fitted in an Amicon pressure cell, has a nominal molecular-size cutoff of 500 daltons. After ultrafiltration this second aliquot was titrated with copper.

Results

A complexometric titration of 110 mL of filtered, defined medium in which 110 organisms had been incubated for 72 h revealed a substantial degree of complexation compared to the blank titration curve (Figure 2). The complexometric curve exhibited a reduction in the cupric ion activity by several orders of magnitude at the lower total copper concentrations. Although this titration demonstrates the presence of complexing agents in the collection medium, these ligands could not be attributed to Daphnia excretions without considering the effects of bacterial ligand production. The results of the complexometric titrations in the control experiments indicated that such effects are minimal.

The titrations of the control media demonstrated the absence of copper complexation by Daphnia body fluids, by the exudates of bacteria which could grow in the collection beakers, or by breakdown products resulting from a mixture of antibiotics and tissue fluids. The results of the titration of the antibiotic solution revealed only a small degree of metal complexation relative to the blank (Figure 2). In contrast, the titration of material collected from live Daphnia in the presence of antibiotic under the same conditions showed significant complexation of

Figure 2. Cu(II) titration of blank medium (—▽——▽—), blank medium with antibiotic added (—o—o—), medium after incubation for 72 h with 1.0 Daphnia per mL and no antibiotic (—△——△—), medium after 72 h with 1.0 Daphnia per mL with antibiotic treatment (—□——□—). Dashed line is for reference and indicates pCu (-log $[Cu^{2+}]$) equal to Cu_T (total added copper).


4
5
6
7
8
9
10
pCu
8
7
6
5
4
-log Cu$_T$

copper and results in a titration curve which is virtually identical to that of the collection made without antibiotic (Figure 2). The medium from the "axenic" collection was tested for bacterial activity as well as complexing ability. After 72 h the agar test-plate contained only two colonies of rod-shaped bacteria, evidence of minimal bacterial presence in the collection medium. A complexometric titration of this collected material revealed the same degree of copper binding as in non-axenic collections.

Although none of these controls, taken individually, completely eliminate the possibility of artifacts, collectively they provide strong evidence that the complexing phenomena observed here are due to the excretory products of living Daphnia.

As mentioned earlier, Daphnia are known to excrete both ammonia and orthophosphate. Data published by Smith and Martell (1976) indicate that these inorganic ligands form weak complexes with copper. Calculations show that orthophosphate and ammonia could affect the copper activity only at ligand concentrations much higher than was found in these experiments. Titrations of phosphate and ammonia solutions verified these calculations. These observations indicate that the major complexing agents produced by the Daphnia are not inorganic ligands.

The apparent concentration of ligands in the collection medium is obtained from the titration data by examining the complex formation curves in which the concentrations of complexed copper (CuL) are plotted against the total copper concentrations (Cu_T). The CuL concentration at which this curve levels off is the total equivalent ligand concentration (L_T). Using this value the ligand production rate and the metal-complex stability constants can be determined. The formation curves can also be

normalized by dividing the data on both axes by L_T in order to compare the shapes of curves from different collections.

The copper-complex concentration is determined as the difference between the total added copper (Cu_T) and the free copper measured (Cu^{2+}) by the electrode, taking into account the inorganic hydroxo and carbonato complexes. This method is quite accurate over most of the titration but, near the endpoint, the value of CuL (= L_T) may be only 5% of the total copper. Measuring this small difference from the logarithmically scaled electrode data introduces a significant margin of error in determining L_T. This effect is illustrated by plotting the normalized complex formation functions for all of the titrations (Figure 3). Such a plot reveals highly consistent data from all titrations in the low Cu_T range. As the ratio of Cu_T to L_T excedes a value of 1, there is substantial scatter in the data, both among titrations and within a titration. In the end region (Cu_T/L_T = 3 to 4) the value of L_T can be determined only within approximately ±20%. A similar estimate of error is obtained from replicate titrations in which the reproducibility of the cupric ion activity measurements is about ±0.1 log units (i.e., equivalent to ±20%).

In Table 2 the L_T values are normalized for both population density and collection period, resulting in total ligand production rates per organism. Because of the measurement errors discussed above, these values should be considered mean values, significant within an error range of ±20%.

The production rates presented in Table 2 show no consistent pattern of variation detectable with respect to either population density or time of confinement. Nor is there a significant difference

Figure 3. Complex formation data for Daphnia excretions. Copper-complex concentrations (CuL) are plotted as a function of total added copper (Cu_T) after both values are normalized by dividing by the total ligand concentration (L_T) for each titration. Data are shown for all titrations documented in Table 2.

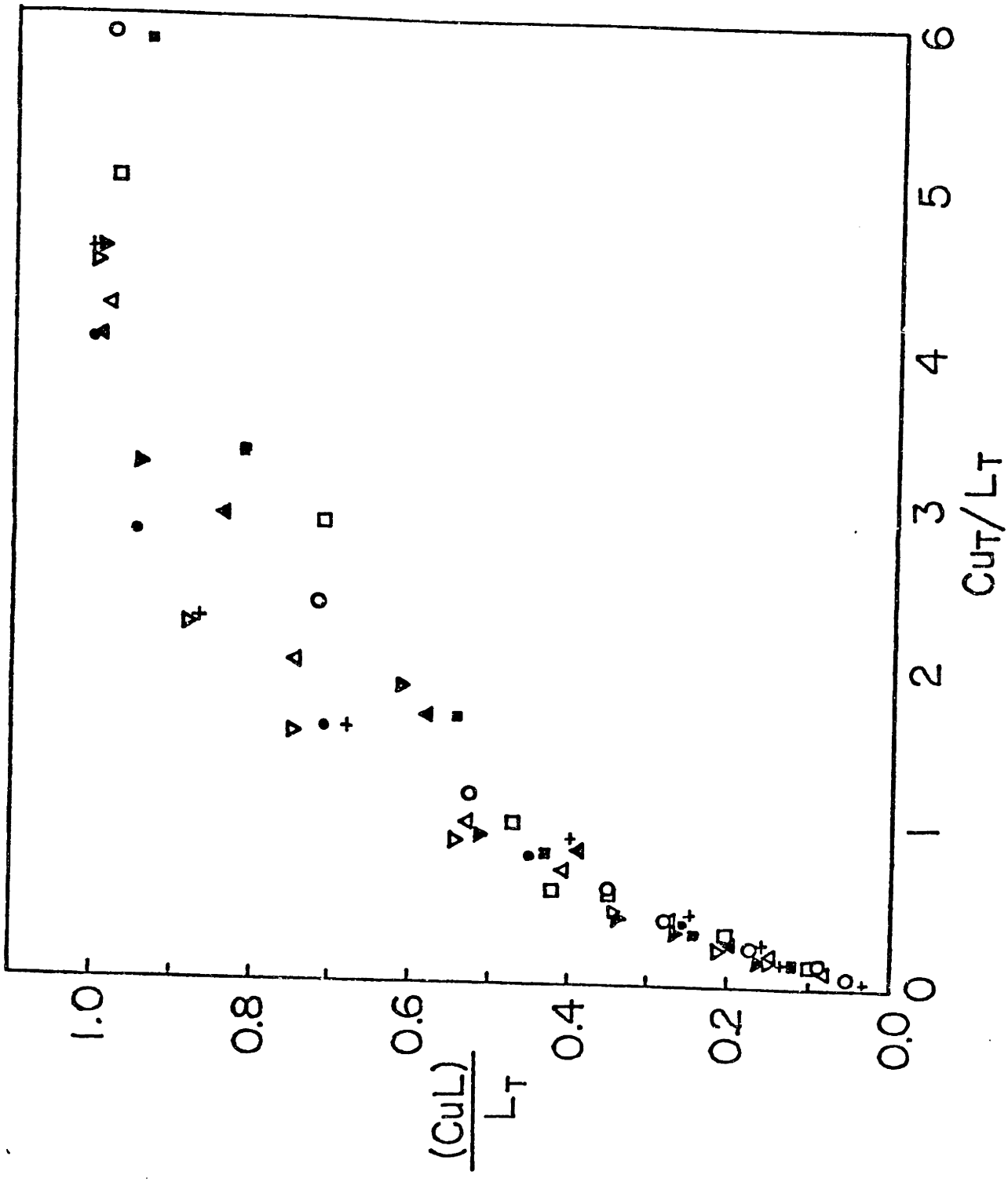

1.0
0.8
0.6
0.4
0.2
0.0
(CuL)/LT
0
1
2
3
4
5
6
CuT/LT

TABLE 2. Total equivalent ligand production rates for Daphnia magna.

Density of organisms (per 100 mL)	Collection period (h)	Total ligand (umol/L)	Ligand production rate (pmol/org·h^{-1})
15	10	0.25	170
33[a]	84	1.2	43
50	48	7.0	29
50	72	1.0	28
50	120	2.4	39
70	120	4.5	52
100	48	1.7	36
100	72	4.4	61
100[b]	72	2.2	31

a - Organisms fed during experiment.

b - Furan-2 antibiotic added to medium.

between the collection in which the Daphnia were fed and those in which they are not. Any variation due to these factors apparently is small enough to be included within the margins of error for the detection method. The overall mean production rate is 40 pmol/organism·h^{-1} with an error of ±8 pmol/org·h^{-1}.

Titrations indicated that passing the Daphnia excretion solution through an ultrafilter with a nominal molecular-weight cutoff of 500 daltons effectively removes all of the ligands from solution.

Using the copper titration data, stability constants for the formation of the copper-ligand complex can be obtained. As is conventional in the characterization of natural organic ligands, conditional stability constants for the metal complex are defined. These do not explicitly account for the pH dependence of the formation equilibrium and are strictly valid only at the pH used in the titration, i.e., pH 6.30.

The copper titration data were analyzed using the graphical method of Scatchard (1948). For a multiligand system, Scatchard plots take on a characteristic concave shape in which two approximately linear regions can be distinguished. These regions do not, in general, correspond to two chemical species but instead may be thought of as regions of binding strengths within the total ligand concentration. In these plots, the slope of each linear section is equal to $-K_i$ and the intercept on the horizontal axis corresponds to n_i, where K_i is the equilibrium binding constant for the i-th binding region and n_i is the relative proportion of that region. These constants may then be used for modelling chemical speciation via equilibrium calculations, utilizing the binding regions as if they represented discrete ligands.

Scatchard plots for all of the titrations yield consistent values for K_1 and K_2. The values obtained with least-squares linear regression are log K_1 = 8.6 and log K_2 = 6.4. The relative proportions of the two binding regions are n_1 = 0.20 and n_2 = 0.80. Accordingly, the complexing material released by D. magna can be modelled by a smaller proportion of relatively strong metal-binding sites and a larger proportion of weaker sites. Data from five representative titrations have been combined into a single Scatchard plot in Figure 4.

It is impossible to attribute specific physical characteristics to the ligand molecules (such as functional group arrangement or number of actual site-types) but the titration data can be well modelled by two ligands with the K and n values given above. In Figure 5 a titration curve is compared to a plot generated by equilibrium calculations using this two-ligand model.

To characterize fully metal-organic interactions, we must consider the weak-acid properties of the ligands as well as their metal affinities. Because of these acid properties, the complexation of copper is a function of pH as well as copper and ligand activities. The proton affinity of the ligands is demonstrated by the pH titration. For this procedure a 50 mL aliquot of the axenic, 120 h collection was used.

As the pH of the medium increases due to NaOH additions, the copper ions compete more effectively with protons for the available binding sites on the ligands (Figure 6). This competition is evident in the pH titration from the sharp decrease in copper ion activity in the higher pH range. The effect of inorganic hydroxo complexes is shown in the blank titration.

Figure 4. Scatchard plot of Cu(II) titration data; $v = [CuL]/L_T$. Dashed lines indicate linear regressions on the data in the two regions.

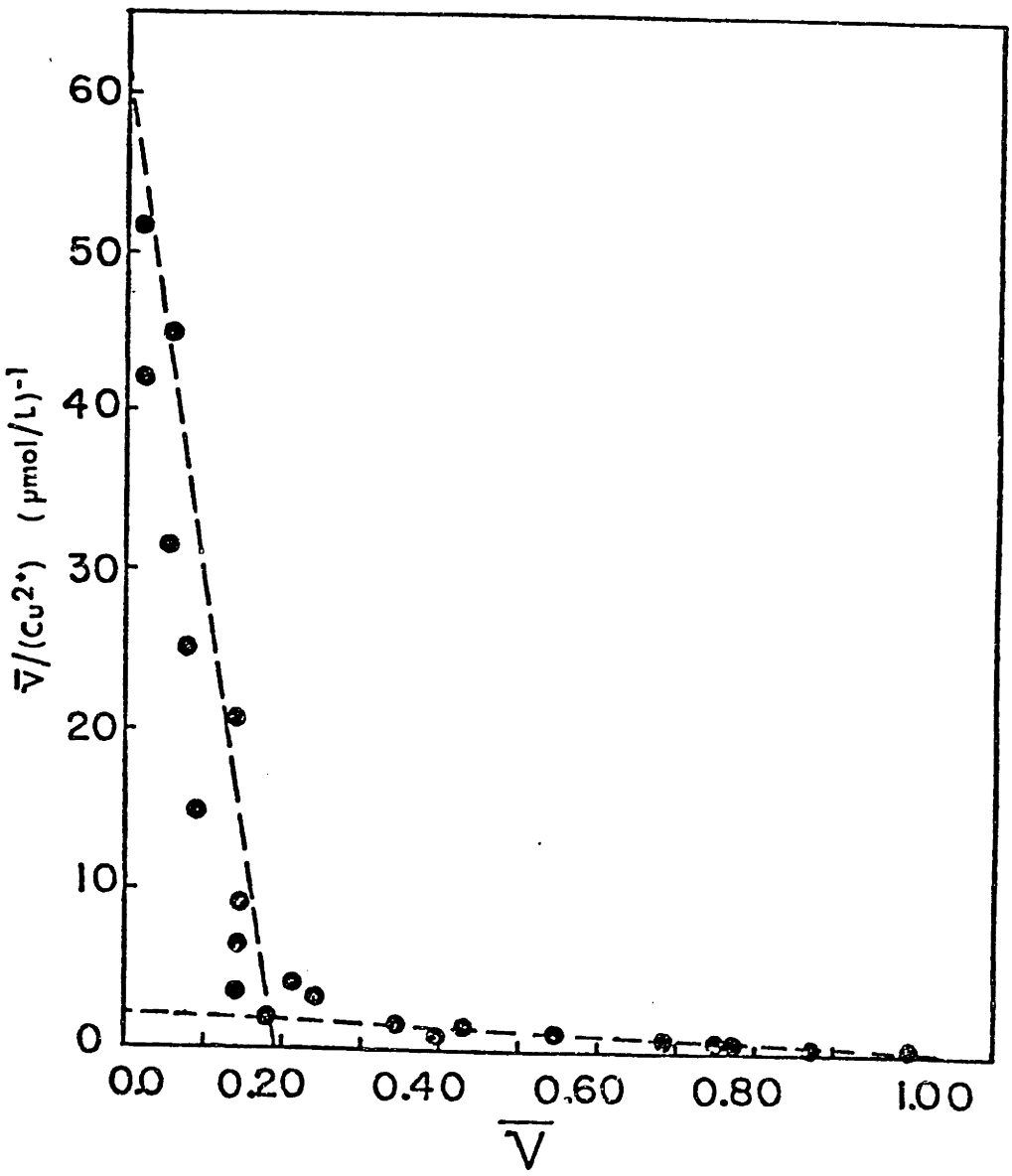
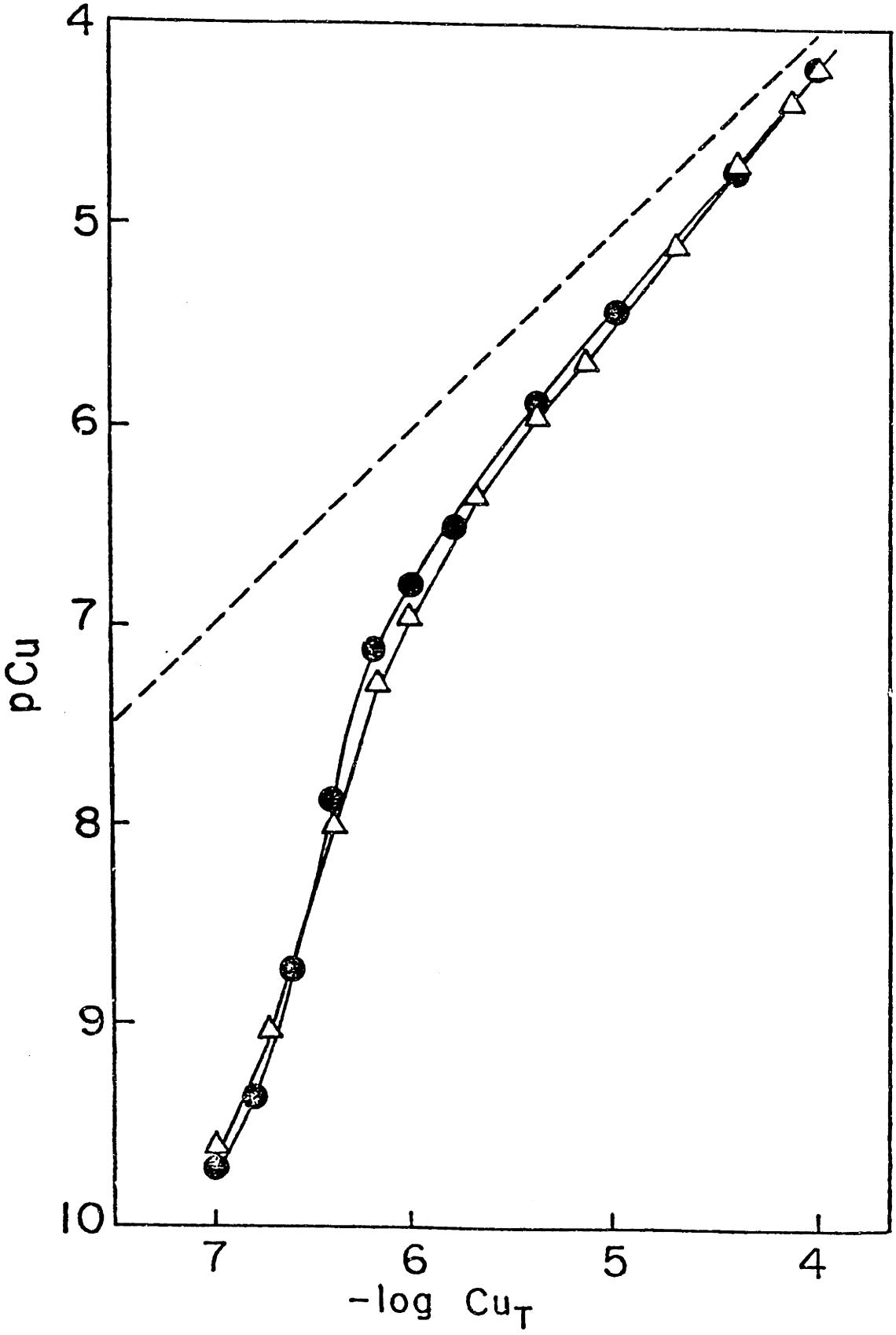

60
50
40
30
20
10
0
0.0
0.20
0.40
0.60
0.80
1.00
$\bar{V}/(Cu^{2+})$ (μmol/L)$^{-1}$
$\bar{V}$

Figure 5. Calculated Cu(II) titration curve using two model ligands: L_1 = 0.90 umol/L with log K_1 = 8.6 and L_2 = 3.6 umol/L with log K_2 = 6.1 (—△——△—). Experimental titration curve for 120 h Daphnia collection at a density of 0.7 organism/mL (—●——●—).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9
10
pCu
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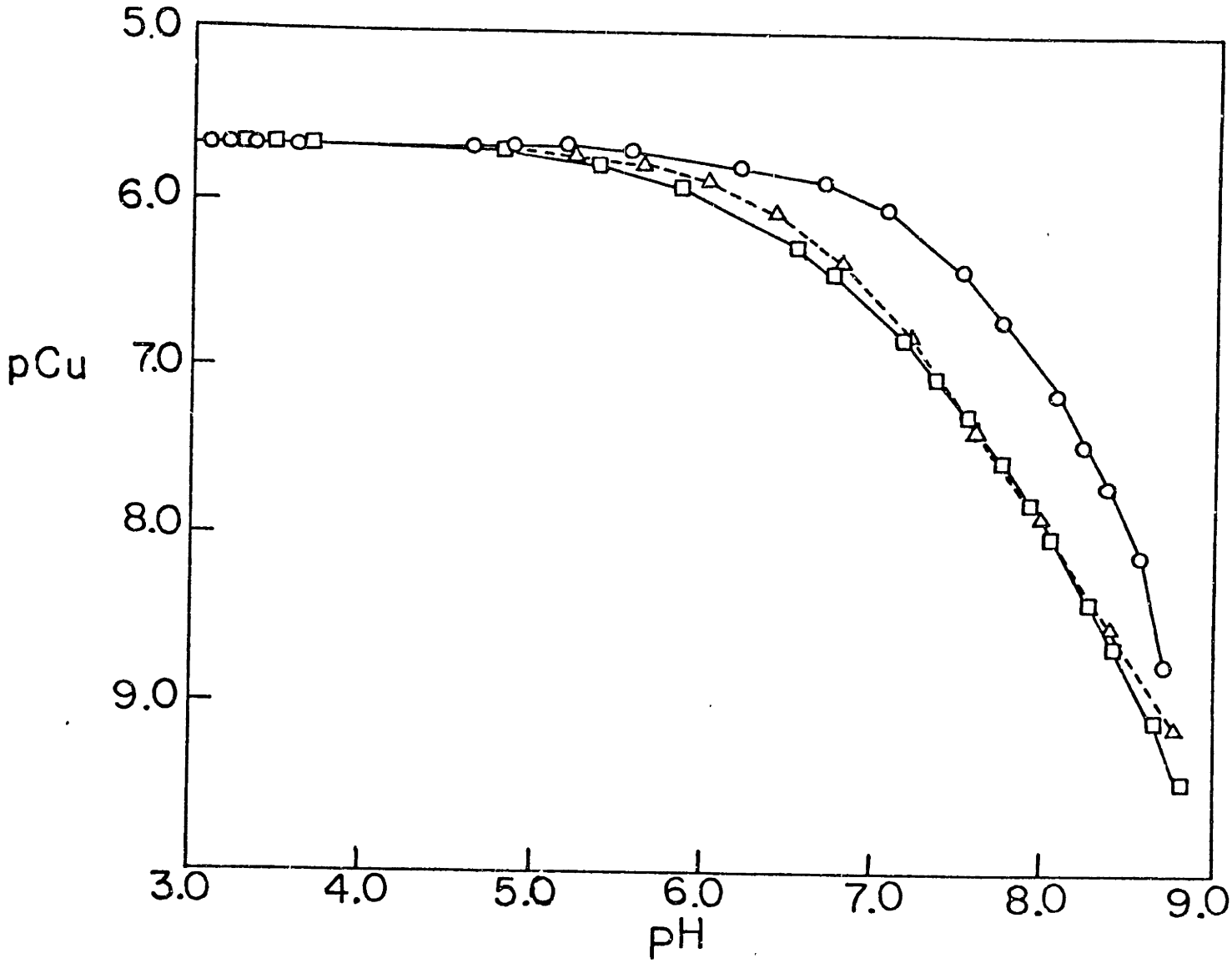
6
5
4
-log Cu$_T$

Figure 6. NaOH titrations of blank medium (—○——○—) and Daphnia exudate (—□——□—) at a fixed total copper concentration (= 2 umol/L). Calculated titration curve using the conditional constants defined in the text (—△——△—).


5.0
6.0
7.0
8.0
9.0
pCu
3.0
4.0
5.0
6.0
7.0
8.0
9.0
pH

The slope of the curve plotted in Figure 6 can be interpreted as the average number of protons removed when a copper ion is bound, as in the reaction:

$$Cu^{2+} + H_xL = CuL + \underline{x}H^+$$

The stability constants found at pH 6.30 can be extended over a wider pH range by incorporating the slope $\underline{x}$ in a conditional stability constant that describes this proton exchange reaction.

$$K_i^* = (K_i)(H^+)^{\underline{x}}$$

The data can be fitted by defining $\underline{x}$ in three pH regions: $\underline{x}$ = 0.5, pH 5.0 to pH 6.5; $\underline{x}$ = 1.0, pH 6.5 to pH 7.5 and $\underline{x}$ = 1.8, pH 7.5 to pH 9.0. When these ionization constants are used in the computer-based chemical equilibrium program MINEQL (Westall et al. 1976), the experimental titration curve can be closely modelled (Figure 6).

Discussion

The experimental results presented here characterize the copper-complexing capacity of organic material released by D. magna in laboratory culture. Analysis of complexometric titration data indicates that this material can be modelled as a mixture containing a large proportion of a weak ligand and a smaller proportion of a moderately strong ligand. This description is only a practical model, as the composition and structure of the material is unknown.

Gardner and Miller (1981) reported the release of amino acids by Daphnia magna at rates comparable to the excretion rates noted in our work. Amino acids form stable complexes with copper but do not fully explain the observed metal binding. The proportion of relatively strong complexing agents found in our experiments is substantially higher than in the amino acid mixture observed by Gardner and Miller (1981). Histidine, which forms the strongest copper complexes of any in that mixture, has an effective binding constant of log K = 7.0 at pH 6.3 (Martell and Smith 1974). This is over an order of magnitude weaker than the log K = 8.6 found in our study. The removal of the Daphnia complexing material by ultrafiltration suggests that it consists of substantially larger molecules than amino acids, although ultrafilters can also adsorb small organic molecules, causing error in the apparent molecular-weight cutoff. Amino acids are rapidly utilized by bacteria (Hobbie and Crawford 1969), yet the presence or absence of bacteria has little effect on the accumulation of the Daphnia ligands.

One source of the complexing agents may be the release of proteinaceous metal-binders, such as metallothioneins, by the Daphnia. Another explanation is that Daphnia may release an assortment of amino acids, polysaccharides and peptides that combine and are chemically altered in the medium, perhaps by photochemical reactions, to form higher molecular weight metal-complexing compounds. This would be analogous to the reaction mechanisms suspected in the formation of marine humic matter from autochthonous organic carbon sources (Gagosian and Stuermer 1977).

The results of this research are directly transferable to laboratory-scale experiments in which Daphnia are confined in small volumes of

medium for periods of up to several days. Trace metal toxicity tests commonly use a defined medium to minimize the variability of chemical speciation, but the results presented here show that the organisms themselves can alter the speciation of trace metals in the test medium. Daphnia excretory products can reduce the cupric ion activity by an order of magnitude within 48 h at the population densities described. Spurious results in toxicity tests can be avoided by either reducing population densities in the tests or by directly accounting for the effects of the excreted ligands on the metal speciation. The latter can be achieved by measuring the metal speciation with ion-selective electrodes or by conducting the experiments in a metal-buffer system designed so as to prevent a significant change in the free metal activities when ligands are released.

The significance of these excretions to trace metal speciation in natural waters depends on the total concentrations of ligands and trace metals. Natural ligand concentrations are not known and estimates of these values depend on several assumptions. The use of only females in this work was a reasonable simplification because males of this genus are an insignificant part of natural populations on a seasonal basis. However, the data presented here were collected at the unnaturally high population densities of 50 to 100 organisms per 100 mL. By comparison, Hall (1964) found natural Daphnia populations in Lake Mendota reaching densities of about 5 per 100 mL during early summer and fall. Thus, the experimental densities were about an order of magnitude higher than those found in active natural populations. However, it should also be noted that the collection periods spanned only 5% to 10% of a typical D. magna lifetime.

Experiments conducted at several population densities suggested no significant variation in production rates over a twofold density change. It is not certain that these rates can be extrapolated to populations in the natural environment where the densities will be an order of magnitude lower. Similar arguments can be made concerning the type of feeding regime used. The data do not show a significant variation between fed and unfed organisms but the effects of continuous (rather than discrete) feeding are not known. In natural water conditions, excretion rates may be either greater or less than those presented here. Well-fed, uncrowded organisms may metabolize at higher rates, resulting in more excretion, or, such unstressed organisms may lack some necessary stimulus to produce these complexing materials. The only experiment to yield an atypically high production rate was a short term, low density collection. This may indicate that unstressed organisms produce more ligands or that initial ligand production is higher than the mean, long-term rate.

A knowledge of ligand decay rates is needed to estimate steady state concentrations. Data from the essentially axenic collection suggested minimal abiotic degradation on the time-scale of one week. To test this, an aliquot of collected excretions was retained at room temperature for 5 d. The titration curves of freshly-titrated material did not differ from those of the stored excretions. A decay coefficient for the complexing material in a natural system would depend on the bacterial population and cosubstrate availability. Without this decay constant, it is not possible to estimate a steady-state concentration of complexing material in a natural system.

It is interesting to note, however, that the stability constants obtained for this material are in approximately the same range as those reported by a number of workers for humic substances (Mantoura et al. 1978; Buffle et al. 1977). In a highly productive lake supporting a zooplankton bloom, epilimnetic concentrations of excreted ligands could reach 1 $\mu mol \cdot L^{-1}$ or more. If the concentration of humic matter is relatively low (e.g., less than 2 $mg \cdot L^{-1}$) calculations using the humic data of Mantoura et al.(1978) indicate that a 1.0 $umol \cdot L^{-1}$ concentration of Daphnia excretions would influence the speciation of copper the water.

The organic compounds described here are part of the pool of autochthonous organic material that is often a significant proportion of the dissolved organic carbon in natural freshwater systems (Thurman and Malcolm 1981). The release of organic carbon by phytoplankton, bacteria, fungi, macrophytes and zooplankton within the water column is the source of such a non-humic pool. Our work, as well as the work of others, indicates that dissolved organic compounds generated by all of these sources can form complexes with trace metals and that some of these compounds may dominate the metal speciation in natural waters. A better understanding of metal speciation in eutrophic waters will require characterization of the autochthonous complexing agents and consideration of the effect of the sum of all these non-humic organic materials. Production rates and metal-complex stability constants have been estimated for a number of these substances. Further work is needed to quantify the total production and to characterize the decay and conversion processes of such material in particular aquatic systems.

Acknowledgements

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APPENDIX TWO:

EFFECTS OF NATURAL AND SYNTHETIC CHELATORS ON TOXIC METAL TRANSPORT IN A POROUS MEDIUM

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ABSTRACT

A series of laboratory experiments was performed to study interactions among dissolved toxic metals, adsorbent porous medium surfaces and natural and synthetic complexing agents. In these experiments a silica sand column was used as the solid medium with copper as the model heavy metal. One set of tests verified the retardation of metal transport due to simple adsorption of metals onto the silica surface. The addition of the non-adsorbing complexing agents glycine or EDTA reduced retardation. This effect can be predicted by incorporating chemical equilibrium constants in the mass transport equation. Naturally-occuring humic organic compounds adsorb onto silica surfaces as well as binding to metals. Humic matter is shown here to either accelerate or retard the transport of metals in a groundwater system depending on its affinity for a particular medium surface. Linearized forms of the adsorption and equilibiurm equations allow for a convenient first-order approximation of the observed phenomena. A one-dimensional, quasi-equilibrium, finite difference transport model is developed which incorporates the chemical behavior into a useful, predictive form.

INTRODUCTION

Modelling and understanding pollutant behavior in groundwater systems can be achieved through several approaches. Analytical models are useful for quick approximations in well-defined systems. Computer models are capable of almost anything, given enough data and computer time (both are non-trivial considerations). A third type of model is the physical model. For flow-regime modelling, these engender many problems, but for understanding chemical behavior they are nearly indispensible. Basic data on adsorption and chemical interactions with surfaces can only be obtained from a physical representation of the medium-solute-solvent system.

This paper describes the use of a bench-scale porous medium column for the purpose of obtaining contaminant breakthrough curves under varying conditions. The results obtained from this physical modelling are then compared with the predictions made by simple analytical and computer models of similar systems. The purpose of this work is to demonstate how surface and solution chemistry can dramatically affect the transpot of toxic metals in porous medium flows.

The interactions under consideration are:

- Interactions between metal ions in solution and the adsorbing surfaces of the porous medium.

- The effects of non-adsorbing organic compounds which chelate (bind) the metal ions.

- The effect on the system when both the free metal ions and the chelated metal complexes adsorb onto surfaces.

These effects were examined empirically using quartz (silica) sand as the medium, copper solutions as the metal contaminant and several synthetic and natural organic metal-complexing agents. Equilibrium adsorption and complexation theory is then used to analyze and predict the effect on the metal transport of varying the chemical parameters.

EXPERIMENTAL

The experiments were carried out using a 15 cm glass chromatography column. The column holds a gross volume of 27 cm^3 and is fitted with a porous Teflon support plate at the bottom. There is about 0.5 ml free volume below this support plate and about 0.1 ml head space above the sand surface. The small volumes minimize extraneuos dispersion. See figure 1.

The medium was a fine silica sand sold by Mallinckrodt Chemical. The sand has a porosity of 0.30 as determined by the volume of water required to saturate the sand. Due to the relative coarseness and

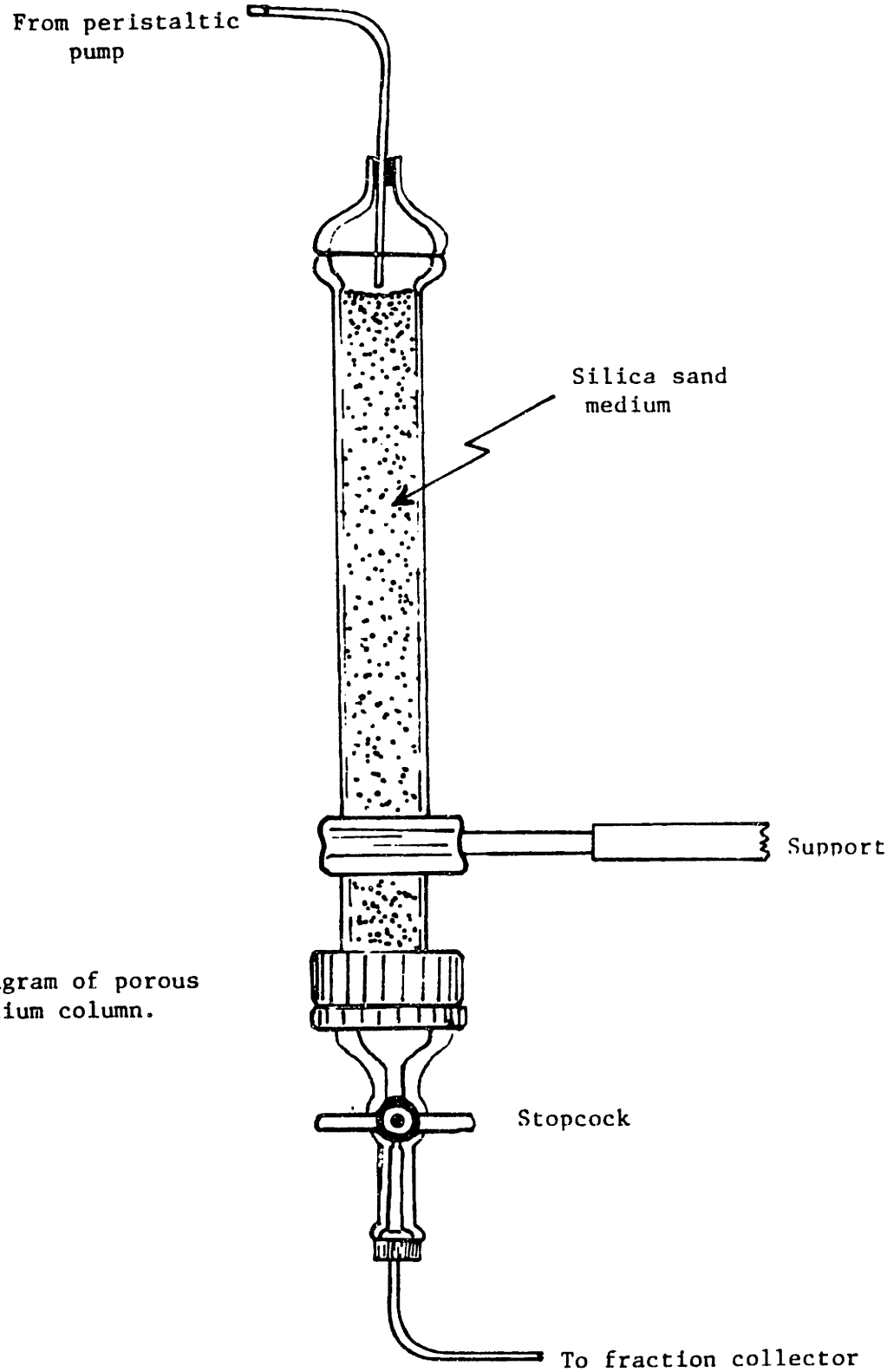

Figure 1. : Diagram of porous medium column.

uniformity of the sand, dead-end pore space is neglected. The sand was prepared by stirring vigorously in dilute nitric acid to remove trace metals and then washed with deionized water until no excess acidity remained in the wash water.

The sand in all experiments was packed into the column as a slurry. The water level was kept above the sand surface at all times to eliminate trapped air in the medium. Fresh sand was used for each run.

A high-purity, deionized water was used in the experiments. Since such water is normally about pH 5, 2×10^{-4} mol/liter of sodium bicarbonate was added to raise the pH to 6.3 and to buffer the pH near that value throughout the experiment. Additional bicarbonate was added if acidic ligands were added.

Solutions were pumped through the column using a peristaltic pump. All experiments were conducted at a flow rate of 0.5 ± 0.05 ml/min. The pH 6.3 solution was run through the column while flow calibrations were made. Usuallly about 10 void volumes of solution were allowed to pass through to enable the surface to equilibrate to this pH. (One void volume = 8.0 cm^3). Using a porosity value of $n = 0.3$ we obtain a seepage velocity of 0.85 cm/min. Since sand is packed to a depth of 25 cm in the column, the breakthrough time for an ideal tracer is about 18 min. This was verified with a dye tracer.

Trial runs determined the necessary metal concentrations and flow rates to get breakthroughs at times on the order of a few hours. Flow rate and total metal content were held constant in all runs. Copper was used as the metal since it is easily analyzed and since its behavior is representative of a number of transition metals. Total copper was fixed at 1.0 ppm (= 1.0 mg/l = 1.5×10^{-5} mol/l).

Three types of organic complexing agents were used in the experiments. Ethylenedinitrilo-tetraacetate (EDTA) was used as a non-adsorbing, strong complexing agent which binds virtually all of the added copper. Glycine was used as a non-adsorbing, weak complexing agent which binds approximately 95% of the added copper at the concentrations and pH used. Humic acid was used as representative of the organic complexing agents found in natural systems. This material is the refractory end-product of lignin and cellulose decomposition and is a complex mixture of high-molecular weight, polymeric phenolic and carboxylic acids (Gjessing 1976). These compounds are present in both surface waters and shallow aquifers and are capable of binding substantial amounts of toxic metals. Unlike EDTA and glycine, they adsorb strongly onto inorganic oxide surfaces.

The protocol for the experiments was as follows:

1. Run a solution of copper through the column to determine the effects of adsorption onto the silica surface, i.e., to note the amount of retardation in breakthrough.

2. Run through the column a solution of copper to which excess EDTA has been added.

3. Run a mixture of copper and a weak complexing agent, glycine, through the column.

4. Run a humic acid solution through the column to measure adsorption on the silica surface.

5. Run a mixture of copper and natural humic acid through the column.

The column effluent was collected in polycarbonate sample vials mounted on a Technicon sample collector. The sampler was adjusted to collect a one minute sample every 6 minutes (10 per hour). The samples were analyzed on a Perkin-Elmer Model 372 atomic absorption spectrophotometer equipped with a graphite furnace. The data were plotted as total copper eluting as a function of time. The copper concentration is presented in the normalized form C/C_o where C is the effluent copper concentration and C_o is the copper concentration which is fed into the column.

RESULTS AND DISCUSSION

The calibration run with a dye tracer confirmed the theoretical breakthrough time for the column of 18 min. By comparison the breakthrough curve for the first copper run (figure 2) shows significant retardation due to interaction with the silicate surface sites. This type of adsorptive interaction is predicted from the results of Vuceta (1976) who studied copper adsorption on finely divided a-quartz. At pH 6.3 over 90% of the copper in a Cu/SiO_2 system will be in the form of adsorbed copper at equilibrium.

The breakthrough curve for the unchelated copper is retarded by approximately a factor of five relative to the ideal-tracer breakthrough time. The retardation factor is determined from the point where C/C_o =

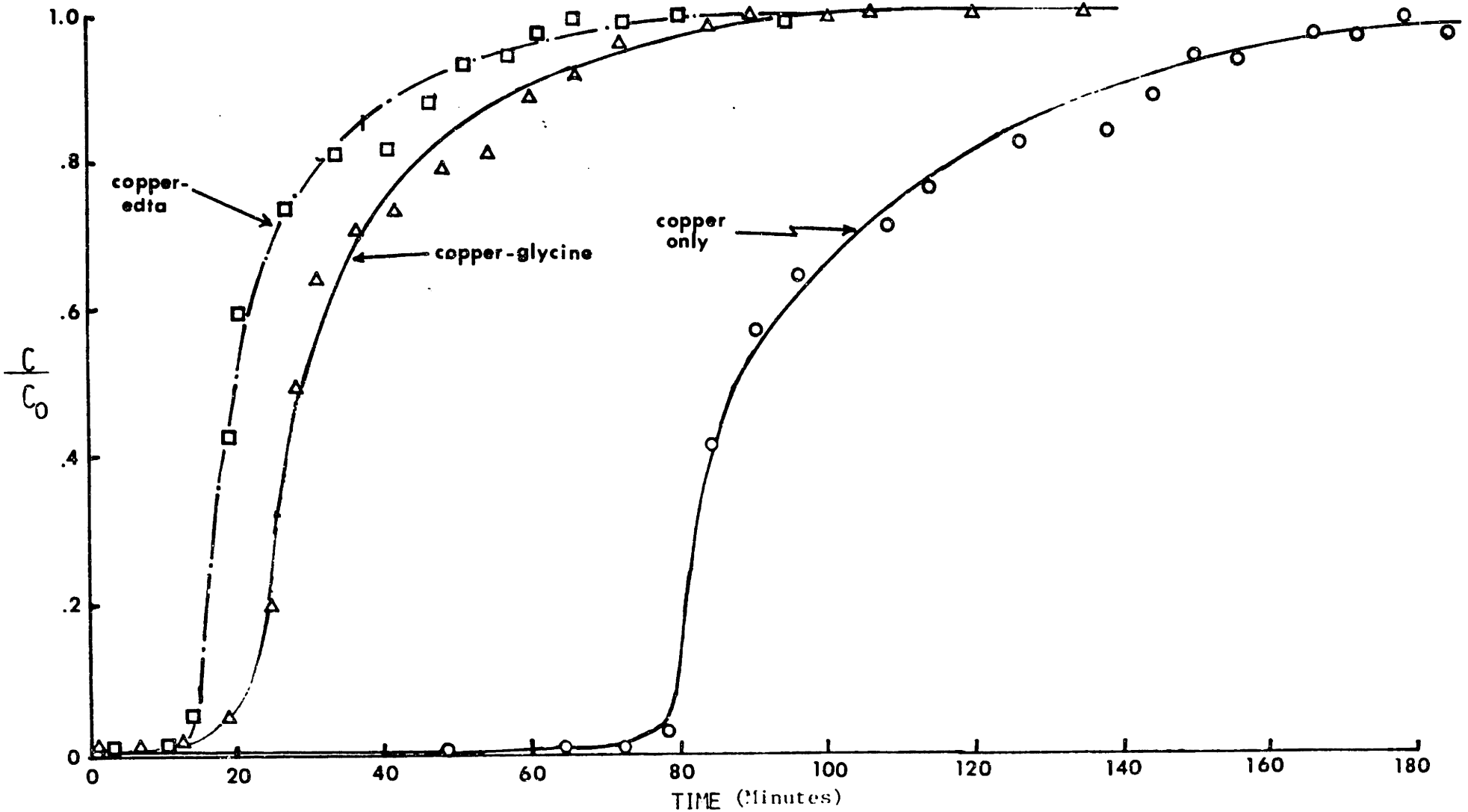

Figure 2. : Breakthrough curves for copper and copper-ligand mixtures.

0.50. In this curve there is a sharp rise in the concentration followed by a long tailing off toward C_o. This tailing off may be attributed to kinetic (i.e., non-equilibrium) factors . That is, at higher surface coverage, the rate of sorption may be sufficiently hindered so as to "fall behind" the rate of fluid movement. Alternatively, it is possible to explain these results with equilibrium assumptions if the equilibrium adsorption constant is non-linear at higher adsorption levels (e.g., a Freundlich isotherm). Benjamin and Leckie (1979) suggested nonuniformity of surface sites as an explanation of nonlinear adsorption at higher surface coverage. However, as will be demonstrated later with the computer model, some of this tailing-off phenomenon is an intrinsic effect of adsorption on the transport process. It is not possible at this stage of model development to precisely assign the curve shape to any particular factor or combination of factors.

Treating these effects as negligible in the first half of the curve, we can estimate the retardation coefficient R_d as about 4.8. If we assume a linear exchange of copper for surface-bound protons (reasonable in this region and at pH 6.3) we can write:

$$R_d = 1 + \frac{\rho_b Q}{n C_o} = 4.8$$

$$\frac{\rho_b Q}{n C_o} = 3.8 \qquad \rho_b = 2.65 \text{ g/cm}^3 \quad n = 0.3 \quad C_o = 5 \times 10^{-5} \text{ meq/cm}^3$$

$$Q \cong \frac{(3.8)(0.3)(5 \times 10^{-5} \text{ meq/cm}^3)}{2.65 \text{ g/cm}^3} = 2.1 \times 10^{-5} \text{ meq/g}$$

By comparison, Vuceta, working with finely divided silica, obtained Γ_{max} (= Q) of $\sim 6 \times 10^{-4}$ meq/g or about an order of magnitude higher. Since the present experiments were conducted with a coarser form of silica (sand) there should be less surface area per gram. For spherical particles the surface to volume ratio is roughly equal to 1/d. Thus it could be assumed that Vuceta's grain size was an order of magnitude smaller than that of the sand used here, although this information was not given.

For the EDTA-copper system we expect the complexing capability of the EDTA to inhibit copper adsorption by drastically reducing the amonut of free copper available to adsorb. Vuceta found this in her batch data for quartz and the breakthrough curve in figure 2 shows that the copper in the presence of excess EDTA behaves virtually as a ideal tracer with essentially no adsorption effects ($R_d \cong 1.0$).

For the glycine-copper system, the complexation of the metal should reduce the adsorption of copper, as with the EDTA. However, glycine is a much weaker ligand than EDTA and about 5% of the total coppper should remain as free cupric ion according to equilibrium calculations at this pH. Because a small amount of the copper is available to adsorb onto the stationary phase, a small amount of retardation in copper breakthrough should be detected. This is shown to be the case in the curve for the glycine-copper system in figure 2. The position of C/C_o gives a value $R_d = 1.6$.

In principle we can model the change in R_d as a function of the ligand strength through its effect on the mass transport equation (MTE). Consider the one-dimensional MTE for a three-compartment system: free copper ion, complexed copper and adsorbed copper.

$$\frac{\partial(Cu)}{\partial t} + \frac{\rho_b}{n}\frac{\partial \Gamma_{cu}}{\partial t} + \frac{\partial(CuL)}{\partial t} + v\frac{\partial(Cu)}{\partial x} + v\frac{\partial(CuL)}{\partial x} = 0$$

Where: Cu = concentration of free cupric ion

CuL = concentration of copper-ligand complex

Γ_{cu} = concentration of adsorbed copper

v = seepage velocity

And the zero on the RHS indicates that mechanical dispersion is neglected and that there is no decay of the contaminant.

To simplify the chemistry a bit, assume a linear, ion-exchange adsorption isotherm (as stated earlier) enabling us to write:

$$\Gamma_{cu} = k_1(Cu)$$

where k_1 is the isotherm slope (linear adsorption coefficient). Considering the partition of the copper between the free and complexed states, the chemical equilibrium is written as:

$$\frac{[CuL][H^+]}{[Cu^{2+}][HL]} = \text{stability constant}$$

For a large excess of ligand (HL) and a constant pH (H^+) this becomes:

$[CuL] = k_2[Cu^{2+}]$ or in our MTE notation

$CuL = k_2Cu$

Make these substitutions into the MTE to get:

$$\frac{\partial Cu}{\partial t}\left(1 + \frac{\rho_b}{n}k_1 + k_2\right) + v(1 + k_2)\frac{\partial Cu}{\partial x} = 0$$

Since we are interested in the breakthrough of total copper, make the substitution:

$$Cu_T = Cu + CuL = Cu\ (1 + k_2) \rightarrow Cu = (1/(1+k_2))\cdot Cu_T$$

The MTE then becomes:

$$\frac{\partial Cu_T}{\partial t}\cdot\left(1 + \frac{\rho_b}{n}\cdot k_1 + k_2\right)/(1 + k_2) + V\cdot\frac{\partial Cu_T}{\partial x} = 0$$

Then:

$$R_d = [\ 1 + (\rho_b/n)\cdot k_1 + k_2] \ /\ (1 + k_2)$$

This value could be determined directly from the various constants. For this case the only unknown value is the adsorption coefficient k_2. We can derive a value for this constant by backcalculating from the R_d obtained from the pure copper system. That is, when no complexors are present $k_2 = 0$ and the R_d equation reduces to the usual form

$$R_d = 1 + (\rho_b/n)\cdot k_1$$

For the pure copper system $R_d = 4.8$ so $(\rho_b/n)\cdot k_1 = 3.8$. Using the standard valus of the copper-glycine complex formation constant (from Sillen and Martell (1971))

$$\frac{[CuL2][H^+]^2}{[Cu^{2+}][HL]^2} = 10^{-3.8}$$

$[HL]^2 = 10^{-7.6}$ and $[H^+]^2 = 10^{-12}$

$[CuL_2] = 10^{0.60} = 4.0$

$R_d = (1 + 3.8 + 4.0) \ /\ (1 + 4.0) = 1.76$

The observed value for the glycine-copper system is $R_d = 1.6$, so for this system there is good agreement with the model.

This approach is quite adequate for systems in which the organic does not adsorb on the silica surface. However, some organic ligands display an affinity for surfaces as well as for metals. These ligands have a very different effect on metal adsorption. As Davis (1977) notes:

> The functional groups of an adsorbing ligand may serve as new adsorption sites for trace metals at the surface or may simply stabilize specific oxide sites by their proximity.

Davis and Gloor (1981) found substantial adsorption of naturally occuring humic compounds onto oxide surfaces. This adsorption is controlled by such factors as the oxide surface type, the molecular weight of the humic compounds and the pH of the solution. Anionic compounds such as humic acids appear to adsorb to protonated (positively charged) surface oxide functional groups. For this reason, they exhibit the greatest adsorption onto relatively basic oxides which retain surface protons in the middle pH range. Silica is a fairly acidic oxide surface and therefore shows a relatively small amount of humic adsorption.

When a mixture of copper and humic acid passes through a silica sand column the system must be modelled with four compartments rather than three. In addition to the free copper, complexed copper and adsorbed copper compartments, we must consider the presence of adsorbed copper-humic complexes. A more mechanistic model would consider a fifth compartment, the adsorbed ligand, and then modify the fourth compartment to be copper which attaches to the adsorbed ligands. However, we will follow the approach of Benjamin and Leckie (1981) who combine these two processes and model it as the adsorption of a copper-ligand complex with its own adsorption coefficient. This approach is highly appropriate for the system described here in which the pH, total ligand and total metal are all fixed. That is to say, when these parameters are not variable,

the two-step process of ligand adsorption followed by metal complexation is thermodynamically equivalent to a single-step adsorption of a metal-ligand complex. It also reduces the number of adjustable parameters in the model and means one less "knob" which can be manipulated to fit the data.

In the experimental system, humic acid is added to the copper solution to produce a total concentration of 10 mg/l which is equivalent to 5 mg-C/l. This is a concentration which is in the range typical of surface waters. A copper titration of this material indicates that approximately 90% of the total copper in solution should be bound by the humic acid at these concentrations and pH. Our laboratory lacked the facilities to do precise measurements of the adsorption of humic organics on silica surfaces in batch methods. In lieu of this, the adsorption was estimated from the retardation of a humic solution passed through the silica column. From the breakthrough curve, $R_d = 1.12$ which yields an adsorption coefficient of approximately 0.15. For a linearly adsorbing system, this can be interpreted as meaning that about 13% of the total humic acid in the system will adsorb on the surface while the remainder will be in solution.* This value is in line with the relatively low adsorption predicted from the acidity of the silicate surface groups. If we make the naive assumption that copper-humate complexes adsorb on the same order of magnitude as the humic acid alone, we would predict

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* : $\Gamma_{lig} = 0.15(L)$ and $L_{tot} = \Gamma_{lig} + (L)$. Hence $\Gamma_{lig} = 0.15/1.15(L_{tot})$ or $\Gamma_{lig} = 0.13(L_{tot})$.

breakthough for the copper-humic mixture which lies somewhere in between that of the copper-EDTA mixture (no adsorption) and that of the purely copper solution (strong adsorption).

Looking at the actual breakthrough curve (figure 3) we see that this is in fact the case. However, the most notable feature of this curve, in comparison with those previously obtained, is its distictive shape. Instead of a relatively sharp breakthrough, there is a much more gradual increase in the total copper concentration from zero to C_o. This type of curve is indicative of the more complicated nature of this system where both the free copper ions and the complexed copper are adsorbing and with different adsorption coefficients.

NUMERICAL MODELLING

The primary emphasis of this research was an empirical determination of the effects of chemical reactions on metal transport processes in a porous medium. Chemical equilibrium theory was then applied to the mass transport equation to show how simple analytical solutions can predict these effects. No attempt is made here to develop a full-scale numerical simulation of metal transport which incorporates the chemistry discussed. However, a very simplified 1-D finite difference model was developed to demonstrate how chemical equilibria calculations can be linearized for easier incorporation into transport models. The model presented here does not account for any mechanical dispersion and is intended only to indicate the chemical effects on transport which will be superimposed on the mechanical transport phenomena. The model results show fairly good

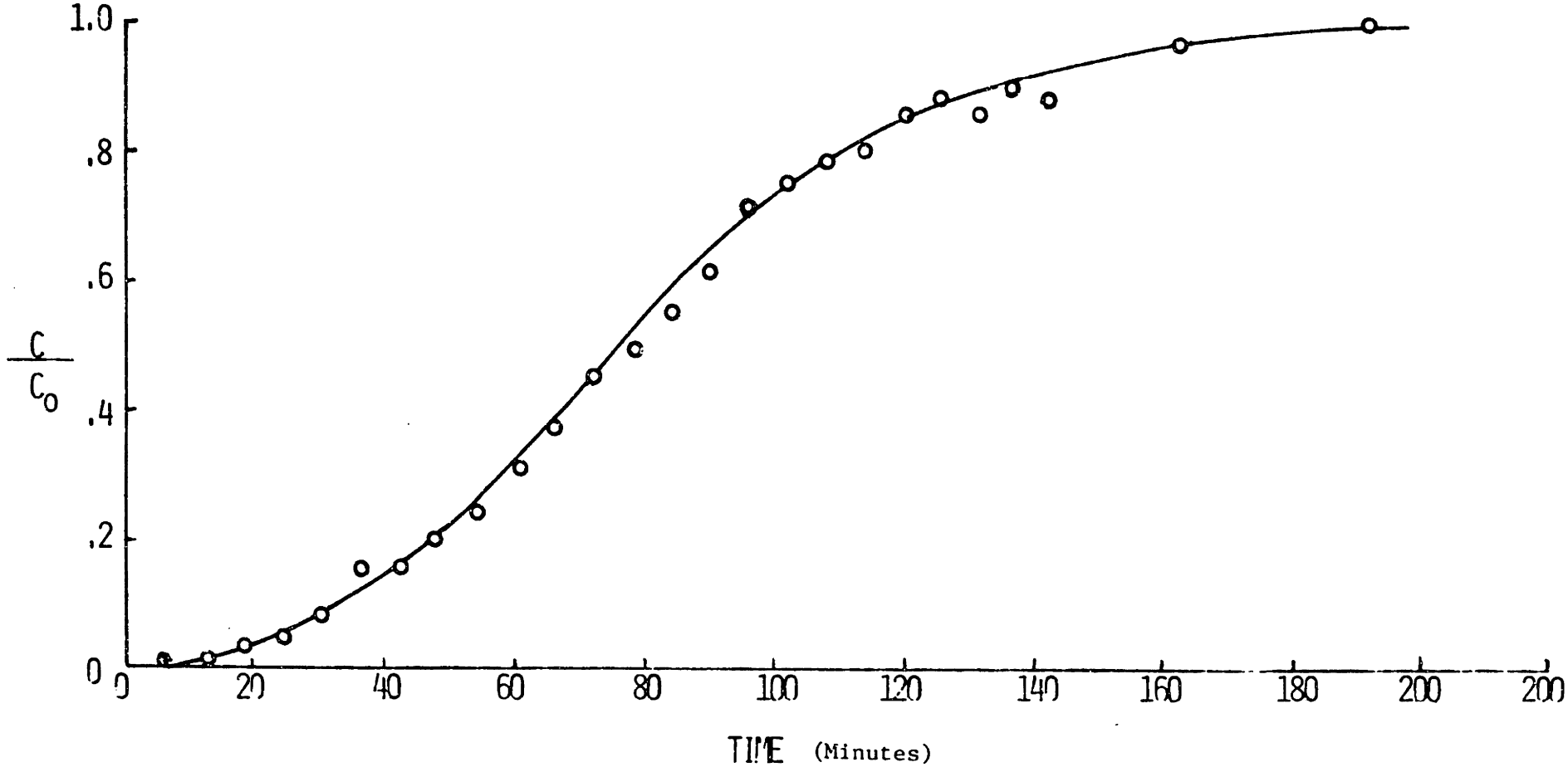

Figure 3. : Copper breakthrough curve for a copper-humic mixture.

correspondence to the empirical data and also elucidate some of the underlying chemistry which is not evident in the breakthrough curves.

The main feature of this model is the linearization of the chemical equilibrium equations. In the previous section the linearized metal-surface adsorption and copper-complex formation equations were developed. They are:

$:SCu = k_1 \cdot Cu$ where :SCu indicates copper bound to the surface

$CuL = k_2 \cdot Cu$ where CuL is the copper bound as the soluble complex

In addition we need to develop the linear equilibrium equation for the copper-ligand complex which is bound to the surface. Consider the reaction

$$ML + :S = :S(ML)$$

Where : ML is the soluble metal-ligand complex

:S is the adsorbing surface site

:S(ML) is the surface-bound metal-ligand complex

The equilibrium can be defined from the mass law

$$K_{sml} = \frac{:S(ML)}{(ML) \cdot (:S_T)}$$

Where there are assumed to be excess surface sites so that total surface site concentration ($:S_T$) is used. If this value is constant then we can write $k_3 = K_{sml}(:S_T)$ so that

$$:S(ML) = k_3 \cdot ML = k_2 \cdot k_3 \cdot Cu = k_4 \cdot Cu$$

Thus all of the copper species can be represented as linear functions of the free copper concentration. By dividing through by $(1 + k_2)$ all species can be linear functions of the total soluble copper concentration.

In the model, a porous medium column is represented as a 1-D series of boxes. Initially there are no metal species in any of the boxes except the first which represents the input contaminant solution (C_o). With each successive time step, the equilibrium concentrations of the various copper species are calculated in each box and the mobile species (soluble) are transported to the next box where a new equilibrium is calculated.

Computationally, the model operates by setting up a 2-D matrix for the free copper (or total soluble copper) for all the spatial elements at all of the time steps. At each time step the amount of each copper species in a given box is known for the previous time step. To these are added the soluble (mobile) species from the previous box in the previous time step. These species then re-equilibrate to give the concentrations in this box in the present time step. The simplest way to compute this is to consider the input of metal to a given box as the total of the free and complexed metal from the previous box, previous time step plus the adsorbed metal and the adsorbed complex from this box in the previous time step. Calculating the new equilibrium is then simply a matter of finding the value of the free copper which satisfies all of the linear equilibrium equations subject to the constraint of the total amount of the copper in the box. In equation form this becomes

(for time step j, spatial element i)

$$\text{Input } {}^{j}M_{Ti} = ({}^{j-1}M_{i-1}) + (k_2 \times {}^{j-1}M_{i-1}) + (k_1 \times {}^{j-1}M_{i}) + (k_4 \times {}^{j-1}M_{i})$$

Free metal | Metal complex | Adsorbed metal | Ads. complex

prior box, prior time-step | this box, this time-step

The program to perform this was small enough to be set up on an Apple II Plus microcomputer. The input values of the linear constants were varied and plots were generated in a format showing the spatial distribution of the metal in a given time step. In all of the runs shown in this paper, the total input copper concentration was fixed at 10^{-5} M, the total ligand concentration was fixed at 10^{-4} M and the system was divided into 20 spatial elements.

Two general trends are detected in the output. First, that the shape of the total metal concentration curve is most strongly dependent on the amount of adsorption in the system. The second is that the ligand strength only controls the distribution of total soluble copper between the free and complexed forms. The strength of the complexing ligand for the metal affects the total transport of metal only insofar as it controls the major soluble species. That is, if a weak ligand is present, most of the copper will be uncomplexed and vice versa for a strong ligand. But this affects transport only in relation to which species are most strongly adsorbed. If the complex does not adsorb, a strong ligand will inhibit adsorption. If the complex is highly adsorbed then the strong ligand will increase the overall adsorption of the metal.

This is illustrated by two comparative runs where the ligand is assigned the relatively strong stability constant of 10^{5}. In one run the metal adsorbs weakly to the surface while the copper-ligand complex

adsorbs strongly. In the second run the adsorption affinities are reversed. The resulting plots show that the complexed metal is always in much higher concentration relative to the free copper, but in the first run (figure 4a), the total copper transport is greatly retarded due to the adsorption of the complex. In the second run (figure 4b) the copper moves rapidly through the system even though the free copper is adsorbing as strongly as the complex was in the first run. In a practical application, such as a contaminated aquifer, it is obviously important to understand which copper species are adsorbing in order to predict the movement of the metal.

The effect of adsorption alone on the distribution of metal in the medium at a give time step is shown in Figures 5a, 5b and 5c. In these plots the parameters are set so as to model metal adsorption in the absence of chelator effects. In three successive runs the value of k_1, the adsorption coefficient, is assigned the values of 0.1, 0.3 and 0.5. As k_1 increases the movement of the metal throgh the system is strongly retarded. However, as discussed earlier, the effect of strong metal adsorption can be completely reversed if a strong, non-adsorbing complexing agent is added. This is evident in Figure 6 where the conditions are identical to the run in Figure 5c ($k_1 = 0.5$) but in which a strong, non-adsorbing chelator is included. In this case, the bulk of the metal, in complexed form, moves quickly through the medium.

CONCLUSIONS

It is hoped that the reader will now have some appreciation of the ways in which chemical interactions between toxic metals and the surfaces

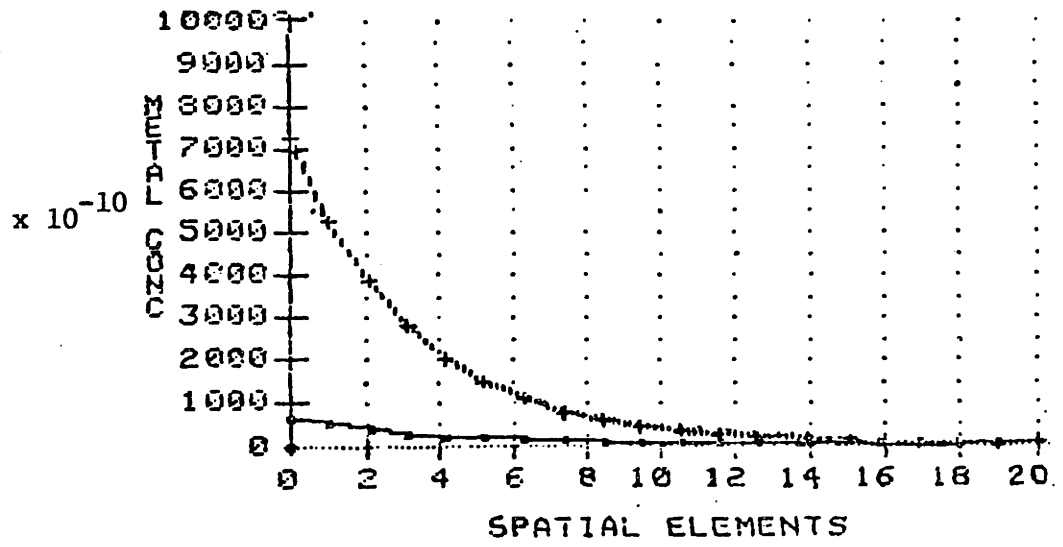

Figure 4(a). : Computer simulation of the spatial distribution of total copper (+···+··) and free cupric ion (-·-----) in the porous medium column.
$k_1 = 0.10$, $k_3 = 0.90$ and $K_{ML} = 10^5$.

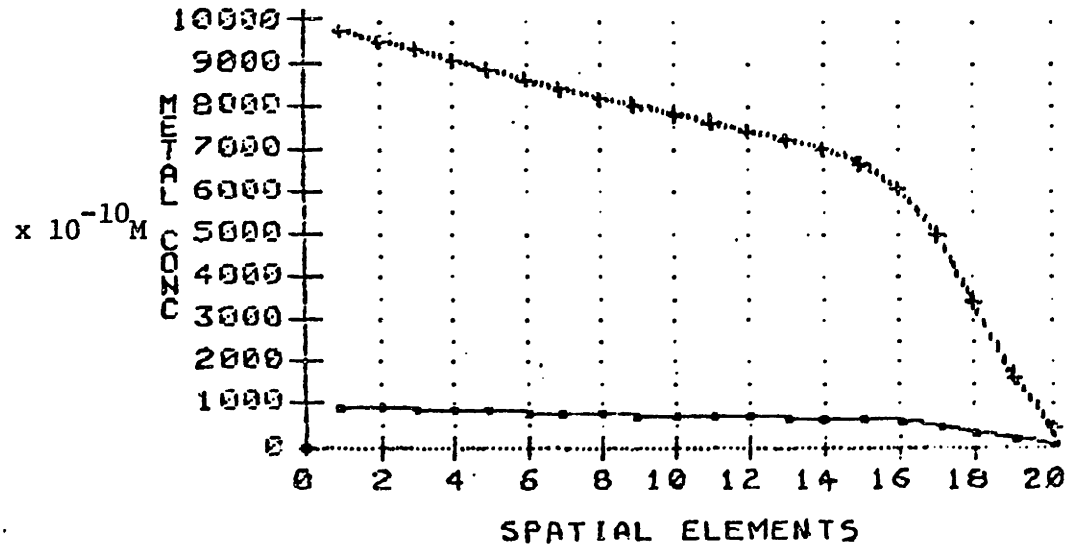

Figure 4(b). : As above except with $k_1 = 0.90$, $k_3 = 0.10$, $K_{ML} = 10^5$.

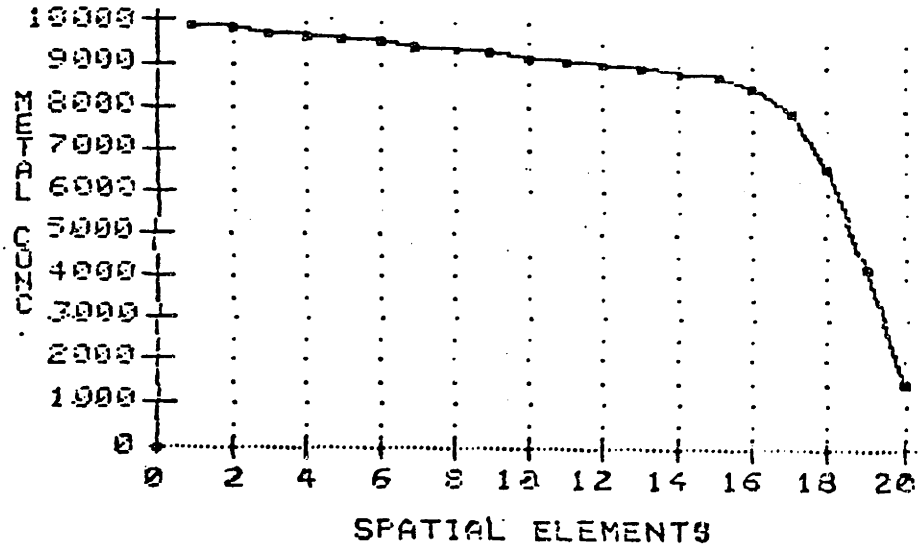

Figure 5(a).
Computer simulation of the spatial distribution of free cupric ion in the PM column.

$k_1 = 0.10$

$k_3 = 0.01$

$K_{ML} = 1$

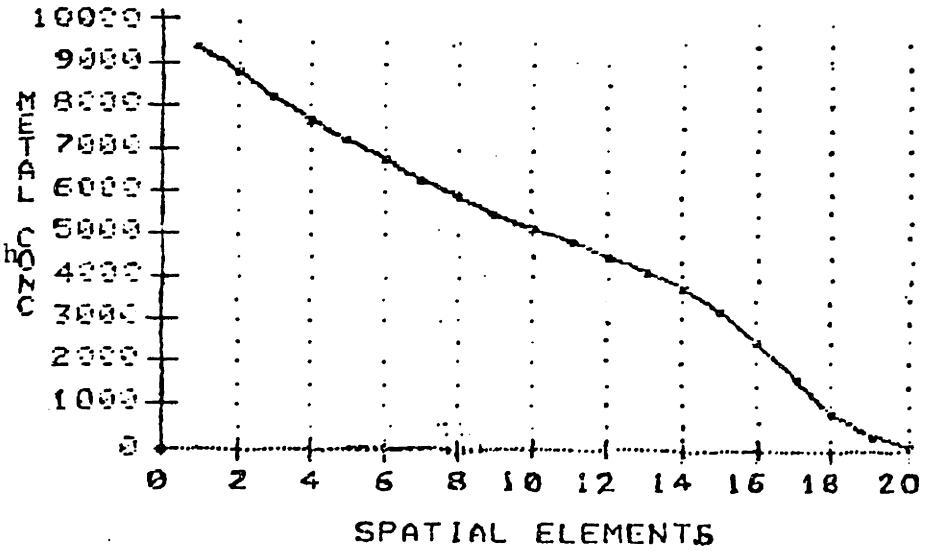

Figure 5(b).

As above except with $k_1 = 0.30$

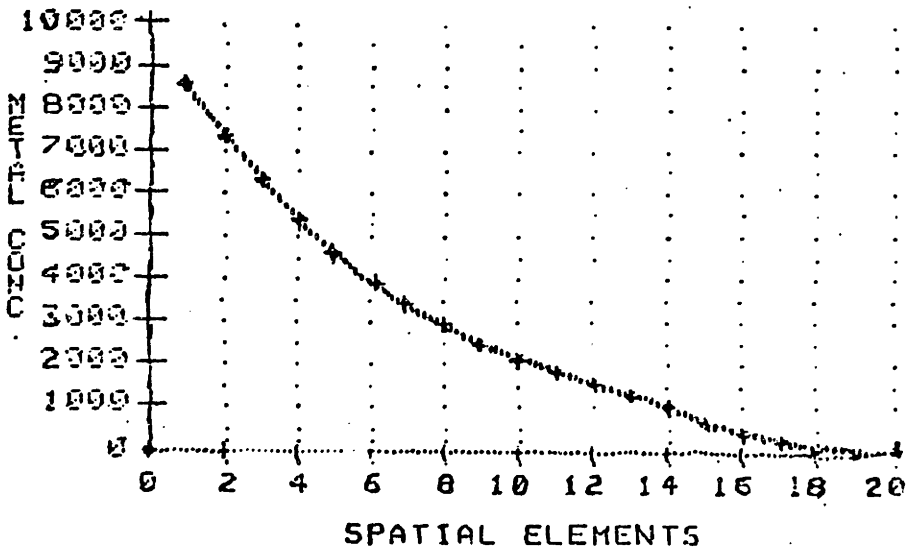

Figure 5(c).

As above except $k_1 = 0.50$

of porous media can dramatically affect the rate of metal transport. In modelling the movement of a toxic metal through an aquifer it is important to consider whether synthetic chelators are present in the contaminant source, whether the groundwater contains natural chelating agents such as humics and what sort of adsorption phenomena are occuring in the groundwater matrix. It is not sufficient to model only the adsorption of the metal on the substrate. The possibility of metal-complex adsorption is critical and must be investigated if accurate results are expected. The present research is by no means exhaustive in this field and is meant only as highly simplified first approach to the problem. It is hoped that a more detailed understanding of these phenomena is soon forthcoming.

ACKNOWLEDGEMENTS

Special thanks are given to Mr. Kevin Farley for his valuable discussions and suggestions concerning this research. I am also indebted to Prof. John Wilson for guiding me through the mysteries of porous medium hydraulics. This work was supported in part by the International Copper Research Association, (INCRA) Project # 346.

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APPENDIX THREE:

FRACTIONATION OF DISSOLVED ORGANIC ACIDS

BY XAD-8 RESIN AND DIETHYLAMINOETHYL (DEAE) CELLULOSE

Detailed studies of the properties of aquatic humic materials and other organic acids require the isolation of these compounds from dilute aqueous solution. Thurman and Malcolm (1981) have described a procedure for extracting humic and fulvic acids from natural waters by adsorption onto XAD-8 resin. This procedure has been advanced as operationally defining that portion of the dissolved organic carbon which is to be considered humic matter. In essence, aquatic humic materials are defined as the hydrophobic organic acids, and the isolation technique is based on the hydrophobicity of the compounds.

An alternative procedure for isolating humic materials from natural waters uses diethylaminoethyl (DEAE) cellulose, as described by Miles et al., 1983. DEAE-cellulose is a weak anion exchanger that reversibly binds essentially all weak acids from water at neutral or basic pH. The procedure of Miles et al. (1983) is faster and easier than the XAD-8 method but the extract obtained is a mixture of all organic acids in the sample and is not a well-defined humic fraction.

The differences in the XAD-8 and the DEAE procedures suggest that the methods could be combined to conveniently fractionate the acidic components of dissolved organic carbon (DOC). Leenheer (1981) developed a detailed fractionation scheme for DOC based on the bulk properties of each fraction (e.g., hydrophobicity, acidity, basicity). The work described here is indebted to the concepts of Leenheer, but is a simpler procedure with a narrow focus.

In this study we isolated and fractionated the humic and non-humic organic acids to study their roles as metal-bidning agents in natural

waters. Humic materials are described as controlling factors in the speciation of trace metals in many aquatic systems but little is known of the metal binding potential of naturally occurring non-humic (hydrophilic) organic acids. Isolating both humic and non-humic acids permits an evaluation of the relative effects of the two groups on trace metal speciation.

The study of non-humic organic acids was stimulated by the observation that XAD-8 procedures typically remove less than 60% of the DOC from most waters. The remaining organic matter is presumably a mixture of acidic, neutral and basic hydrophilic compounds. Only the acidic compounds act as ligands for metal ions and only this fraction needs to be isolated to obtain any non-humic metal-binding agents. Thus we employed a simple sequence of extractions in which a water sample was first acidified and passed through an XAD-8 column as per the method of Thurman and Malcolm (1981). This water, now stripped of the humic (hydrophobic) acids, was neutralized and mixed with DEAE-cellulose. The DEAE-cellulose retained any remaining weak acids, which could then be eluted in concentrated form. Cu(II) titrations, as monitored by a cupric ion-selective electrode, were performed on the whole water sample as well as reconstituted solutions of the humic and non-humic acids. The significance of non-humic aquatic acids to metal speciation could then be evaluated.

The water used for this study was taken from Grassy Pond in Bedford, MA. All glassware was washed in 4N HNO_3 and metal-clean techniques used in all procedures. All chemicals were reagent grade. The Cu(II) titrations were performed with a Radiometer F3000 Selectrode according to methods reported earlier (Fish and Morel, 1984). All solutions were titrated in a background electrolyte of 2×10^{-2}M $NaClO_4$. Solution pH was adjusted with 1N NaOH or HCl to pH 6.3 and monitored with a Fisher E5-A glass electrode coupled to an Orion 401 Ionanalyzer.

All XAD-8 extractions were carried out according to the methods of Thurman and Malcolm (1981). The clean XAD-8 resin was generously provided by Diane McKnight, USGS, Denver. The DEAE-cellulose was obtained from Sigma Chemicals.

The DEAE-cellulose procedures are a slight modification of those described by Miles et al. (1983). The DEAE-cellulose was pretreated by suspending about 50g of dry cellulose in 1L of 1.0M HCl for 1 h. The fines were decanted and the remaining slurry filtered onto a Whatman GFC glass fiber filter. The cellulose was resuspended in deionized water and refiltered several times. The cellulose was then suspended for 1h in 0.5N NaOH followed by filtration and repeated rinsing with deionized water. The cellulose was finally suspended in a 0.02N phosphate buffer ($NaH_2PO_4/NaHPO_4$) adjusted to pH 6.80 and allowed to equilibrate overnight.

A 10L sample of Grassy Pond water was acidified with HCl and passed through 75cm x 4 cm column of XAD-8 resin. The material adsorbed to the resin was eluted with NaOH and reconcentrated on a 150 mm x 15 mm XAD-8

column, re-eluted, desalted, and freeze-dried, all according to the method of Thurman and Malcolm (1981).

The effluent of the first column was collected in a glass carboy and 1N NaOH added to bring the pH to about pH 7. Phosphate buffer was added to the effluent to a total concentration of 0.02N to buffer the solution at pH 6.80. The pretreated DEAE-cellulose was filtered from the buffer solution in which it was stored and mixed with the column effluent. The cellulose/effluent mixture was stirred for 2h and then filtered through a Whatman GFC filter. The DEAE-cellulose was removed from the filter and resuspended in 100 mL of 1M $NaClO_4$ and stirred for 20 min. Preliminary studies showed that more complete elution of organic matter was obtained if the cellulose/$NaClO_4$ slurry was sonicated for 1 min in the 60% pulse mode. The slurry was vacuum filtered through a GFC filter to maximum dryness (barely moist), the filter cake rewetted with 2 mL of 1M $NaClO_4$ and vacuum filtered again to near-dryness. The filtrate was a pale amber solution and was stored at 4°C. The entire sequential extraction procedure is shown schematically in Fig. 1.

RESULTS AND DISCUSSION

A Cu(II) titration of filtered whole water from Grassy Pond at pH 6.3 indicates the typical range of weak to moderately strong metal binding by organic ligands (Fig. 2).

The XAD-8 extraction of 10L of Grassy Pond water yielded 45.8 mg of fulvic acid and 3.6 mg of humic acid. We conservatively estimated the recovery of humic materials to be 80% based on the work of Mantoura and

Figure 1.

Protocol for the sequential extraction of hydrophobic and hydrophilic organic acids from a natural water sample. Details of procedure are given in the text.

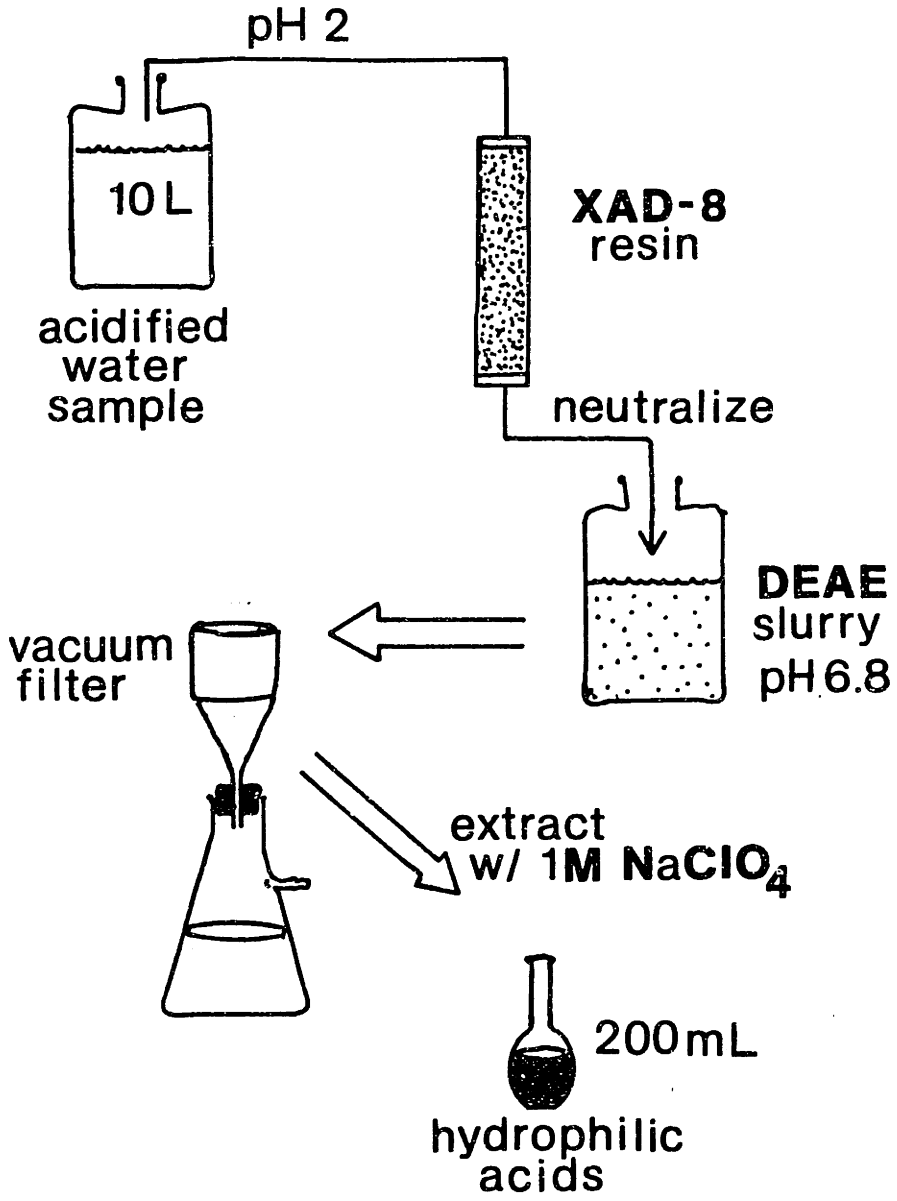
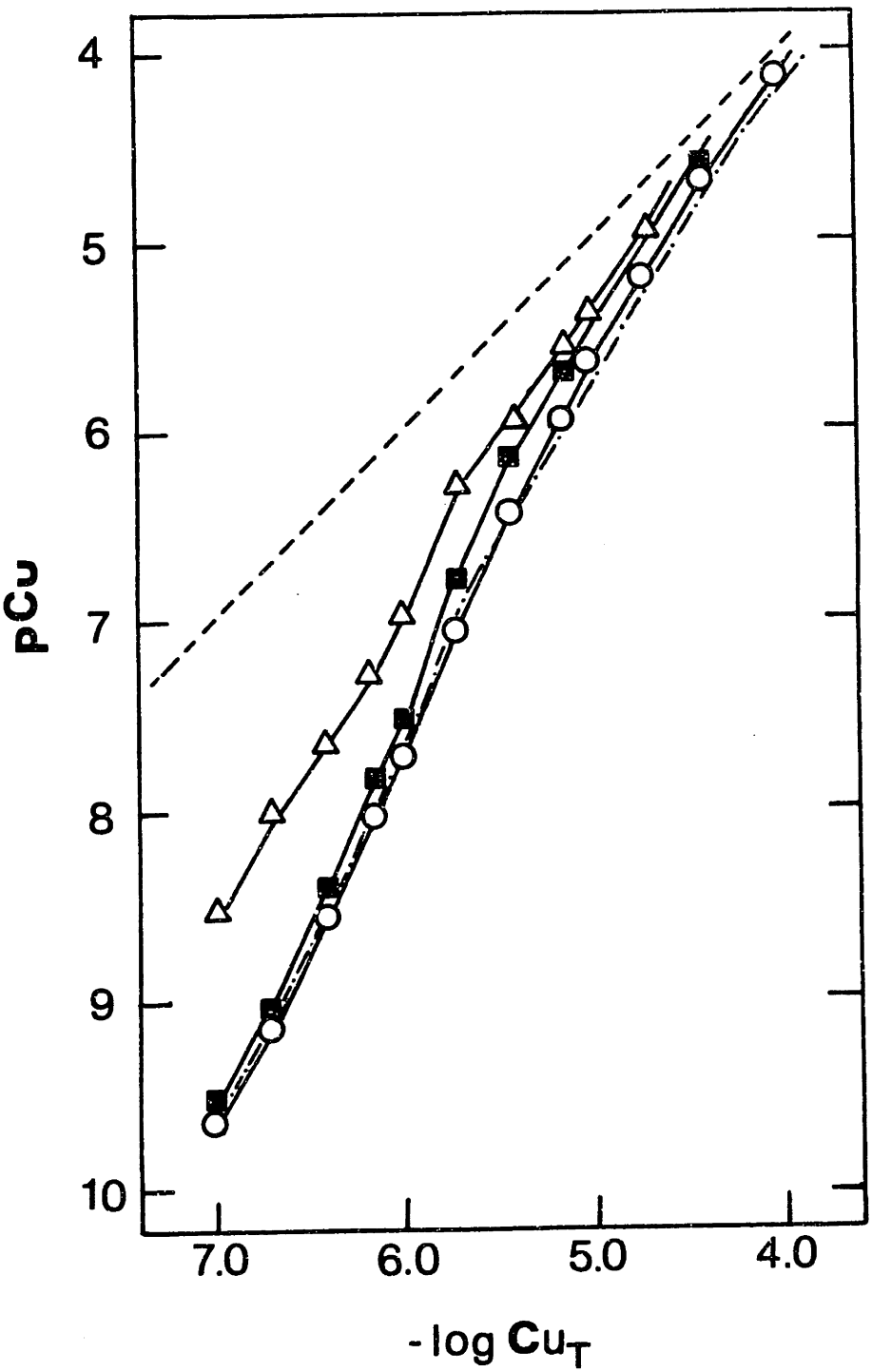

pH 2
10 L
acidified
water
sample
XAD-8
resin
neutralize
DEAE
slurry
pH 6.8
vacuum
filter
extract
w/ 1M NaClO4
200 mL
hydrophilic
acids

Figure 2.

Cu(II) titration data in the form pCu (= -log Cu^{2+}) vs. -log total copper concentration (Cu_T). Upper dashed line is the calibration line for pCu = -log Cu_T. Grassy Pond whole water (filtered) (O); Grassy Pond fulvic acid (XAD-8 extract) (■); DEAE-cellulose extract of Grassy Pond water after XAD-8 extraction (hydrophilic acids) (△); Calculated titration based on combination of discrete ligands in Table 1 (ligands individually fitted to fulvic fraction and hydrophilic acid fraction titrations) (—·—·). All titrations performed at pH 6.3, 0.02 M $NaClO_4$.


4
5
6
7
8
9
10
pCu
7.0
6.0
5.0
4.0
- log Cu$_T$

Riley (1975) who obtained recoveries in the range of 90% using XAD-8 resin. Using this assumpition, the 10L Grassy Pond sample contained about 100 mg of humic matter for a concentration of 6 mg/L, about 90% of which is fulvic acid.

Based on this calculation, a 6 mg/L solution of Grassy Pond fulvic acid was prepared in 2 x 10^{-2}M $NaClO_4$ electrolyte and titrated with Cu(II) at pH 6.3. The titration curve is similar to that of the whole water sample but indicates less binding of Cu(II) at higher metal concentrations (Fig. 1). At the lowest total Cu(II) (Cu_T) concentrations, there is only a slight difference in the cupric ion activity of the whole water and that of the fulvic acid solution. This titration curve can be modeled by three discrete ligands fitted to the data with non-linear regression program (FITEQL, Westall, 1982). The apparent stability constraints and ligand concentrations are given in Table 1.

The Cu(II) titration of the hydrophilic acids not adsorbed on the XAD-8 resin was performed on a 1:50 dilution of the concentrated DEAE-cellulose eluant. The fifty-fold dilution factor reversed the concentration factor of the extraction assuming 100% recovery of these organic acids. For this case, the assumption of 100% recovery is a conservative one because it leads to a more dilute solution for the titration. Any complexation observed in the titration will thus be a minimum estimate for this fraction of the DOC. Note that this dilution results in the same background concentration of $NaClO_4$ as in the other titrations.

The Cu(II) titration of the hydrophilic acid fraction at pH 6.3 indicates the presence of a range of moderate to weak ligands (Fig. 2). The strongest ligands, those observable at low Cu_T concentrations, are about an order of magnitude weaker than those observed in the whole

water sample or in the fulvic acid titration. The weaker binding by the hydrophilic acids, however, is similar in magnitude to that observed in the other titrations. These observations are quantified in the apparent stability constants and ligand concentrations fitted to the hydrophilic acid titration (Table 1).

The titrations indicate that XAD-8 resin does not isolate all of the metal complexing material from a natural water. The XAD-8 procedure in essence defines aquatic humic material as the hydrophobic acids retained on the resin at low pH. Our results do not contradict the usefulness of that definition but do suggest that metal ions are bound by organic compounds other than humic or fulvic acids.

Because of the operational definition of humic matter, it may be argued that the DEAE-cellulose-bound fraction of the DOC is a mixture of non-chelating weak acids along with a small fraction of humic matter that was not adsorbed (perhaps for kinetic reasons) on the XAD-8 resin. For example our recovery assumptions for the XAD-8 resin suggests that 20% of the humic matter could pass through the column. The metal complexation in the so-called hydrophilic acid fraction could be attributed to simply a small proportion of fulvic acid. This hypothesis is contradicted by the fact that the titration curve for the hydrophilic acid fraction reveals ligands that are similar in total concentration to those in the fulvic acid titration. The hydrophilic ligands differ from the fulvic ligands in their binding strength, not in concentration. Therefore, there is a qualitative difference in the organic acids that adsorb to the XAD-8 and those that pass through the column.

The source and nature of these hydrophilic acids is not known. They may be forms of fulvic acid that cross the hydrophilicity "thresh-

TABLE 1. Conditional stability constants and total concentrations of discrete ligands fitted to Cu(II) titrations data t pH 6.3

Fraction	Ligand	$\log K_i$	L_i^T, μM
Fulvic Acid	1	9.73	0.24
	2	7.35	1.71
	3	5.95	10.0
DEAE-Cellulose	1	8.50	0.21
	2	6.61	2.0
	3	5.04	13.0

old" for XAD-8 adsorption. This is reasonable in that humic molecules are viewed as a continuum of possible sizes and structures. At a certain point in the hydrophilicity spectrum the compounds are no longer defined as "humic" (by the operational definition of the separation technique) but still retain humic properties such as metal binding.

Alternatively, the hydrophilic acids fraction may include the weak metal complexing agents known to be excreted from aquatic biota such as eukaryotic algae (McKnight and Morel, 1979), Daphnia (Fish and Morel, 1983) or bacteria (Bitton and Freihofer, 1978). The XAD-8 adsorptive behavior of these excretions has not been investigated but the Cu(II) binding behavior of the materials collected by McKnight and Morel (1979) and Fish and Morel (1983) is strikingly similar to the moderately weak binding observed in the material extracted by the DEAE-cellulose in this study.

The titrations of the two subfractions (fulvic acid and hydrophilic acids) are essentially additive: A combination of the discrete ligands that model the subfractions will closely model the whole water titration results (Fig. 1).

The effect of neglecting the non-humic fractionn of the DOC when modeling metal speciation depends on the metal species of greatest interest. The free cupric ion activity is largely controlled by the strongest binding agent present. At low ratios of Cu(II) to ligand, fulvic acid appears to dictate the cupric ion activity. At higher ratios of copper to ligand, the hydrophilic acid fraction has an increasingly large effect of the cupric ion activity, but the difference between the fulvic acid solution and the whole water is still rather small. If, however, interest centers on the amount of metal bound to

organic matter (for example, in a study of transport and cycling of metals), then the Cu(II) bound to non-humic DOC can be a major component of the system.

CONCLUSIONS

The results of this study confirm the findings of Miles et al. (1983) that DEAE-cellulose is a simple and efficient means of concentrating aquatic organic acids. We have shown that DEAE-cellulose will isolate a DOC fraction that is not collected in the XAD-8 defined humic matter but does possess metal binding properties. The moderately weak metal binding characteristics of the DEAE-isolated fraction can be quantitatively added to those of the fulvic acid fraction to reproduce the metal binding properties of a whole water sample.

The DEAE-isolated (hydrophilic) organic acids had only a minor effect on the cupric ion activity in the tirations of Grassy Pond water. At Cu(II) concentrations greater than 5 μM, however, this non-humic fraction appeared to constitute a significant portion of the organically bound Cu(II). The proportions of humic and non-humic complexing agents may vary in different natural waters and the non-humic fraction may be the result of excretions from aquatic biota.

ACKNOWLEDGEMENTS

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APPENDIX FOUR:

CONCENTRATIONS OF MARINE ORGANIC ACIDS

USING DEAE-CELLULOSE

INTRODUCTION

The use of diethylaminoethylcellulose (DEAE-cellulose) for the isolation of freshwater humic materials was introduced by Miles et al. (1983). This note describes an extension of that procedure to marine systems. Isolating humic and other organic acids from ocean water is generally more difficult than the isolation of freshwater humus because the concentration of dissolved organic carbon (DOC) is frequently an order of magnitude or more lower than in terrestrial aquatic systems (< 0.1 mg-C/L for open ocean water vs. 1-10 mg-C/L for lakes and streams).

Concentration of even a few milligrams of marine humus requires processing tens or hundreds of liters of water. A researcher has the option of transporting this large quantity of water back to the laboratory in an uncontaminated state, or else can use a mobile extraction set-up at the coast, or on board a ship.

The most common procedure for isolating aquatic humus is by adsorption at low pH onto XAD resins. XAD-8 resin is preferred for freshwater use (Thurman and Malcolm, 1981) while XAD-4 has been used for marine humus (Harvey et al., 1984). The major drawback to this method is that large volumes of seawater must be pumped at moderate pressure (approximately 70 psig) through large glass columns of resin. The resin must be carefully cleaned of all leachable organics by extensive solvent refluxing. A typical procedure requires several days of refluxing in nitriloacetate and processing kilogram quantities of resin in costly, oversized Soxhlet extractors (D. McKnight, U.S.G.S., Denver, pers. comm.). Furthermore, all water to be extracted must be acidified at pH 2. Thus, equipping and operating an XAD extraction apparatus in the field,

particularly for marine application, is a major undertaking requiring a significant commitment of money, time and personnel.

The procedure described here is an alternative to the XAD method. The DEAE-cellulose method extracts a fraction of the DOC that is operationally different than the fraction isolated by XAD resin. But the DEAE-cellulose procedure is far simpler and should be of great use to researchers interested in concentrating organic acids and metal-complexing agents from seawater with less preparation, equipment and expense than the XAD procedure.

EXPERIMENTAL

Coastal marine water was obtained from the Massachusetts Bay at East Point Rocks, Nahant, Mass. The pH adjustments were made with 1m NaOH or 1m HCl and a phospate buffer ($NaH_2PO_4/NaHPO_4$) used to buffer pH at 6.8. DEAE-cellulose was obtained from Sigma Chemicals.

The isolation procedure is essentially the batch method described by Miles et al. (1983) for isolation of freshwater humus. The DEAE-cellulose was washed and pretreated as described in that work.

A 17.5L sample of Nahant seawater was filtered and pumped into a 20L glass carboy using a polypropylene peristaltic pump and a Whatman GFA glass fiber filter. The pH was adjusted to near neutral with HCl and about 20mL of phosphate buffer added to fix the pH at 6.8. A wet portion of DEAE-cellulose (20 g dry weight) was added to the seawater and mixed thoroughly. The mixture was allowed 30 min to equilibrate and settle. The cellulose flocculated in the seawater and quickly settled

out. The supernatant water was poured off and the cellulose was retained in about 0.5 L of water. Another 17.5L of fresh seawater was filtered and pumped into a separate glass carboy and the pH adjusted and buffered as before. This sample was then poured into the carboy containing the DEAE-cellulose and equilibrated for another 30 min and allowed to settle. This procedure allowed a total of 35L of seawater to be extracted in about 90 min of field time. The procedure could be repeated for greater volumes of water or else several carboys could be simultaneously equilibrated with the cellulose.

After the second equilibration, the supernatant was again decanted and the DEAE-cellulose with about 0.5L of residual seawater was transferred to a 1L glass bottle for transport back to the laboratory.

At the laboratory the cellulose was filtered onto a Whatman GFC filter, rinsed with 100 mL of 0.01N phosphate buffer, pH 6.8. The cellulose pellet was transferred to a 250mL Erlenmyer flask and 100mL of 1M NaOH was added. The slurry was thoroughly mixed and allowed to stand for about 3h. The slurry was again filtered through a GFC filter and rinsed with about 10mL of 1M NaOH. The filtrate was a clear amber color.

The NaOH filtrate was then passed through a cation exchange column (Rexyn 100, H^+ form) that was previously washed with methanol and 1M HCl to remove impurities. The cation exchange column was washed with 40mL of deionized water so that a total of 150mL of eluant was obtained. The eluant pH was monitored to assure that Na^+ was completely removed.

The desalted eluant was then freeze-dried and 18 mg of dry material obtained. A 10mg/L solution of this material was prepared using deionized water with a 0.001m $NaClO_4$ background electrolyte. This solution

was titrated with Cu(II) and monitored with a cupric ion selective electrode, as described elsewhere (Fish and Morel, 1983).

RESULTS AND DISCUSSION

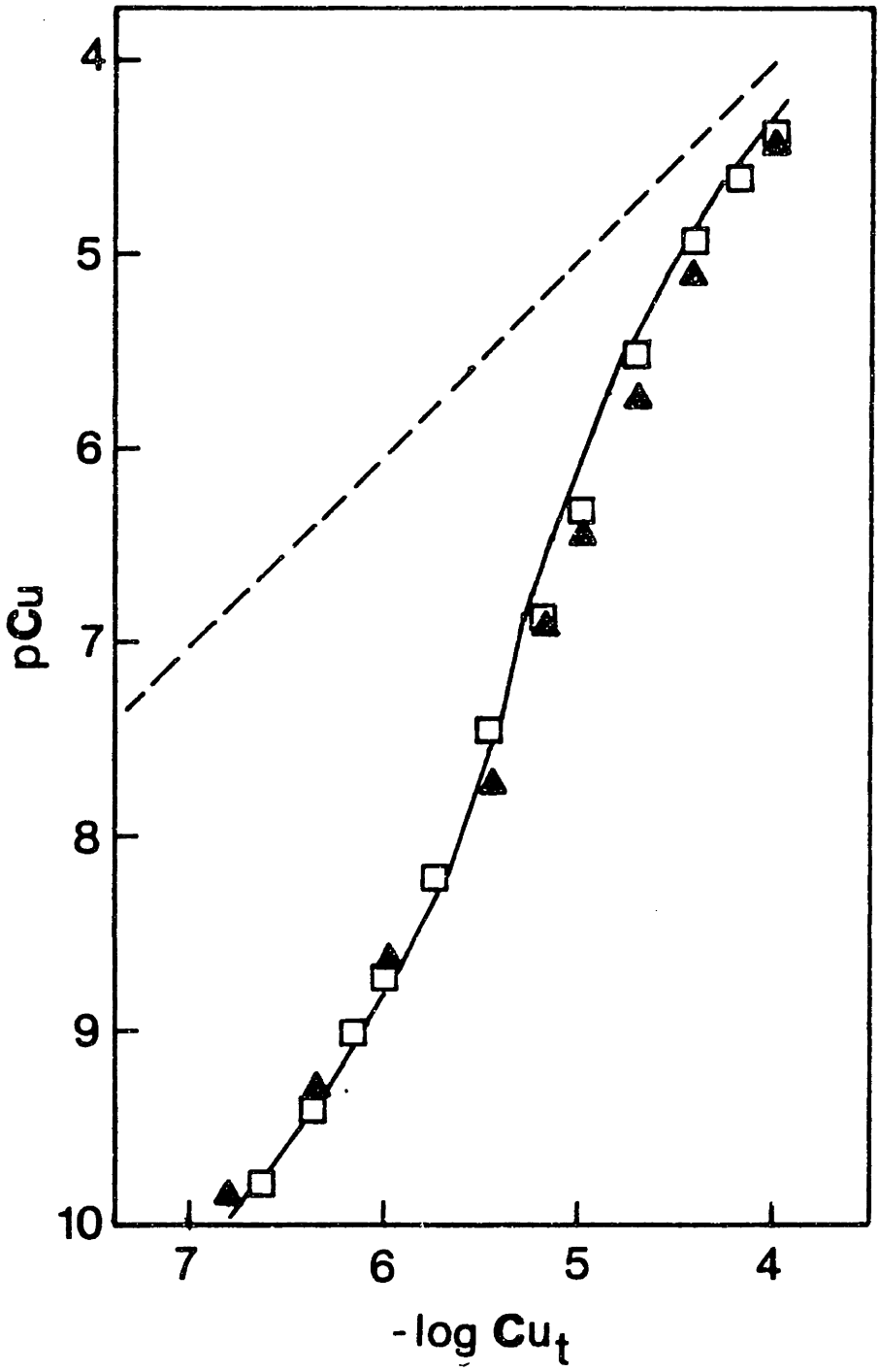
The titration of the isolated marine organic matter with Cu(II) at pH 7.0 indicates metal binding similar to that of humic materials from freshwater (Fig. 1). For comparison, a titration curve of a 5mg-C/L solution of a terrestrial fulvic acid (Grassy Pond, Fish and Morel, 1984) is also shown. Assuming that the Nahant organic matter is of comparable composition to most humus (about 50% carbon), the two concentrations should be about the same (5mg-C/L).

The isolated material in this titration could be characterized by three discrete ligands: L_1^T = 0.90 μM, $\log K_1$ = 10.27; L_2^T = 1.4 μM, $\log K_2$ = 7.95; L_3^T = 8.8 μM, $\log K_3$ = 6.16. These parameters were fitted by a trial and error variation of the graphical Scatchard method. A better approach would be a non-linear fit of all data points but the present example provides a reasonable fit.

We conclude that this procedure is a fast and effective means of isolating the organic metal-complexing agents found in seawater. Further work needs to be done to quantify the yields obtained by DEAE-cellulose extraction and to determine its comparibility to the XAD procedure for seawater. Our experience with both methods indicates that the DEAE-cellulose batch procedure is vastly less complicated than the XAD resin column methodology.

Figure 1.

Cu(II) titration of two fulvic acid samples at concentrations of 5 mg-C/L. Marine fulvic acid extracted by DEAE-cellulose from Nahant seawater (□); Freshwater fulvic acid extracted by XAD-8 from Grassy Pond (▲). Solid line is the calculated titration of the Nahant organic matter using the constants given in the text.


4
5
6
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pCu
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APPENDIX FIVE:

FLUORESCENCE SPECTRA OF GRASSY POND FULVIC ACID

INTRODUCTION

Before the quenching of fluorescence can be used to study the binding of metals by humic materials it is important to examine the fluorescence excitation and emission spectra of such materials. The most sensitive quenching analysis is obtained when measurements are made at wavelengths and slit widths that yield a maximum signal to noise ratio. These wavelengths are typically the emission maximum and the excitation maximum.

If several peaks are observed in fluorescence spectra it is important to distinguish artifacts from chemically significant peaks. This is particularly true of humic materials which tend to have relatively undifferential spectra with only a few broad peaks.

This appendix shows excitation and emission spectra for Grassy Pond fulvic acid. Several unusual features are noted in the excitation spectra that are not explained as artifact peaks. Examples of an artifact peak (Raman scatter peaks) are also demonstrated.

MATERIALS AND METHODS

All spectra were measured on solution of Grassy Pond fulvic acid (GP-FA). This material was isolated from Grassy Pond in Bedford, Mass. using the XAD-8 extraction procedure of Thurman and Malcolm (1981). Solutions were filtered through a Whatman GFA glass fiber before analysis. Fluorescence measurements were made with a Perkin-Elmer LS-5 fluorescence spectrophotometer fitted with a 1 cm sample cuvette. All measurements were made at ambient laboratory temperature (20°-22°C).

Reference to earlier studies of fulvic acid fluorescence (Ewald et al., 1983; Visser, 1983) and preliminary studies in this lab indicated that the excitation band for GP-FA should lie in the range of 300nm and the emission band should be at about 450nm. For the initial examination of excitation spectra, the emission wavelength was fixed at 450nm and slit widths of 10nm used on both monochromators.

The excitation spectrum of a 1mg-C/L solution of GP-FA revealed the expected peak in the 300nm range, but extending the spectrum into the near-UV range (λ < 280nm) revealed a large excitation band between 200nm and 270nm (Fig. 1A). Previous publications of fulvic acid fluorescence spectra have not reported excitation behavior in this range of wavelengths.

A common source of error in the interpretation of fluorescence spectra is the presence of Raman scatter peaks. These peaks are due to molecular scattering by the solvent molecules and occur at harmonics of the excitation wavelength. For example, we expect (and observe) a sharp peak of apparent fluorescence at an excitation wavelength of 200nm when the emission is monitored at 400nm (2 x 200). Because these peaks are optical rather than chemical in nature, they can be revealed by adjusting one of the two wavelengths. Chemical fluorescence peaks only change height when an excitation or emission wavelength is shifted, while a Raman peak will shift position so as to remain a harmonic of the excitation wavelength. An example of this is shown in Fig. 2 in which the emission spectrum was optimized to avoid Raman artifacts.

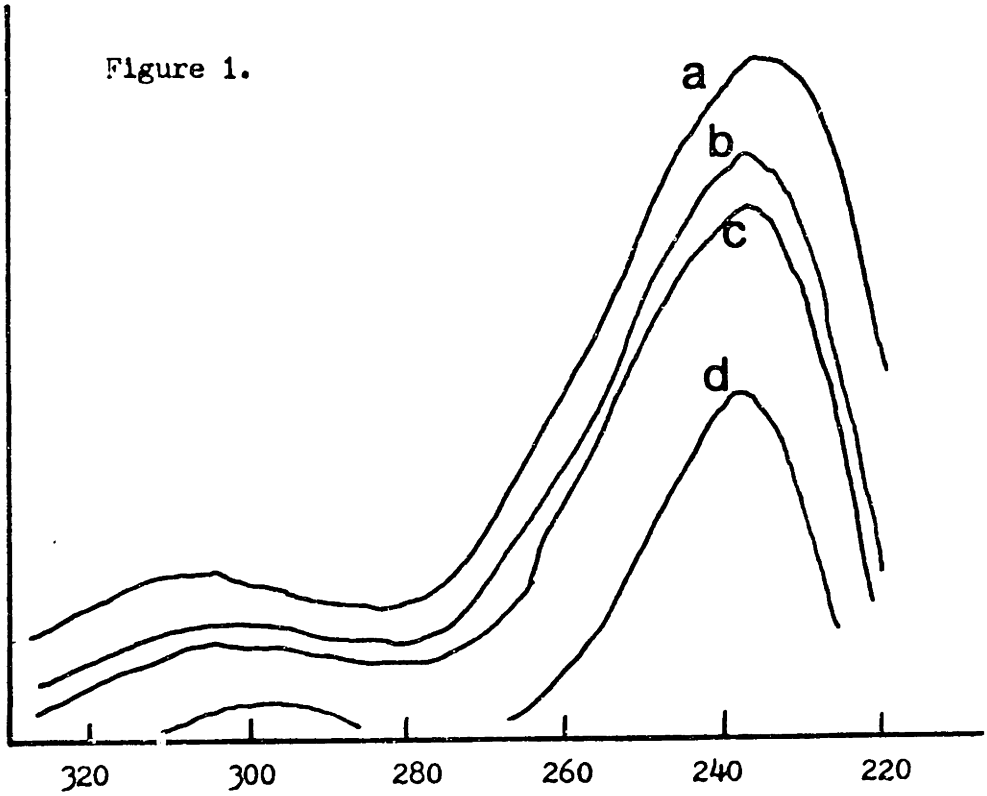
Figure 1.

Excitation spectra for Grassy Pond fulvic acid (GP-FA) as measured at an emission wavelength of 405 nm. The vertical axis on this and all subsequent figures is an arbitrary relative fluorescence scale. a. 1 mg-C/L GP-FA with no added Cu(II). b. Same solution with 1 μM Cu(II). c. 3 μM Cu(II). d. 40 μM Cu(II). All spectra measured at pH 5.0.

Figure 2.

Emission spectra for GP-FA, 1 mg-C/L. A. Spectrum taken when excitation wavelegth at 250 nm; i. true fluorescence peak, ii. Raman scatter peak, iii. and iv. Raman (secondary) peaks.
B. Same emission spectrum for excitation at 300 nm. i. Fluorescence peak, ii. Raman peak.

Figure 1.



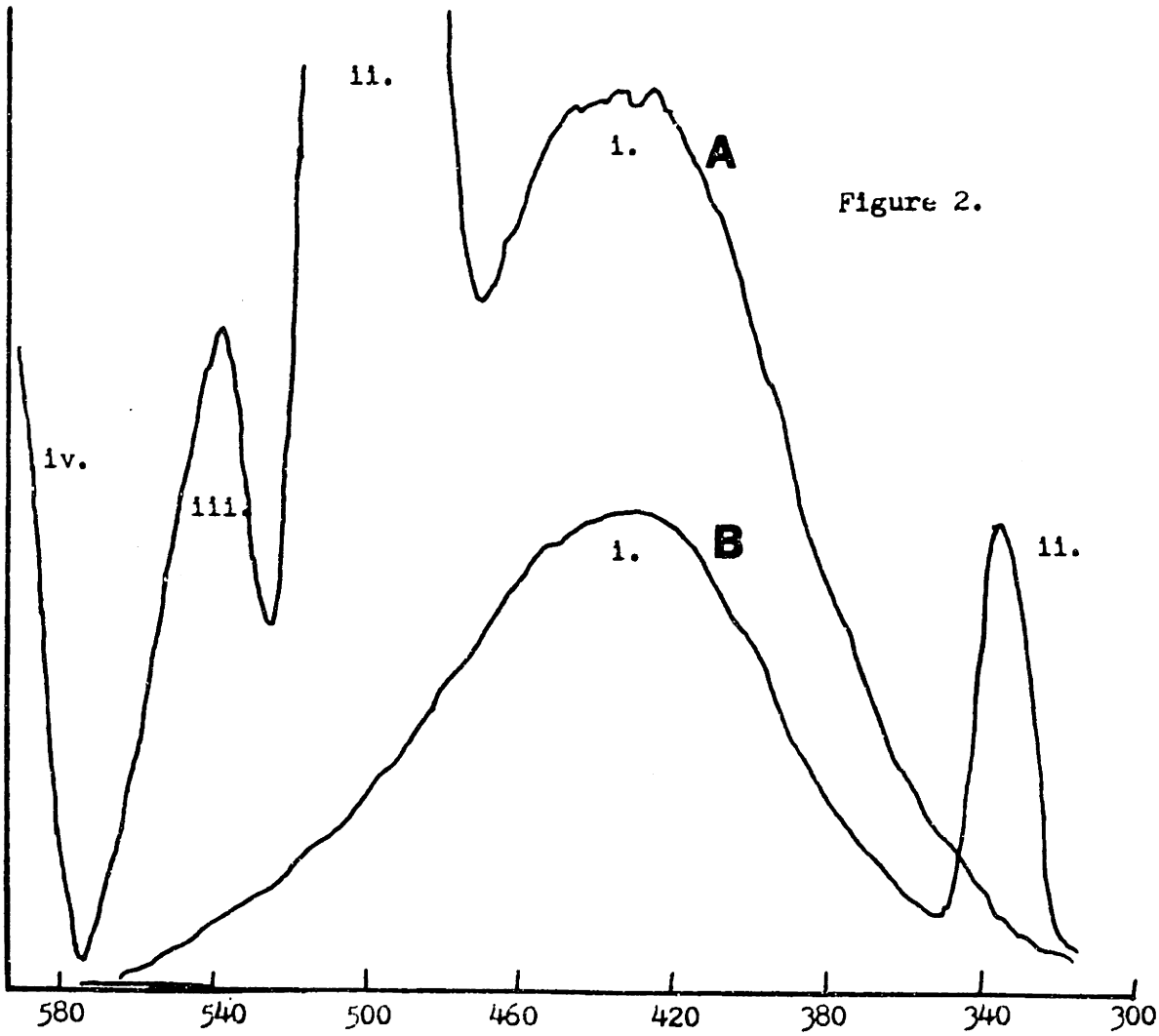


Figure 2.

250nm for the emission spectrum in Fig. 2A. There is a fluorescence peak (i.) at 420-460nm followed by off-scale emission at about 500nm (ii.) and several smooth symmetric peaks at 550nm and 600nm (iii. and iv.). We expect a Raman peak at 500nm because that is twice the excitation wavelength of 250nm. The peaks at 550nm and 600nm are probably "overtones" of this Raman scatter as attested by their smooth symmetry (chemical fluorescence peaks are more rough and asymmetric). These Raman peaks are confirmed by moving the excitation wavelength to 300nm (Fig. 2B). The emission at 420-460nm retains the same shape and position (iB) but the intensity has decreased because we have moved away from the peak excitation frequency. By contrast, the Raman peaks have completely shifted beyond the range of this spectrum (a principal Raman peak will now appear at 600nm). Note, however, that an overtone from the 300nm Raman peak is now moving into view at 330nm (iiB). Accurate measurement of fluorescence requires that the specra be checked to be certain that artifact Raman scatter has not moved into the emission band that is being monitored.

From studies such as those illustrated in Fig. 2, we determined that excitation spectra over the range of 200nm to 360nm could be obtained without Raman interference if the emission was monitored at a wavelength in the range of 390nm to 410nm. For this study our interests centered on the excitation spectra because we wished to investigate the undocumented near-UV excitation peaks. The emission spectrum (Fig. 2) had only a single, broad peak that corresponded to published spectra, and was not studied in detail.

The ultimate purpose of these spectral studies was the use of fluorescence quenching by Cu(II) ion as a tool in metal binding studies.

spectra is shown in Fig. 1. Curve a. is the spectrum for a Cu(II)-free system. Curve b. is the same solution after the addition of 1 μM total Cu(II). Curves c. and d. are, respectively, the results of 3 μM and 40 μM additions of Cu(II). This series of spectra suggests that all wavelengths in the excitation spectra are equally quenched by the binding of Cu(II) to the fulvic molecule. We interpret this to mean that there is no distinction in the binding preference among fluorophores.

This implies that fluorescence peaks do not correspond to functional groups that can be chemically distinguished in the course of metal titration. Apparently, weak metal-binding at high Cu(II) quenches the same fluorophores as does the strong binding observed in the first Cu(II) addition. This is an important result for the use of fluorescence quenching in titration studies. Ideally, quenching should be directly proportional to complex formation throughout the titration. While the spectral study does not prove this assumption, it does at least show that the choice of wavelengths is not critical: either peak in Fig. 1 would yield the same titration curve.

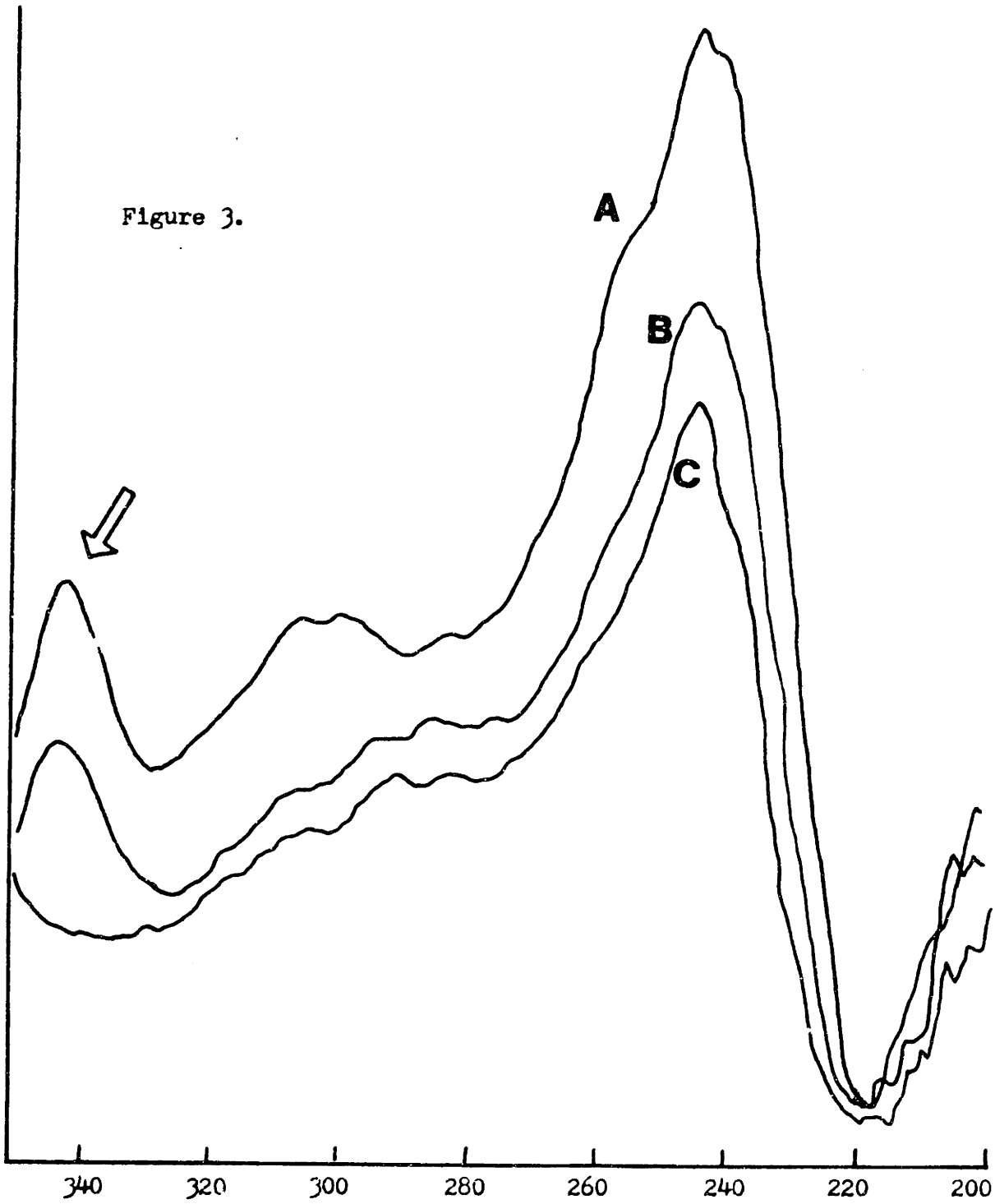
However, the identification of strong metal-binding groups by spectrofluorometry would be a very useful tool for improving models of metal-humic interactions. We performed a more detailed examination of the excitation spectra to determine if quenching were truly uniform at all excitation wavelengths. By changing to a 5nm excitation slit width coupled with slower scanning speeds, we obtained spectra of higher resolution.

Fig. 3 shows excitation spectra obtained in this way, using an emission wavelength of 390nm. Fig. A is the spectrum of a 1 mg-C/L

Figure 3.

Excitation spectra of 1mg-c/L GP-FA. A. Emission measured at 390 nm, no Cu(II) added. B. Emission measured at 390 nm with 4 μM Cu(II) added. C. Same as B. except emission measured at 410 nm, causing the Raman peak (arrow) to shift out of spectrum.

Figure 3.



solution of GP-FA at pH 8.5. The noisy peak near 200nm is simply a Raman peak (½ of 390nm) and is noisy because of the poor photomultiplier response at very low wavelengths. The large peak at 250nm is clearly distinguished as is the well-known peak near 310nm. The arrow indicates a peak at 345nm which is beyond the spectral range of the previous spectrum.

With the addition of 4 μM Cu(II) (Fig. 3B) all three peaks have decreased by the same proportion. However, changing the emission wavelength to 410nm (Fig. 3C) shifts the 345nm off to the left; it was only a Raman peak. This example illustrates the care needed in identifying spectral features. In this case, even the Raman peak at 345nm appeared to be quenched by the addition of Cu(II). In reality, the decrease in the peak was due to the quenching of the underlying chemical fluorescence in that wavelength region.

Aside from the artifact peak at 345nm, close examination of the curves in Fig. 3 reveals an interesting phenomenon. While the fluorescence at the peak values at 250nm and 310nm is quenched proportionally, the shoulder at 260nm drops off faster than the peak values. The shape of the peak is changing as quenching advances. This suggests that the poorly defined shoulder at 260nm may be an occluded peak that corresponds to a fluorophore associated with strong metal-binding sites. The limit of resolution precluded further study of this feature at this time.

A more clearly defined, although puzzling, example of disproportional quenching was found in studies of filtered whole water from Grassy Pond. In general, the fluorescence characteristics of the whole water were identical to the isolated fluvic acid fraction. A notable exception was the expansion of the near-UV excitation peak into a double

peak with maxima at 226nm and 218nm. The previously observed peak at 245nm is only a shouler on the double peak of the whole water spectrum (Fig. 4).

Of particular interest is the apparently high sensitivity of the 218nm peak to quenching by Cu(II). Fig. 4A is the unquenched spectrum. After the addition of 0.02 μM and 0.08 μM Cu(II), the 218 μM peak begins to diminish rapidly (Figs. 4B and 4C). When the Cu(II) concentration is raised to 0.2 μM and 0.4 μM (Figs. 4d and 4e) the peak at 218nm almost completely disappears. One possible interpretation is the existence of strong binding sites that fluoresce at 218nm excitation and which are quickly saturated in the titration. These binding sites could be either part of a non-fulvic fraction (hence their apparent absence in GP-FA extract) or else the extract contains small amounts of contaminating metal sufficient to quench these fluorophores in the presumed blank.

Titration data and fluorescence intensity measurements indicated that Grassy Pond water contained approximately 5 mg-C/L of fulvic acid. This five-fold difference from the 1 mg-C/L GP-FA solutions previously examined was a possible explanation of the lack of a 218nm excitation peak in the 1 mg-C/L GP-FA: A small background level of metal contamination would have a greater effect on a more dilute fulvic acid solution, quenching the 218nm peak.

A spectrum of 5 mg-C/L solution of GP-FA did in fact reveal a 215-230nm excitation band not observed in 1 mg-C/L GP-FA but virtually identical to that of the whole water (Fig. 5A). This band exhibited the same Cu(II) quenching as in the whole water; i.e., sharp initial loss of excitation at 218nm.

However, we found that the same quenching behavior could be induced by simply lowering the pH. Studies in this and other laboratories have

Figure 4.

Excitation spectra of a whole water sample of Grassy Pond water, emission measured at 405 nm.

a. No Cu(II) added. b. 0.02 μM Cu(II). c. 0.08 μM Cu(II). d. 0.2 μM Cu(II). e. 0.4 μM Cu(II). Titration performed at pH 6.0.

Figure 5.

Excitation spectra of 5 mg-C/L GP-FA. Emission at 390 nm.

a. No CO_2 bubbling, pH 7.4. b. CO_2 added, pH 5.2. c. CO_2 added, pH 5.02. No Cu(II) added to any.

Figure 4.

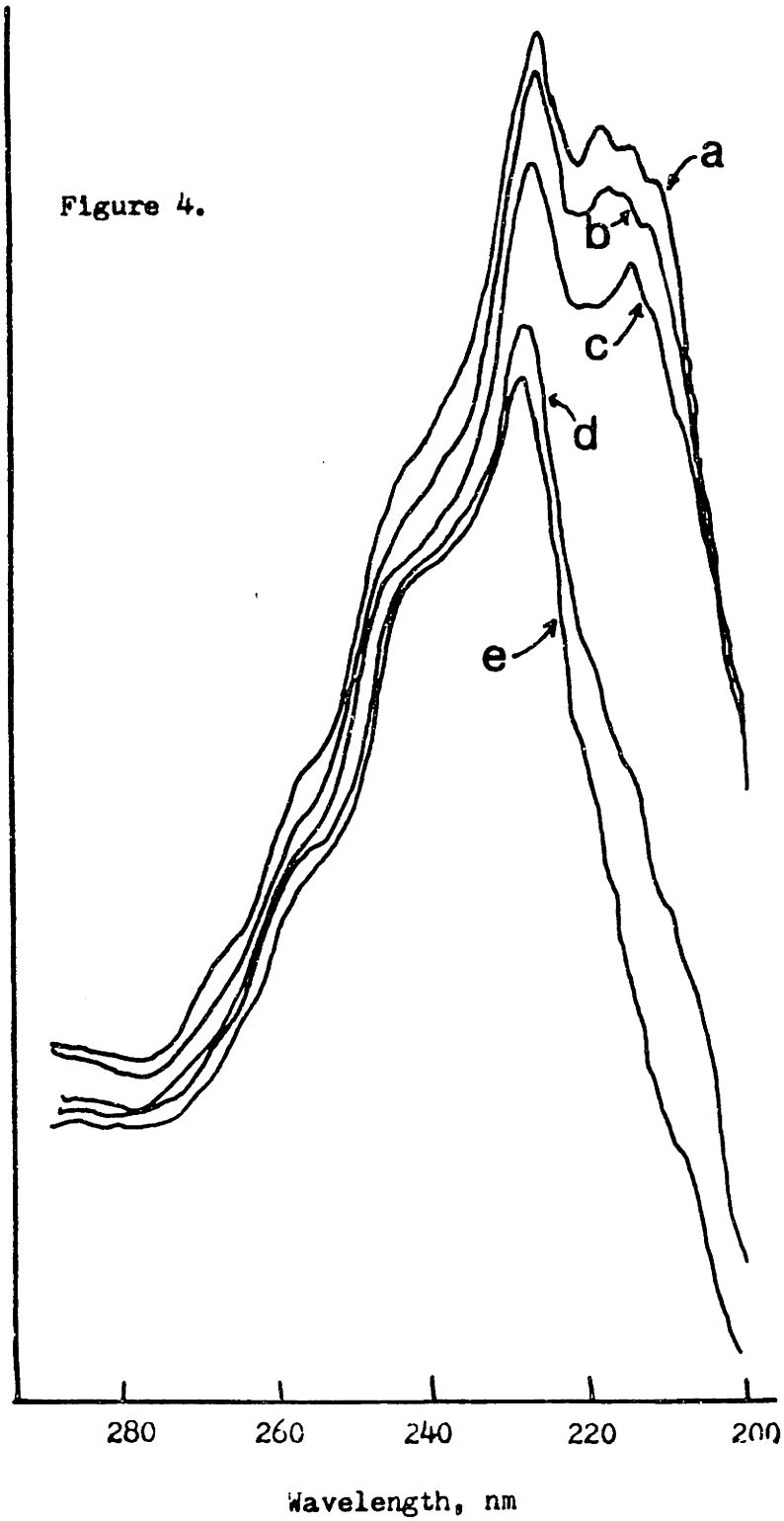
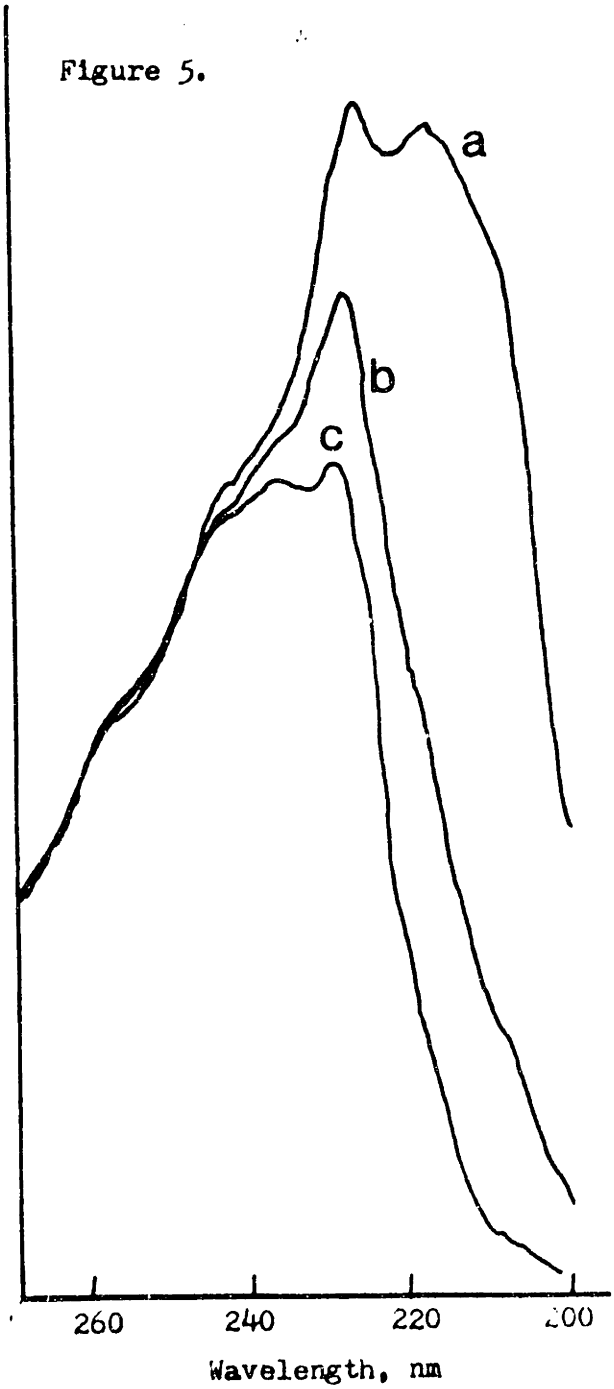


Figure 5.



shown that fluorescence of humic materials is largely unaffected by solution pH or degree of protonation over the range pH 3 to pH 11. Protons only affect quenching if they displace a quenching metal ion. In contrast to these findings, we observed that the 215nm-230nm excitation band diminished sharply when the solution pH was lowered from 7.4 to 5.02 by bubbling with CO_2 (Fig. 5B, 5C). In Fig. 5A (pH 7.4) the entire excitation band is present. In Fig. 5B (pH 5.2) most of the band below 225nm has disappeared and at pH 5.02 (Fig. 5C) even the 227nm peak has sharply diminished leaving the 245nm peak to predominate.

Curiously, in several repeated analyses of 1 mg-C/L GP-FA solutions, the peaks at wavelengths less than 220nm were never observed at either pH 6.9 and pH 8.5.

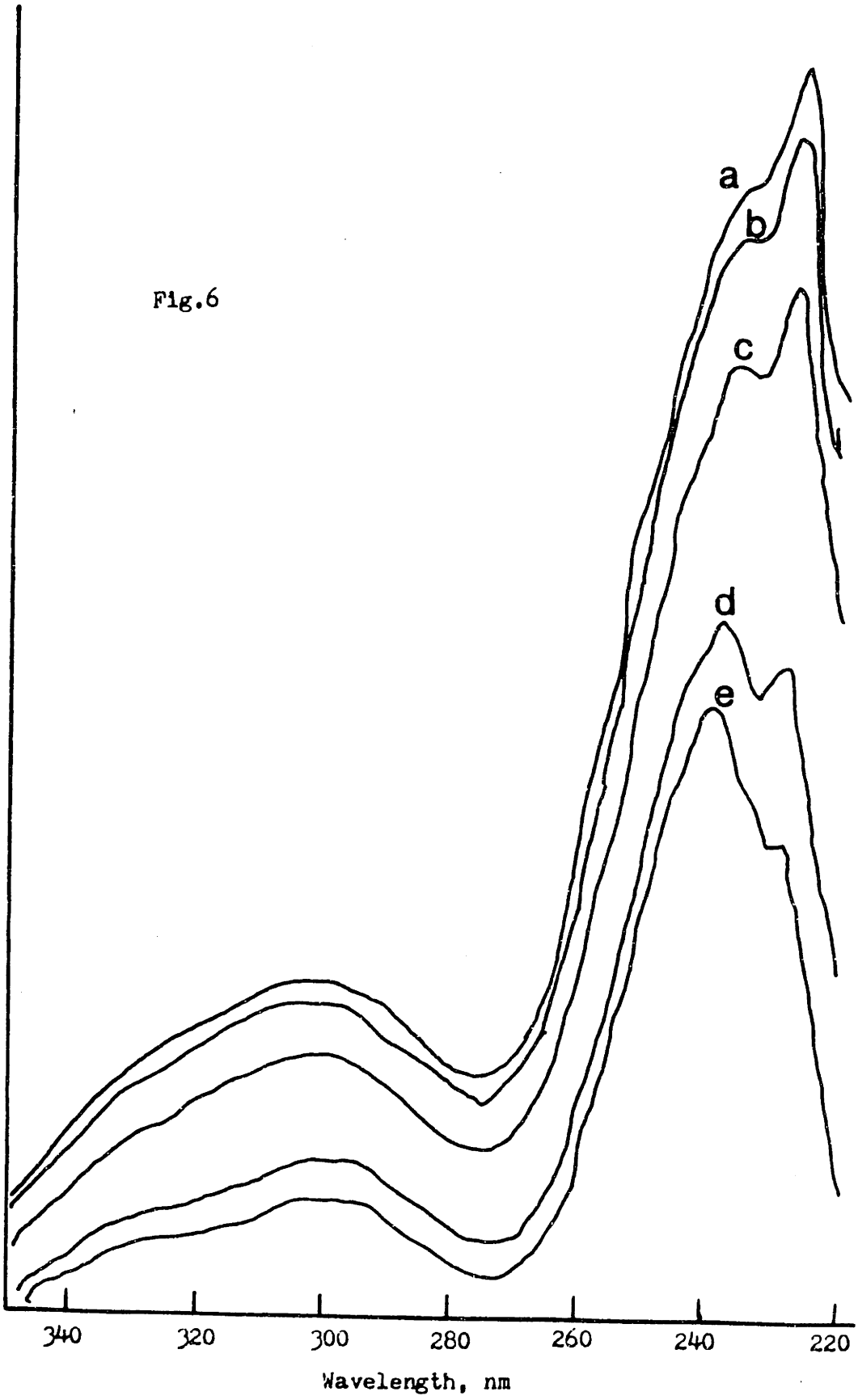
Although there is an apparent concentration and pH dependence of the sub-240nm excitation, the conditions under which these peaks appear and the factors controlling their shape are not well known. Fig. 6 depicts successive excitation spectra of a 1 mg-C/L solution of GP-FA at pH 6.2. In this instance the 227nm peak was observed in a 1 mg-C/L sample, but there was no evidence of the 218nm peak. In addition the 245nm peak observed in all samples appears more prominently. As Cu(II) is added, the 227nm peak drops away as before, leaving only the 245nm peak.

In this study we were unable to conclusively document the reasons for the variability of the shapes and magnitudes of the excitation peaks of humic materials. It is possible that the excitation phenomena observed in the 215nm-230nm range are very sensitive to small quantities of contaminating trace metals and pH variation. Further work in this area may provide new insights into the mechanisms of metal binding by aquatic humic matter.

Figure 6.

Excitation spectra of Grassy Pond fulvic acid, 1 mg-C/L at pH 6.2. a. No Cu(II) added. b. 0.4 μM Cu(II). c. 4.0 μM Cu(II). d. 40 μM Cu(II). e. 100 μM Cu(II).

Fig.6



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