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### CADMIUM(II), LEAD(II), AND COPPER(II) COMPLEXATION BY FULVIC ACIDS DERIVED FROM SOIL AND WATER: ION-SELECTIVE ELECTRODE AND SPECTROFLUOROMETRIC STUDIES

ROBERT A. SAAR

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SAAR, ROBERT A.

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*University of New Hampshire*

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FULVIC ACIDS DERIVED FROM SOIL AND WATER:  
ION-SELECTIVE ELECTRODE AND SPECTROFLUOROMETRIC STUDIES

BY

Robert A. Saar

B. A., Yale University, 1973

A DISSERTATION

Submitted to the University of New Hampshire  
In Partial Fulfillment of the Degree of  
Doctor of Philosophy  
Department of Chemistry

May, 1980

This dissertation has been examined and approved.

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Date

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## ABSTRACT

CADMIUM(II), LEAD(II), AND COPPER(II) COMPLEXATION BY  
FULVIC ACIDS DERIVED FROM SOIL AND WATER:  
ION-SELECTIVE ELECTRODE AND SPECTROFLUOROMETRIC STUDIES

by

Robert A. Saar

University of New Hampshire, May, 1980

This study describes complexation of metal ions by fulvic acid, the acid soluble fraction of organic matter found in soils (SFA) and water bodies (WFA). Such complexation affects the toxicity and geochemical mobility of heavy-metal ions. All the work was performed with two previously isolated and characterized fulvic acids: one from a podzol soil and the other from a freshwater river. The work is divided into three major sections.

I. Complexation of  $\text{Cd}^{2+}$  by fulvic acid. The conditional stability constants for complexes between aqueous  $\text{Cd}^{2+}$  and the two fulvic acids were studied by  $\text{Cd}^{2+}$  ion-selective electrode potentiometry. A temperature of  $25^{\circ}\text{C}$  and a medium of  $0.1 \text{ M KNO}_3$  was used throughout. The best results arose when  $\text{Cd}^{2+}$  (rather than fulvic acid) was the titrant. The experiments show that (1) stability constants increased with increasing pH, and (2) stability constants decreased as the fulvic acid concentration rose toward  $70 \text{ mg/L}$ . The second effect does not occur for the

$\text{Cu}^{2+}$ -fulvate system. In relatively concentrated solutions, interactions among fulvic acid molecules apparently block sites otherwise available to  $\text{Cd}^{2+}$ . From pH 4.0 to 8.0, the logarithm of the 1:1 conditional stability constant increases from 3.15 to 4.08 for WFA and from 3.23 to 4.63 for SFA. Fewer oxygen-containing complexation sites per mole of WFA is a reason for the lower WFA constants found during this study and in the next one with  $\text{Pb}^{2+}$ .

II. Complexation of  $\text{Pb}^{2+}$  by fulvic acid.  $\text{Pb}^{2+}$  forms much stronger complexes with SFA and WFA than does  $\text{Cd}^{2+}$ , as measured by  $\text{Pb}^{2+}$  ion-selective electrode potentiometry at 25°C in 0.1 M  $\text{KNO}_3$ . From pH 4.0 to 6.0, the logarithm of 1:1  $\text{Pb}^{2+}$ -SFA conditional stability constants increases from 4.0 to 6.3. The corresponding constants for  $\text{Pb}^{2+}$ -WFA in the range pH 4.5 to 6.0 increase from 3.7 to 5.1.

$\text{Pb}^{2+}$ -fulvate precipitates at very low mole ratios of metal ion to fulvic acid. This precipitation limits the range of reagent concentrations that can be used for solution-phase studies of  $\text{Pb}^{2+}$ -fulvate complexes.  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  form similar strength complexes with fulvic acid until  $\text{Pb}^{2+}$ -fulvate precipitation begins. At that point, removal of hydrated  $\text{Pb}^{2+}$  is more complete than is the removal of hydrated  $\text{Cu}^{2+}$  as demonstrated in parallel experiments. The extra  $\text{Pb}^{2+}$  removal from solution appears to occur through physical association with  $\text{Pb}^{2+}$ -fulvate solids as well as by chelation by sites still available in the precipitates. Only at much higher mole ratios of metal ion to fulvic acid

does  $\text{Cu}^{2+}$ -fulvate precipitate.

III. Fluorescence studies. These fulvic acids have a broad, featureless fluorescence peak at 445-450 nm upon excitation at 350 nm. The work shows that (1) fluorescence intensity varies with pH, being the greatest near pH 5.0 and dropping off most notably below pH 4. (2) Metal ions at concentrations of approximately  $10^{-3}$  M or less that are not complexed by fulvic acid do not quench the fluorescence. (3) paramagnetic ions such as  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$ , when complexed by fulvic acid quench the fluorescence. A comparison of ion-selective electrode and fluorescence studies for  $\text{Cu}^{2+}$ -SFA complexes shows a direct proportionality between the amount of complex and the percentage of fluorescence quenched from pH 3.0 to 6.0. With further development, fluorescence offers promise as a means of measuring stability constants for complexes between fulvic acid and paramagnetic metal ions.

## CHAPTER 1

### AN OVERVIEW OF METAL-ION COMPLEXATION BY FULVIC ACID

#### BACKGROUND ON FULVIC ACID

The pale yellow-brown color in many rivers and lakes indicates the presence of dissolved organic matter. The organic matter comes largely from soils covering the surrounding areas, and is derived originally from the wastes of plants and animals and the by-products of their decay. The material is of two major types: biopolymers (including carbohydrates, protein fragments, fats, and pigments) and geopolymers, which are commonly called humic substances (Reuter and Perdue, 1977).

When isolated, humic substances are dark brown or nearly black. They are derived from biological processes just as biopolymers are, but the humic substances have been altered so much by weathering and biological degradation that they lose the commonly known structures of organic and biological molecules (Schnitzer and Khan, 1972). Many usual enzymes and microbes cannot efficiently break down these

unusual structures, so humic substances degrade more slowly than do the more usual biological molecules. It is estimated that 60-80% of dissolved and particulate organic carbon in river water is in humic substances (Reuter and Perdue, 1977).

Humic substances have been studied since the eighteenth century (Schnitzer and Khan, 1972), but they are so complicated and difficult to study that there is no shortage of research possibilities even now. The material is a mixture of many hydrophilic compounds that have the characteristics of polyelectrolytes. The molecular weights of the compounds in the mixture range from a few hundred to tens of thousands of daltons.

It is possible to divide humic substances into several subgroups. The most common separation is based on solubility in acid and base:

Humic acid. Soluble in base, but precipitates when acidified.

Fulvic acid. Soluble in both acid and base.

Humic. Soluble in neither acid nor base.

Fulvic acid (FA) is the fraction of humic substances that participates in the chemistry discussed in this dissertation. It shares many chemical characteristics of humic substances in general, having a wide variety of aromatic and aliphatic structures; indeed, there may not be two molecules in the mixture that are identical. Molecular weights of the various compounds making up FA vary from

hundreds to thousands of daltons. Fulvic acid is considered a polyelectrolyte (Gamble, 1970), largely because it has many oxygen-containing functional groups, particularly -COOH and -OH. Many of these functional groups can be protonated and deprotonated in the pH range common in natural waters (pH 3-9), and, therefore, FA may alter the pH buffering capacity of soils and water bodies (Brosset, 1979). Hydrogen ion, though, is not the only cation that may attach to fulvic acid molecules. Metal ions, notably alkali, alkaline earth, and transition-metal ions may be complexed by anionic groups on the fulvic acid molecules.

The metal-ion complexation by fulvic acid has important implications. First, dissolved organic matter in a water body may form soluble complexes with metal ions that were precipitated or adsorbed onto sediments. In addition, some fraction of the metal ions newly entering a water body will be taken up by the fulvic acid molecules. In a sense, fulvic acid, as well as other classes of soluble organic matter, can act as metal-ion buffers. Metal-ion buffers, like hydrogen-ion buffers, have limits: at some point, the capacity of dissolved organic matter to complex added metal ion will be reached. This limit is called the complexing capacity of the water sample. Complexing capacities differ not only from one fulvic acid sample to the next, but also from one metal ion to the next. Although the thermodynamic strength of a complex and the number of complexation sites in a fulvic acid sample are not directly related, one must



consider both factors when trying to determine the amount of free (hydrated) metal ion and the amount of complexed metal ion in a water or soil sample.

A second implication of complexation between fulvic acid and metal ions relates to the toxicity of certain metal ions. Ions such as  $\text{Cu}^{2+}$  or  $\text{Cd}^{2+}$  have lower toxicity when they are part of a complex than when they are free, hydrated metal ions (Jenne and Luoma, 1977). Even though the total concentration of a toxic metal ion (both complexed and uncomplexed) may be higher in a water body with high fulvic acid concentration than in a water body with low fulvic acid concentration, the toxicity of the fulvic acid rich water may be lower, because a relatively small fraction of the toxic metal ion is in its most toxic (hydrated) form.

The fact that fulvic acid is a class of compounds rather than a single substance distinguishes its study from other chemical pursuits. For individual compounds, we talk about molecular weights, crystal structures, discrete dissociation constants and distinct spectra. Those studying individual compounds are often rewarded by the precision, completeness, and finality of their studies.

Such is not the case for fulvic acid or for the other classes of naturally occurring organic substances, humic acid and humin. At best, we speak of average molecular weights and ranges of dissociation constants for the class; the spectra are featureless and broad. The effort to characterize fulvic acid as completely as has been done for

single compounds drives researchers to ever more complicated mathematics and models.

A problem may arise from this type of effort. Are we trying to apply the vocabulary and methods used for single compounds to classes of compounds? Might we need a new approach? Or is it admitting defeat if we do not pursue the investigation of relatively ill-defined classes of substances to a high level of completeness?

The fact remains that describing and working with fulvic acid is frequently difficult. But over the years, an understanding of this class of compounds has begun to develop. There is no shortage of contradictory data, but the general trend is toward clarity rather than confusion.

#### COMPLEXES BETWEEN METAL IONS AND FULVIC ACID

Measuring the strength of complexes between metal ion and fulvic acid is frequently the goal of fulvic-acid research, and it is the subject of this dissertation. In the environment, many different metal ions may compete in solution for fulvic acid binding sites. However, the many reactions between the various metal ions and fulvic acid cause the natural system to be so complicated that it must be studied in simpler pieces: stability constants for FA complexes are calculated for one type of metal ion at a time. Not only are there these solution-phase reactions, but the dissolved species can also adsorb onto solids and colloids. The hope is that the results of many research

groups may collectively form a reasonably close picture of what actually occurs.

There are several ways to measure stability constants for complexes between metal ions and fulvic acid. One way is to measure the free metal-ion concentration after adding a known amount of metal ion. The difference between the concentrations of total and free metal ion (the concentration of bound metal ion) can be measured potentiometrically by ion-selective electrodes, which do not disturb the equilibrium. The method is limited to the metal ions for which electrodes exist (copper(II), cadmium(II), lead(II), and calcium(II)) and limited by the detection limits of these electrodes, which are not as low as for some other electrochemical methods. Measuring the quantity of hydrogen ion released as metal ion-fulvic acid complexes form is another potentiometric method that does not disturb the equilibrium (Stevenson, 1976, 1977).

Researchers have used several voltammetric methods, including anodic stripping voltammetry and polarography, for stability-constant and complexing-capacity determinations (Bresnahan et al., 1978; Greter et al., 1979; Shuman and Cromer, 1979). Anodic stripping voltammetry has a very low detection limit, but it has two major problems:

- (1) Free metal ion and an unknown fraction of the bound metal ion can be plated into the electrode (Shuman and Cromer, 1979). The free metal-ion reading, therefore, is higher than it would be if only free metal-ion were

electroactive.

(2) Surface-active agents such as fulvic acid adsorb onto hanging mercury-drop or thin-film mercury electrodes and distort the free metal-ion reading (Brezonik et al., 1976; Ernst et al., 1975; Batley and Florence, 1976; Benes et al., 1979). Glassy carbon or wax-sealed graphite electrodes apparently do not have these problems (Weber and Cheng, 1979), but the linear calibration range for  $\text{Cu}^{2+}$  is narrow (Smart and Weber, 1980). Polarography also suffers from these two problems (Bond and Hefter, 1971; Jacobson and Lindseth, 1976).

A second way to study the binding of metal ions is to separate free and bound metal ions and then measure the total concentration of metal ions in the fraction that contains only free metal ions. Free and bound metal ions can be separated by small-pore dialysis tubing (Benes and Steinnes, 1974; Guy and Chakrabarti, 1976) or by small-pore ultrafiltration membranes. Dialysis tubing has nominal molecular weight cutoffs as low as 1000 daltons, and ultrafiltration membranes can have a nominal cutoff as low as 700 daltons (Amicon Corporation, 1977). The dialyzed or filtered solution, which ideally contains only free metal ions (metal ion-fulvate complexes are too large to pass through the membrane), may be analyzed by atomic absorption spectrophotometry or by anodic stripping voltammetry if the organic matter is destroyed (Gardiner and Stiff, 1975). Again, knowledge of free and total metal-ion concentration

allows one to calculate bound metal-ion concentration, and ultimately, stability constants.

A third approach to measuring stability constants would be to distinguish between fulvic acid molecules that have metal ions attached (complexed ligand) and fulvic acid molecules not having a metal ion attached (uncomplexed ligand). One promising method for this type of analysis is spectrofluorometry. Chapter 4 contains descriptions of the work needed to develop this technique for analysis of ligand-metal complexes.

Other methods for determination of stability constants between metal ions and organic matter include ion exchange (Schnitzer and Hansen, 1970; Randhawa and Broadbent, 1965), gel filtration chromatography (Mantoura and Riley, 1975), and electron spin resonance spectroscopy (Gauer et al., 1976; Templeton, 1980).

Potentiometric, voltammetric and fluorescence analysis have advantages and disadvantages relative to each other and relative to other techniques. Ion-selective electrode analysis is quick and does not disturb the system's equilibrium, but the detection limit is not as low as it is for anodic stripping voltammetry. Anodic stripping voltammetry and polarography have disadvantages: organic matter adsorbs onto the electrode, and some bound metal ion may be electroactive, that is, voltammetry does not strictly distinguish between free and bound metal ion. Fluorescence analysis may vary from ligand to ligand, depending on the

ligand's fluorescing properties. Furthermore, unless special flow apparatus is available, separate samples with different mole ratios of metal ion to ligand must be prepared and their fluorescence measured separately. However, the information obtained is different from that provided by any of the electrochemical or separation methods. Any extra perspective on a system as complicated as the metal ion-fulvic acid one is helpful.

Chapter 2 of this dissertation contains a detailed description of cadmium(II) complexation by two types of fulvic acids, one derived from a soil and the other from a freshwater river. There is a brief discussion of a complexation study using ultrafiltration/atomic absorption spectrophotometry, but ion-selective electrode was the method of analysis for most of this work. The  $pK_h$  for cadmium(II) hydrolysis is 11.70 (Huheey, 1978), so  $Cd^{2+}$  hydrolysis is not a problem even for experiments up to pH 8.5, two pH units higher than those of the lead(II) and copper (II) work.

Chapter 3 contains detailed information on lead(II) complexation by SFA and WFA. Also included there is a description of copper(II) complexation by SFA. An ion-selective electrode was again the means of analysis. Although copper (II)-fulvic acid studies were done earlier in this group (Bresnahan et al., 1978), those experimental conditions were different from the conditions of these lead(II) titrations, so that results can not be compared.

The new copper(II)-fulvate experiments were designed to be comparable to this lead(II) work and to the fluorescence work that followed.

The development of fluorescence as a technique for measuring stability constants for complexes between metal ions and fulvic acid is the subject of Chapter 4. The work shows the different quenching ability of several divalent cations, including copper(II), lead(II), cadmium(II), cobalt(II), and nickel(II). Further discussion appears there on the possibilities for fluorescence in this application.

#### CHARACTERISTICS OF THE SOIL-DERIVED AND WATER-DERIVED FULVIC ACIDS USED IN THIS RESEARCH

During 1973-1977, workers in this research group isolated fulvic acid from two sources: from the B<sub>2</sub> horizon of a podzol soil (Conway, New Hampshire) and from fresh water flowing in the Oyster River, Lee, New Hampshire (Weber and Wilson, 1975). They performed elemental analysis, functional-group analysis and pH titrations, and measured the number-average dissociation-corrected molecular weight (Wilson and Weber, 1977a). These molecular weights are 644 daltons for the soil-derived fulvic acid and 626 daltons for the water-derived fulvic acid. These values and the results of elemental and group analyses appear in Table 1. Wilson and Weber (1977b, 1979) also investigated the semiquinone free-radical nature of these fulvic acids.

TABLE 1

Characteristics of the fulvic acids used in this research<sup>a</sup>

	Soil-derived fulvic acid (SFA)	Water-derived fulvic acid (WFA)
number-average dissociation-corrected molecular weight (daltons)	644	626
elemental analysis		
% carbon	53.1	51.1
% nitrogen	0.90	1.13
% hydrogen	3.24	3.62
% ash	0.8	1.0
functional groups (milliequivalents per gram)		
total acidity	13.4	10.6
carboxyl	8.2	6.3
phenolic <sup>b</sup>	5.2	4.3
carbonyl	3.5	4.3

<sup>a</sup> From Weber and Wilson, 1975.

<sup>b</sup> Difference between total acidity and carboxyl values.



Fulvic acid has long been studied because of its ability to complex metal ions (Gamble and Schnitzer, 1973; Reuter and Perdue, 1977; Jackson et al., 1978). This complexation probably occurs through oxygen-atom donors, because of the prevalence of oxygen-bearing functional groups and the very low concentration of nitrogen, or any other atom that can donate electron pairs to a metal ion (Beckwith, 1959; Khanna and Stevenson, 1962). The possibility exists that an average fulvic acid molecule has more than one binding site and that a metal ion can be surrounded by more than one bidentate ligand. These possibilities raise questions about the proper stoichiometry of metal ion-fulvic acid complexes and about the mathematics needed to calculate the stability constants. Cadmium(II), lead(II), and copper(II) ions have rather different chemistries, and as the following discussions show, different assumptions and methods of calculation may be best suited for each of the metal-ion complexes.

## CHAPTER 2

### Cd<sup>2+</sup> COMPLEXATION BY FULVIC ACIDS DERIVED FROM SOIL AND WATER

#### INTRODUCTION

Although several transition metals such as cobalt, molybdenum and iron are essential micronutrients, no need for cadmium has been demonstrated. Indeed, Cd<sup>2+</sup> is a poison that accumulates in plants and animals exposed to it. In humans, renal arterial hypertension and Itai-Itai disease may result from over-exposure to Cd<sup>2+</sup>. To prevent Cd<sup>2+</sup> poisoning, a panel from the National Academy of Sciences recommended a limit of no more than 0.01 mg/L of Cd<sup>2+</sup> in drinking water (National Academy of Sciences, 1972).

Cadmium(II) toxicity in water and soils is not necessarily in direct proportion to total Cd<sup>2+</sup> concentration. The toxicity of different Cd<sup>2+</sup>-bearing species (hydrated Cd<sup>2+</sup>, Cd<sup>2+</sup>-organic matter complexes, and inorganic Cd<sup>2+</sup> complexes) may vary, either because such species are picked up by plants and animals to different extents, or because, once in the organism, they are

assimilated to varying degrees.

Naturally occurring organic matter may chelate  $\text{Cd}^{2+}$ . Humic acid diminishes  $\text{Cd}^{2+}$  availability to Daphnia magna in Lake Superior water (Poldoski, 1979). Aqueous organic fractions also reduced the toxicity of  $\text{Cd}^{2+}$  toward Simocephalus serrulatas and toward Gambusia affinis (Giesy et al., 1977).

It is clear that the various  $\text{Cd}^{2+}$ -bearing species, particularly those containing organic matter ligands, have different biological availability and move differently in geochemical systems. There are a number of ways to measure the strength of association between  $\text{Cd}^{2+}$  and this organic matter. Several researchers measured the speciation of  $\text{Cd}^{2+}$  (Batley and Gardner, 1978; Mantoura et al., 1978; Gardiner, 1974a, 1974b; Florence and Batley, 1977; Blustein and Smith, 1978). Others have taken the analysis one step further by determining the complexing capacity of a given body of water for  $\text{Cd}^{2+}$  (Giesy et al., 1978).

Calculation of stability constants for  $\text{Cd}^{2+}$ -organic matter complexes represents an even more detailed attempt to understand  $\text{Cd}^{2+}$  transport. Most often reported is the conditional stability constant, that is, a stability constant at a specified pH and ionic strength. The total concentration of fulvic acid ligand is usually used in the equilibrium quotient rather than the fraction that is deprotonated and available for complexation. The fraction of deprotonated fulvic acid changes with pH, and hence, the

conditional stability constant also varies with pH. By definition, the thermodynamic stability constant does not vary with pH; it expresses in a fundamental way the affinity of a deprotonated ligand for the hydrated metal ion. The extent of deprotonation needed for complex formation, however, varies depending on the metal ion. Further discussion of this issue appears below.

Workers in at least seven research groups have published stability constants measured in several ways. The key results appear in Table 2. Mantoura et al. (1978) extracted humic acid from several samples of fresh and saltwater and extracted fulvic acid from peat. They employed gel filtration chromatography to calculate the stability constants. This technique, based on the Hummel-Dreyer procedure (Mantoura and Riley, 1975; Hummel and Dreyer, 1962), is very time consuming and might well not have worked for our low-weight fulvic acids. Such small molecules would require the smallest pore Sephadex resin (G-10); irreversible interactions between aromatic groups in the fulvic acid and the tightly cross-linked G-10 resin could easily ruin the analysis (Gellote, 1960).

Guy and Chakrabarti (1976) employed dialysis/atomic absorption spectrophotometry to separate and measure free and complexed  $\text{Cd}^{2+}$ . The organic matter they used was commercially available humic acid (Aldrich Chemical Company), and they analyzed their data by the Scatchard method (Scatchard, 1949). Again, the molecular size of our

TABLE 2

Cadmium(II) stability constants with humic materials

Complex <sup>a</sup>	pH	I	Log K	Method	Reference
Cd-(peat FA) <sup>b</sup>	8.0	0.02	4.83	1	1
Cd-(lake HA) <sup>b</sup>	8.0	0.02	4.57	1	1
Cd-(lake HA) <sup>b</sup>	8.0	0.02	4.70	1	1
Cd-(loch HA) <sup>b</sup>	8.0	0.02	4.95	1	1
Cd-(loch HA) <sup>b</sup>	8.0	0.02	4.87	1	1
Cd-(sea HA) <sup>b</sup>	8.0	0.02	4.69	1	1
Cd-SFA	4.9	0.1	3.04	2	2
Cd-SFA	5.95	0.1	3.64	2	2
Cd-SFA	5.7	0.01	5.3	2	3
Cd <sub>2</sub> -SFA	5.7	0.01	9.8	2	3
Cd <sub>3</sub> -SFA	5.7	0.01	14.0	2	3
Cd-SFA	6.7	0.01	5.6	2	3
Cd <sub>2</sub> -SFA	6.7	0.01	10.6	2	3
Cd <sub>3</sub> -SFA	6.7	0.01	15.5	2	3
Cd-SFA	7.7	0.01	6.0	2	3
Cd <sub>2</sub> -SFA	7.7	0.01	10.7	2	3
Cd <sub>3</sub> -SFA	7.7	0.01	15.4	2	3
Cd-(coal HA)	5.5	0.1	4.9 <sub>±</sub> 0.2	2	4
Cd-(coal HA)	6.0	0.1	5.3 <sub>±</sub> 0.2	2	4
Cd-(coal HA)	7.0	0.1	5.9 <sub>±</sub> 0.2	2	4

Table 2, continued

Cd-(coal HA)	7.5	0.1	6.3 $\pm$ 0.2	2	4
Cd <sub>2</sub> -(coal HA)	6.0	0.1	9.2 $\pm$ 0.2	2	4
Cd <sub>2</sub> -(coal HA)	7.0	0.1	10.6 $\pm$ 0.2	2	4
Cd <sub>2</sub> -(coal HA)	7.5	0.1	11.7 $\pm$ 0.2	2	4
Cd <sub>3</sub> -(coal HA)	6.0	0.1	13.7 $\pm$ 0.2	2	4
Cd <sub>3</sub> -(coal HA)	7.0	0.1	15.7 $\pm$ 0.2	2	4
Cd <sub>3</sub> -(coal HA)	7.5	0.1	16.5 $\pm$ 0.2	2	4
Cd-(peat HA)	c	0.1	5.07	3	5
Cd-(lignite HA)	c	0.1	5.32	3	5
Cd-(lignite HA)	c	0.1	6.01	4	5
Cd-SHA	c	0.1	5.14	3	5
Cd-SHA	c	0.1	6.06	4	5
Cd-(peat HA)	c	0.01	6.39	3	5
Cd-(lignite HA)	c	0.01	6.69	4	5
Cd-SHA	c	0.01	6.38	3	5
Cd-SHA	c	0.01	6.92	4	5
Cd-SHA	6.0	0.1	3.46 <sup>d</sup>	2	6
Cd-(SHA) <sub>2</sub>	-	0.1	e	2	6
Cd-AHA	6.8	0.01	5.04 <sup>f</sup>	5	7
Cd-SFA	-	0.1	2.78	3	8

Table 2, continued

- a Abbreviations. FA (fulvic acid); HA (humic acid); SFA (soil-derived fulvic acid); SHA (soil-derived humic acid); AHA (Aldrich humic acid).
- b Humic materials isolated by adsorption onto Amberlite XAD-2 resin and eluted with 1:1 v/v methanol and 2 M ammonia.
- c Average of pH 4 and 5.
- d Average of 13 different soil samples.
- e  $6.25 + 0.63(\text{pH} - 5)$ .
- f Value for the strongest class of sites as calculated by the Scatchard method.

Methods. (1) Gel filtration compleximetry. (2) Ion-selective electrode potentiometry. (3) Conventional hydrogen-ion potentiometry. (4) Modified hydrogen-ion potentiometry. (5) Dialysis/atomic absorption spectrophotometry.

References. (1) Mantoura et al., 1978. (2) Cheam and Gamble, 1974. (3) Brady and Pagenkopf, 1978. (4) Whitworth and Pagenkopf, 1979. (5) Stevenson, 1977. (6) Takamatsu and Yoshida, 1978. (7) Guy and Chakrabarti, 1976. (8) Ramamoorthy and Manning, 1974.

SFA and WFA is smaller than the pores of dialysis tubing that is available. The Scatchard method of data treatment was somewhat unsuitable because the complexing sites in our SFA and WFA do not fall into discrete classes.

Stevenson (1976, 1977) and Ramamoorthy and Manning (1974) used hydrogen-ion potentiometry to measure the stability constants of  $\text{Cd}^{2+}$  with various organic fractions. All of Stevenson's samples were humic acids; Ramamoorthy and Manning's organic matter was soil-derived fulvic acid. The method works because protons are released as  $\text{Cd}^{2+}$ -organic matter complexes form. It is not an easy method of analysis; precise pH control is essential.

To avoid this need for strict control of pH, several groups have chosen  $\text{Cd}^{2+}$  potentiometry. An ion-selective electrode sensitive to  $\text{Cd}^{2+}$  provides data for stability constant calculations. Cheam and Gamble (1974) calculated 1:1 stability constants for  $\text{Cd}^{2+}$ -SFA. Brady and Pagenkopf (1978) also experimented with SFA, but they calculated stability constants for  $\text{Cd}$ -SFA,  $\text{Cd}_2$ -SFA, and  $\text{Cd}_3$ -SFA complexes. Workers in the same group (Whitworth and Pagenkopf, 1979) calculated stability constants for the same three stoichiometries for complexes containing  $\text{Cd}^{2+}$  and humic acid derived from coal. Takamatsu and Yoshida (1978) derived humic acid from 13 soil samples and calculated conditional stability constants for 1:1 and 1:2 complexes.

When one considers the toxicity of  $\text{Cd}^{2+}$ , the work cited above does not form a long list, especially when compared



with work done on  $\text{Cu}^{2+}$ . A major reason may be that analytically,  $\text{Cu}^{2+}$  is much easier to handle, in part because its associations with organic matter are much stronger than are those of  $\text{Cd}^{2+}$ , and therefore, effects are much easier to measure. The result is that much work remains to be done on the interaction between  $\text{Cd}^{2+}$  and naturally occurring organic matter.

Available information indicates that conditional stability constants for complexes containing  $\text{Cd}^{2+}$  and any of several different organic matter fractions increase with increasing pH. However, no group has analyzed the conditional stability constant for  $\text{Cd}^{2+}$ -WFA complexes, and no information is available on the effect, if any, of different organic-matter concentrations on the conditional stability constant.

The work described here compares the complexation ability of fulvic acids derived from soil and from fresh water. I calculate the 1:1 conditional stability constant at pH values ranging from 4.0 to 8.0 and determine the effect of FA concentration and ionic strength on the stability constants.

## EXPERIMENTAL

### Dialysis

Separation techniques dominated some of the early work with the cadmium(II)-fulvic acid system. Available for

separating bound and free metal ions were dialysis tubing (Spectrum Medical Industries, Los Angeles, California) with a nominal molecular weight cutoff of 3500 daltons. The same company began manufacturing 1000-dalton cutoff tubing, but they could not deliver any with the proper porosity until the ion-selective electrode work was well under way. I did not return to the dialysis method.

Testing the dialysis tubing involved measuring the darkness of the dialysate (ultraviolet-visible absorption) or measuring how much free copper(II) the dialysate could bind compared with known dilutions of the full-strength solution. All work was done in 0.1 M potassium nitrate at room temperature (20-25°C). If these tests of dialysis tubing had been satisfactory, the next step would have been to prepare external solutions with varying mole ratios of metal ion to fulvic acid (probably with the fulvic acid concentration constant and the metal-ion concentration varying) and dialyzing against an inner solution containing 0.1 M  $\text{KNO}_3$ . The total metal ion concentration in the inner solution, once it reaches equilibrium, should equal the unbound (free) metal-ion concentration in the external solution. Atomic absorption spectrophotometry would be suitable for analyzing the metal-ion concentration of the inner solution. Anodic stripping voltammetry would also be appropriate if no organic matter passed through the tubing or if the organic matter were destroyed before analysis (Gardiner and Stiff, 1975).

## Ultrafiltration

Ultrafiltration/atomic absorption spectrophotometry provided a means for analysis of cadmium(II)-SFA binding. The apparatus was an Amicon Corporation (Lexington, Massachusetts) model 52 stirred cell fitted with Amicon UM05 membranes that have a nominal molecular weight cutoff of 500 daltons. Unpurified nitrogen gas at a pressure of 40 to 45 pounds per square inch ( $3 \times 10^5$  Pa) pressurized the ultrafiltration cell for all runs. Early experiments with this system involved

(1) measuring metal-ion losses to the ultrafiltration cell parts.

(2) measuring the amount of ligand passing through the membrane.

(3) developing a procedure for cleaning the ultrafiltration cell between samples.

Metal-ion losses. I determined the fraction of total  $\text{Cd}^{2+}$  that appeared in the filtrate for solutions containing 0.0, 0.1, 0.5, 1.5, and 5.0 parts per million (ppm)  $\text{Cd}^{2+}$ , prepared in 0.1 M  $\text{KNO}_3$ . This experiment was similar to one that examined losses of 1.3 and 13 ppm copper(II) solutions in this cell.

Ligand passage. I wished to see how much of the fulvic acid binding power passed through the membrane. The nine solutions tested were  $3 \times 10^{-5}$  M,  $15 \times 10^{-5}$  M and  $78 \times 10^{-5}$  M SFA, each at pH 4, 5, and 6. All the samples had 0.1 M  $\text{KNO}_3$ . Because there was no metal ion in these samples,

there was need only for a 15-minute KOH wash and a 15-minute  $\text{KNO}_3$  wash between samples. I pipeted 3-4 mL of solution out of the ultrafiltration cell before filtration; this sample served as the full-strength reference. I discarded the first 2 mL of filtrate and collected the next 3 mL for analysis. An Orion copper(II) ion-selective electrode (model 94-29) measured the decrease in free  $\text{Cu}^{2+}$  concentration in a 0.65 ppm solution upon addition of 1 mL of filtrate or 1 ml of a reference solution.

The UM05 membrane, unlike the other Amicon membranes, has a net negative charge (Amicon, 1977), which could affect its separation ability. To check the membrane, I prepared  $5 \times 10^{-4}$  and  $5 \times 10^{-5}$  M solutions of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) in 0.1 M  $\text{KNO}_3$ , and then filtered two replicates at three pH values (2.0, 4.5, and 10). At pH 2.0, 60% of the phosphate is  $\text{H}_3\text{PO}_4$  and 40% is  $\text{H}_2\text{PO}_4^-$ . At pH 4.5, there is nearly 100%  $\text{H}_2\text{PO}_4^-$ , and at pH 10, nearly all the phosphate is in the  $\text{HPO}_4^{2-}$  form. Forming the phosphorus molybdate complex (Brown et al., 1970) allowed analysis of phosphate concentrations in the filtrate at 882 nm with a Beckman DU spectrophotometer. Unfiltered reference samples pipeted from the ultrafiltration cell before filtration served as references for each filtered sample.

Cleaning the cell. For a titration to be successful, the ultrafiltration cell must be cleared of ligand and metal-ion residues before a new sample is added to the cell. I tested various washing sequences: pushing  $10^{-3}$  M  $\text{HNO}_3$ ,

$10^{-3}$  M KOH, ethanol, and  $\text{KNO}_3$  through the cell in several combinations and orders. Generally, the total cleaning time was one hour. After a washing/drying sequence, I placed 30 mL of 1.3 ppm  $\text{Cu}^{2+}$  in the ultrafiltration cell and collected six 1-1.5 mL aliquots to be analyzed by means of the Orion  $\text{Cu}^{2+}$  ion-selective electrode. I compared the concentration of  $\text{Cu}^{2+}$  in each aliquot with the concentration in an unfiltered sample.

Ultrafiltration complexation study. I collected two series of complexation data for  $\text{Cd}^{2+}$  and SFA at pH 6.0. The samples, prepared on two different days, have an SFA concentration of approximately  $1.5 \times 10^{-4}$  M at the start of filtration, and the concentration of  $\text{Cd}^{2+}$  is varied to achieve  $C_{\text{SFA}}/C_{\text{Cd}} = 50, 30, 20, 15, 12, 10, 8, 6, 4, 2$ , and 30 for the first series and  $C_{\text{SFA}}/C_{\text{Cd}} = 50, 40, 30, 20, 17, 14, 11, 8, 5$ , and 3 for the second series. All solutions were 50 mL and prepared in 0.1 M  $\text{KNO}_3$ . To allow metal ions to saturate sites in the cell system, I discarded the first 6 mL of filtrate and collected the next 5 mL for analysis by atomic absorption spectrophotometry. Scatchard (1949) developed a method for calculating the stability constants for a complex and the number of binding sites on the ligand. I used this method to produce a Scatchard-type plot for this ultrafiltration study. I later stopped using the Scatchard method of stability constant calculation, and calculated the stability constant for a 1:1 complex.

### Ion-selective electrode studies

There are two major ways to do metal ion/fulvic acid titrations with a metal-ion-selective electrode. The first is to prepare a metal-ion solution and titrate it with a fulvic acid solution (FA-into- $M^{2+}$  titration). The second method is to prepare a fulvic acid solution and titrate it with a metal-ion solution ( $M^{2+}$ -into-FA titration). Bresnahan et al. (1978) performed FA-into- $Cu^{2+}$  titrations; the procedures in that paper served as a pattern for the early  $Cd^{2+}$ -fulvic acid work. The later  $Cd^{2+}$ -fulvic acid titrations and most of the useful results arose from  $M^{2+}$ -into-FA titrations. Common to both types of titrations are the materials and apparatus.

Materials. A description of the soil-derived and water-derived fulvic acids appears in Chapter 1; there are further details in Weber and Wilson (1975). The source of  $Cd^{2+}$  ion was Fisher SO-C-118 1000 ppm certified atomic absorption standard, diluted as necessary in 0.1 M  $KNO_3$ . The  $Cu^{2+}$  ion source, used in some experiments to compare the response of  $Cd^{2+}$  and  $Cu^{2+}$  separately in the presence of fulvic acid, came from Orion 0.1000  $\pm$  0.0005 M copper(II) nitrate, diluted as necessary in electrolyte. The electrolyte was Mallinckrodt purified granular potassium nitrate, prepared in doubly deionized water. The electrolyte concentration in nearly all experiments was 0.1 M; it was 0.01 M for the few others.

The acid solutions used for pH adjustment were prepared

from dilutions of Baker reagent grade (15.9 M) nitric acid. The base solutions were prepared from Baker reagent grade 45% KOH solution. Acid and base solutions having concentrations of 0.01 M or less were prepared in 0.1 M  $\text{KNO}_3$ , rather than in water. All water used for the experiments is doubly deionized.

Apparatus. An Orion solid-state ion-selective electrode model 94-48 measured free  $\text{Cd}^{2+}$  concentration. The corresponding electrode sensitive to  $\text{Cu}^{2+}$  is Orion model 94-29. The reference portion of a Corning model 476050 combination electrode was the reference for the ion-selective electrode; a second Corning combination electrode monitored pH. Two Orion model 701A pH/mv meters recorded simultaneously the concentrations of free metal ion and hydrogen ion.

The titration vessel was a Princeton Applied Research (Princeton, New Jersey) model 9301 water-jacketed cell. A P. M. Tamson model T9 circulating water bath maintained the temperature at  $25 \pm 0.2^\circ\text{C}$ , and a magnetic stir bar and stirrer maintained solution homogeneity. All small solution volumes were delivered through Gilson variable pipets and Eppendorf fixed-volume micropipets.

Various FORTRAN programs running on the University of New Hampshire DECsystem 1090 computer helped calculate and tabulate results for the titrations using raw millivolt and concentration data as input. The output from some of these programs went to the Calcomp 936 plotter to be graphed.

Depending on the range of free  $\text{Cd}^{2+}$  concentration to be measured, I fitted either two straight lines or a polynomial to the calibration curve. Such a polynomial was needed because the electrode response flattened below about  $10^{-6}$  M free metal ion. The polynomial has the form

$$\log[M^{2+}] = \sum_{i=0}^n a_i (\text{mv})^i$$

where  $[M^{2+}]$  is the free metal ion concentration, mv is the millivolt reading,  $a_i$  are the polynomial regression coefficients and n is the order of the polynomial.

FA-into- $\text{Cd}^{2+}$  titrations. The concentrations of  $\text{Cd}^{2+}$  and fulvic acid for these experiments appear in Table 3. The procedures include placing 25.0 mL of 0.1 M  $\text{KNO}_3$  into the titration cell and adding several aliquots of 10 and 100 ppm  $\text{Cd}^{2+}$  solutions to form a calibration curve. The final  $\text{Cd}^{2+}$  concentration depended on the pH of the experiment--a higher  $\text{Cd}^{2+}$  concentration at higher pH and a lower  $\text{Cd}^{2+}$  concentration at lower pH. During preparation of this calibration, KOH additions maintained the pH at the desired value. After addition of the final aliquot of  $\text{Cd}^{2+}$ , I began titrating with a concentrated fulvic acid solution (typically 35 mg of FA in 10 mL of 0.1 M  $\text{KNO}_3$  and KOH added to raise the pH) without changing the solution in the cell. Again, additions of KOH maintained the pH at the value of the experiment. I varied the size of FA aliquots to cause the reading from the ion-selective electrode to change by



TABLE 3  
Conditions for fulvic acid-into-Cd<sup>2+</sup> titrations

Date	pH	FA titrant		Initial Cd <sup>2+</sup>	Replicates
		(M x 10 <sup>3</sup> )		in cell (M x 10 <sup>5</sup> )	
8/15/77	7.0	5.45	SFA	3.5	2
8/18/77	7.0	5.05	SFA	3.6	2
8/20/77	6.0	5.47	SFA	1.97	2
8/21/77	8.0	4.89	SFA	3.55	2
8/22/77	5.0	5.44	SFA	1.62, 1.30	2
8/24/77	5.0	5.44	SFA	1.27	2
8/25/77	6.0	5.20	SFA	1.91	2
8/26/77	6.0	5.27	WFA	1.91, 1.60	2
8/27/77	7.0	5.24	SFA	3.5	2
8/31/77	8.0	5.04	SFA	4.9	2
9/01/77	8.0	5.91	WFA	4.9	2
10/21/77	6.0	5.48	SFA	1.9	2
10/22/77	6.0	5.40	WFA	1.6	2
10/23/77	8.5	5.37	SFA	5.6, 5.5, 5.3	3
10/24/77	8.5	5.50	WFA	5.2, 5.0	2
3/29/78	6.0	5.33	SFA	2.6	2

approximately 2 mv with each addition.

Cd<sup>2+</sup>-into-FA titrations. Experimental conditions appear in Table 4. On most days, I performed two titrations and three calibrations in the following order: calibration 1, titration 1, calibration 2, titration 2, and calibration 3. I frequently used both the calibration before and after a titration to calculate the results for that titration. After initial calibration, the first titration began with 25 mL of FA solution in the cell. The Cd<sup>2+</sup> concentration of the titrant varied depending on the pH of the experiment and the concentration of the FA, but was as high as possible to cause only a small change in the overall solution volume. Again, a typical millivolt change per aliquot was 2 mv.

Initially, the data were calculated by the Scatchard method. Difficulties with this method led to the search for a simpler model, such as that of 1:1 complexation.

TABLE 4  
Conditions for Cd<sup>2+</sup>-into-fulvic acid titrations

Date	pH	Cd <sup>2+</sup> titrant (M x 10 <sup>3</sup> )	Initial FA		Replicates
			in cell (M x 10 <sup>4</sup> )		
1/05/78	6.0	6.58	5.82	SFA	1
1/08/78	5.0	3.44	5.15	SFA	1
3/26/78	6.0	2.67	5.60	SFA	2
4/06/78	7.0	2.67	5.90	SFA	2
4/09/78	5.0	1.67	5.43	SFA	2
4/11/78	6.0	1.99	5.94	WFA	2
4/19/78	6.0	0.209	0.607	SFA	1
4/21/78	6.0	0.108	0.302	SFA	1
4/21/78	6.0	0.395	1.33	SFA	1
4/26/78	6.0	1.07	2.35	SFA	1
4/26/78	6.0	1.68	4.05	SFA	1
5/05/78	8.0	4.15	5.36	SFA	2
5/07/78	4.0	1.67	5.40	SFA	2
5/09/78	7.0	2.69	5.70	WFA	2
5/11/78	6.0	1.53	5.54	WFA	2
5/23/78 <sup>a</sup>	6.0	0.268	0.59	SFA	2
5/25/78 <sup>a</sup>	6.0	3.29	5.71	SFA	2
6/06/78	8.0	3.30	5.76	WFA	2
6/09/78	4.0	1.03	5.46	WFA	2

Table 4, continued

8/14/78	5.0	0.122	0.327	WFA	1
8/14/78	5.0	0.245	0.65	WFA	1
8/15/78	5.0	0.473	1.02	WFA	1
8/15/78	5.0	1.34	3.05	WFA	1
8/16/78	7.0	0.215	0.279	WFA	1
8/16/78	7.0	0.357	0.559	WFA	1
8/18/78	7.0	0.756	1.06	WFA	1
8/18/78	7.0	2.14	3.18	WFA	1
8/29/78	5.0	0.303	0.585	SFA	1
8/29/78	5.0	1.05	2.26	SFA	1
4/27/79	5.0	0.722	0.60	SFA	1
		7.22			
4/27/79	5.0	0.722	6.5	SFA	1
		7.22			
8/04/79	4.5	0.844	2.0	WFA	1
		8.44			

<sup>a</sup> Electrolyte was 0.01 M  $\text{KNO}_3$ ; for all other titrations, the electrolyte was 0.1 M  $\text{KNO}_3$ .

## RESULTS AND DISCUSSION

### Ultrafiltration

Metal-ion losses. Losses of  $\text{Cu}^{2+}$  in the ultrafiltration cell were greater than were the losses of  $\text{Cd}^{2+}$ . The filtrate concentration rose to 96% of the original 13 ppm  $\text{Cu}^{2+}$  and to 91% of the original 1.3 ppm solution after 4 mL of filtrate had passed. A check of the solution above the membrane, however, revealed that the unfiltered solution had also lost copper ion, and to the same extent as the filtrate. Subsequent tests showed that, of all the parts of the cell, the base and the top were best at adsorbing  $\text{Cu}^{2+}$ . They are made of glass-filled nylon. Losses of metal ion from  $\text{Cd}^{2+}$  solutions ranging from 0.1 ppm to 5 ppm were less than 3%, which is substantially better than the results for  $\text{Cu}^{2+}$ .

Ligand passage. The filtered fulvic acid solutions had typically 5-10% of the ability to complex  $\text{Cu}^{2+}$  that an unfiltered fulvic acid solution had. There appeared to be no particular trend based on concentration of fulvic acid or on pH.

The permeability of the UM05 membrane to phosphate changes dramatically with pH. The average percentages of phosphate allowed through the membrane for the  $5 \times 10^{-4}$  M solution were 84% at pH 2.0, 70% at pH 4.5, and 14% at pH 10. For the  $5 \times 10^{-5}$  M solution, the values were 93% at pH 2.0, 48% at pH 4.5, and 3% at pH 10. The phosphate ion is

small enough to pass easily through the membrane. In contrast, the charge on the ion appears to have a major effect. These results agree with tests of the amino acid permeability of the UM05 membrane at pH 5 and 10 (Amicon, 1977).

The phosphate tests have important implications for complexation studies between fulvic acid and metal ions if ultrafiltration with the UM05 membrane is used. It appears that the net charge on either the complexed or uncomplexed fulvic acid will alter the ability of that entity to pass through the membrane. Whereas uncomplexed fulvic acid may have several negative charges, complexed fulvic acid may have fewer negative charges and be freer to pass through the membrane. A change in pH will also change the average charge on the fulvic acid molecules and might make results of ultrafiltration studies done at different pH values hard to compare. Furthermore, it is possible that the conformation and hence the size and filterability of our fulvic acids change with pH (Lingvist, 1974; Gjessing, 1971; Ghassemi and Christman, 1968).

Cleaning the cell. The cell-washing sequence of acid / base / electrolyte (20 minutes each for a total wash time of one hour) appeared to work slightly better at reducing  $\text{Cu}^{2+}$  losses than any other sequence. This may occur because any residual fulvic acid will pass most easily through the membrane and out of the cell when it is least anionic--at low pH. An ethanol solution did not clean the cell any

better than the acid and base. Therefore, I used the acid / base / electrolyte washing sequence for all work.

Ultrafiltration complexation study. Figure 1 shows the results of the ultrafiltration study for  $\text{Cd}^{2+}$ /SFA at pH 6.0. Also included in the figure are the results of ion-selective electrode titrations at pH 5.0 and 6.0 for  $\text{Cd}^{2+}$ /SFA. The SFA concentrations are  $2 \times 10^{-4}$  M for the ultrafiltration experiment,  $2.2 \times 10^{-4}$  M for the pH 6.0 and  $5 \times 10^{-4}$  M for the pH 5.0 ion-selective electrode titrations. The y-axis for the figure is the logarithm of K, where K is the 1:1 conditional stability constant, which equals

$$(C_{\text{Cd}} - [\text{Cd}^{2+}]) / ([\text{Cd}^{2+}] (C_{\text{SFA}} - (C_{\text{Cd}} - [\text{Cd}^{2+}])))$$

where  $C_{\text{Cd}}$  is the total (analytical) concentration of  $\text{Cd}^{2+}$ ,  $[\text{Cd}^{2+}]$  is the free (hydrated)  $\text{Cd}^{2+}$  concentration, and  $C_{\text{SFA}}$  is the total (analytical) concentration of soil-derived fulvic acid. The x-axis is the mole ratio of bound  $\text{Cd}^{2+}$  to FA ( $[\text{Cd-SFA}] / C_{\text{SFA}}$ ).

The first observation to make is that the ultrafiltration data are much more scattered than are the ion-selective electrode data. This is not surprising because the ultrafiltration samples are prepared separately. Some variability may arise in the handling of these separate samples. In contrast, each ion-selective electrode titration involves only one fulvic acid sample. Also, the ultrafiltration cell and especially the membrane may not be in the same condition for each sample. The scatter in the

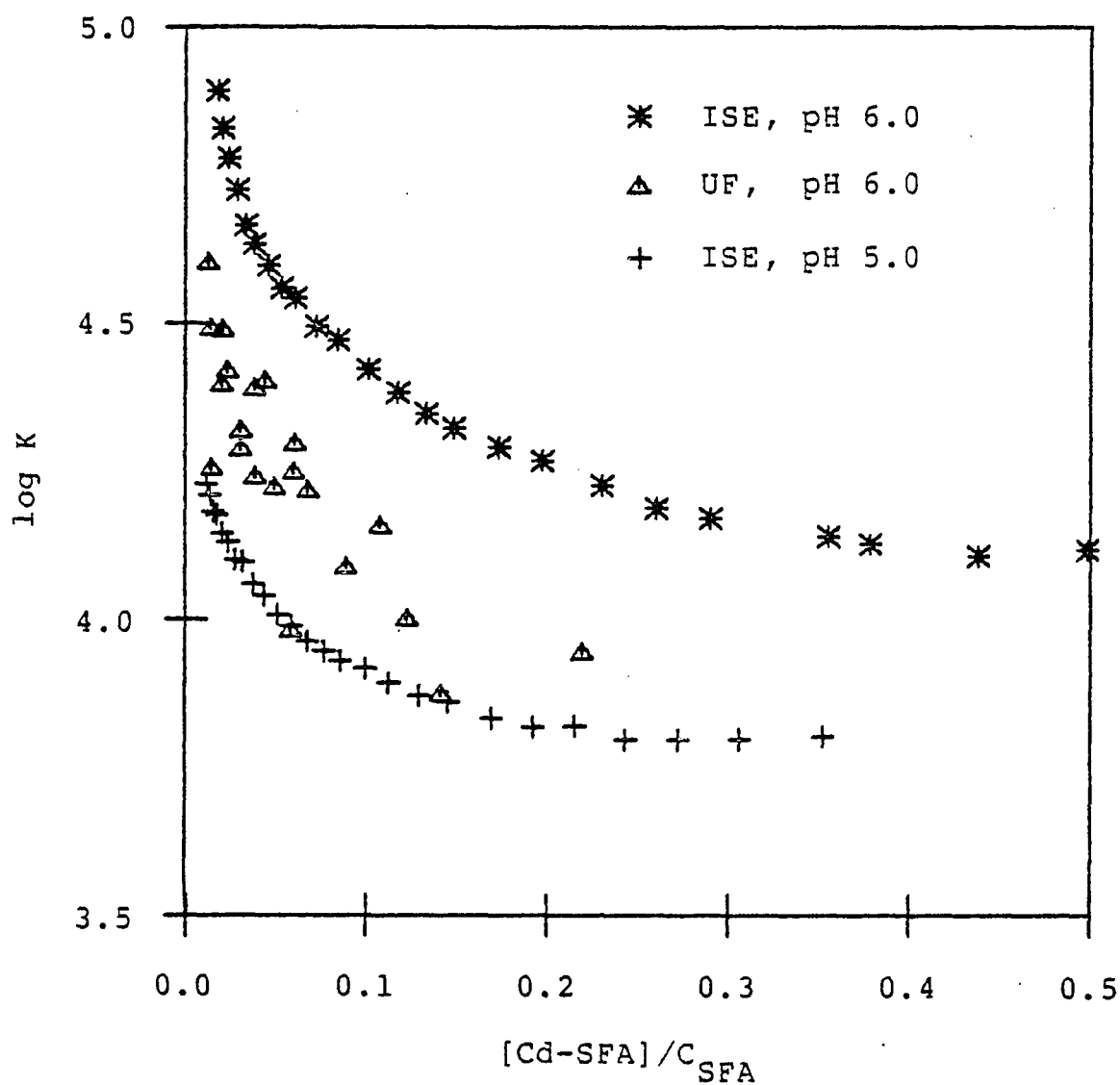


Figure 1. Comparison of titrations of  $Cd^{2+}$  and soil-derived fulvic acid (SFA) as performed by ion-selective electrode potentiometry (ISE) and by ultrafiltration/atomic absorption spectrophotometry (UF).  $K$  is the conditional stability constant. Ionic strength ( $I$ ) = 0.1 M and temperature ( $T$ ) = 25°C.



data might have been reduced by inserting a new membrane for each data point (thereby simplifying the cleaning procedure), but the membranes are expensive and possibly variable, so this was not possible.

The ultrafiltration data does not go to high values of  $[Cd-SFA]/C_{SFA}$  ( $= \bar{v}$ ). The values for  $\log K$  must be compared at approximately equal values of  $\bar{v}$  because the conditional stability constant is a function of the extent to which the fulvic acid is loaded with metal ion. For  $\bar{v} = 0.2$ ,  $\log K = 3.9$  for the ultrafiltration experiment at pH 6.0;  $\log K = 4.3$  for the pH 6.0 ion-selective electrode titration and  $\log K = 3.8$  for the pH 5.0 ion-selective electrode experiment.

Considering that the ultrafiltration/atomic absorption spectrophotometry and the ion-selective electrode experiments are so different, the results are reasonably in agreement. As noted above, the UM05 membrane lets through about 5-10% of the chelation power of fulvic acid. Any  $Cd^{2+}$  bound to this fraction of fulvic acid will show up as free metal when analyzed by atomic absorption spectrophotometry. The effect is an apparently diminished concentration of bound metal ion and, hence, a lower conditional stability constant than would arise if no bound metal appeared in the filtrate.

In the final analysis, the ultrafiltration method for determination of stability constants is time-consuming and subject to larger errors and data scatter than the ion-selective electrode experiment. It is reassuring to

note, however, that the results from these two types of experiments are comparable.

### Ion-selective electrode studies

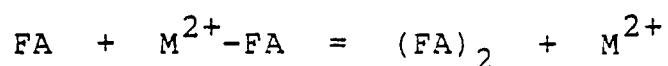
All early titrations were of the FA-into- $\text{Cd}^{2+}$  type, the experimental procedure used earlier in this group for  $\text{Cu}^{2+}$ /fulvic acid studies (Bresnahan et al., 1978). All data were initially calculated and plotted by the Scatchard method (Scatchard, 1949). The equation used to generate such a plot is based on the Adair equation (Van Holde, 1971), which, when simplified, becomes

$$\bar{v}/[\text{M}^{2+}] = -\bar{v}K + nK$$

where  $\bar{v} = [\text{bound } \text{M}^{2+}]/C_{\text{FA}}$ ,  $K$  is the stability constant for the class of sites and  $n$  is the number of sites in the class. Graphing  $\bar{v}/[\text{M}^{2+}]$  vs.  $\bar{v}$  gives a Scatchard plot, whose slope is  $-K$  and x-intercept is  $n$ . A system with  $i$  classes of sites may have a more complicated curve. If the system is simple enough, it is possible, mathematically, to dissect the curve into  $i$  lines, having slopes  $-K_i$  and x-intercepts  $n_i$  which are the constants for each of the  $i$  classes. However, all parts of such a curve should have a negative slope, corresponding to positive values of  $K_i$ . A positive slope on any part of a Scatchard plot indicates a negative value for one or more of the  $K_i$ , a situation that is difficult to interpret. Two interpretations are consistent with a positive slope on a plot:

(1) Addition of metal ion to a solution containing ligand causes dissociation of some complexes already formed, resulting in a greater increase in the number of free metal ions than were added by the titrant aliquot.

(2) Addition of ligand to a solution containing metal ion causes some previously bound metal ion to become free metal ion, with the net result that there are more free metal ions after addition of an aliquot of ligand titrant than before the addition. For such an equilibrium to arise, a reaction such as



would have to occur.

Anomalous Scatchard plots arose from several of the FA-into- $\text{Cd}^{2+}$  titrations. For titrations at pH 5.0 and 6.0, a substantial portion of the plots had a positive slope. Figure 2 contains an example; it is a titration at pH 5.0 with SFA titrant and  $\text{Cd}^{2+}$  in the titration cell. Even the Scatchard data for some higher pH experiments show a small amount of upward curvature at high values of  $\bar{v}$ .

The reason for the unusual Scatchard plots became clear after I performed many  $\text{Cd}^{2+}$ -into-FA titrations. Scatchard plots for all these titrations were of the more usual form: values for  $\bar{v}/[\text{Cd}^{2+}]$  always decreased as  $\bar{v}$  increased. Furthermore,  $\text{Cd}^{2+}$ -into-FA titrations with a very low concentration of fulvic acid gave different results from comparable titrations with higher fulvic acid concentration.

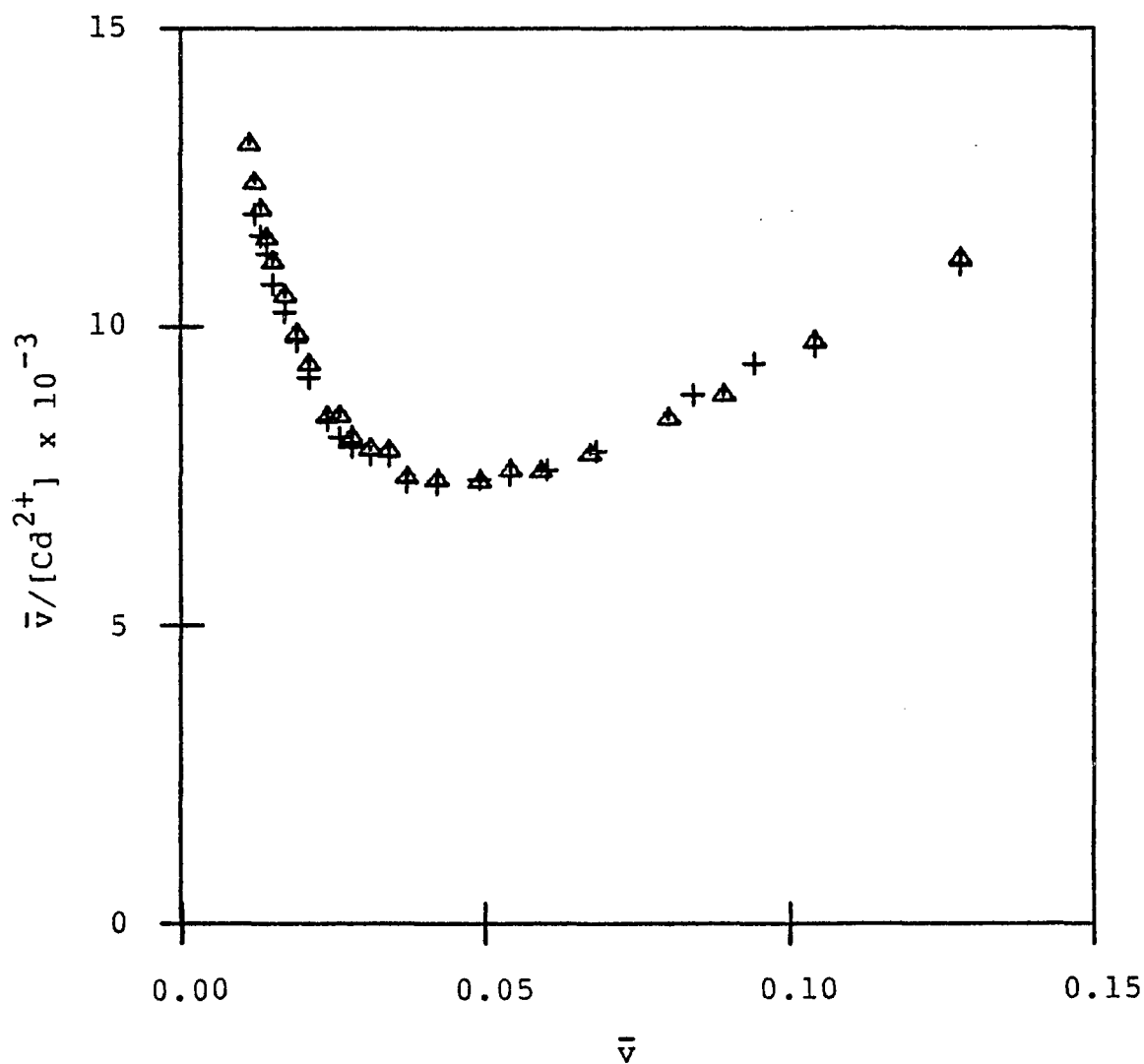


Figure 2. Scatchard plot of an SFA-into- $\text{Cd}^{2+}$  ion-selective electrode titration at pH 5.0.  $\bar{v} = (C_{\text{Cd}} - [\text{Cd}^{2+}]) / C_{\text{SFA}}$ . The two symbol types represent replicate titrations.  $I = 0.1 \text{ M}$  and  $T = 25^\circ\text{C}$ .

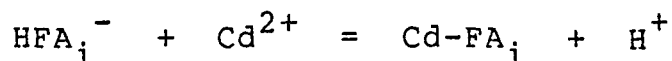
The key difference between FA-into- $\text{Cd}^{2+}$  and  $\text{Cd}^{2+}$ -into-FA titrations is the different range of FA concentrations. During an FA-into- $\text{Cd}^{2+}$  titration, the FA concentration increased 100-fold or more. For  $\text{Cd}^{2+}$ -into-FA titrations, the fulvic acid concentration was nearly constant, changing by approximately 20% because added titrant aliquots and pH-adjusting solutions diluted the fulvic acid. The conclusion was that fulvic acid concentration influenced the conditional stability constant of  $\text{Cd}^{2+}$ -fulvate complexes. Further discussion of this effect of concentration appears after the following section on calculation methods.

Calculations. I first tried using the Scatchard method of calculation to divide complexation sites on fulvic acid into classes of sites. There were typically  $1.0 \pm 0.3$  sites per average fulvic molecule, but the sites did not fall into well-defined classes with an equilibrium constant for each. A value for a stability constant comes from the slope of a line drawn through sets of points on the Scatchard plot. However, the points on the plots were not colinear; the slope of the line, and hence, the values of  $K$  and  $n$ , depended on which points the line was drawn through. It is very difficult to establish any objective criteria for selecting which points are to be included in the least squares calculation. Indeed, the ever-present curvature in the Scatchard plots indicates a continuous variation in site strength with respect to  $\text{Cd}^{2+}$ . The Scatchard method,

therefore, appeared poorly suited for presenting the data and calculating the stability constants. I then turned to a simpler model, based on formation of 1:1  $\text{Cd}^{2+}$ -fulvate complexes.

Gamble and Schnitzer (1973) reported that fulvic acid contains acid functional groups with similar but not identical  $\text{pK}_a$  values. The acid strength of these groups decreases as the degree of ionization increases. Work by several groups has shown that salicylic-acid-type structures are the sites of  $\text{Cu}^{2+}$  chelation (Beckwith, 1959; Khanna and Stevenson, 1962; Schnitzer and Skinner, 1963). I assume that such sites are available for  $\text{Cd}^{2+}$  complexation as well.

The extent of complexation and hence the stability constant for the  $\text{Cd}^{2+}$ -fulvate system will depend on pH and the degree of  $\text{Cd}^{2+}$ -fulvate association. The salicylic-type sites are similar but not identical, so the overall stability constant is a weighted average of the stability constants for each increment of chelating sites. The reaction at the  $i$ th type of chelating site may be represented as



where  $\text{HFA}_i^-$  is the  $i$ th type of fulvic acid binding site without metal ion,  $\text{Cd}^{2+}$  is the free cadmium(II) ion, and  $\text{Cd-FA}_i$  is the  $i$ th type of cadmium-fulvate 1:1 complex. Subsequent calculation, outlined below, showed that much less than one hydrogen ion was released for each  $\text{Cd}^{2+}$  that

became bound. For the simple case, where one hydrogen ion is released, the incremental conditional stability constant  $K_i$  is

$$K_i = [\text{Cd-FA}_i] / [\text{HFA}_i^-] [\text{Cd}^{2+}]$$

The overall conditional stability constant  $K$  is

$$K = \frac{\sum_{i=1}^k [\text{HFA}_i^-] K_i}{\sum_{i=1}^k [\text{HFA}_i^-]}$$

The weighting factors  $[\text{HFA}_i^-]$  will change with pH and possibly with the concentration of fulvic acid. Furthermore, the overall conditional stability constant  $K$  will vary depending on the proportion of sites occupied by metal ion. With  $\text{Cd}^{2+}$  occupying the strongest sites at low mole ratios of  $\text{Cd}^{2+}$  to FA, each added increment of  $\text{Cd}^{2+}$  will go to successively weaker chelation sites and  $K$  will drop. I calculated the overall stability constant  $K$  for various levels of  $\text{Cd}^{2+}$  loading, that is, for various values of  $[\text{Cd-FA}] / C_{\text{FA}}$ , where  $C_{\text{FA}}$ , the total fulvic acid concentration, equals  $[\text{HFA}^-] + [\text{Cd-FA}]$ . The Scatchard analysis showed approximately one site per fulvic acid molecule, so I assumed that 1:1 complexes predominate. The expression

$$K = [\text{Cd-FA}] / [\text{HFA}^-] [\text{Cd}^{2+}]$$

represents the conditional stability constant. Since total  $\text{Cd}^{2+}$  concentration,  $C_{\text{Cd}}$ , is

$$C_{\text{Cd}} = [\text{Cd-FA}] + [\text{Cd}^{2+}],$$

$$K = [\text{Cd-FA}] / (C_{\text{FA}} - [\text{Cd-FA}]) [\text{Cd}^{2+}]$$

Effect of pH. Figure 3 shows the results of calculations for  $\text{Cd}^{2+}$ -SFA complexes; Figure 4 shows the corresponding results for  $\text{Cd}^{2+}$ -WFA. Several important aspects of  $\text{Cd}^{2+}$ -fulvate chemistry show up in these figures. The first is that the conditional stability constant increases with pH. This finding agrees with the general pattern for  $\text{M}^{2+}$ -fulvate complexation as observed in this laboratory (Bresnahan et al., 1978) and elsewhere (for example, Takamatsu and Yoshida, 1978). Second, the log K values for SFA are somewhat larger than they are for WFA. This may be explained by the difference in total acidity of the two fulvic acids: 13.4 meq/g for SFA and 10.6 meq/g for WFA (Weber and Wilson, 1975). The acidity comes from carboxylic acid and phenol groups, which are the likely sites of  $\text{Cd}^{2+}$  complexation.

A third feature that shows well in these figures is the smooth change in log K (for any of the pH values) as the value of  $[\text{Cd-FA}] / C_{\text{FA}}$  increases. This indicates a continuum of site strengths with respect to  $\text{Cd}^{2+}$ . The Scatchard method for data treatment is most applicable when there are relatively few and largely discrete classes of sites. Such is clearly not the case here.

A fourth feature that shows up in the graphs is the leveling off of K values as  $[\text{Cd-FA}] / C_{\text{FA}}$  increases and the fact that this leveling off occurs at lower  $[\text{Cd-FA}] / C_{\text{FA}}$  for the lower pH titrations.



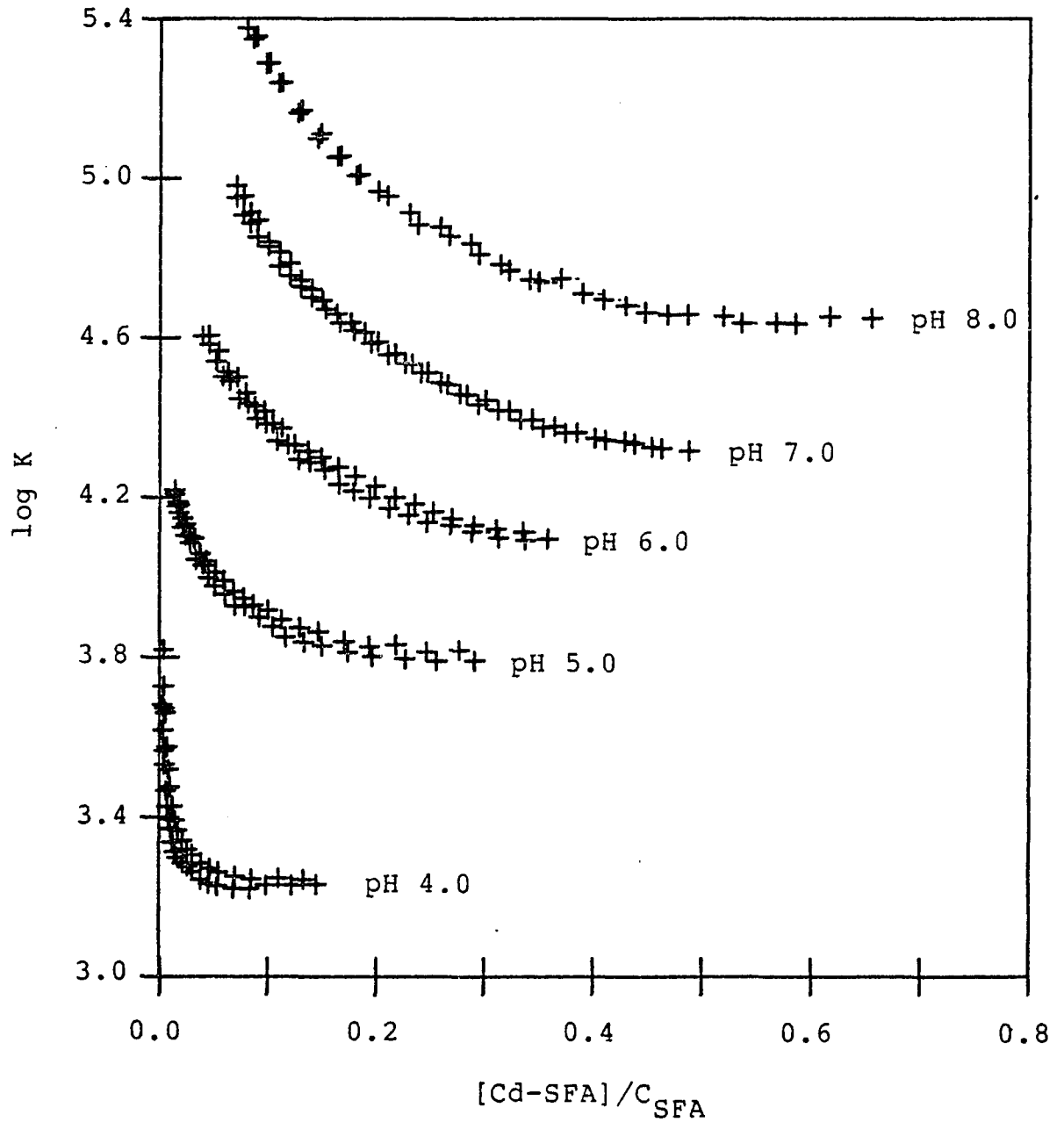


Figure 3. Conditional stability constants (K) for replicate  $Cd^{2+}$ -into-SFA titrations at each of five pH values. The soil-derived fulvic acid (SFA) concentration was  $5 \times 10^{-4}$  M.  $I = 0.1$  M and  $T = 25^{\circ}C$ .

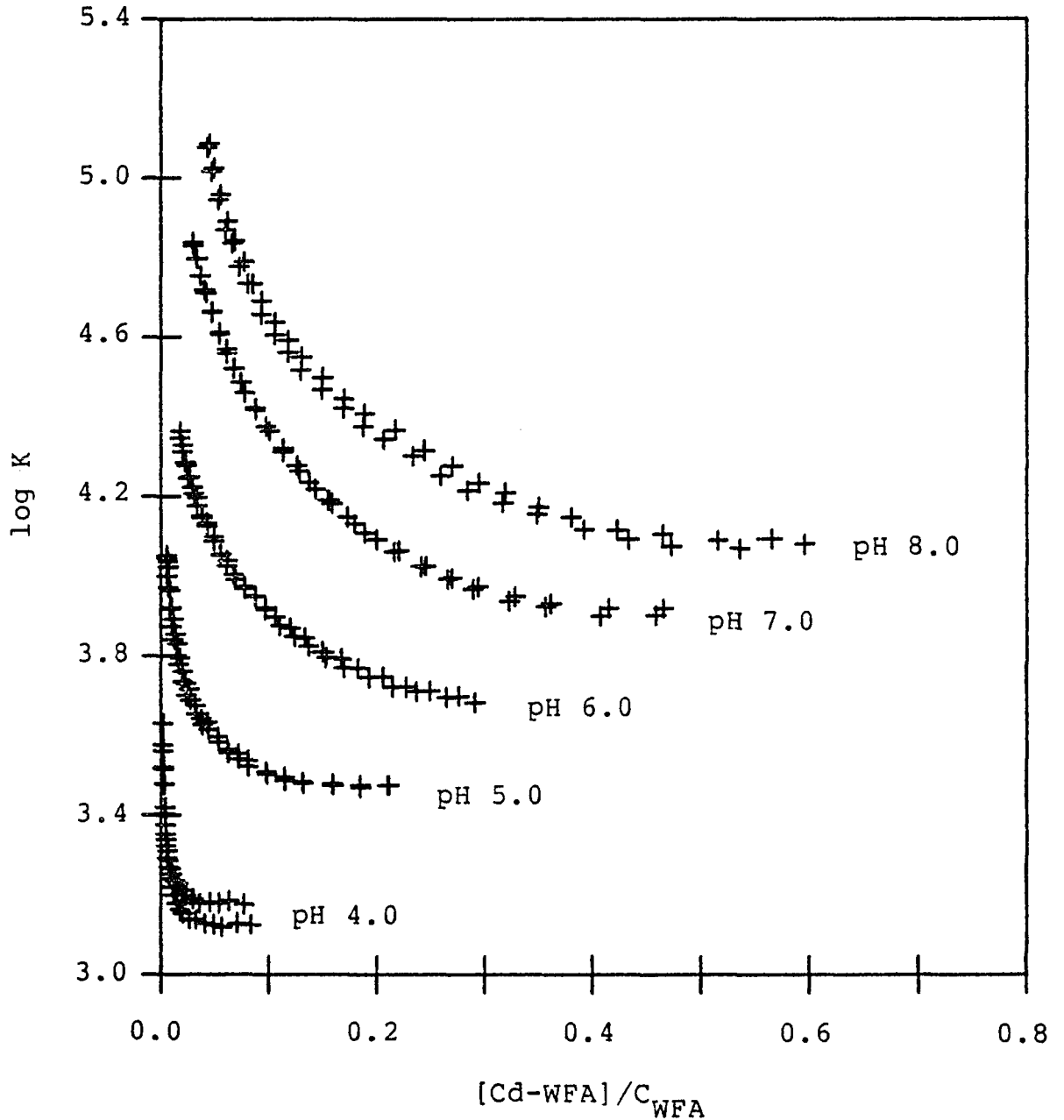
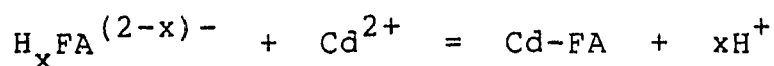


Figure 4. Conditional stability constants ( $K$ ) for replicate  $\text{Cd}^{2+}$ -into-WFA titrations at each of five pH values. The water-derived fulvic acid (WFA) concentration was  $5 \times 10^{-4}$  M.  $I = 0.1$  M and  $T = 25^{\circ}\text{C}$ .

As indicated above, the conditional stability constants arise from a 1:1 complexation model. The high  $[Cd-FA]/C_{FA}$  values represent an average of complexation for all sites involved during the titrations, and these values for the conditional stability constant  $K$  appear in Table 5.

These are conditional stability constants because the concentration of total ligand rather than the concentration of deprotonated ligand is used in the calculation. The values are useful for environmental planning, but they hide the details of the  $Cd^{2+}$ -FA complexation reaction. Potentiometric titrations by Stevenson (1976, 1977) show that protons are released as various metal ions, including  $Cd^{2+}$ , bind to soil-derived fulvic acid. The number of protons coming off as each  $Cd^{2+}$  ion is complexed may be calculated. The initial assumption needed is that the thermodynamic (intrinsic) stability constant,  $K'$ , for  $Cd^{2+}$ -FA complexation does not change over several pH units. The explicit equilibrium to be checked is



The equilibrium expression for this is

$$K = \frac{[Cd-FA][H^+]^x}{[H_x FA^{(2-x)-}][Cd^{2+}]}$$

The only difference between  $K'$  and the previously defined conditional stability constant  $K$  is a factor of  $[H^+]^x$ , that is

TABLE 5  
Effect of pH on cadmium(II)-fulvic acid  
conditional stability constants<sup>a</sup>

pH	<u>log K</u>	
	WFA	SFA
4.0	3.15	3.23
5.0	3.48	3.80
6.0	3.68	4.08
7.0	3.91	4.32
8.0	4.08	4.63

<sup>a</sup> All titrations have fulvic acid concentrations of 5 to 6 x 10<sup>-4</sup> M and 0.1 M KNO<sub>3</sub> as supporting electrolyte. The temperature for all titrations was 25°C.

$$K' = K[H^+]^x.$$

If  $K'$  is constant as it is for simple ligands, it should be possible to select a value for  $x$  such that  $K[H^+]^x$  from pH 4 to 8 will equal a constant ( $K'$ ). Table 6 shows that for pH 5.0 to 8.0,  $x = 0.28$  for SFA gives constant values for  $K'$ ;  $x = 0.20$  for WFA also gives constant values for  $K'$ . These values mean that, on average, 0.28 protons are released as each  $Cd^{2+}$  becomes bound to SFA and 0.20 protons are released as each  $Cd^{2+}$  becomes bound to WFA. Buffle et al. (1977) calculated  $x$  values for  $Cu^{2+}$  and  $Pb^{2+}$  complexes of humic substances. Their values range from 0.6 to 0.8. Schnitzer and Skinner (1963) also report  $x$  values for SFA in experiments ranging from pH 3 to 10 for  $Fe^{3+}$ ,  $Al^{3+}$ ,  $Ni^{2+}$ , and  $Cu^{2+}$ . For the pH range of the work described in this dissertation (pH 4.0 to 8.0), their  $x$  values vary from 1.6 to 2.5 for  $Fe^{3+}$ , 1.3 to 2.9 for  $Al^{3+}$ , 0.4 for  $Ni^{2+}$ , and 0.8 to 1.1 for  $Cu^{2+}$ . All these values taken together indicate that the greater the strength of a complex between a metal ion and fulvic acid, the greater the number of protons released upon complexation. This work lends support to the notion that carboxyl and phenol groups are the sites of complexation, with high inner-sphere character (close association of metal ion and ligand causing much proton displacement) for strongly bound metal ions, and high outer-sphere character (loose association causing relatively little proton displacement) for weakly bound metal ions.

For neither SFA nor WFA, do the data for pH 4.0 fit

TABLE 6  
Determination of x for Cd<sup>2+</sup>-fulvic acid reaction<sup>a</sup>

pH	K' for SFA	K' for SFA	K' for WFA	K' for WFA
	x = 0.20	x = 0.28	x = 0.20	x = 0.28
4.0	270	130	220	110
5.0	630	250	300	120
6.0	760	250	300	100
7.0	840	230	320	90
8.0	1100	250	300	70

<sup>a</sup> The variable x is the average number of protons released from fulvic acid when a Cd<sup>2+</sup> ion becomes bound.

well into the pattern (Table 6). There are two possible reasons. First, the data at pH 4.0 are the least reliable of all the data. Relatively little complexation occurs, so that there is only a small difference between free  $\text{Cd}^{2+}$  concentration and  $C_{\text{Cd}}$ , the total  $\text{Cd}^{2+}$  concentration. Second, it is possible that there is some fundamental change in the fulvic acid that occurs between pH 4 and 5. This would be difficult to prove without much more experimentation in that pH range.

Effect of fulvic acid concentration. Figures 5 and 6 show the effect of fulvic acid concentration on its ability to complex  $\text{Cd}^{2+}$ . Table 7 contains the conditional stability constants for titrations with different fulvic acid concentrations. For both types of fulvic acid, the more dilute solutions have higher conditional stability constants. The dependence of stability constant occurs for solutions below about  $1 \times 10^{-4}$  M (60-70 mg/L of FA). This concentration range corresponds well to the concentrations (below 100 mg/L) where Reuter (1977) found that reduced viscosities of aquatic humus change with concentration. Reduced viscosity is the viscosity per unit concentration of the solute. The higher reduced viscosity of dilute solutions may imply that humus molecules are in open conformations or that they are interacting with each other in concentrated solutions but not in dilute solutions.

Studies in this group (Bresnahan et al., 1978) and elsewhere (Stevenson, 1976, 1977; Cheam and Gamble, 1974;

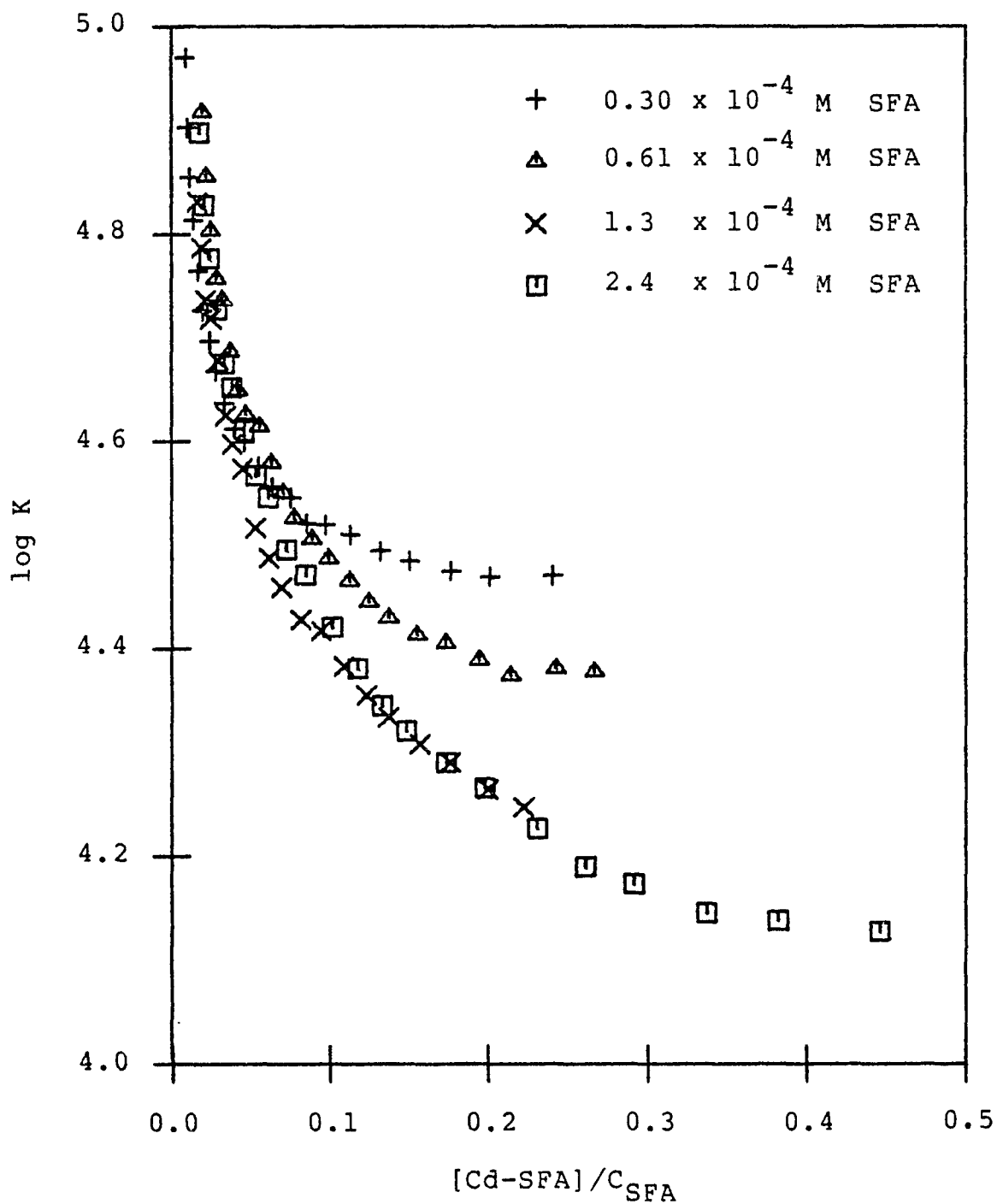


Figure 5. Conditional stability constants (K) for Cd<sup>2+</sup>-into-SFA titrations at four concentrations of soil-derived fulvic acid (SFA). I = 0.1 M and T = 25°C.



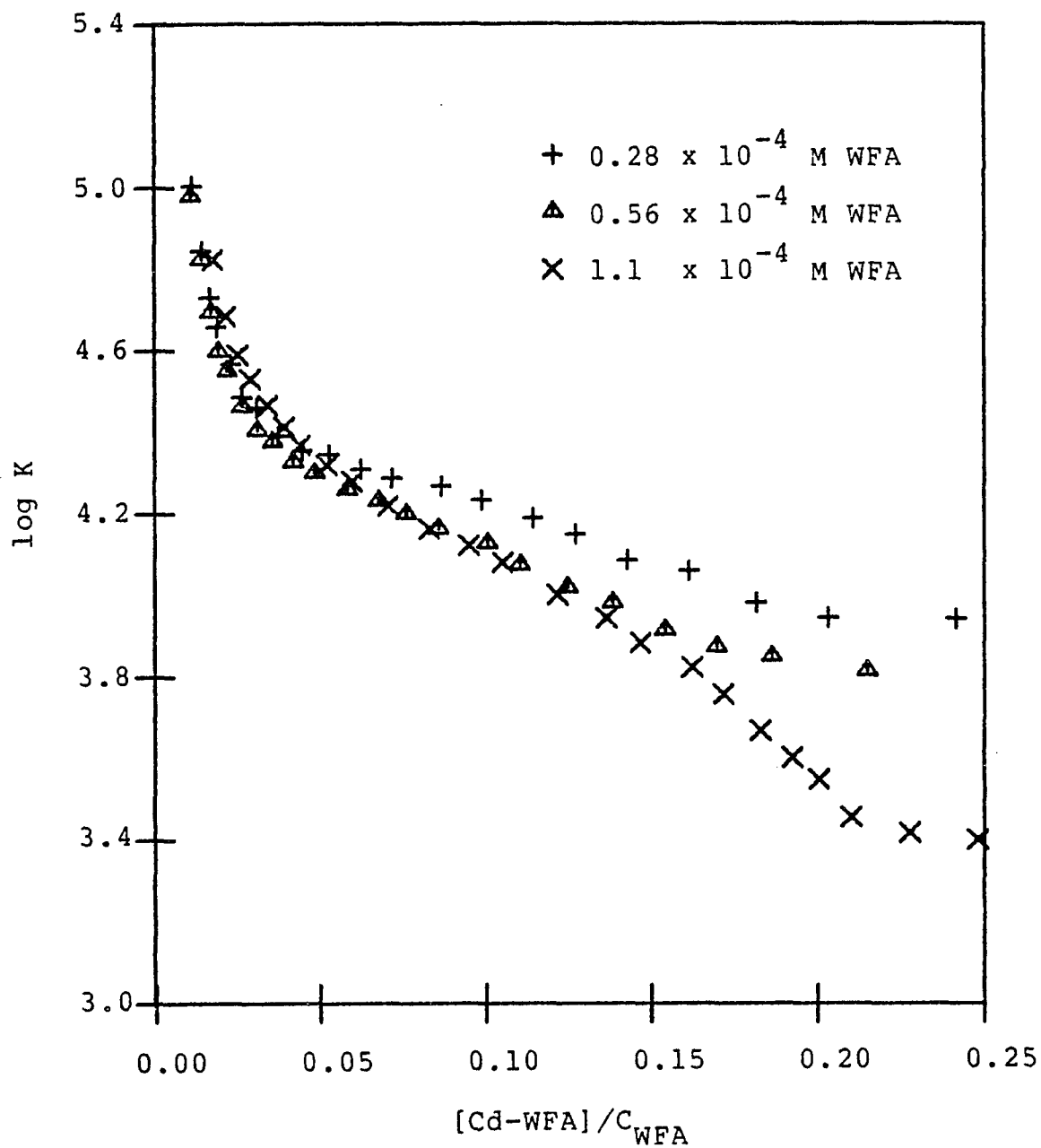


Figure 6. Conditional stability constants (K) for  $Cd^{2+}$ -into-WFA titrations at three concentrations of water-derived fulvic acid (WFA).  $I = 0.1$  M and  $T = 25^{\circ}C$ .

TABLE 7  
Effect of fulvic acid concentration on Cd<sup>2+</sup>-fulvic acid  
conditional stability constants

[WFA] x 10 <sup>4</sup> M <sup>a</sup>	log K	[SFA] x 10 <sup>4</sup> M <sup>b</sup>	log K
0.28	3.94	0.30	4.46
0.56	3.81	0.61	4.38
1.06	3.40	1.3	4.26
3.18	3.64	2.4	4.11
		4.1	4.15
		5.6	4.08

<sup>a</sup> Titrations done at pH 7.0 in 0.1 M KNO<sub>3</sub> at 25°C.

<sup>b</sup> Titrations done at pH 6.0 in 0.1 M KNO<sub>3</sub> at 25°C.

Takamatsu and Yoshida, 1978; Mantoura et al., 1978; Guy and Chakrabarti, 1976) show that  $\text{Cu}^{2+}$  binds more strongly to fulvic and humic acids than  $\text{Cd}^{2+}$  does. Furthermore, the concentration of fulvic acid does not affect the  $\text{Cu}^{2+}$ -FA stability constant. This clearly shows in Figure 7:  $\text{Cu}^{2+}$ -into-FA and FA-into- $\text{Cu}^{2+}$  titrations at pH 4.0 give nearly identical results. The two types of titrations for the  $\text{Cu}^{2+}$ -SFA system at pH 3.0 also give the same curve shape.

In contrast, results from  $\text{Cd}^{2+}$ -into-FA and FA-into- $\text{Cd}^{2+}$  experiments can be very different. Results from one titration of each type for  $\text{Cd}^{2+}$ -WFA at pH 6.0 appear in Figure 8. A similar plot for two  $\text{Cd}^{2+}$ -SFA titrations at pH 6.0 appears in Figure 9. Although this is not a Scatchard plot, its form is quite similar to one, so that the curve shapes for the FA-into- $\text{Cd}^{2+}$  titrations shown in Figures 8 and 9 are very similar to the curve shape shown in Figure 2 for a pH 5.0 FA-into- $\text{Cd}^{2+}$  experiment.

The complicated curve shape for the FA-into- $\text{Cd}^{2+}$  titrations can now be explained. Early in such a titration, the stability constant drops after each addition of FA, because of the concentration effect described above. Curve A in Figure 10 represents this effect. However,  $C_{\text{FA}}/C_{\text{Cd}}$  is always increasing, so that  $\text{Cd}^{2+}$  ions have an ever widening choice of sites on the FA for complexation. The metal ions will bind preferentially to the strongest sites, so as more and more FA is added, the average K value increases (curve B

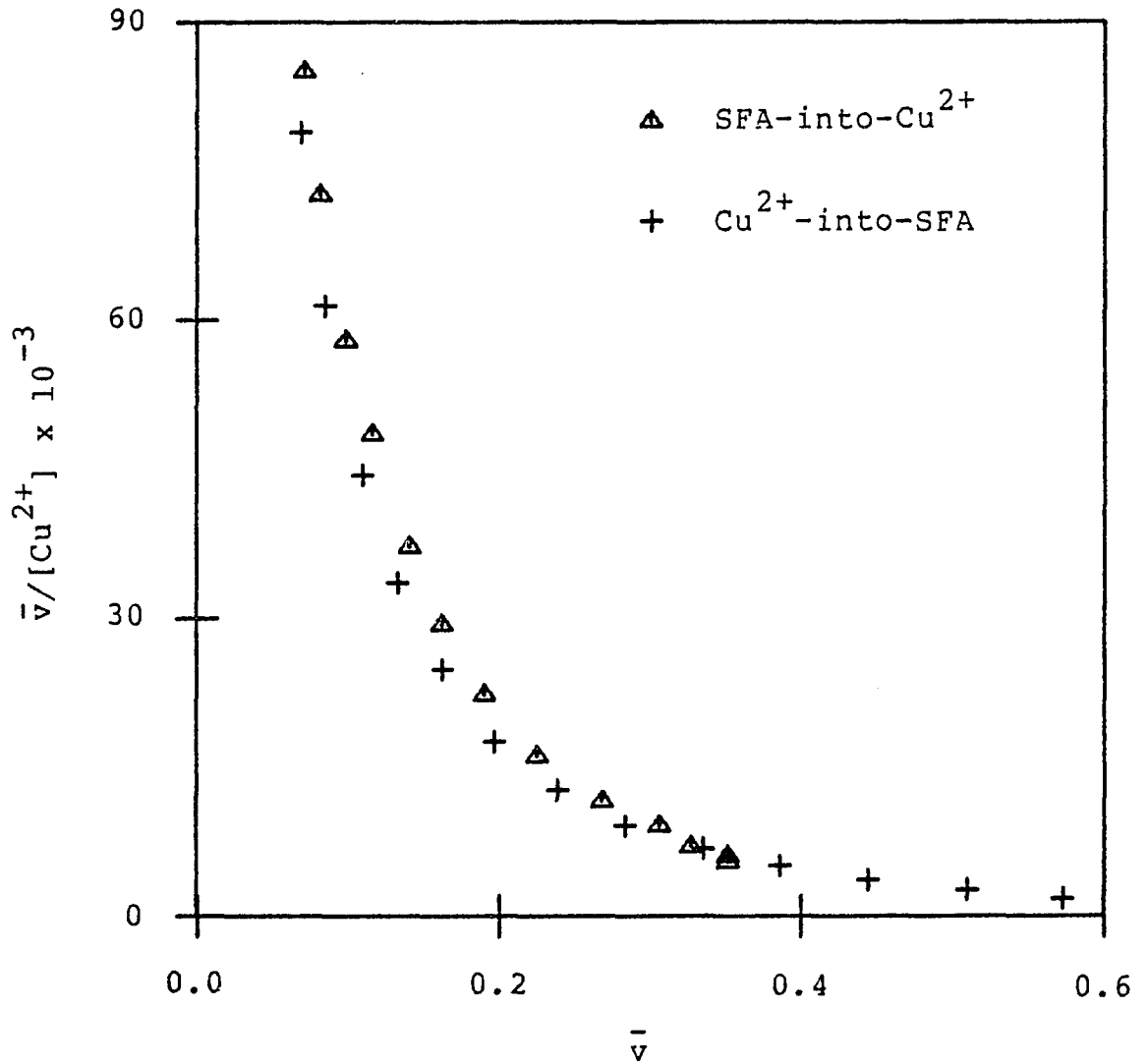
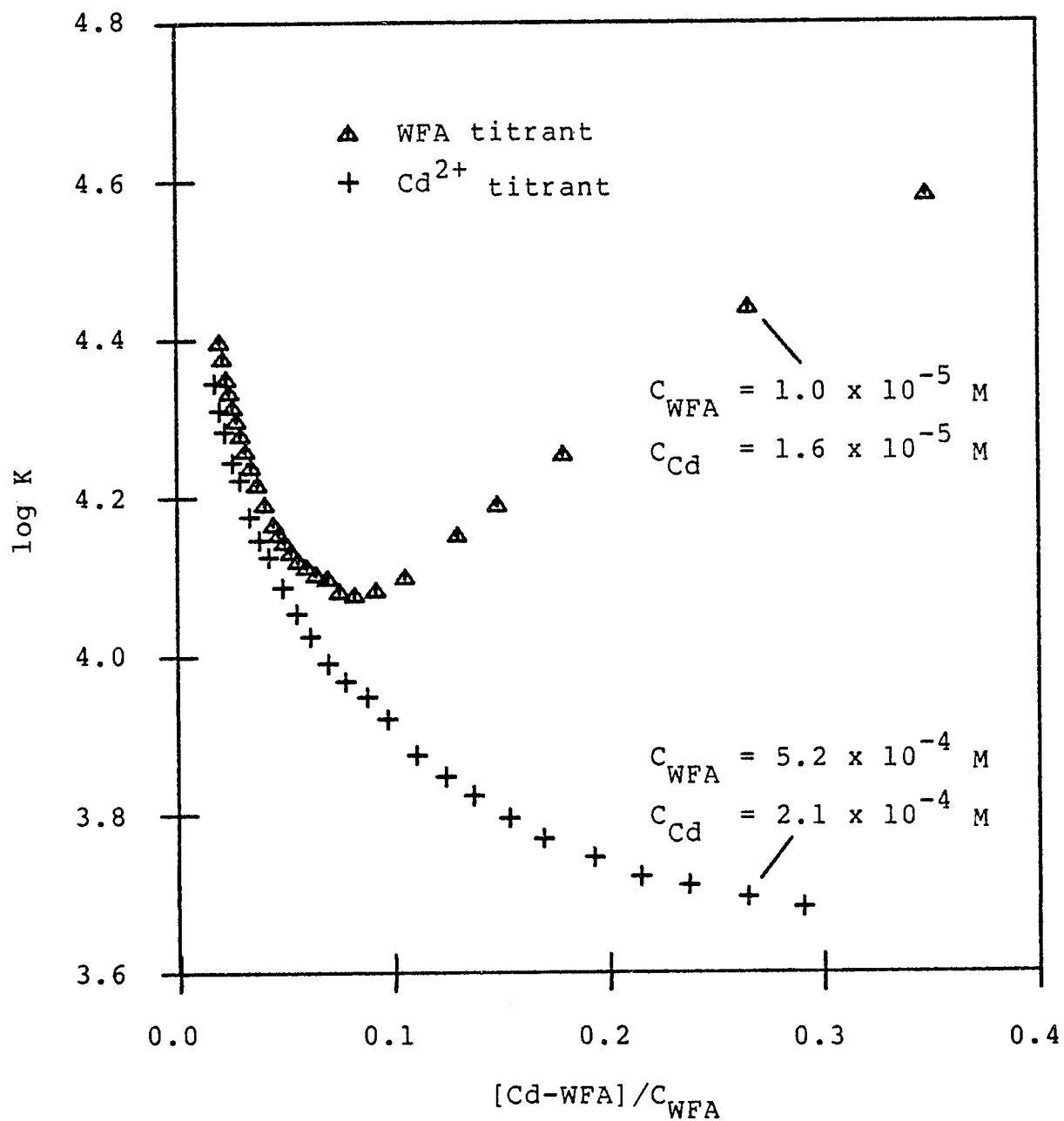


Figure 7. Scatchard plot comparing  $\text{Cu}^{2+}$ -into-SFA and SFA-into- $\text{Cu}^{2+}$  titrations at pH 4.0. SFA is soil-derived fulvic acid and  $\bar{v}$  is  $(C_{\text{Cu}} - [\text{Cu}^{2+}])/C_{\text{SFA}}$ .  $I = 0.1 \text{ M}$  and  $T = 25^\circ\text{C}$ .



**Figure 8.** A comparison of  $\text{Cd}^{2+}$ -into-WFA and WFA-into- $\text{Cd}^{2+}$  titrations at pH 6.0. WFA is water-derived fulvic acid and K is the conditional stability constant.  $I = 0.1 \text{ M}$  and  $T = 25^\circ\text{C}$ . Reagent concentrations are shown for one experimental point in each titration with approximately equal  $[\text{Cd-WFA}] / C_{\text{WFA}}$ .

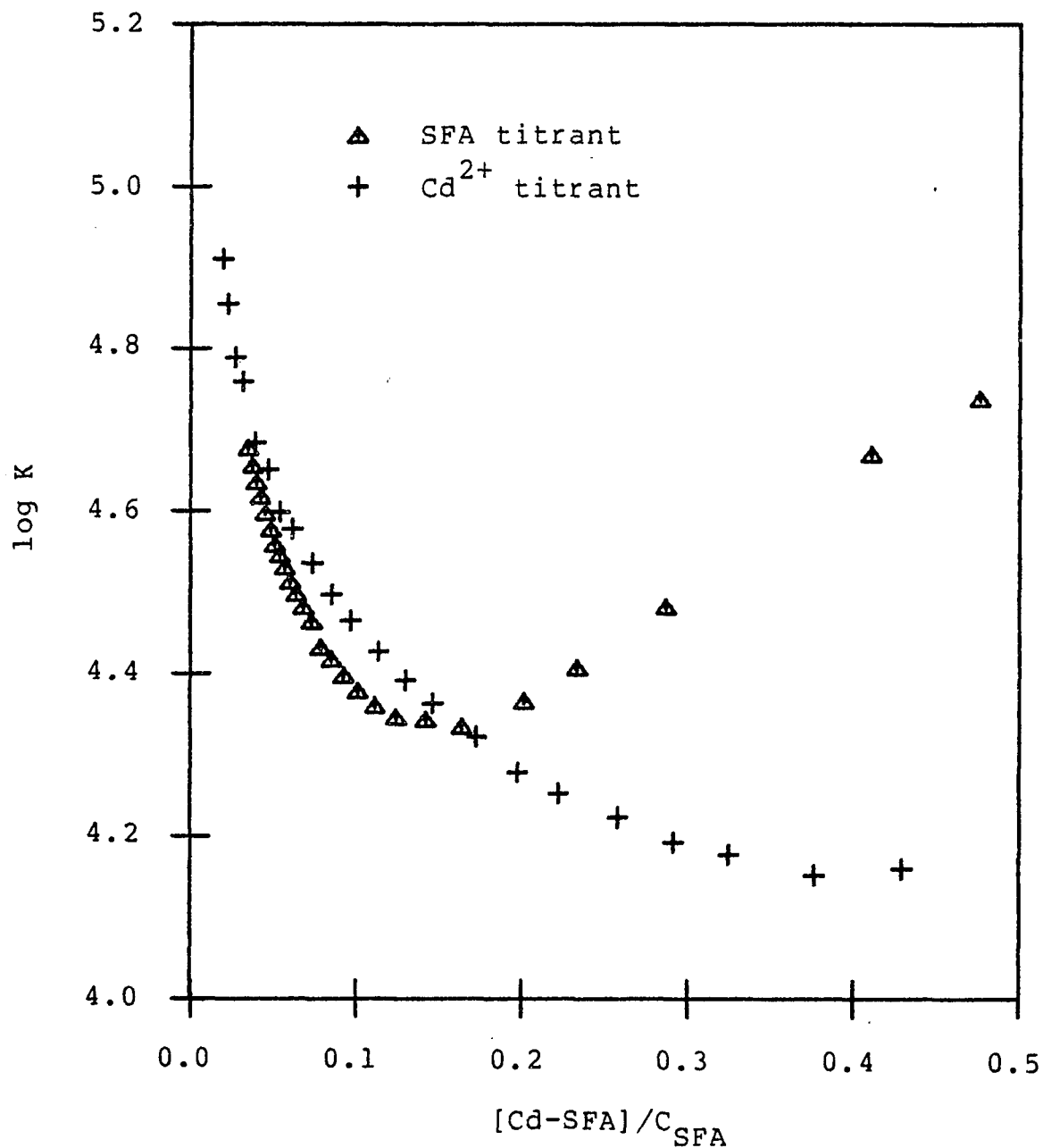


Figure 9. A comparison of  $Cd^{2+}$ -into-SFA and SFA-into- $Cd^{2+}$  titrations at pH 6.0. SFA is soil-derived fulvic acid and K is the conditional stability constant.  $I = 0.1 M$  and  $T = 25^{\circ}C$ .

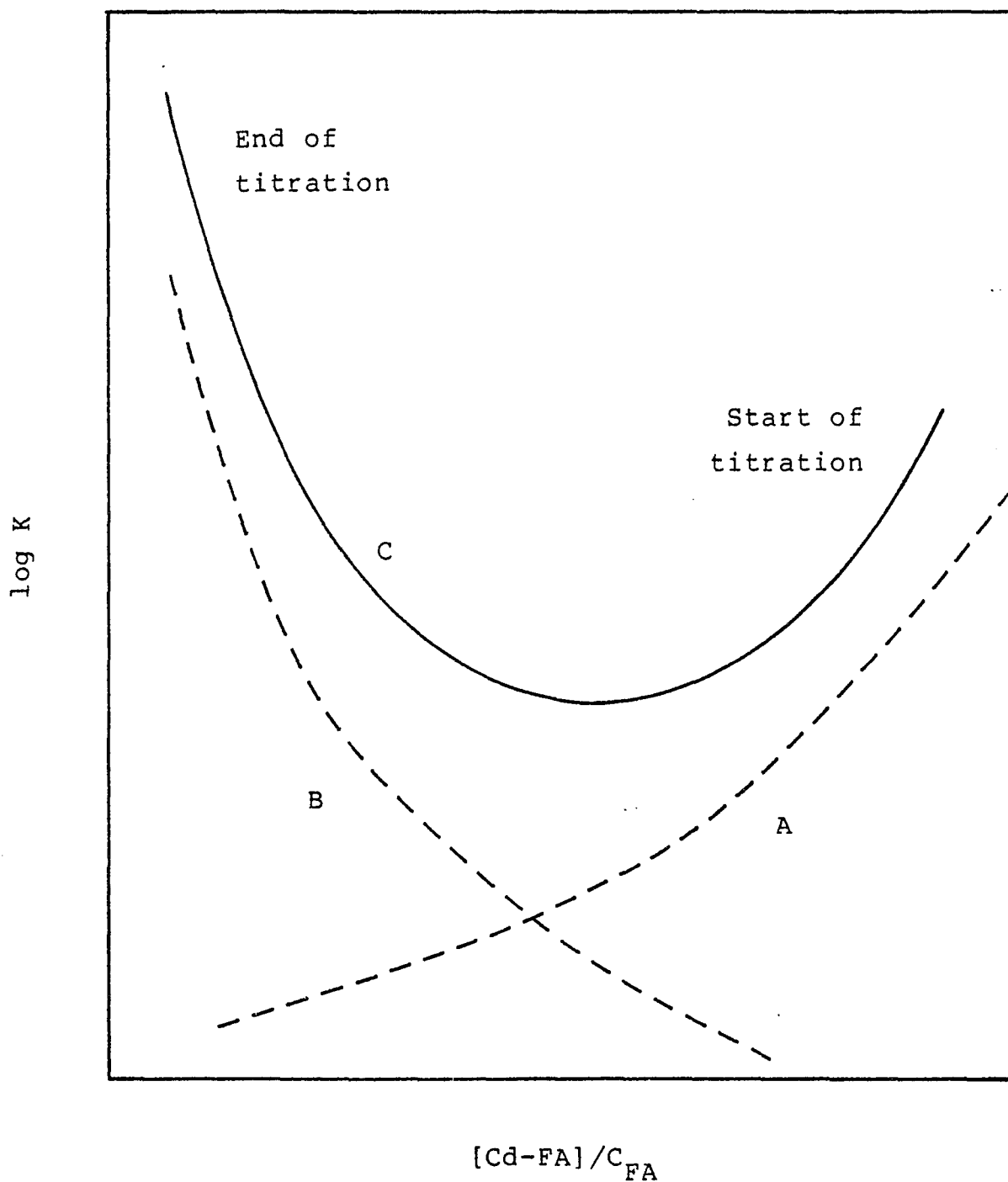


Figure 10. Idealized graph showing the change in conditional stability constant  $K$  with changing  $[\text{Cd-FA}]/C_{\text{FA}}$  for FA-into- $\text{Cd}^{2+}$  titrations. Curves A and B represent two factors that add to form the observed curve shape C. Details on these effects appear in the text.

in Figure 10). The sum of curves A and B is curve C, which is the shape of the FA-into- $\text{Cd}^{2+}$  titration curves as shown in Figures 2, 8, and 9.

The result is that the selection of titrant (FA or  $\text{Cd}^{2+}$ ) makes a large difference in the shape of the  $\text{Cd}^{2+}$ -FA complexation curve. As previously noted, during an FA-into- $\text{Cd}^{2+}$  titration, the FA concentration starts very low and increases 100-fold or more. Such low concentrations of FA and such a wide range of FA concentrations do not occur for  $\text{Cd}^{2+}$ -into-FA titrations.

It appears that interactions between fulvic acid molecules resulting from increased concentration of fulvic acid are forceful enough to block some potential  $\text{Cd}^{2+}$  complexation sites. However, the stability constant for  $\text{Cu}^{2+}$ -FA is great enough to overcome this relatively weak blocking. The FA-FA intermolecular forces appear to be stronger than those between  $\text{Cd}^{2+}$  and FA, but weaker than those between  $\text{Cu}^{2+}$  and FA. Buffle et al. (1978) note that such intermolecular associations occur in humic substances at concentrations greater than 80 mg/L.

Predictions are possible from the comparison of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  complexation by fulvic acid. A metal ion like  $\text{Pb}^{2+}$  that complexes with simple organic ligands much as  $\text{Cu}^{2+}$  does (Martell and Smith, 1977) would not have stability constants that depend on FA concentration. Results in Chapter 3 confirm this prediction. In contrast, a weakly complexing metal ion like  $\text{Zn}^{2+}$  might well have concentration dependent



K values.

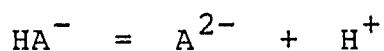
Another possibility may explain the FA concentration effect. Burch et al. (1978) note that the weighted average of acid functional group ionization constants in fulvic acid will generally change when the concentration of such groups changes. It could be argued that the radically different FA concentrations in the FA-into- $\text{Cd}^{2+}$  experiment cause different dissociation constants for the FA and hence alter its ability to bind metal ions. It appears that higher concentrations of acid groups increase the amount of  $\text{H}^+$  dissociation (Burch et al., 1978). This model would agree with the  $\text{Cd}^{2+}$ -FA findings presented here if the increase in FA concentration also increases the dissociation constant for  $\text{Cd}^{2+}$ -FA. However, such a fundamental change in the acid-base properties of fulvic acid should also influence the complexation of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$ . The work summarized in this dissertation shows that it does not.

Effect of electrolyte concentration. Table 8 shows the effect of ionic strength on  $\text{Cd}^{2+}$ -FA complexation. The  $I = 0.01$  M titrations resulted in higher conditional stability constants than for the  $I = 0.1$  M titrations. The FA concentration effect for  $\text{Cd}^{2+}$  holds in either 0.1 or 0.01 M electrolyte. It is not surprising that stability constants are higher in the more dilute electrolyte, where  $\text{K}^+$  competition for FA complexing sites is relatively low (Gamble, 1973). Furthermore, an increase in ionic strength increases the acidity of neutral or negatively charged

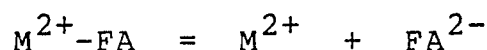
TABLE 8  
Effect of ionic strength on Cd<sup>2+</sup>-soil fulvic acid  
conditional stability constants at pH 6.0

<u>[SFA] x 10<sup>4</sup> M</u>	<u>[KNO<sub>3</sub>] M</u>	<u>Log K</u>
5.6	0.10	4.08
5.7	0.01	4.76
0.61	0.10	4.38
0.59	0.01	4.89

acids, such as fulvic acids, through lowered activity coefficients. For the general acid dissociation reaction



a change in ionic strength from 0.01 M to 0.1 M will lower the  $\text{pK}_a$  by 0.22 units (Stumm and Morgan, 1970). The higher ionic strength would probably also favor the forward reaction



resulting in the lower stability constants at  $I = 0.1$  M than at  $I = 0.01$  M.

### CONCLUSIONS

Fulvic acid concentration appears to be an important variable when investigating the transport of weakly bound metal ions, like  $\text{Cd}^{2+}$ , in natural systems. On a per-mole basis, FA complexes most strongly when FA is in low concentration. Graphs such as those in Figures 3-6 can help calculate the proportion of free and complexed  $\text{Cd}^{2+}$  for a given pH, for a range of fulvic acid concentrations, and at different  $[\text{Cd-FA}]/C_{\text{FA}}$  values.

The low  $[\text{Cd-FA}]/C_{\text{FA}}$  parts of Figures 3-6 apply best to average river water in the United States, which has 10-13 mg/L of dissolved humic substances (Reuter and Perdue, 1977)

and about 0.001 mg/L of  $\text{Cd}^{2+}$  (Durum et al., 1971). However, areas with much heavy-metal pollution might be best represented by the high  $[\text{Cd-FA}]/C_{\text{FA}}$  portions of these graphs. Finally, this work shows that fulvic acids derived from soil and water respond to  $\text{Cd}^{2+}$  very similarly in all aspects investigated here.

## CHAPTER 3

### Pb<sup>2+</sup> COMPLEXATION BY FULVIC ACID AND HOW IT COMPARES TO FULVIC ACID COMPLEXATION OF Cu<sup>2+</sup> AND Cd<sup>2+</sup>

#### INTRODUCTION

Lead(II) differs chemically and toxicologically from cadmium(II). Lead(II) is nearly 100 times as concentrated in igneous rocks as is cadmium(II); the difference in concentration favors lead(II) even more in sedimentary rocks (Hem, 1970). Cadmium is a group IIB metal in period 5. Lead appears in group IVA in period 6. Cadmium's only common oxidation states are 0 and +2. Lead can appear as 0, +2, and +4. The discussion here relates to the +2 oxidation state of both metals.

Despite the relatively high levels of lead in rocks, there is not much of it in natural waters. Lead(II) is released only slowly by weathering (Lovering, 1976) and even if it is released, Pb<sup>2+</sup> concentration can be limited by adsorption and by the low solubility of such inorganic

species as  $\text{PbCO}_3$ ,  $\text{PbSO}_4$ , and  $\text{PbS}$ . The median concentration of  $\text{Pb}^{2+}$  in lakes and rivers in the United States is 0.002 mg/L. This value is only twice the estimated average  $\text{Cd}^{2+}$  concentration in United States waters (Durum et al., 1971).

Transportation vehicles and industrial products and byproducts can cause relatively high concentrations of  $\text{Pb}^{2+}$  to arise, and therein lie potential health problems. Older structures have lead-based paints and lead plumbing (plumbum is Latin for lead). Water with low pH flowing in such pipes can mobilize  $\text{Pb}^{2+}$ . Lead can enter the air from automobiles that burn leaded fuel and from metal smelting and coal burning; coal averages 10 ppm lead (Lovering, 1976). These factors cause lead to be concentrated in urban, industrial, and highway areas. Soils and water bodies in such areas can receive far more lead than occurs naturally.

Human activity puts large amounts of lead into the environment perhaps because lead has been regarded as only moderately toxic, certainly less toxic than  $\text{Cd}^{2+}$  and various mercury-containing species. A National Academy of Sciences panel on water quality criteria suggests that no more than 0.05 mg/L of lead appear in drinking-water supplies. The same panel suggests limits of 0.01 mg/L for cadmium and 0.002 mg/L for mercury (National Academy of Sciences, 1972). However, lead poisoning has received increasing attention, so that lead plumbing and lead-based paints are now used less, and lead anti-knock agents such as tetraethyllead are being phased out of gasoline. Excess lead can cause

anorexia, vomiting, malaise, and convulsions (due to intracranial pressure) in children; in adults, chronic lead poisoning appears as problems in the gastrointestinal tract and in the central nervous system (Merck Index, 1976).

The chemistry of  $Pb^{2+}$  in natural waters and soils is not easy to unravel. There are many possible soluble organic and inorganic complexes of lead, as well as many opportunities for lead and lead-containing species to precipitate out of solution and adsorb onto solids (Jenne and Luoma, 1977). The relative concentrations of the various species will affect the availability and toxicity of  $Pb^{2+}$  to plants and animals, and will also affect the movement of lead in geochemical processes.

Speciation studies may be divided into those applying to salt water (Batley and Gardner, 1978; Zirino and Yamamoto, 1972), to mixed salt and fresh water (Duinker and Kramer, 1977; Blustein and Smith, 1978), and to fresh water (Giesy et al., 1978; Kubota et al., 1974; Ramamoorthy and Kushner, 1975; Beneš et al., 1979). O'Shea and Mancy (1978) prepared model solutions to test the effect of pH and hardness-metal ions on heavy-metal speciation.

Although papers have appeared showing only the inorganic speciation of  $Pb^{2+}$  (Zirino and Yamamoto, 1972), much attention has been concentrated on the interaction between organic matter and  $Pb^{2+}$ . For example, Bondarenko (1968) found that a 0.01% solution of soil-derived fulvic acid could dissolve 10 to 60 times as much galena ( $PbS$ )

during one year as could a solution without fulvic acid. Several studies have gone further to calculate stability constants for  $Pb^{2+}$  with various fraction of organic matter (Table 9).

Of the entries in Table 9, some of the earliest work on the stability constants of  $Pb^{2+}$  and humic substances was done by ion exchange and by the method of continuous variation (Schnitzer and Skinner, 1967; Schnitzer and Hansen, 1970). Hope was high at one time that anodic stripping voltammetry (with its extremely low detection limits) and polarography could provide better data to calculate conditional stability constants. Ernst et al. (1975) obtained dramatically different results from differential pulse polarography and differential pulse anodic stripping voltammetry when they tried to calculate stability constants for  $Pb^{2+}$ -humic acid complexes. They note that ligand adsorption onto the mercury electrode ruined the technique. However, groups continue to use modified voltammetric techniques to determine stability constants (Buffle and Greter, 1979).

Several published stability constants for  $Pb^{2+}$ -humic matter complexes result from potentiometric titrations, which can be done by measuring free (hydrated)  $Pb^{2+}$  with an ion-selective electrode (Takamatsu and Yoshida, 1978; Buffle et al., 1977; Ramamoorthy and Kushner, 1975), or by measuring released hydrogen ion (Stevenson, 1976, 1977; Ramamoorthy and Manning, 1974). One group separated free



TABLE 9

Lead(II) stability constants with humic materials

Complex <sup>a</sup>	pH	I	log K	Method	Reference
Pb-SFA	3.5	0.1	3.1	1	1
Pb-SFA	5.0	0.1	6.1	1	1
Pb-SFA	3.0	0.1	2.6	2	2
Pb-SFA	5.0	0.1	4.1	2	2
Pb-SFA	3.0	0.1	2.7	1	2
Pb-SFA	5.0	0.1	4.0	1	2
Pb-(SHA) <sub>2</sub>	-	0.1	b	3	3
Pb-AHA	6.8	0.01	6.53 <sup>c</sup>	4	4
Pb-AHA	6.8	0.01	5.30 <sup>c</sup>	4	4
Pb-(peat HA) <sub>2</sub>	4.0	0.1	7.08	5	5
Pb-(peat HA) <sub>2</sub>	d	0.1	7.00	6	5
Pb-(lignite HA) <sub>2</sub>	4.0	0.1	7.36	5	5
Pb-(lignite HA) <sub>2</sub>	d	0.1	7.03	6	5
Pb-(SHA) <sub>2</sub>	4.0	0.1	7.14	5	5
Pb-(SHA) <sub>2</sub>	d	0.1	7.03	6	5
Pb-(peat HA) <sub>2</sub>	4.0	0.01	8.14	5	5
Pb-(peat HA) <sub>2</sub>	d	0.01	8.11	6	5
Pb-(lignite HA) <sub>2</sub>	4.0	0.01	8.18	5	5
Pb-(lignite HA) <sub>2</sub>	d	0.01	7.80	6	5

Table 9, continued

Pb-(SHA) <sub>2</sub>	4.0	0.01	8.38	5	5
Pb-(SHA) <sub>2</sub>	d	0.01	7.65	6	5
Pb-SFA	3.0	0.1	2.6	3	6
Pb-SFA	5.0	0.1	4.1	3	6
Pb-river water	6.7	0.1	6.0	3	6
Pb-river water	6.8	0.1	5.5	3	6
Pb-(river water) <sub>2</sub>	6.8	0.1	10.4	3	6
Pb-WFA	6.0	0.1	5.1	8	7
Pb-(WFA) <sub>2</sub>	6.0	0.1	9.7	8	7
Pb-river water	7.3	0.001	3.95	3	8
Pb-SFA	-	-	3.64	3	8
Pb-SFA	3.0	0.1	3.21	3, 5	9
Pb-(SFA) <sub>2</sub>	3.0	0.1	5.58	3, 5	9
Pb-HA	-	0.1	4.0	7	10
Pb-HA	-	0.1	6.1	7	10
Pb-HA	-	0.1	14.8	8	10

Table 9, continued

- a Abbreviations: SFA (soil-derived fulvic acid); SHA (soil-derived humic acid); AHA (Aldrich humic acid); HA (humic acid); WFA (water-derived fulvic acid).
- b  $8.35 + 0.30(\text{pH} - 5)$ .
- c The authors used the Scatchard treatment to generate  $\log K = 6.53$  for the strong (first) class of sites and  $\log K = 5.30$  for the weak (second) class of sites.
- d Average of titrations at pH 4 and 5.

Methods. (1) Ion exchange. (2) Continuous variation. (3) Ion-selective electrode potentiometry. (4) Dialysis / atomic absorption spectrophotometry. (5) Conventional hydrogen-ion potentiometry. (6) Modified hydrogen-ion potentiometry. (7) Differential pulse anodic stripping voltammetry. (8) Differential pulse polarography.

References. (1) Schnitzer and Skinner, 1967. (2) Schnitzer and Hansen, 1970. (3) Takamatsu and Yoshida, 1978. (4) Guy and Chakrabarti, 1976. (5) Stevenson, 1977. (6) Buffle et al., 1977. (7) Buffle and Greter, 1979. (8) Ramamoorthy and Kushner, 1975. (9) Ramamoorthy and Manning, 1974. (10) Ernst et al., 1975.

and bound  $Pb^{2+}$  with dialysis tubing and analyzed the metal ion concentration with atomic absorption spectrophotometry (Guy and Chakrabarti, 1976).

The work described in this dissertation adds to the existing literature in several ways. First, the experiments involve two fulvic acids, one derived from a soil and the other from a freshwater river. Any comparisons of the  $Pb^{2+}$  complexing ability of these two fulvic acids will help our understanding of  $Pb^{2+}$ -organic matter interactions in water by enabling us to draw upon the large amount of information gathered on interactions between soil-derived materials and heavy metals.

This study also includes results of  $Pb^{2+}$  complexation by fulvic acids at four pH values, a larger number of hydrogen-ion concentrations than are discussed in any paper except one (Takamatsu and Yoshida, 1978); that paper describes complexation only for soil-derived humic acid (SHA), and the constants are listed for 1:2 ( $Pb-(SHA)_2$ ) complexes only. A comprehensive knowledge of the pH effect on  $Pb^{2+}$  complexation by fulvic acid will allow more accurate calculations of speciation.

Finally, this work carefully describes the conditions needed to cause precipitation of  $Pb^{2+}$ -FA complexes. It is important to know when solids begin to form during a metal-ion titration, so that data after precipitation begins are not included in calculations of stability constants, which apply strictly to solution-phase reactions.

## EXPERIMENTAL

The  $\text{Pb}^{2+}$  ion-selective electrode experiments use many of the same materials and procedures used in the  $\text{Cd}^{2+}$  ion-selective electrode work. This experimental section will emphasize the differences between these two sets of experiments. Included here also is a description of  $\text{Cu}^{2+}$  ion-selective electrode titrations. These titrations, performed under the same conditions of pH and concentration as the  $\text{Pb}^{2+}$  titrations, allowed comparison of the reactions of fulvic acid with  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$ . I performed scattering tests on solutions with various mole ratios of total metal ion to total fulvic acid ( $C_M/C_{FA}$ ). This allowed determination of what ion-selective data were applicable to calculation of stability constants in the solution phase.

### Materials

The fulvic acids, derived from soil (SFA) and from a freshwater river (WFA), are the same type used in the  $\text{Cd}^{2+}$  complexation experiment. Chapter 1 contains a brief discussion of these materials; their chemical characteristics are listed in Table 1. The source of lead(II) ion was either Fisher SO-L-21 1000 ppm atomic absorption standard (nitrate anion), or Orion 94-82-06 0.1000±0.0005 M lead perchlorate. The source of copper(II) ion was either Fisher SO-C-194 1000 ppm atomic absorption standard (nitrate anion), or Orion 94-29-06 0.1000±0.0005 M

cupric nitrate. The electrolyte for all experiments was 0.1 M  $\text{KNO}_3$ , prepared from Mallinckrodt or Baker purified crystals. The medium for the work was doubly deionized water.

The acid solutions used for pH adjustment were known dilutions of Baker reagent-grade (15.9 M) nitric acid; the base solutions were prepared from Baker reagent-grade 45% KOH solution. All acid and base solutions of 0.01 M concentration or less were prepared in 0.1 M  $\text{KNO}_3$ .

### Apparatus

The titration cell, as in the  $\text{Cd}^{2+}$  titrations, was a Princeton Applied Research (PAR) model 9301 water-jacketed cell. A P. M. Tamson model T9 circulating water bath maintained the solution temperature at  $25 \pm 0.2^\circ\text{C}$ ; a stir bar and magnetic stirrer mixed the solution continuously. The electrode arrangement for  $\text{Cu}^{2+}$  was the same as for  $\text{Cd}^{2+}$ : the reference portion of a Corning model 476050 combination electrode was the reference for an Orion model 94-29  $\text{Cu}^{2+}$  ion-selective electrode. A second Corning combination electrode (also model 476050) monitored pH. The electrodes were attached to two Orion model 701A pH/mv meters, which allowed simultaneous reading of hydrogen ion and free metal ion concentrations. Light affects the reading from the  $\text{Cu}^{2+}$  electrode, so during titrations, the cell was covered with aluminum foil and black cloth.

The electrode arrangement for  $\text{Pb}^{2+}$  analysis is somewhat

different. An Orion model 94-82 electrode is sensitive to  $Pb^{2+}$ , and an Orion model 910100 Ag/AgCl glass electrode is sensitive to hydrogen ion. These two electrodes shared a reference: a PAR model K77 saturated calomel reference electrode, isolated from the test solution by a PAR model K65 reference electrode bridge tube with a Vycor tip. The system requires this double-junction reference to avoid chloride interference with the  $Pb^{2+}$  electrode. These titrations also use the two pH/mv meters.

The surface of the  $Pb^{2+}$  electrode can be oxidized, and indeed, the response deteriorated rapidly over the course of successive calibration titrations. The manufacturer recommends that one part of a formaldehyde-methanol solution (0.002 M formaldehyde in methanol) be mixed with one part of the test solution (Orion, 1977). This mixture lowers the solubility of the electrode membrane and provides a reducing environment that slows oxidation. Such a mixed solvent would not be appropriate for this work, which is designed to study the interactions between fulvic acid and metal ions in aqueous solution. Alternatively, membrane oxidation can be avoided by purging the titration cell with nitrogen gas that has been passed through a vanadium(III) chloride oxygen scrubber.

A Perkin-Elmer model 204 fluorescence spectrophotometer with excitation and emission wavelengths set at 400 nm, allowed determination of solution scattering. Fulvic acid solutions to be tested for scattering were filtered with a

Nuclepore polycarbonate filtration apparatus, fitted with 0.4 micrometer Nuclepore polycarbonate filters.

### Procedures

The concentrations of  $\text{Pb}^{2+}$  and fulvic acids are listed for FA-into- $\text{Pb}^{2+}$  titrations in Table 10 and for  $\text{Pb}^{2+}$ -into-FA titrations in Table 11. Table 12 contains experimental conditions for  $\text{Cu}^{2+}$ -into-FA and FA-into- $\text{Cu}^{2+}$  titrations. For the  $\text{Pb}^{2+}$ -into-FA titrations, which gave the most usable  $\text{Pb}^{2+}$  results, I placed 20-25 mL of the FA solution into the titration cell and then inserted the electrodes. I polished the  $\text{Pb}^{2+}$  electrode once at the start of each day of experimentation. Thirty minutes of purging with the oxygen-free nitrogen preceded the first addition of  $\text{Pb}^{2+}$  titrant. This period allowed the  $\text{Pb}^{2+}$  electrode to become conditioned by the organic matter in the solution. I then added  $\text{Pb}^{2+}$  aliquots to cause 2-4 millivolt changes in the output of the ion-selective electrode. The electrode response was somewhat slower than was the response of the  $\text{Cd}^{2+}$  or  $\text{Cu}^{2+}$  electrodes. Thus, a 3-minute wait per data point was needed. I added acid or base as necessary to hold the pH to within 0.02 units of the desired value, which was 4.0, 4.5, 5.0, or 6.0.

The final step of the titrations was calibration of the electrode in 0.1 M  $\text{KNO}_3$ . For the calculations, I chose not to use calibrations done before a titration because the fulvic acid conditioned the electrode during a titration;



TABLE 10  
Conditions for fulvic acid-into-Pb<sup>2+</sup> titrations<sup>a</sup>

Date	pH	FA titrant (M x 10 <sup>3</sup> )		Initial Pb <sup>2+</sup> in cell (M x 10 <sup>5</sup> )
12/04/78	5.0	5.88	SFA	48.
12/06/78	5.0	6.04	SFA	3.6
12/17/78	5.0	3.88	SFA	5.2
12/18/78	6.0	3.52	SFA	8.6
12/19/78	4.0	3.57	SFA	3.3
1/26/79	5.0	3.9	SFA	5.2
1/28/79	6.7	3.4	SFA	15.
3/21/79	5.0	0.42	SFA	0.38
3/21/79	5.0	4.2	SFA	3.7
3/28/79	5.0	0.42	SFA	0.19
3/30/79	4.0	4.4	SFA	3.3

<sup>a</sup> All titrations are done in 0.1 M KNO<sub>3</sub> at 25°C.

TABLE 11  
Conditions for Pb<sup>2+</sup>-into-fulvic acid titrations<sup>a</sup>

Date	pH	Pb <sup>2+</sup> titrant (M x 10 <sup>2</sup> )	Initial FA in cell (M x 10 <sup>4</sup> )	
12/07/78	5.0	1.0 10.0	7.8	SFA
12/11/78	5.0	0.1 1.0	8.8	SFA
12/20/78	4.0	0.048 0.48	0.79	SFA
3/30/79	4.0	0.048	7.0	SFA
5/04/79	6.0	0.048 0.48	0.53	SFA
5/04/79	6.0	0.48 10.	5.6	SFA
6/24/79	5.0	0.047 0.47	0.54	WFA
6/24/79	5.0	0.47 10.	5.5	WFA
6/26/79	6.0	0.047 0.47	0.53	WFA
6/26/79	6.0	0.47 10.	5.3	WFA
7/03/79	5.0	0.047 0.47	0.52	SFA
7/03/79	5.0	0.47 10.	5.3	SFA
7/05/79	6.0	0.047 0.47	0.52	SFA
7/05/79	6.0	10.	4.9	SFA

Table 11, continued

7/11/79	4.0	0.047 0.47	0.52	SFA
7/11/79	4.0	0.47 10.	2.0	SFA
7/13/79	5.0	0.047 0.47	0.51	SFA
7/13/79	5.0	0.47	2.0	SFA
7/15/79	6.0	0.47	0.51	SFA
7/15/79	6.0	0.47	1.9	SFA
7/17/79	4.5	0.047 0.47	0.51	SFA
7/17/79	4.5	0.47	1.9	SFA
7/31/79	4.0	0.047 0.47	0.51	WFA
7/31/79	4.0	0.047 0.47	1.9	WFA
8/02/79	4.5	0.047 0.47	0.51	WFA
8/02/79	4.5	0.47	1.9	WFA

<sup>a</sup> All titrations were done in 0.1 M KNO<sub>3</sub> at 25°C.

TABLE 12  
Conditions for Cu<sup>2+</sup>-fulvic acid titrations<sup>a</sup>

Date	pH	Cu <sup>2+</sup> titrant (M x 10 <sup>2</sup> )	Initial FA in cell (M x 10 <sup>4</sup> )	
6/19/78	4.0	0.59	5.46	SFA
6/21/78	3.0	0.30	5.54	SFA
6/30/78	6.0	0.10	7.35	SA <sup>b</sup>
7/03/78	6.0	1.00	7.23	SA <sup>b</sup>
4/11/79	5.0	0.157 1.57	0.63	SFA
4/11/79	5.0	1.57 10.	7.0	SFA
5/02/79	6.0	1.00	0.60	SFA
5/02/79	6.0	1.00 10.0	6.2	SFA
8/04/79	4.5	0.10 1.00	1.9	WFA
11/29/79	4.0	0.15	0.53	SFA
11/29/79	3.0	1.51	0.52	SFA

(continued)

Table 12, continued

Date	pH	FA titrant (M x 10 <sup>3</sup> )	Initial Cu <sup>2+</sup> cell (M x 10 <sup>4</sup> )
6/19/78	4.0	4.88 SFA	0.667
6/21/78	3.0	4.73 SFA	0.30
6/22/78	3.0	4.94 SFA	1.13

<sup>a</sup> All titrations done in 0.1 M KNO<sub>3</sub> at 25°C.

<sup>b</sup> Salicylic acid.

this conditioning results in a shift of several millivolts in reading.

Copper(II) ion-selective electrode experiments were performed in much the same way, except that the nitrogen purging and the 30-minute equilibration period before the start of a titration were not needed. It usually took one minute to obtain a steady millivolt reading.

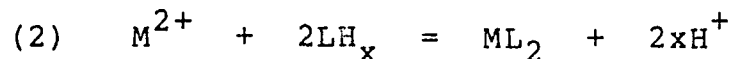
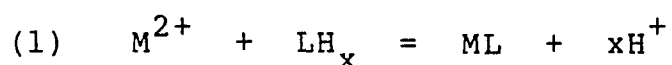
Changes in solution scattering proved to be a sensitive indicator of the formation of  $M^{2+}$ -FA precipitates. Before addition of metal ion, all solutions were passed through the 0.4 micron filter. Several sets of 6-mL samples contained  $Pb^{2+}$  and FA, or  $Cu^{2+}$  and FA, or  $Cd^{2+}$  and FA. Each set had one pH value and a specific type and concentration of FA, but each member of a set had a different  $C_M/C_{FA}$ . A sample with a small increase in scattering compared with a sample with no metal ion had solids that could be centrifuged by 30 minutes of spinning at 10,000 rpm.

### Calculations

There are several possible ways to calculate conditional stability constants for  $Pb^{2+}$ -FA complexes. One could assume formation of only 1:1 complexes; this is the assumption used for the  $Cd^{2+}$ -FA work. However, this assumption may break down because  $\bar{v}$  values ( $\bar{v} = (C_{Pb} - [Pb^{2+}])/C_{FA}$ ) can be greater than 1.0 (especially at pH 6.0), implying a stoichiometry such as  $Pb_2$ -FA. However, the high  $\bar{v}$  values occur at  $C_{Pb}/C_{FA}$  where precipitation begins,

so "Pb<sub>2</sub>-FA" complexes (or complexes with even more metal ions) may not exist in the solution phase.

The Scatchard method of data treatment (Scatchard, 1949; Van Holde, 1971) can handle complexes with several stoichiometries, so it may overcome the weakness of the 1:1 method. However, there is evidence in the literature that 1:1 and 1:2 complexes between Pb<sup>2+</sup> and humic materials can form (Takamatsu and Yoshida, 1978; Buffle et al., 1977). For fulvic acid, these complexes may be represented by PbFA (1:1) and Pb-(FA)<sub>2</sub> (1:2). The possibility of 1:2 complexes prevents proper use of the 1:1 and the Scatchard calculation schemes. A possible alternative is a method derived by Buffle and coworkers (Buffle et al., 1977). The method involves calculation of constants for two equilibria:



The equilibrium constant for (1) is  $B_1^*$  and the constant for (2) is  $B_2^*$ . The conditional stability constants are  $B_1 (= B_1^*/[H^+]^x)$  and  $B_2 (= B_2^*/[H^+]^{2x})$ . The Buffle method requires calculation by computer, because it is necessary to minimize a complicated function that involves these constants, the total metal-ion concentration, the ratio of total to free metal ion, the ligand molecular weight, and the ligand concentration. That is, the best values for  $B_1$  and  $B_2$  are supposed to give the lowest value for the function. The minimization process may be straightforward

(though lengthy) for a simple system, but for a complicated ligand mixture like fulvic acid, the minimum is hard to find. In some cases there is no minimum (see Results and Discussion), but overall, this method of data treatment appears to have worked. A FORTRAN program (BUF4.FOR, Appendix), running on the University of New Hampshire DECSYSTEM 1090 computer, performed the calculations.

### RESULTS AND DISCUSSION

Using the  $\text{Pb}^{2+}$  ion-selective electrode in an aqueous solution with fulvic acid was a major challenge. Even without fulvic acid in the solution, the electrode response was slow and deteriorated after several calibrations. Nitrogen purging helped tremendously in this respect. Not only was there less deterioration in response, but the calibration curves were also steeper. However, even at best, the  $\text{Pb}^{2+}$  electrode is not nearly as sensitive as the  $\text{Cu}^{2+}$  or  $\text{Cd}^{2+}$  electrodes.  $\text{Pb}^{2+}$  response was one-third to one-half the Nernstian slope at  $10^{-6}$  M  $\text{Pb}^{2+}$ , compared with a two-thirds Nernstian slope for the  $\text{Cd}^{2+}$  electrode at  $10^{-7}$  M  $\text{Cd}^{2+}$ . The  $\text{Cu}^{2+}$  electrode performs even better than the  $\text{Cd}^{2+}$  electrode. The calibrations of the  $\text{Pb}^{2+}$  electrode were quite reproducible, especially above  $10^{-5}$  M  $\text{Pb}^{2+}$ , if the bubbles of nitrogen impinged vigorously on the electrode surface. The vigorous agitation caused by the bubbles



breaking against the electrode appears to provide an homogeneous zone that allowed the reproducible response. Reproducibility for this electrode means  $\pm 1$  millivolt, which causes an error of  $\pm 10-15\%$  in the free  $\text{Pb}^{2+}$  reading. The  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  electrodes are reproducible to  $\pm 0.2$  millivolts.

The FA-into- $\text{Pb}^{2+}$  titrations did not appear to be particularly useful, because the high initial  $C_{\text{Pb}}/C_{\text{FA}}$  values caused precipitation of some  $\text{Pb}^{2+}$ -FA solids of unknown stoichiometry. Some of these solids were resolubilized by the end of the titration, but one could not count on resolubilization happening. In fact, test-tube tests showed that excess fulvic acid added to a solution containing some  $\text{Pb}^{2+}$ -FA precipitates did not always redissolve the precipitates, even after weeks had passed.

The  $\text{Pb}^{2+}$ -into-FA titrations were at first fairly uneventful, except for the initial lack of reproducibility that preceded development of the nitrogen purging technique. The graphs of  $\text{Pb}^{2+}$  titration results looked much like those from  $\text{Cu}^{2+}$  titrations. Indeed, reports in the literature show that  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  stability constants with natural organic matter are similar (Stevenson, 1977; Guy and Chakrabarti, 1976; Takamatsu and Yoshida, 1978).

The binding curves for  $\text{Pb}^{2+}$  with SFA and with WFA show the expected effect of pH: the higher the pH, the greater the amount of binding. The binding curves for pH 4.0, 4.5, 5.0, and 6.0 appear in Figure 11 for SFA and in Figure 12

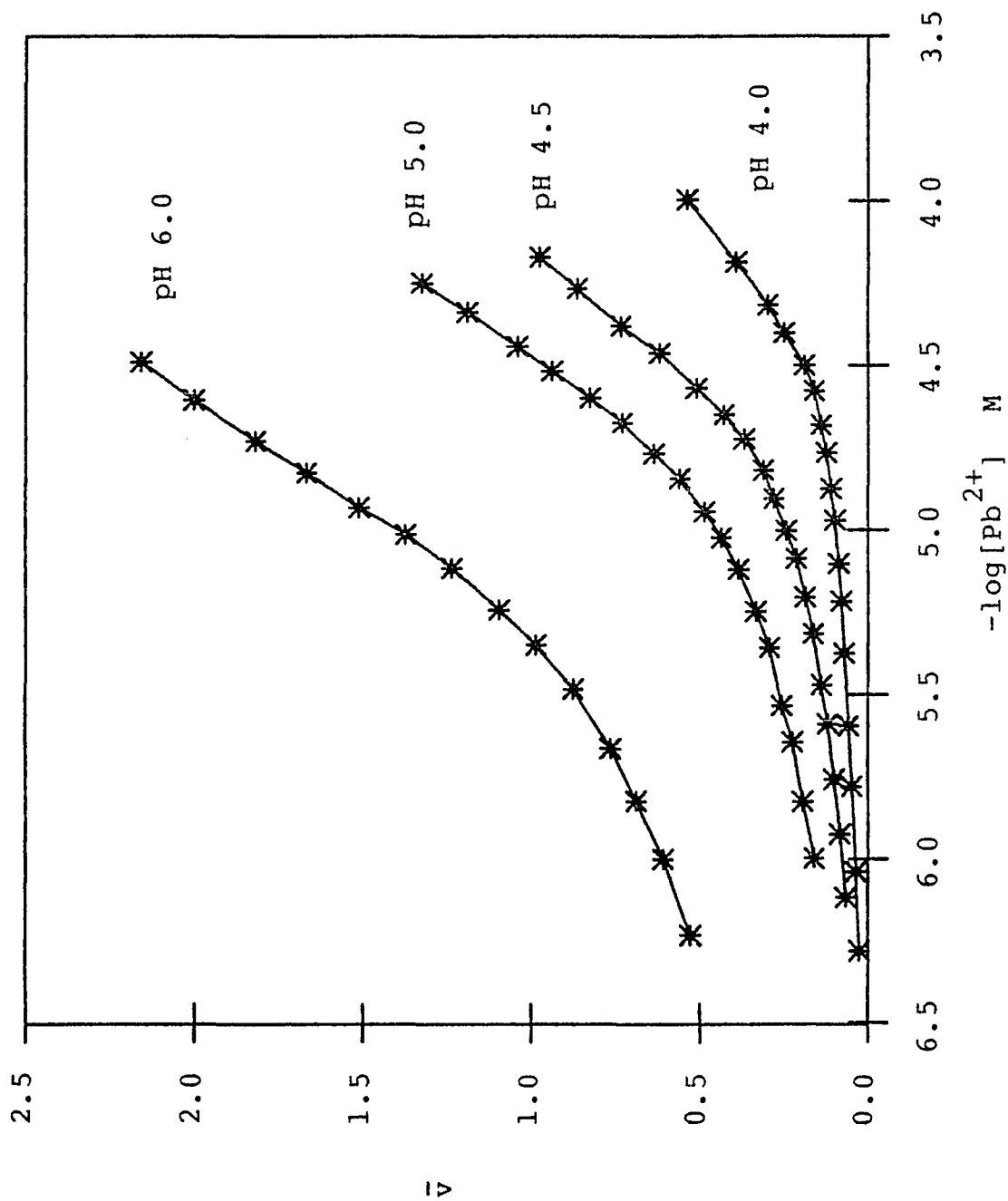


Figure 11. Formation curves for  $\text{Pb}^{2+}$  complexes with soil-derived fulvic acid (SFA).  $[\text{SFA}] = 5 \times 10^{-5} \text{ M}$ ,  $I = 0.1 \text{ M}$ ,  $T = 25^\circ\text{C}$ , and  $\bar{v} = (C_{\text{Pb}} - [\text{Pb}^{2+}]) / C_{\text{SFA}}$ . Precipitation of solid Pb-SFA aggregates occurred before the end of these titrations.

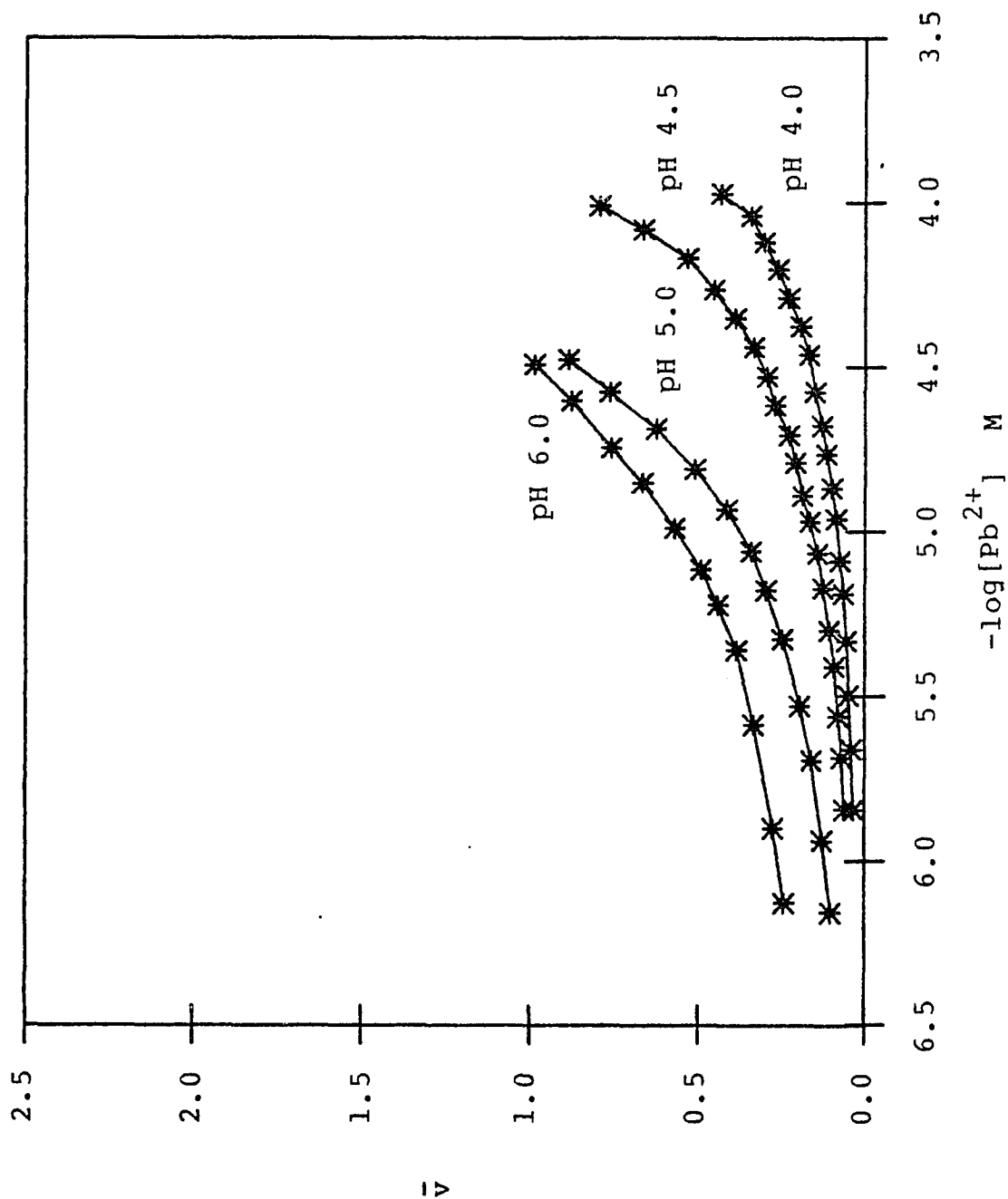


Figure 12. Formation curves for  $\text{Pb}^{2+}$  complexes with water-derived fulvic acid (WFA).  $[\text{WFA}] = 5 \times 10^{-5} \text{ M}$ ,  $I = 0.1 \text{ M}$ ,  $T = 25^\circ\text{C}$ , and  $\bar{v} = (\text{C}_{\text{Pb}} - [\text{Pb}^{2+}]) / \text{C}_{\text{WFA}}$ . Precipitation of solid Pb-WFA aggregates occurred before the end of these titrations.

for WFA. As with  $\text{Cd}^{2+}$ , more complexation occurs with SFA than with WFA. An explanation for at least part of this difference is that the total acidity of SFA (13.4 meq/g) is greater than is the total acidity of WFA (10.6 meq/g) (Weber and Wilson, 1975). Under natural conditions, however, the difference in complexing ability between SFA and WFA will be less than is indicated by these figures. The ionic strength of fresh waters is usually lower than that of soil-pore waters. The reduced competition of  $\text{K}^+$  for fulvic acid binding sites in dilute electrolyte (as in fresh water) will allow more  $\text{Pb}^{2+}$  to become bound.

The results of the calculations for conditional stability constants appear in Table 13. These values of  $B_1$  and  $B_2$  gave the lowest value for the function derived by Buffle et al. (1977). The  $\log B_1$  values increase as rapidly as the pH, indicating displacement of approximately one proton for each  $\text{Pb}^{2+}$ -FA complex formed. This value is somewhat larger than the value of 0.6 protons released during a  $\text{Pb}^{2+}$  complexation reaction as reported by Buffle et al. (1977) and several times larger than the comparable values calculated in Chapter 2 for  $\text{Cd}^{2+}$ -FA complexation.

A sample of the output from the minimization program along with the program itself appears in the Appendix. Early versions of the minimization program showed that the minimum value for the function often depended on the initial values supplied for  $B_1$  and  $B_2$ . That is, there were many local minima, and the procedure initially used could not

TABLE 13

Lead(II) conditional stability constants with fulvic acids derived from soil and water

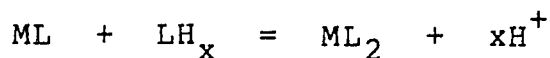
pH	<u>Pb-SFA</u>		<u>Pb-WFA</u>	
	$\log B_1^a$	$\log B_2^b$	$\log B_1^a$	$\log B_2^b$
4.0	4.0	-	-	-
4.5	4.3	9.1	3.7	8.8
5.0	4.9	9.5	4.7	9.3
6.0	6.3	10.1	5.1	10.1

$$^a B_1 = [\text{Pb-FA}] / [\text{Pb}^{2+}] [\text{FA}].$$

$$^b B_2 = [\text{Pb-(FA)}_2] / [\text{Pb}^{2+}] [\text{FA}]^2.$$

find the universal minimum. There are many local minima possibly because fulvic acid is a complicated mixture of ligands. For a simple ligand, a single minimum should be reached with any starting values of  $B_1$  and  $B_2$ . Low data precision caused by difficulties with the  $Pb^{2+}$  ion-selective electrode may also have contributed to difficulties in finding the universal minimum.

To find the best minimum for the fulvic acid system, the the final version of the program (BUF4.FOR) starts with 40 or more combinations of  $B_1$  and  $B_2$  ( $B_2$  always greater than  $B_1$ ) and finds the minimum for each combination. The output from the program lists up to the 10 best minimizations. Often, several of the starting  $B_1$  and  $B_2$  combinations came to virtually the same minimum; this is the case for the example in the Appendix. However, sometimes the program could not minimize well. For example,  $B_2$  might come out nearly equal to or even less than  $B_1$ . This means that the reaction



would have a near zero or negative equilibrium constant ( $B_2/B_1$ ). The failure to reach minimization results in values missing from Table 13. Furthermore, several values in the table show  $B_2/B_1$  that are greater than  $B_1$ . This is an unusual relationship for simple complexation systems and may result from the model being poorly suited to fulvic acid, from the lack of very precise data, or from

deficiencies in the minimization program.

For all these calculations, I used only data with  $C_{Pb}/C_{FA}$  that were low enough so that no precipitates had formed. The values for the precipitation thresholds, as determined by scattering, appear in Table 14. More discussion on precipitation of  $Pb^{2+}$ -FA complexes appears below.

The affinity of both fulvic acids for  $Pb^{2+}$  is clearly much greater than for  $Cd^{2+}$ , as can be seen by comparing conditional stability constants for 1:1 fulvic acid complexes with  $Cd^{2+}$ , listed in Table 5, and with  $Pb^{2+}$ , listed in Table 13. A more subtle problem is to distinguish the difference, if any, between  $Pb^{2+}$ -FA and  $Cu^{2+}$ -FA complexation. In an inorganic sense,  $Cu^{2+}$  and  $Pb^{2+}$  are not very similar, so there may be differences in the complexes they form with fulvic acid.

A clue to this difference appears in Figure 13 for SFA at pH 5.0 and in Figure 14 for WFA at pH 4.5. Each figure results from three titrations, one each with  $Cd^{2+}$ ,  $Cu^{2+}$ , and  $Pb^{2+}$  as the titrant. As noted above, the fulvic acids clearly respond differently to  $Cd^{2+}$  than to  $Cu^{2+}$  or  $Pb^{2+}$ : At any value of  $C_M/C_{FA}$ , there is substantially more free  $Cd^{2+}$  than  $Cu^{2+}$  or  $Pb^{2+}$ . Therefore, if these data are used to calculate stability constants, the  $Cd^{2+}$ -FA stability constant will be substantially smaller than the constants for  $Cu^{2+}$ -FA and  $Pb^{2+}$ -FA. SFA and WFA appear to act similarly toward  $Cu^{2+}$  and  $Pb^{2+}$  until  $C_M/C_{FA}$  equals about

TABLE 14  
Scattering thresholds for Pb-FA complexes

pH	<u>[FA] = 5 x 10<sup>-5</sup> M</u>		<u>[FA] = 2 x 10<sup>-4</sup> M</u>	
	C <sub>Pb</sub> /C <sub>SFA</sub> <sup>a</sup>	C <sub>Pb</sub> /C <sub>WFA</sub> <sup>b</sup>	C <sub>Pb</sub> /C <sub>SFA</sub> <sup>a</sup>	C <sub>Pb</sub> /C <sub>WFA</sub> <sup>b</sup>
4.0	1.2	2.1	0.45	0.55
4.5	0.95	1.4	0.5	0.50
5.0	0.8	1.2	0.55	0.50
6.0	0.90	0.90	0.90	0.65

<sup>a</sup> C<sub>Pb</sub> = total lead(II) concentration; C<sub>SFA</sub> = total soil-derived fulvic acid concentration.

<sup>b</sup> C<sub>WFA</sub> = total water-derived fulvic acid concentration.



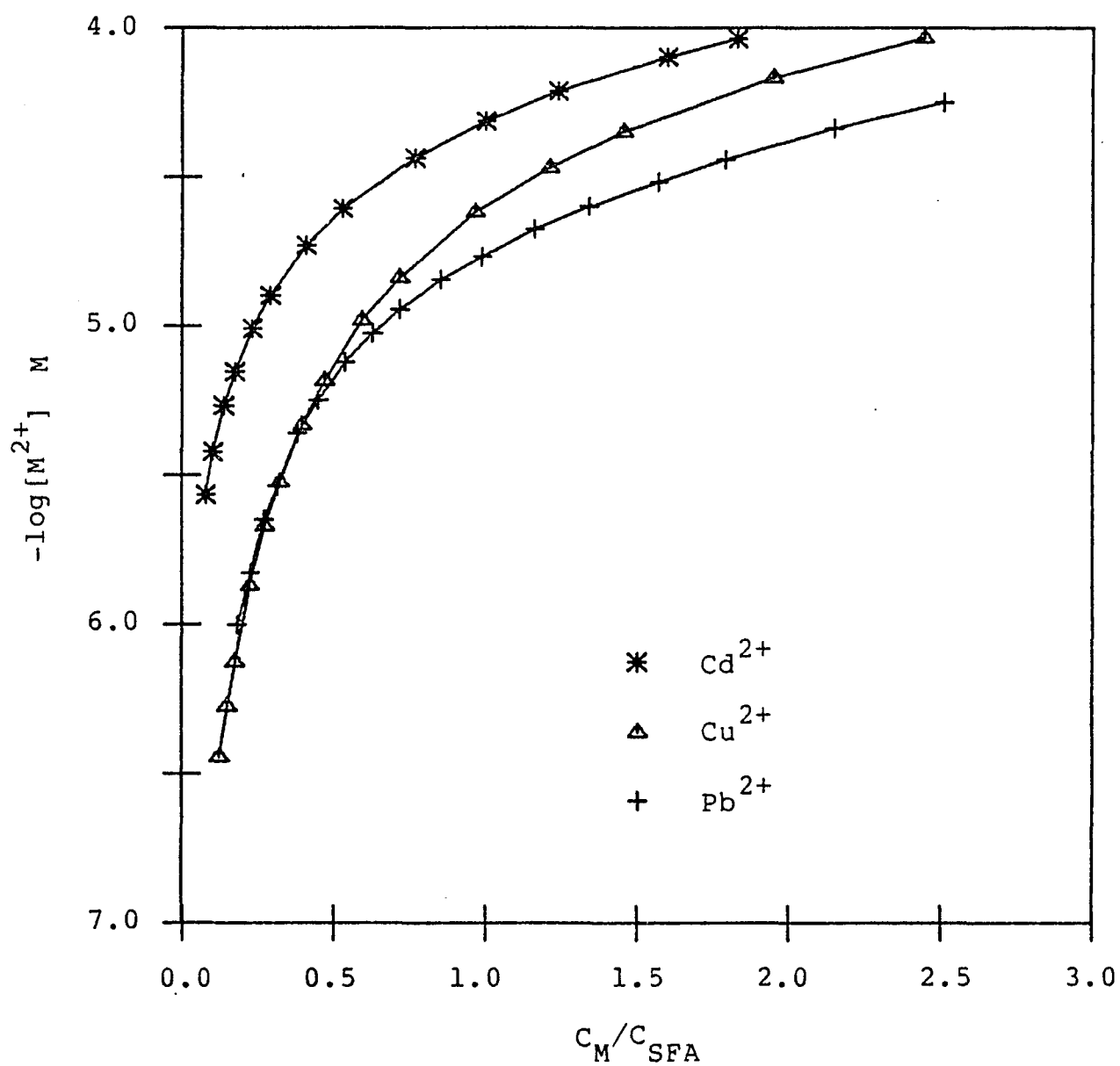


Figure 13. Free metal ion  $[M^{2+}]$  vs. mole ratio of total metal ion to total SFA ( $C_M/C_{SFA}$ ) for separate titrations of  $Pb^{2+}$ ,  $Cu^{2+}$ , and  $Cd^{2+}$  titrants. Soil-derived fulvic acid (SFA) concentration =  $5 \times 10^{-5}$  M, pH = 5.0, I = 0.1 M, and T = 25°C.

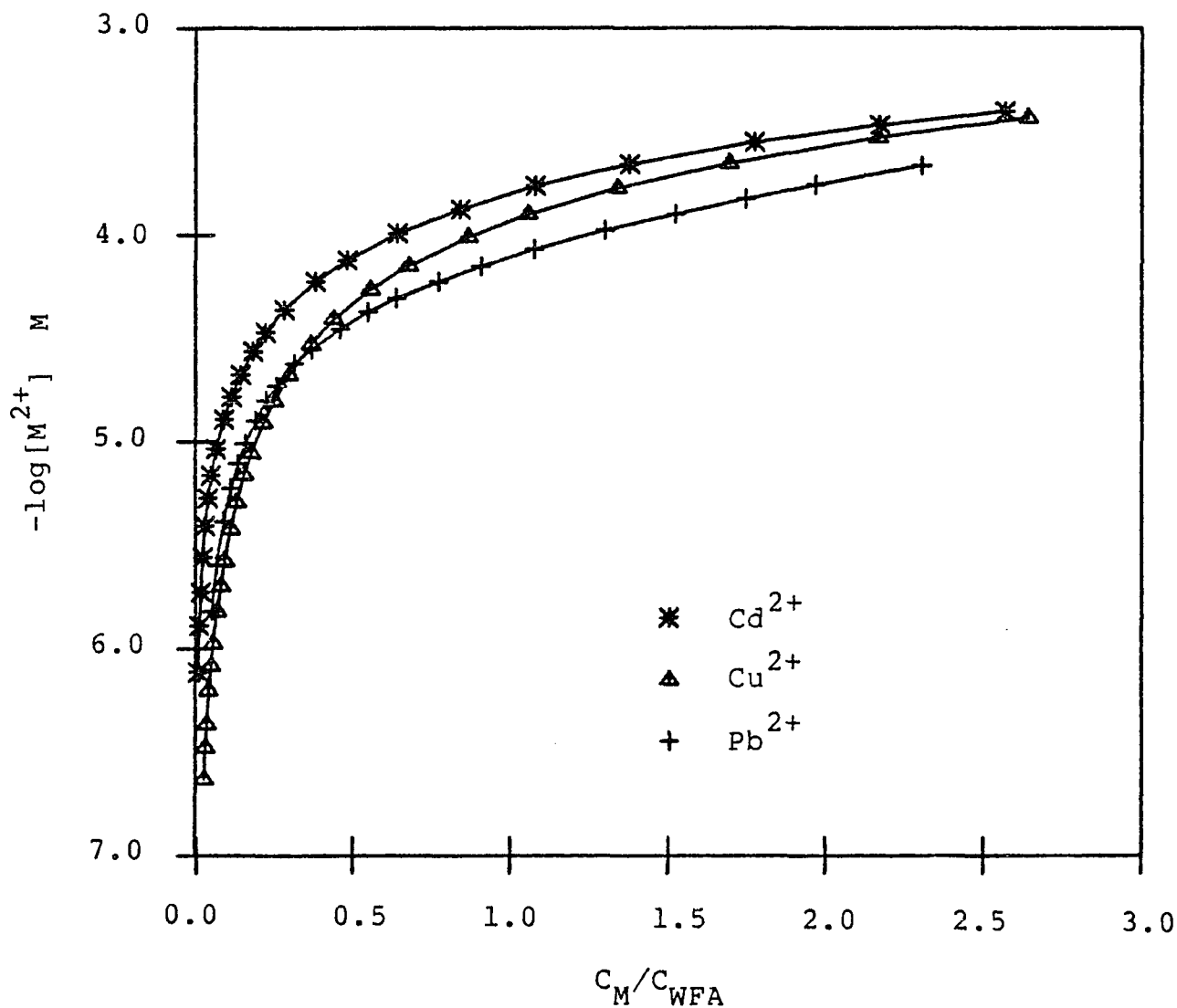


Figure 14. Free metal ion  $[M^{2+}]$  vs. mole ratio of total metal ion to total WFA ( $C_M/C_{WFA}$ ) for separate titrations of  $Pb^{2+}$ ,  $Cu^{2+}$ , and  $Cd^{2+}$  titrants. Water-derived fulvic acid (WFA) concentration =  $5 \times 10^{-5}$  M, pH = 4.5, I = 0.1 M, and T = 25°C.

0.5, after which there is less free  $Pb^{2+}$  in solution at any  $C_{Pb}/C_{FA}$  than there is free  $Cu^{2+}$  at the same value for  $C_{Cu}/C_{FA}$ . The next two figures (Figures 15 and 16) show the difference in  $Cu^{2+}$  and  $Pb^{2+}$  complexation by fulvic acids more dramatically. Before discussing these figures, there is need to discuss the rationale behind the choice of variables in the figures.

Work in our group (Bresnahan et al. 1978; Saar and Weber, 1979) and elsewhere (Burch et al., 1978) shows that FA contains a variety of sites with varying abilities to complex metal ions. Metal ions added early in a titration will bind to the strongest complexing sites. Metal ions in successive  $M^{2+}$  aliquots will have access to an ever smaller selection of FA complexation sites, so FA will bind a diminishing fraction of the added metal ions as a titration continues.

The variables included in Figures 15 and 16 are designed to test this model. The y-axis variable is the fraction of metal ions in each metal-ion aliquot that becomes bound as the aliquot mixes with the fulvic acid solution. This fraction can be expressed as  $d\bar{v}/d(C_M/C_{FA})$ , where  $\bar{v} = (C_M - [M^{2+}])/C_{FA}$ . The x-axis variable is the mole ratio of total metal ion to total fulvic acid ( $C_M/C_{FA}$ ) and is a measure of the progress of a titration. The data included in Figures 15 and 16 are the same as are included in Figures 13 and 14, except that the  $Pb^{2+}$  curve in Figure 15 is the average of three replicate titrations; the raw

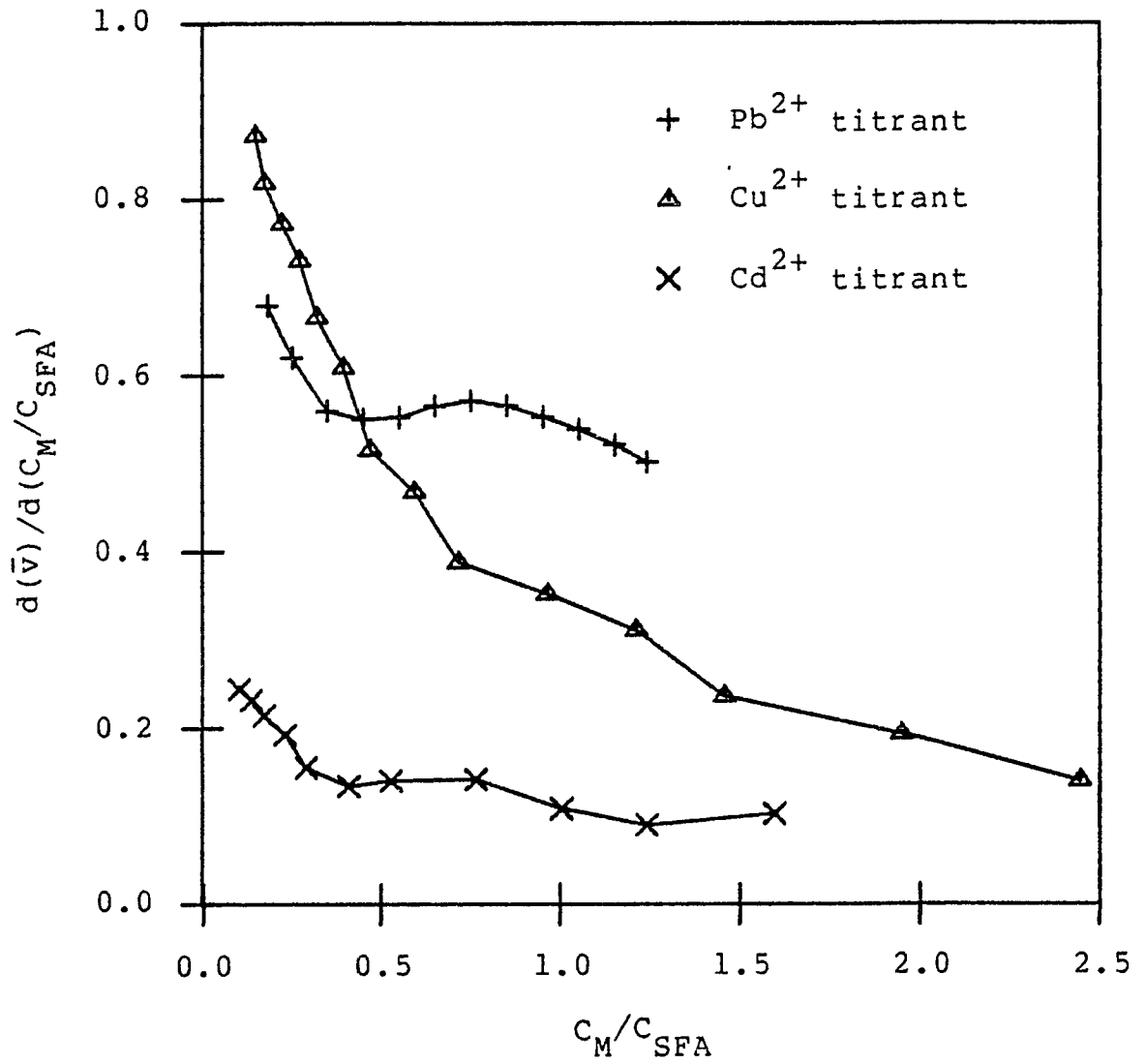


Figure 15. Results for the same titrations as in Figure 13. The y-axis variable is the fraction of all metal ions in each aliquot that becomes bound as it mixes with the soil-derived fulvic acid solution.  $\bar{v} = (C_M - [M^{2+}])/C_{SFA}$ .

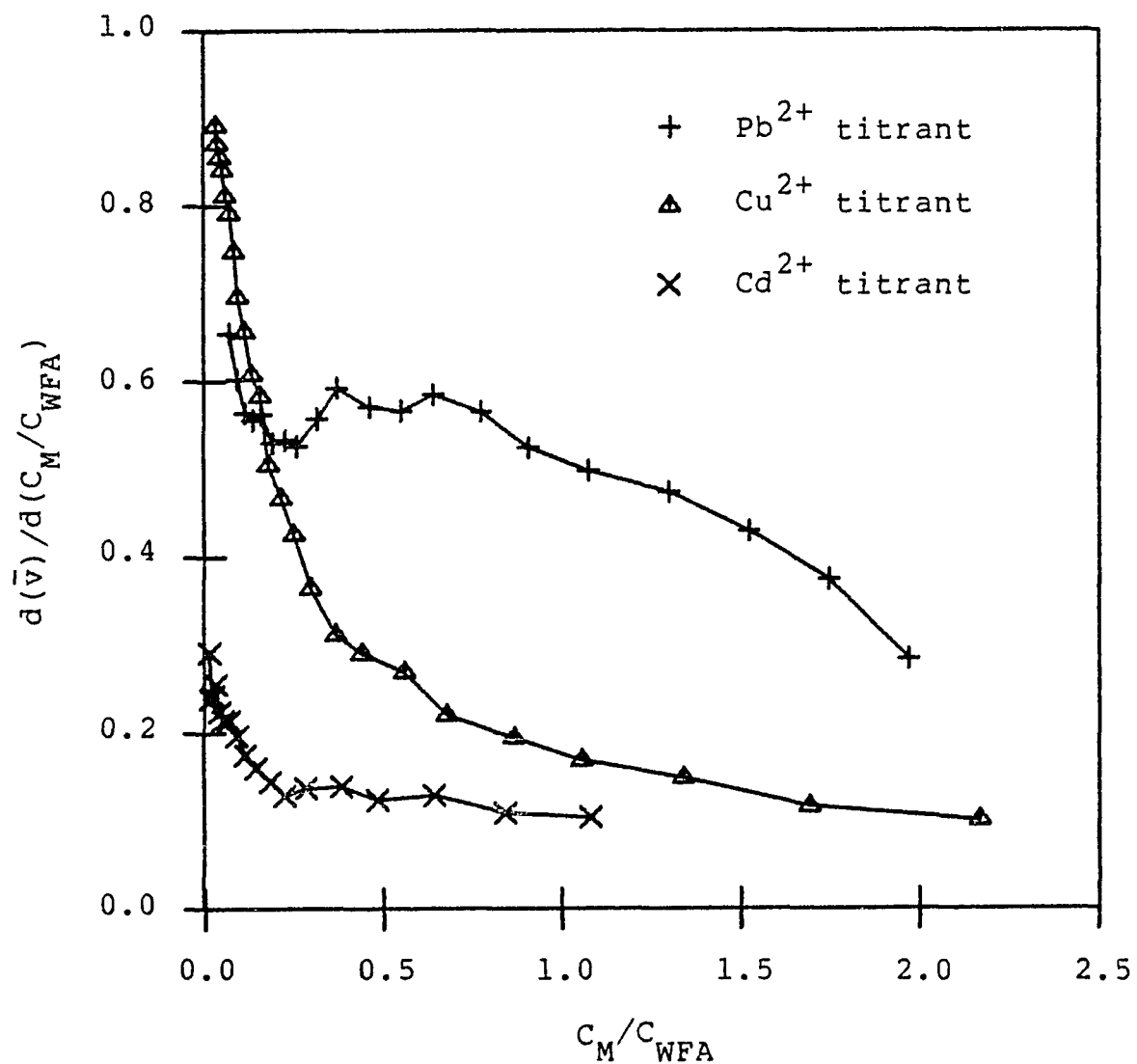


Figure 16. Results for the same titrations as in Figure 14. The y-axis variable is the fraction of all metal ions in each aliquot that becomes bound as it mixes with the water-derived fulvic acid solution.  $\bar{v} = (C_M - [M^{2+}])/C_{WFA}$ .

data for one of these replicates appears as the  $\text{Pb}^{2+}$  line in Figure 13. Within experimental error ( $\pm 2\%$  for  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$ ), the y-axis variable for the  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  titrations declines in all places as the titration proceeds, until the complexing capacity of fulvic acid for the metal ion has been reached. For the range of  $C_M/C_{\text{FA}}$  used here, the complexing capacity for  $\text{Cd}^{2+}$  is nearly reached (hence the leveling off of the  $\text{Cd}^{2+}$  line), whereas it is not reached for  $\text{Cu}^{2+}$ . Thus,  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  act according to the model of fulvic acid complexation described above.

The averaged  $\text{Pb}^{2+}$  results have a relative standard deviation of  $\pm 12\%$  at  $C_{\text{Pb}}/C_{\text{SFA}}$  below approximately 0.65 and  $\pm 5\%$  for  $C_{\text{Pb}}/C_{\text{SFA}}$  above 0.65. Many Pb-into-FA titrations with SFA and WFA at several pH values showed an increase in the y-axis variable during some part of the titration (Figures 15 and 16). Even for titrations in which there was no rise in this variable, after a certain  $C_{\text{Pb}}/C_{\text{FA}}$ , the difference in  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  performance is statistically significant: the fraction of added  $\text{Pb}^{2+}$  that appeared to become bound in  $\text{Pb}^{2+}$ -into-FA titrations dropped more slowly than did the fraction of added  $\text{Cu}^{2+}$  in  $\text{Cu}^{2+}$ -into-FA titrations.

An explanation for this behavior appeared upon comparison of two titrations with  $\text{Pb}^{2+}$  titrant: one with  $2 \times 10^{-4}$  M SFA and the other with  $5 \times 10^{-5}$  M SFA in the titration cell. Figure 17 shows these two titrations. The halt in the decline of the y-axis variable, which signals a

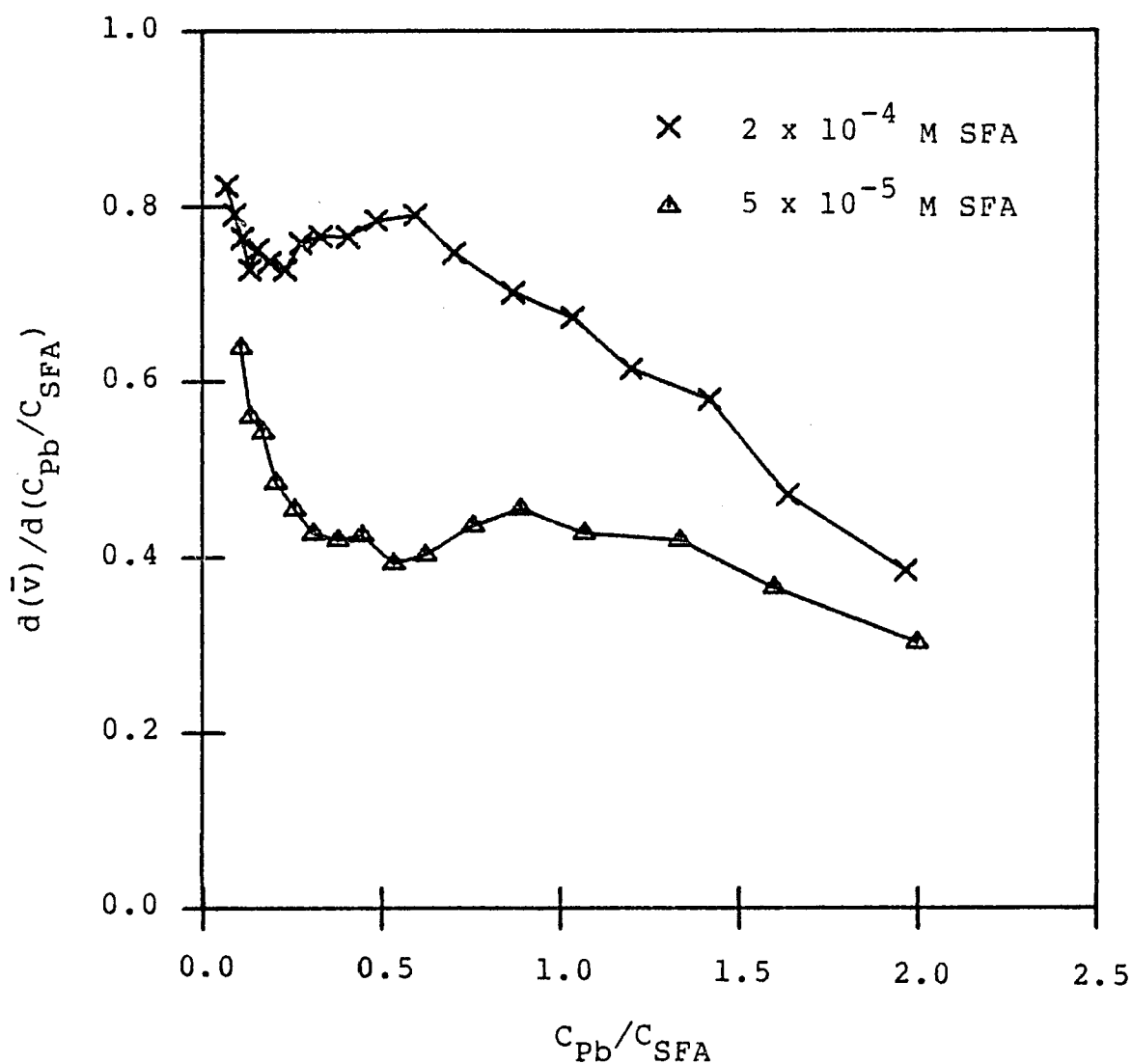


Figure 17. Titrations of  $Pb^{2+}$  into two concentrations of soil-derived fulvic acid (SFA).  $I = 0.1$  M,  $pH = 4.5$ ,  $T = 25^{\circ}C$ , and  $\bar{v} = (C_{Pb} - [Pb^{2+}])/C_{SFA}$ . The y-axis variable is the fraction of all  $Pb^{2+}$  in each aliquot that becomes bound as it mixes with the SFA solution.

change in the complexation behavior of  $\text{Pb}^{2+}$ -FA as compared to  $\text{Cu}^{2+}$ -FA or  $\text{Cd}^{2+}$ -FA, occurs at lower  $C_{\text{Pb}}/C_{\text{SFA}}$  for the more concentrated SFA solution than for the more dilute one. Precipitation of insoluble  $\text{Pb}^{2+}$ -FA complexes also occurs at lower  $C_{\text{Pb}}/C_{\text{FA}}$  for the more concentrated SFA solutions. Figure 18 shows, for the same conditions as in Figure 17, that solution scatter rises abruptly when a certain  $C_{\text{Pb}}/C_{\text{SFA}}$  is reached. These scattering thresholds correspond to nearly the same  $C_{\text{Pb}}/C_{\text{SFA}}$  value where the y-axis variable in Figure 17 stops declining or even starts rising. A list of scattering thresholds for SFA and WFA appears in Table 14.

The data, then, show that  $\text{Pb}^{2+}$  removal from solution increases when  $\text{Pb}^{2+}$ -FA begins to precipitate, implying mechanisms of  $\text{Pb}^{2+}$  removal other than complexation. These could be adsorption on or entrapment within developing aggregates (Ling Ong et al., 1970). The occurrence of such aggregates and the increase in FA molecular weight upon addition of metal ions are noted frequently in the literature (Stevenson, 1976, 1977; Sipos et al., 1978; Ramunni and Palmieri, 1975; Jackson and Skippen, 1978). Even so, the  $\text{Pb}^{2+}$  binding curve begins to drop again as usable complexation sites become occupied and possibly as the amount of new  $\text{Pb}^{2+}$  adsorption or entrapment declines.

The unusual curve shape for the  $\text{Pb}^{2+}$ -FA system occurs for both our soil-derived and water-derived fulvic acids (Figures 15 and 16), so the results are not just a peculiarity of one FA sample. However, this unusual



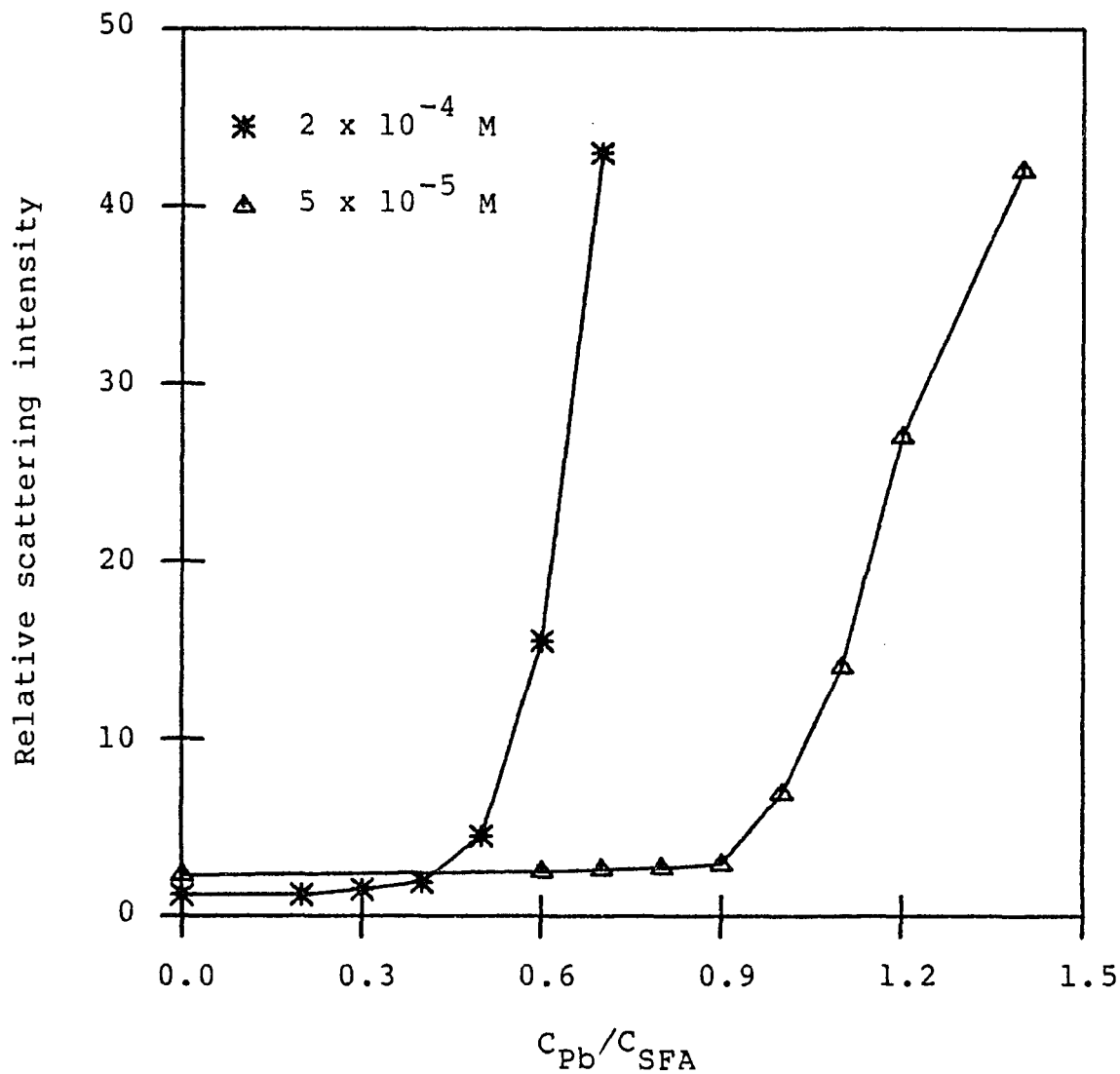


Figure 18. Scattering at 400 nm of pH 4.5 solutions containing  $Pb^{2+}$  and two different concentrations of soil-derived fulvic acid (SFA).  $I = 0.1$  M and  $T = 25^{\circ}C$ .

behavior partly disappears at pH 6.0. It seems reasonable that hydrogen ion aids aggregate formation, perhaps in hydrogen-bonding bridges (Green and Manahan, 1977). Even so, the value of  $d\bar{v}/d(C_M/C_{FA})$  for  $Pb^{2+}$  at pH 6.0 still decreases more slowly than it does for  $Cu^{2+}$ , so  $[H^+]$  is not the only important factor.

Insoluble complexes of FA and either  $Cu^{2+}$  or  $Cd^{2+}$  can also form (MacCarthy and O'Connell 1974; Whitworth and Pagenkopf 1979), but only when the metal ions are in large excess: a 4-fold excess for  $Cu^{2+}$  and a 20-fold excess for  $Cd^{2+}$  in a  $5 \times 10^{-5}$  M solution of our SFA. These scattering thresholds are shown along with that for  $Pb^{2+}$  in Figure 19. The unusual curve shape we see for the  $Pb^{2+}$ -FA system in Figures 15 and 16 may occur for the  $Cu^{2+}$ -FA and conceivably for the  $Cd^{2+}$ -FA systems, but to see such effects for  $Cu^{2+}$  or  $Cd^{2+}$ , one would have to add large quantities of metal ion. Data from the latest part of such a titration would have large errors because the FA complexing capacity would be far exceeded and  $[M^{2+}]$  would be only slightly smaller than  $C_M$ .

### CONCLUSIONS

Lead ion, then, is unique among these three metal ions: Only  $Pb^{2+}$ -FA begins precipitating before the FA complexing capacity for the metal ion has been reached. Work in this laboratory with  $Cu^{2+}$ -FA complexes shows there are two or

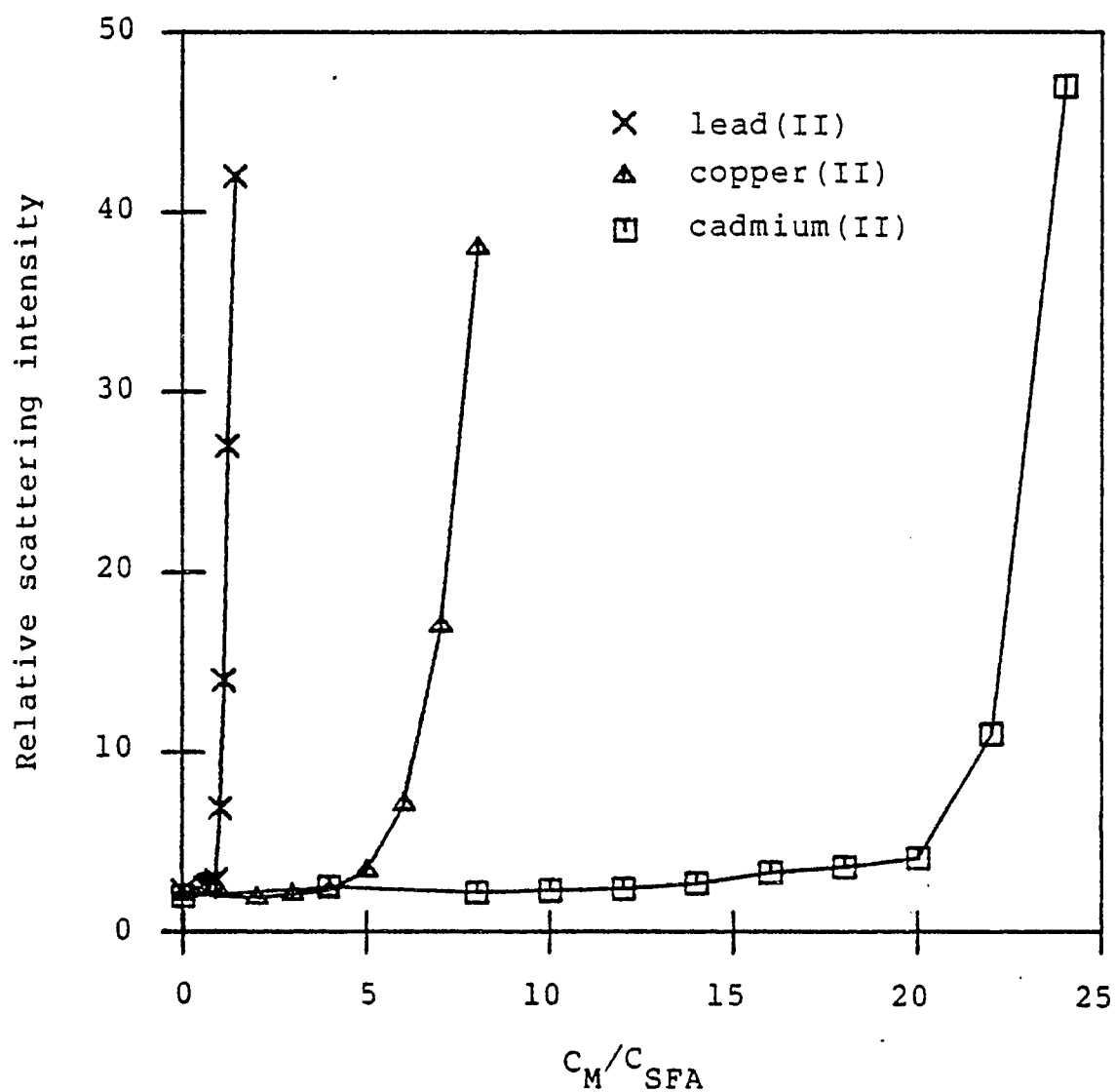


Figure 19. Scattering at 400 nm of pH 4.5 solutions containing  $5 \times 10^{-5}$  M SFA and either  $Pb^{2+}$ ,  $Cu^{2+}$ , or  $Cd^{2+}$ . SFA is soil-derived fulvic acid.  $I = 0.1$  M and  $T = 23^\circ C$ .

more  $\text{Cu}^{2+}$  complexation sites per fulvic acid molecule (Bresnahan et al., 1978). That is, during  $\text{Cu}^{2+}$ -into-FA titrations,  $\bar{v}$  values rise above 2.0 and no  $\text{Cu}^{2+}$ -FA precipitation results. If we assume that most of the complexation sites available to  $\text{Cu}^{2+}$  are available to  $\text{Pb}^{2+}$ , it appears that Pb-FA solids settle out with complexation sites on the FA unfilled. It is not clear whether formation of the precipitate blocks these unfilled sites. It would not be unreasonable to speculate that some of these sites are still available, thereby making Pb-FA solids unusually good at sequestering free metal ions--both through surface adsorption to the solid and through chemical chelation. Indeed, the fraction of  $\text{Pb}^{2+}$  ions in each aliquot that becomes bound remains significantly higher than does the fraction of  $\text{Cu}^{2+}$  ions that become bound for high  $C_M/C_{FA}$ .

The work described in Chapter 2 showed an unusual property of  $\text{Cd}^{2+}$ -FA complexes. Their conditional stability constants increase as the FA concentration drops below about  $1 \times 10^{-4}$  M, in contrast to  $\text{Cu}^{2+}$ -FA complexes, whose stability constants vary little with changing FA concentration. It appears that  $\text{Pb}^{2+}$  binds to FA much the way  $\text{Cu}^{2+}$  does at low  $C_M/C_{FA}$ : FA concentration does not influence complexation. But at higher  $C_M/C_{FA}$ , a change in FA concentration may noticeably alter the degree to which  $\text{Pb}^{2+}$  is removed from solution. The extra removal of  $\text{Pb}^{2+}$  from solution may be important in the study of  $\text{Pb}^{2+}$  movement in waters that are polluted or rich in organic matter, and

in soils to which sludges containing metal ions have been added.

## CHAPTER 4

### FLUOROMETRIC ANALYSIS OF COMPLEXES BETWEEN METAL IONS AND FULVIC ACID

#### INTRODUCTION

The primary method of analysis described in Chapters 2 and 3 is ion-selective electrode potentiometry. The electrode measures the concentration of free metal ion; the concentration of bound metal ion is the difference between the total metal ion concentration and the free metal ion concentration. The stability constants for metal ion complexes with fulvic acid can be determined from the concentrations of bound metal ion, free metal ion, and fulvic acid (both free and complexed), and from the stoichiometry or stoichiometries of the complexes.

Several books describe the theory behind fluorescence of organic compounds (Parker, 1968; Hercules, 1966; Guilbault, 1973). Generally, compounds with aromatic structures or delocalized electrons can fluoresce.

Fluorescence emission is only one of several ways in which a molecule can give off excess energy it has absorbed. Excess energy may be dissipated nonradiatively, as through collisions between molecules, or by radiative means other than fluorescence such as phosphorescence. Heavy metal ions and paramagnetic metal ions (those with unpaired electrons) enhance phosphorescence emission and therefore reduce the amount of fluorescence.

Fluorescence analysis should complement ion-selective electrode analysis of complexes between fulvic acid and metal ions if fulvic acid fluoresces, and if changes in fluorescence are proportional in some way to the amount of complex formed. The work described in this chapter shows that both of these requirements are met and that fluorescence analysis of fulvic acid complexes with certain metal ions is possible.

The literature contains ample evidence that humic materials fluoresce. Seal and coworkers (1964) excited various fractions of soil organic matter at 365 nm and found emission peaks at 520 to 540 nm. Mueller-Wegener (1977) recorded fluorescence spectra from three soil-derived humic acids and found that pH had a different effect on the spectra of the three samples. In addition, for a given emission peak, the excitation wavelength was different. The author concludes that the fluorescence properties of humic acid depend in large part on the phenolic -OH content.

Several groups have reported the fluorescence of

organic matter dissolved in water. Many of these studies aim to show a relation between concentration of dissolved organic matter and intensity of fluorescence (Black and Christman, 1963; Ghassemi and Christman, 1968; Smart et al., 1976). Brun and Milburn (1977) describe an automated technique for determining such fluorescence.

The amount of fluorescence work on interactions between metal ions and humic materials is small. This lack of data is surprising because fluorescence has been used extensively to probe the binding of metal ions to biological molecules (Chen, 1976). For example, Argauer and White (1964) analyzed zirconium-flavenol and thorium-morin complexes with fluorescence. The binding of  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  to human transferrin has also been studied this way (Lehrer, 1969). One group (Banerjee and Mukherjee, 1972), studying the effect of metal ions on soil-derived humic and fulvic acids, found that  $\text{Cu}^{2+}$  and  $\text{Fe}^{2+}$  quenched the humic fluorescence most, and  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  quenched less.  $\text{Zn}^{2+}$  enhanced the fluorescence emission at 470 nm. Lévesque (1972) notes that humic fractions of high molecular weight containing iron have little fluorescence.

Cline and Holland (1977) published an extensive study on the reactions of  $\text{Cu}^{2+}$  and  $\text{Co}^{2+}$  with pore waters extracted from lake sediments. They found a large decrease in organic matter fluorescence when  $\text{Cu}^{2+}$  was added and a much smaller decrease when  $\text{Co}^{2+}$  was added. They also note an increase in light absorption by the solution upon addition of  $\text{Cu}^{2+}$ , but



no increase in absorption when  $\text{Co}^{2+}$  was added. They conclude that  $\text{Cu}^{2+}$  ions form strong, reversible complexes with the organic matter; in contrast,  $\text{Co}^{2+}$  is adsorbed on the surface and is not complexed.

The pore-water organic matter that Cline and Holland used is evidently rather different from the fulvic acids studied in this dissertation. Their sediment organic matter has a high  $\text{Ca}^{2+}$  concentration; organic matter complexes containing  $\text{Ca}^{2+}$  may be positively charged and flocculate when the pH is raised (when there is enough hydroxide to neutralize the positive charge on the complex). The fulvic acids discussed here do not respond in this way. The ash content of both SFA and WFA is very low (Weber and Wilson, 1975), and the fulvic acids stay in solution over a wide range of pH values--at least 1.4 to 10.

The work described in this chapter is more quantitative than earlier work on humic fluorescence. It first confirms that our fulvic acid does fluoresce and then endeavors to show that the reduction in fluorescence in the presence of metal ions is proportional to the concentration of bound metal ion. I tried to show this relation by comparing fluorescence results with those from ion-selective electrode experiments for any of the three metal ions studied in this group by ion-selective electrode:  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ , and  $\text{Pb}^{2+}$ . Once this relation between fluorescence quenching and metal binding was established, it remained only to apply the technique to complexation studies for metal ions such as

$\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Mn}^{2+}$  for which there are no electrodes.

## EXPERIMENTAL

Materials. The two fulvic acids, one derived from a podzol soil and the other from a freshwater river are described briefly in Chapter 1 and in more detail elsewhere (Weber and Wilson, 1975; Wilson and Weber, 1977a, 1977b). The electrolyte for all experiments was  $\text{KNO}_3$ , prepared from Mallinckrodt or Baker crystals. Acid solutions were made from Baker reagent grade (15.9 M) nitric acid, and base solutions were made from Baker 45% KOH.

The sources of metal ion were Fisher 1000 ppm atomic absorption standards:  $\text{Cu}^{2+}$  (SO-C-194),  $\text{Pb}^{2+}$  (SO-L-21),  $\text{Cd}^{2+}$  (SO-C-118),  $\text{Ni}^{2+}$  (SO-N-70),  $\text{Mn}^{2+}$  (SO-M-81), and  $\text{Co}^{2+}$  (SO-C-193). During this work, three compounds served as models for fulvic acid. They were salicylic acid (Mallinckrodt crystals), 3,5-dinitrosalicylic acid (Baker), and 5-nitrosalicylic acid, prepared by the method of Barany and Pianka (1946) and later obtained from Eastman.

Apparatus. A Nuclepore polycarbonate filtration apparatus and Nuclepore 0.4 micrometer polycarbonate filters removed particulate matter present in the fulvic acid solutions. I measured pH with a Corning model 476050 combination electrode attached to an Orion model 701A pH/mv meter. Small volumes of solution were delivered by Gilson,

Eppendorf, and Oxford pipets. The fluorescence spectra were obtained with a Perkin-Elmer model 204 fluorescence spectrophotometer and recorded on a Heath strip-chart recorder. Fulvic acid samples and the two salicylic acid derivatives were analyzed in a glass cuvette; the salicylic acid samples were analyzed in a quartz cuvette. A Shimadzu Spectronic 200 UV spectrophotometer measured the absorbance of solutions.

Procedures. For nearly all experiments involving fulvic acid, I weighed out a sample of fulvic acid powder and dissolved it in 0.1 M aqueous  $\text{KNO}_3$ . After adjusting the sample pH to the value of the coming experiment, I filtered it through the Nuclepore apparatus. Then, to 5.0 mL fulvic acid aliquots which were in 12-mL Nalgene polyethylene vials, I added various amounts of metal ion, depending on the mole ratio of total metal ion to total fulvic acid to be achieved. I then adjusted the pH of the solution and added electrolyte until the total solution volume was  $6.00 \pm 0.05$  mL. The final fulvic acid concentration for all samples (except those where the effect of fulvic acid concentration on fluorescence was checked) was  $5 \times 10^{-5}$  M, or approximately 32 ppm. The temperature in the spectrofluorometer room was  $22-23^\circ\text{C}$ ; I allowed the samples to equilibrate at that temperature for one hour or more before putting them into the cuvettes for analysis.

Because so little is known about fluorescence analysis of complexes containing metal ions and fulvic acid, there

were many experiments to do.

(1) I needed to determine the best wavelength for excitation and for emission. I checked excitation wavelengths from 320 nm to 500 nm.

(2) The electrolyte might affect fulvic acid fluorescence. I checked FA fluorescence at two levels of electrolyte:  $4.2 \times 10^{-4}$  M and  $7.5 \times 10^{-2}$  M  $\text{KNO}_3$ .

(3) Oxygen is paramagnetic, and as such might quench fulvic acid fluorescence. I purged a pH 4.9 sample of  $6 \times 10^{-5}$  M SFA for 40 minutes with nitrogen and compared its fluorescence intensity with a pH 5.2 unpurged SFA sample of the same concentration. An earlier test showed that purging the carbon dioxide (along with the oxygen) raised a  $6 \times 10^{-5}$  M SFA solution from pH 4.9 to pH 5.2.

(4) To see if fluorescence intensity was proportional to fulvic acid concentration, I prepared solutions that were 0, 10, 20, 30, 40, and 50 ppm in SFA, and measured the fluorescence.

(5) To measure the effect of pH on fulvic acid fluorescence, I prepared series of SFA and WFA samples with pH values ranging from 1.5 to about 8 in steps of 0.3 to 0.5 pH units, and measured the fluorescence for each.

(6) I performed a series of titrations with various ions to see the effect each had on fulvic acid fluorescence. The ions included  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{I}^-$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$ . The important experimental details are listed in Table 15. Experiments were performed with SFA and with WFA.

TABLE 15  
Conditions for fluorescence experiments<sup>a</sup>

Date	Ion	[ligand] <sup>b</sup>		C <sub>M</sub> /C <sub>L</sub>	Number of samples	pH
		(M x 10 <sup>5</sup> )				
9/12/79	Pb	4.9	WFA	0.0-1.3	8	5.0
9/19/79	Cu	4.9	SFA	0.0-5.0	14	5.0
9/20/79	Pb	4.9	SFA	0.0-1.0	6	5.0
10/24/79 <sup>c</sup>	Pb, Cu	4.9	SFA	0.0-1.0	17	5.0
10/25/79 <sup>c</sup>	Pb, Cu	4.9	SFA	0.0-1.0	7	5.0
10/31/79 <sup>c</sup>	Pb, Cu	4.9	SFA	0.0-1.0	28	4.5
11/02/79 <sup>c</sup>	Pb, Cu	4.9	SFA	0.0-1.0	22	6.0
11/06/79	Cu	4.9	SFA	0.0-5.0	19	3.0
11/07/79	Cu	4.9	SFA	0.0-5.0	19	6.0
11/09/79	Cu	4.9	SFA	0.0-4.0	12	1.5
11/14/79	Cu	4.9	SFA	0.0-4.0	17	4.5
	Pb	4.9	SFA	0.0-1.0	10	4.5
11/30/79	Cu	5.1	SFA	0.0-4.0	16	4.0
12/04/79	Cu	4.9	SFA	0.0, 3.0	36	1.5-7.9
12/06/79	I <sup>-</sup>	4.9	SFA	0.0-6.0	5	3.0
	I <sup>-</sup>	4.9	SFA	0.0-8.0	6	5.0
12/12/79	Pb	5.1	WFA	0.0-1.0	10	4.0
	Pb	5.1	WFA	0.0-1.0	10	4.5
12/13/79	Pb	5.1	WFA	0.0-1.0	10	5.0
	Pb	5.1	WFA	0.0-1.0	10	6.0
12/14/79 <sup>d</sup>	Cu	4.9	SFA	0.0, 3.0	30	3.0-6.2
12/17/79	Ni	5.1	SFA	227	1	1.45
	Mn	5.1	SFA	243	1	1.45
	Co	5.1	SFA	227	1	1.41
	Pb	5.1	SFA	65	1	1.44
	Cu	5.1	SFA	209	1	1.47
	Cd	5.1	SFA	119	1	1.42

Table 15, continued

12/18/79 <sup>e</sup>	Ni, Co	5.1	SFA	0.0, 3.0	24	2.0-8.0
12/19/79 <sup>e</sup>	Ni, Co Cu, Pb	15.	SA	0.0, 1.0	12	1.5, 5.0
12/20/79	Cu	15.	SA	0.0-15.6	10	5.0
12/27/79	Cu	15.	SA	0.0-16	10	6.0
1/2/80	Cd	4.9	SFA	0.0-15.0	11	7.5
1/4/80	Cu	13.	NSA	0, 1, 10.4	3	6.4
	Ni	13.	NSA	10	1	6.4
1/8/80	Ni	13.	NSA	0.0-11.0	11	7.0-7.5
1/9/80	Ni	13.	DSA	0.0-10.0	7	6.0
	Cu	13.	DSA	0.0-4.0	8	5.0
1/11/80	Cu	5.0	WFA	0.0, 3.0	37	1.5-8.0
1/18/80	Ni	5.0	SFA	0.0-10.0	12	6.0
	Co	5.0	SFA	0.0-10.0	11	6.0
1/19/80	Ni	5.0	SFA	0.0-14.0	12	4.0
	Co	5.0	SFA	0.0-14.0	11	4.0
1/23/80	Ni	5.6	SA	0.0-178	6	8.0
	Co	5.6	SA	0.0-181	5	8.0
1/25/80 <sup>f</sup>	none	0-8	SFA	0.0	7	5.0
1/28/80	Ni	6.0	SA	0.0-178	6	5.0
	Co	6.0	SA	0.0-181	5	5.0
1/29/80 <sup>g</sup>	none	5.0	SFA	0.0	7	4.0-7.7
1/30/80	Ni	5.6	SA	0.0-145	6	7.9
	Co	5.6	SA	0.0-145	5	7.9
	Ni	5.6	SA	0.0-145	6	5.0
	Co	5.6	SA	0.0-145	5	5.0
2/7/80 <sup>h</sup>	none	6.0	SFA	0.0	2	5.2

Table 15, continued

- <sup>a</sup> All experiments were done in 0.1 M KNO<sub>3</sub> at 23°C.
- <sup>b</sup> Abbreviations: WFA (water-derived fulvic acid); SFA (soil-derived fulvic acid); SA (salicylic acid); NSA (5-nitrosalicylic acid); DSA (3,5-dinitrosalicylic acid).
- <sup>c</sup> Pb and Cu ions mixed together in samples.
- <sup>d</sup> Ten samples from 12/04/79 each excited at 320, 350, and 400 nm.
- <sup>e</sup> No more than one metal-ion type in any single sample.
- <sup>f</sup> Test of the effect of fulvic acid concentration on fluorescence intensity.
- <sup>g</sup> Data used together with data of 12/04/79 to demonstrate effect of pH on SFA fluorescence.
- <sup>h</sup> Test of effect of oxygen on fulvic acid fluorescence.

The goal of this research was to show whether the loss of fluorescence was specific to the amount of metal ion bound to the fulvic acid. If it was, the technique would be very useful. However, if uncomplexed metal ion caused substantial fluorescence quenching, the technique would have limited use.

(7) Checking the correspondence between fulvic acid fluorescence quenching and the amount of bound metal ion required using model compounds. Such a compound must fluoresce and it must be possible to calculate the amount of complex formed at various pH values and metal-ion concentrations. These model compounds were salicylic acid, 5-nitrosalicylic acid, and 3,5-dinitrosalicylic acid. Their proton association constants and stability constants for complexes of these ligands with  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$  appear in the literature (Martell and Smith, 1977) and are included in Table 16.



TABLE 16  
Association constants for  
fulvic acid and model compounds<sup>a</sup>

<u>Ligand</u>	<u>Proton association constant</u>		<u>Log of the stability constant</u> <sup>b</sup>		
	log K <sub>a1</sub>	log K <sub>a2</sub>	Cu <sup>2+</sup>	Ni <sup>2+</sup>	Co <sup>2+</sup>
salicylic acid <sup>c</sup>	2.8	13.4	10.62	6.95 <sup>d</sup>	6.72 <sup>d</sup>
5 nitro-salicylic acid <sup>c</sup>	2.2	10.11	8.40	5.87	5.57
3,5-dinitro-salicylic acid <sup>c</sup>	2.14	7.22	6.85	4.45	4.05
soil-derived fulvic acid	4.4	-	-	-	-
hydroxide <sup>e</sup>	-	-	-7.53	-9.40	-9.60

<sup>a</sup> References: All constants for the salicylic acids (Martell and Smith, 1977); Soil-derived fulvic acid (Truitt, R. E., personal communication); Hydroxide (Huheey, 1978).

<sup>b</sup> For the reaction  $M^{2+} + L^{2-} = ML$ .

<sup>c</sup> At 25°C at 0.1 M ionic strength except where noted.

<sup>d</sup> At 20°C at 0.15 M ionic strength.

<sup>e</sup> For the reaction  $[M(H_2O)_6]^{2+} = [M(H_2O)_5OH]^+ + H^+$ .

## RESULTS AND DISCUSSION

A striking feature of fulvic acid fluorescence is that the peak wavelength of emission moves to a longer wavelength as the wavelength of excitation is lengthened. Such a dependence of the maximum emission wavelength on the excitation wavelength means that fulvic acid is a mixture of fluorescing compounds (Parker, 1968). As the excitation wavelength is lengthened (that is, as the light becomes lower in energy), certain fluorophores are no longer excited, so the range of emissions changes. In the range of 320 nm to 500 nm, the maximum emission occurs upon excitation at 350 nm. This excitation wavelength causes maximum emission at 445-450 nm. Except where noted, the excitation and emission wavelengths of the fulvic acids and their model compounds were as follows:

	<u>Excitation</u>	<u>Emission</u>
SFA, WFA	350 nm	445-450 nm
Salicylic acid	305	403
5-nitrosalicylic acid	370	420
3,5-dinitrosalicylic acid	400	460

The emission intensity of the fulvic acids is not as great as that of salicylic acid, but it is stronger than that of 5-nitrosalicylic acid or 3,5 dinitrosalicylic acid. The fulvic acid emission band is much broader than the emission band for salicylic acid or its derivatives, another indication that fulvic acid contains a mixture of

fluorophores. The fulvic acid emission band is also featureless.

The fluorescence of soil-derived fulvic acid is the same in either  $4.2 \times 10^{-4}$  M or  $7.5 \times 10^{-2}$  M  $\text{KNO}_3$ . Even if changes in ionic strength or  $\text{KNO}_3$  concentration had influenced the fluorescence, the experiments would still have been valid, because all work was done under the same conditions, in 0.1 M  $\text{KNO}_3$ . A change in electrolyte concentration, however, would affect the amount of metal-ion complexation (Stevenson, 1977), which would alter the amount of fluorescence quenching.

There was no difference in fluorescence between the SFA sample that was purged of oxygen and the sample that was not.

Fluorescence intensity is not proportional to fulvic acid concentration over the range of 0 to 50 ppm. The relative peak heights are 0 ppm (0), 10 ppm (39), 20 ppm (70), 30 ppm (93), 40 ppm (110), and 50 ppm (120). The non-linearity indicates that the excitation or emission light or both is being partly absorbed. Experiments with the Shimadzu spectrophotometer show that the absorbance for a  $6 \times 10^{-5}$  M SFA solution in 0.1 M  $\text{KNO}_3$  is 0.055 at 450 nm (near the emission maximum) and 0.25 at 350 nm (the excitation wavelength for fluorescence experiments). This SFA solution is about 15% more concentrated than the solutions used for the fluorescence experiments, which were  $5 \times 10^{-5}$  M in SFA. At 450 nm, the absorbance is just low

enough so that the amount of self absorption would cause minimal curvature in the calibration. However, the absorption at 350 nm is high, so it is the incoming light that is being strongly absorbed. All the experiments were done at one fulvic acid concentration, so that the attenuation of source intensity in the solution is constant and does not affect the results. This self absorption would make it difficult to compare results of experiments having different fulvic acid concentrations.

The intensity of fluorescence emission from SFA and WFA varies with pH. The data represented by triangles in Figure 20 for SFA and in Figure 21 for WFA show the pH effect. The maximum emission intensity occurs at about pH 5, with a fairly steep drop in intensity as the pH drops below 4. The emission at pH 1.5 is two-thirds that at pH 5. Above pH 5, there is a gentle decline in emission intensity so that at pH 7.5-8, the emission is 85-90% that at pH 5. It appears that fulvic acid fluoresces best when the strongest acid groups ( $pK_a = 1-4$ ) are dissociated. Although the effect is not as strong, the deprotonation of weaker groups ( $pK_a > 6$ ) reduces fluorescence; these protons might be coming from weak carboxylic acid groups or from the most acidic phenol groups (Gamble, 1970). These results are somewhat different from those of Black and Christman (1963), who found ever increasing fluorescence intensity as the pH was raised toward 11.

Many experiments involved addition of ions to fulvic

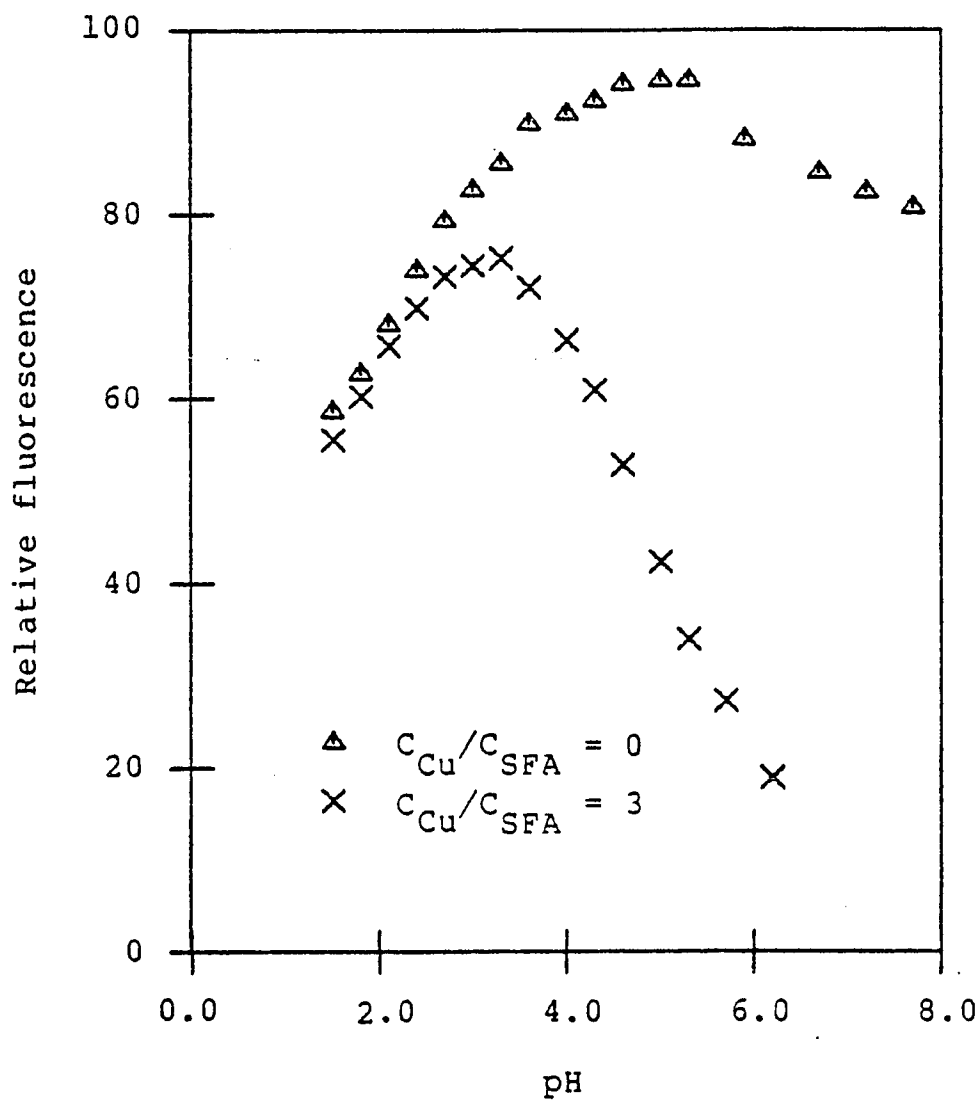


Figure 20. Fluorescence of  $5 \times 10^{-5}$  M soil-derived fulvic acid (SFA) solutions in the presences (crosses) or absence (triangles) of  $\text{Cu}^{2+}$ .  $I = 0.1$  M and  $T = 23^{\circ}\text{C}$ .

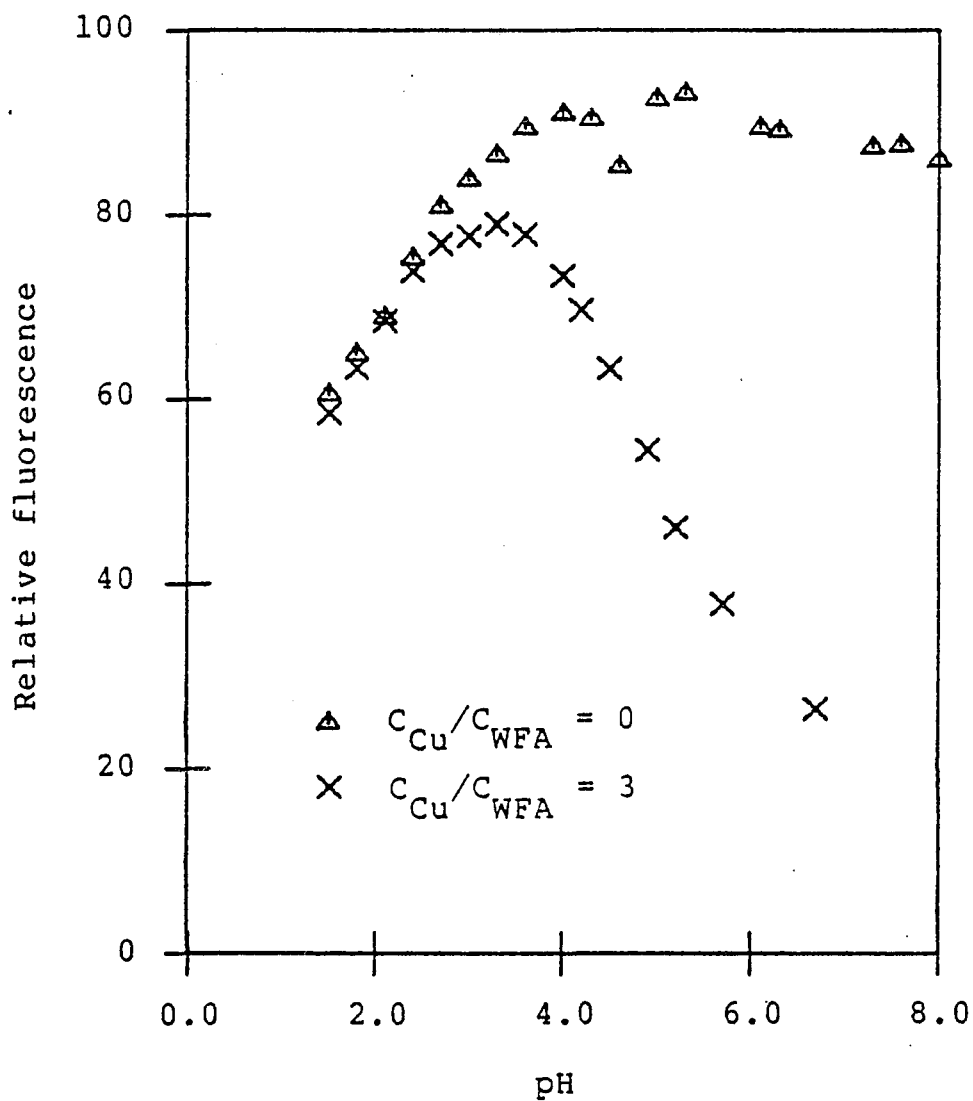


Figure 21. Fluorescence of  $5 \times 10^{-5}$  M water-derived fulvic acid (WFA) in the presence (crosses) or absence (triangles) of  $\text{Cu}^{2+}$ .  $I = 0.1$  M and  $T = 23^\circ\text{C}$ .

acid solutions to see if the FA fluorescence was quenched. The first work was done with  $\text{Cu}^{2+}$ , and, indeed, fluorescence diminished as more  $\text{Cu}^{2+}$  was added. It remained to be seen whether  $\text{Cu}^{2+}$  had to be bound to FA in order to quench the fluorescence.

Figure 22 shows that the percentage of total fluorescence that was quenched increased as the mole ratio of total  $\text{Cu}^{2+}$  to total SFA concentration ( $C_{\text{Cu}}/C_{\text{SFA}}$ ) increased. The data in the figure representing results of fluorescence experiments are depicted as triangles. The amount of quenching at any  $C_{\text{Cu}}/C_{\text{SFA}}$  depended strongly on pH; the most quenching occurred at pH 6.0 and the least at pH 3.0. The work done in this laboratory (Bresnahan et al., 1978; Saar and Weber, 1979) and elsewhere (Takamatsu and Yoshida, 1978) shows that the amount of metal-ion complexation increases with increasing pH. It appeared, then, that metal-ion complexation and fluorescence quenching might be related phenomena.

I originally did parallel ion-selective electrode and fluorescence experiments for  $\text{Cu}^{2+}$  and SFA at pH 5.0. The result was that, for any  $C_{\text{Cu}}/C_{\text{SFA}}$ ,

$$\bar{v} \times 57 = \text{percentage quenched}$$

where  $\bar{v}$ , measured by the ion-selective electrode experiment, equals  $(C_{\text{Cu}} - [\text{Cu}^{2+}])/C_{\text{SFA}}$ , and  $[\text{Cu}^{2+}]$  is the free metal ion concentration. The match between the percentage quenched for the fluorescence experiment and  $\bar{v} \times 57$  for the

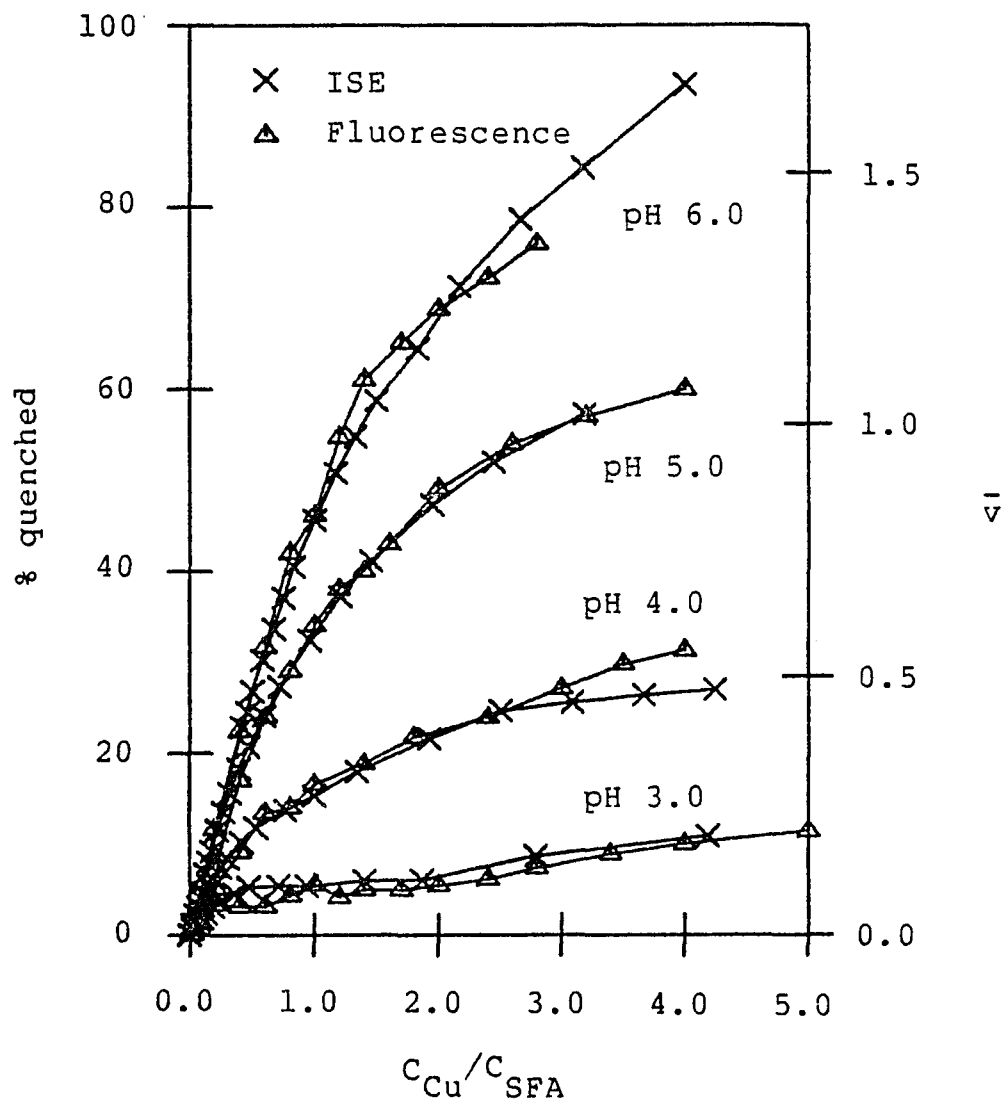


Figure 22. Comparison of  $Cu^{2+}$  titrations performed by ion-selective electrode potentiometry and fluorescence spectrophotometry. The concentration of soil-derived fulvic acid (SFA) was  $5 \times 10^{-5}$  M,  $I = 0.1$  M and  $T = 23-25^{\circ}C$ .



ion-selective electrode experiment is very close. I then repeated the  $\text{Cu}^{2+}$ -SFA fluorescence experiment at pH 3.0, 4.0, and 6.0; I already had ion-selective electrode data for  $\text{Cu}^{2+}$ -SFA at pH 6.0, so I only needed to perform ion-selective electrode titrations at pH 3.0 and 4.0 (experimental details in Chapter 3 and in Table 12). Figure 22 shows the comparison between the fluorescence experiments at the four pH values and the ion-selective electrode experiments done at the same pH values. All  $\bar{v}$  values are scaled by the same factor that caused a match between fluorescence and ion-selective electrode results for pH 5.0. The curves for the two types of experiments coincide well at all pH values.

I conclude that the effect of pH on the extent of  $\text{Cu}^{2+}$  complexation by SFA is the same as the effect of pH on the amount of fluorescence quenching: the higher the pH, the greater the amount of  $\text{Cu}^{2+}$  complexation and the greater the amount of fluorescence quenching. In addition, this conclusion is supported by the results shown in Figure 20 for SFA and in Figure 21 for WFA. The data represented by crosses show the fluorescence intensity of fulvic acid in the presence of a three-fold excess of  $\text{Cu}^{2+}$ . At low pH, where there is little  $\text{Cu}^{2+}$  complexation, there is only a small difference between the fluorescence of fulvic acid with and without  $\text{Cu}^{2+}$ . The difference in fluorescence with and without  $\text{Cu}^{2+}$  increases with increasing pH, as more complexation occurs.

The close correspondence between complexation and fluorescence quenching is not just a peculiarity of excitation at 350 nm in the fluorescence experiment. Table 17 shows, for excitation at 320, 350, and 400 nm, the fluorescence of SFA solutions without  $\text{Cu}^{2+}$  ion and of SFA solutions with a three-fold molar excess of  $\text{Cu}^{2+}$ . The samples have pH values of 3.0, 4.0, 5.0, and 6.0. The percentage quenched at 320 and 350 nm is nearly the same for all pH values (experimental error =  $\pm 2\%$ ). The results indicate that the close correspondence between complexation and fluorescence does not depend on having chosen 350 nm as the excitation wavelength; 320 nm excitation would have worked just as well. The quenching percentages at 400 nm, however, are somewhat different from the percentages at 320 and 350 nm. It is likely that the low-energy 400 nm light does not excite as broad a range of fluorophores as does the higher energy 320 or 350 nm light. It is probably important to excite as many fluorophores as possible in order to have fluorescence quenching correspond as well as possible to metal-ion complexation.

Two further possible conclusions resulting from these data are that  $\text{Cu}^{2+}$  ions cause quenching only when they are bound to fulvic acid and that a bound  $\text{Cu}^{2+}$  ion completely quenches a nearby fluorophore. To verify the first possible conclusion, one must consider whether metal ions may quench fluorescence by merely colliding with or being near a fulvic acid molecule, without forming a complex with it. A

TABLE 17

Fluorescence response of SFA at three wavelengths<sup>a</sup>

pH	$C_{Cu}/C_{SFA}$	<u>Wavelength (nm)</u>		
		320	350	400
3.0	0.0	89.9 %	89.5 %	83.9 %
3.0	3.0	82.0	81.9	74.1
4.0	0.0	100.	100.	100.
4.0	3.0	75.5	74.1	65.7
5.0	0.0	102.9	99.5	109.6
5.0	3.0	52.7	49.3	44.4
6.0	0.0	98.0	96.0	116.3
6.0	3.0	23.5	22.0	31.0

<sup>a</sup> Values in the table are the ratio (expressed in percent) of the height of each peak to the height of the peak for the pH 4.0 solution without copper ion at the same wavelength.

comparison of collision frequency and the lifetime of the fluorescence excited state must be made. A double exponential closely fits the excited-state decay pattern for our soil-derived fulvic acid. The lifetimes corresponding to these exponentials are 1.3 and 7.0 nanoseconds (Seitz, W. R., personal communication). A calculation of collisional frequency at 23°C for  $\text{Cu}^{2+}$  and SFA concentrations of approximately  $1 \times 10^{-4}$  M shows that little collisional quenching would occur (Parker, 1968). It appears that fluorescence quenching is selective for bound metal ion.

More evidence for the observation of no quenching by unbound species comes from experiments with the iodide ion. With its negative charge, iodide would not be expected to bind to anionic sites on fulvic acid, but it is a heavy ion, so some quenching might occur. Fluorescence titrations at pH 3.0 and 5.0 with iodide and SFA resulted in unchanged SFA fluorescence up to an eight-fold molar excess of iodide. The heavy iodide ion does not quench SFA fluorescence.

Further verification that unbound metal ion does not cause appreciable quenching of fulvic acid fluorescence comes from experiments done at pH 1.4-1.5 with high mole ratios of metal ion to fulvic acid. The high hydrogen ion concentration of this experiment prevents any divalent metal ions from binding to fulvic acid, so all quenching is due to free metal ion. The results are listed in Table 18. Except for  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$ , there is no significant quenching of SFA fluorescence by metal ions. The small amount of quenching

TABLE 18

Quenching of soil-derived fulvic acid fluorescence by  
high concentrations of metal ions at low pH<sup>a</sup>

Metal ion	$C_M/C_{SFA}$	pH	% quenched <sup>b</sup>
Ni <sup>2+</sup>	227	1.45	0.9
Mn <sup>2+</sup>	243	1.45	-0.5 <sup>c</sup>
Co <sup>2+</sup>	227	1.41	1.8
Pb <sup>2+</sup>	65	1.44	3.8
Cu <sup>2+</sup>	209	1.47	3.5
Cd <sup>2+</sup>	119	1.42	-0.7 <sup>c</sup>

<sup>a</sup> Fulvic acid concentration was  $5.1 \times 10^{-5}$  M. The experiments were performed in 0.1 M KNO<sub>3</sub> at 23°C.

<sup>b</sup> Uncertainty in % quenched values is +2%.

<sup>c</sup> Negative values indicate fluorescence intensity greater for SFA with metal ion than for the reference sample that had no metal ion.

by  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  is not a problem for the usual complexation study done at high pH, because the amount of metal ion in the samples listed in Table 18 is one to two orders of magnitude greater. Complexes of fulvic acid in a less acidic medium are not soluble at very high  $C_M/C_{\text{SFA}}$ , so such high metal ion concentrations cannot be used in complexation studies.

Use of salicylic acid as a model for fulvic acid helped to verify the second possible conclusion that a bound metal ion completely quenched a nearby fluorophore. I measured the fluorescence of a series of pH 6.0 solutions, all containing  $\text{Cu}^{2+}$  (except the metal-ion blank) and salicylic acid in 0.1 M  $\text{KNO}_3$ , but each sample had a different mole ratio of total  $\text{Cu}^{2+}$  to total salicylic acid. Values in the literature (Martell and Smith, 1977) for the  $\text{Cu}^{2+}$ -salicylic acid stability constant allowed calculation, for any concentration of  $\text{Cu}^{2+}$ , of the fraction of total salicylic acid that was attached to a  $\text{Cu}^{2+}$  ion. The calculated and fluorescence results appear in Figure 23. The correspondence of these results proves that a salicylic acid molecule attached to a  $\text{Cu}^{2+}$  ion does not fluoresce. That is, bound  $\text{Cu}^{2+}$  quenching is complete.

Copper(II) is not the best ion with which to test the capability of fluorescence analysis. It is paramagnetic and hence, it quenches fluorescence efficiently. It also forms strong complexes with fulvic acid; the close association of metal ion and ligand is necessary for

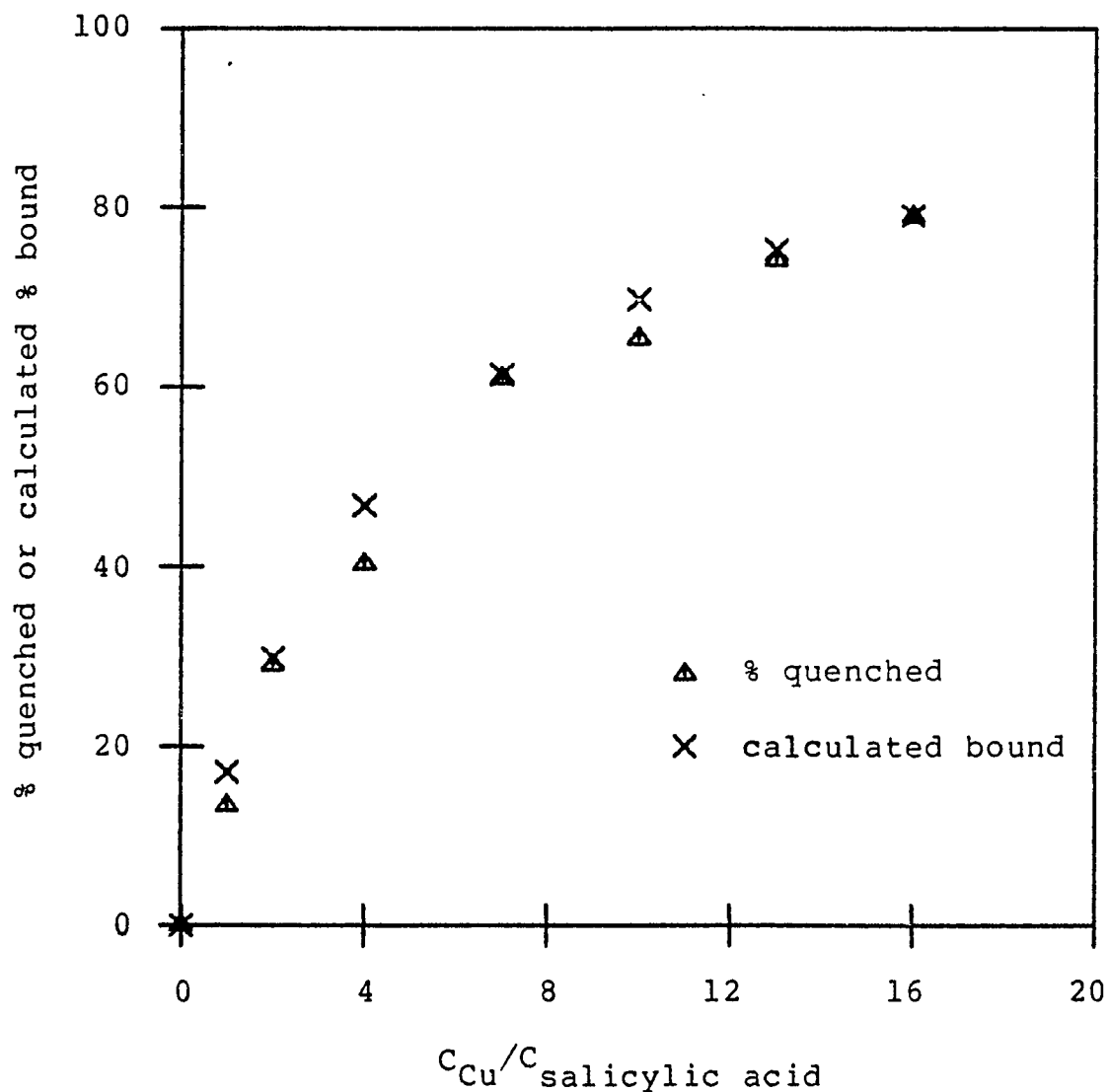
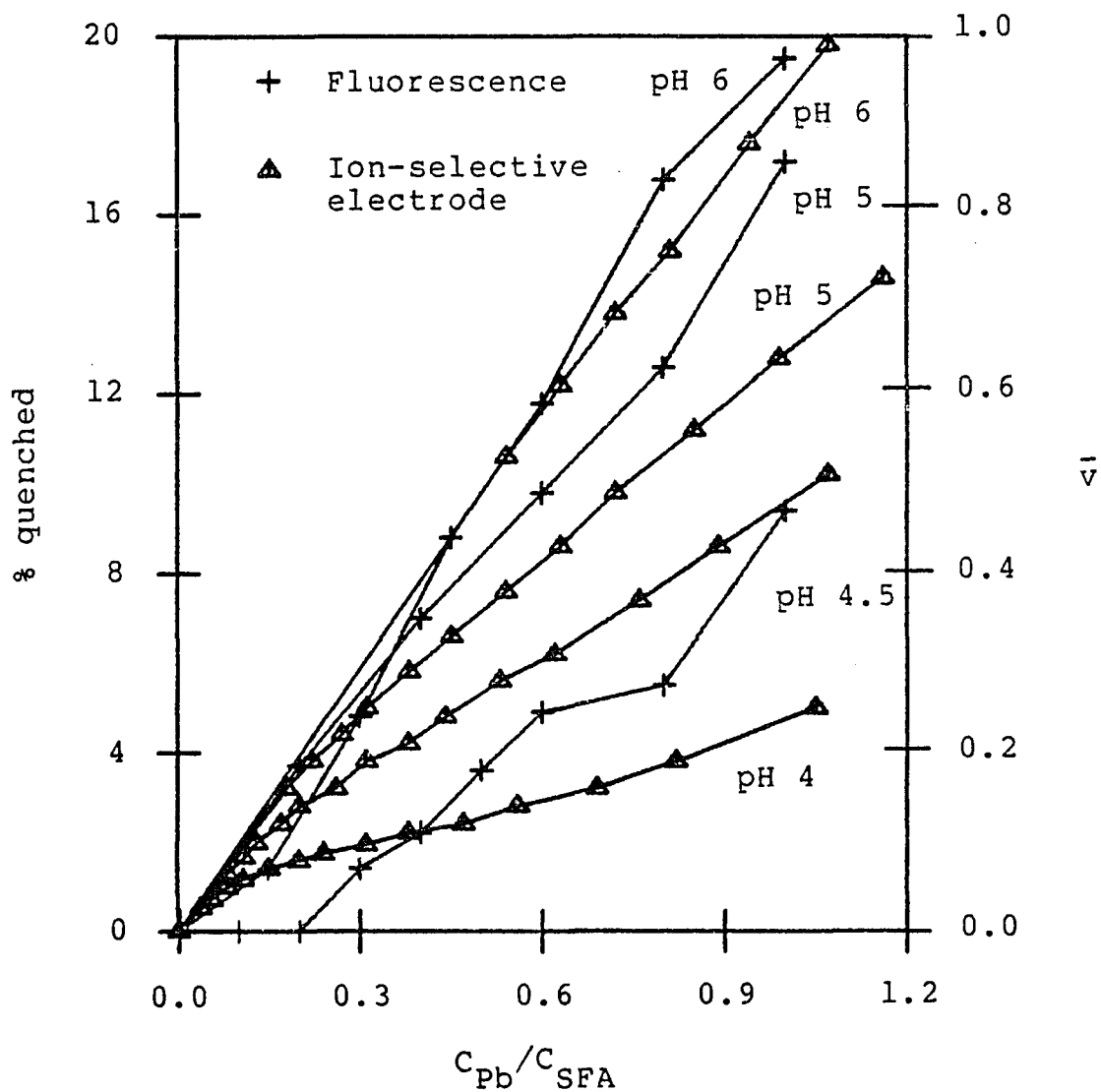


Figure 23. Comparison of the percentage of salicylic acid fluorescence that was quenched and the calculated percentage of salicylic acid complexed with  $\text{Cu}^{2+}$  at pH 6.0. Salicylic acid concentration was  $1.5 \times 10^{-4}$  M,  $I = 0.1$  M, and  $T = 23^{\circ}\text{C}$ .

quenching. I tested other metal ions that were not paramagnetic or did not bind strongly to fulvic acid to gauge the sensitivity of fluorescence for analysis of complexes between fulvic acid and such metal ions.

A comparison of fluorescence and ion-selective electrode results for  $\text{Pb}^{2+}$ -SFA complexes appears in Figure 24. Parallel data for  $\text{Pb}^{2+}$ -WFA complexes appear in Figure 25. The pH values for experiments represented in these figures range from 4.0 to 6.0. There are several differences between the data in these figures and the comparable data for  $\text{Cu}^{2+}$ -SFA complexes shown in Figure 22. First, the  $\bar{v}$  values are scaled by a factor of 20 in order to have the ion-selective electrode results coincide roughly with the percentage-quenched numbers from the fluorescence experiments. Earlier work (Chapter 3) showed that at low  $C_M/C_{FA}$ ,  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  bind with approximately the same strength to fulvic acids. The smaller scaling factor for  $\text{Pb}^{2+}$  compared to that for  $\text{Cu}^{2+}$  indicates that each bound  $\text{Pb}^{2+}$  ion does not quench as much fluorescence as does each bound  $\text{Cu}^{2+}$  ion. The ratio of the  $\bar{v}$  scaling factors for  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  (57/20) is the ratio of fluorescence-quenching efficiency for the two metal ions; each bound  $\text{Cu}^{2+}$  ion quenches almost three times as much fluorescence as does each bound  $\text{Pb}^{2+}$  ion. The probable reason is that  $\text{Pb}^{2+}$  is diamagnetic; it has all paired electrons. Paramagnetic metal ions, those with one or more unpaired electrons, are known to quench fluorescence more effectively than





**Figure 24.** Comparison of  $Pb^{2+}$  titrations performed by ion-selective electrode potentiometry and by fluorescence spectrophotometry. The concentration of soil-derived fulvic acid (SFA) was  $5 \times 10^{-5}$  M,  $I = 0.1$  M, and  $T = 23-25^{\circ}C$ .

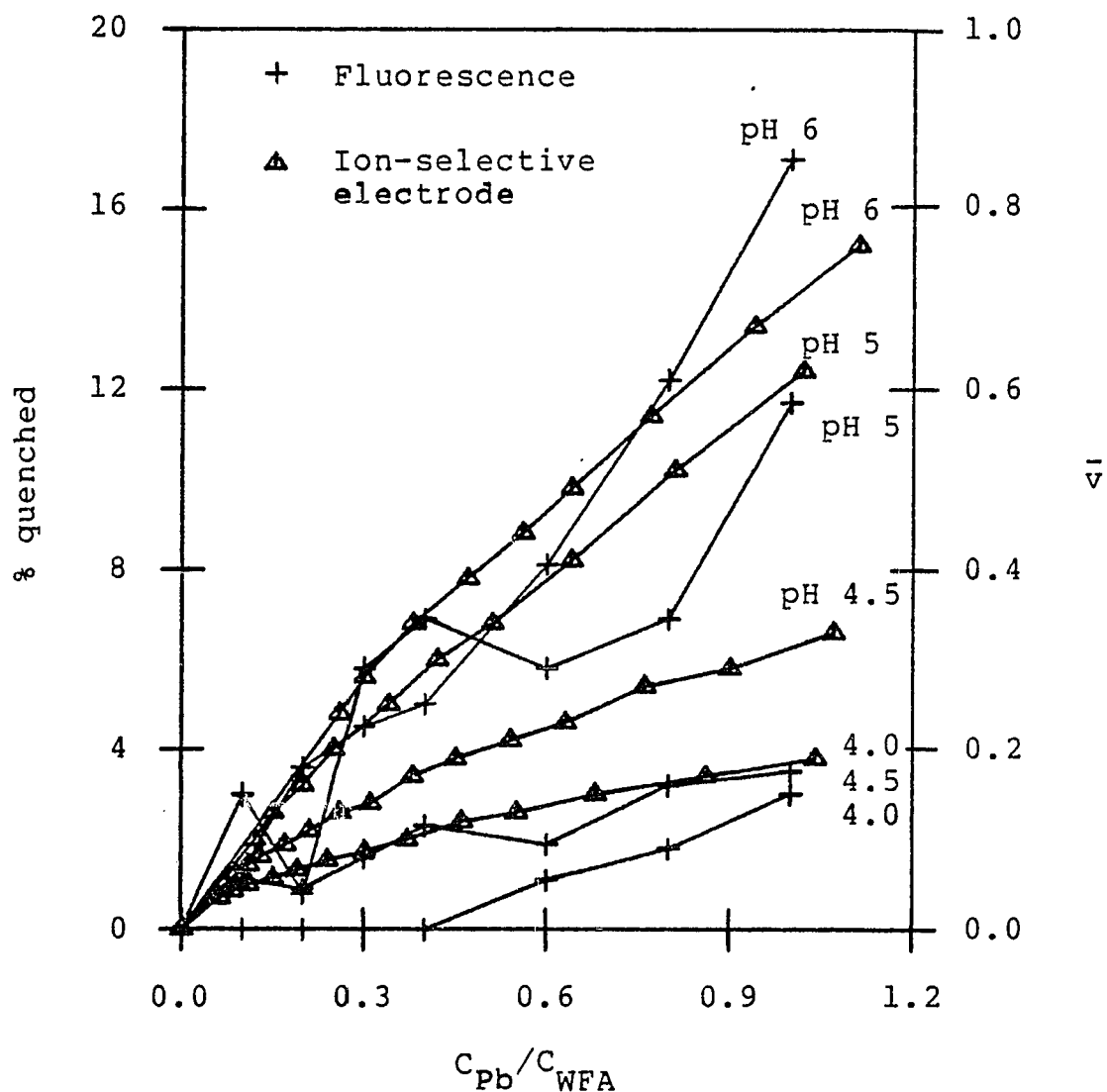


Figure 25. Comparison of  $Pb^{2+}$  titrations performed by ion-selective electrode potentiometry and by fluorescence spectrophotometry. The concentration of water-derived fulvic acid (WFA) was  $5 \times 10^{-5}$  M,  $I = 0.1$  M, and  $T = 23-25^{\circ}C$ .

diamagnetic ions (Parker, 1968). It is also conceivable that  $\text{Pb}^{2+}$  binds to different sites on fulvic acid than  $\text{Cu}^{2+}$  does or in a different way to the same sites. Such  $\text{Pb}^{2+}$  sites would have to be farther from fluorophores and thus less able to quench their fluorescence.

There is a second major difference between the  $\text{Pb}^{2+}$ -FA Figures 24 and 25, and the  $\text{Cu}^{2+}$ -SFA Figure 22. The maximum mole ratio of metal ion to fulvic acid for the  $\text{Pb}^{2+}$  experiments is 1.0 because precipitation of Pb-FA solids occurs at higher ratios. Mole ratios of  $\text{Cu}^{2+}$  to fulvic acid can reach 4 or 5 before precipitation begins (Chapter 3), so a much wider range of data is possible to obtain.

The scattered appearance of the data for  $\text{Pb}^{2+}$  plotted in Figures 24 and 25, in contrast to the orderly data for  $\text{Cu}^{2+}$  in Figure 22, results from the two differences discussed above. The quenching per bound  $\text{Pb}^{2+}$  ion is only one-third that per  $\text{Cu}^{2+}$  ion, and the amount of  $\text{Pb}^{2+}$  that can be added is only one-fifth of the amount of  $\text{Cu}^{2+}$  that can be used. The result is that the fluorescence data in either Figure 24 or 25 cover only about one-fifteenth the area that the fluorescence data for  $\text{Cu}^{2+}$  in Figure 22 do. Considering the magnification of the axes, the scatter in the  $\text{Pb}^{2+}$  figures is not surprising, nor does it weaken the conclusions reached for  $\text{Cu}^{2+}$ .

Because fluorescence analysis does not work well with the  $\text{Pb}^{2+}$ -FA system, complexes with other diamagnetic ions and fulvic acid would probably not be analyzed easily by

fluorescence.  $Pb^{2+}$ , among diamagnetic metal ions, is likely the most amenable to fluorescence analysis because it is very heavy (high atomic weight in addition to unpaired electrons being a factor promoting quenching) and binds strongly to fulvic acid. The possibility of high  $C_M/C_{FA}$  without precipitation is one advantage that some diamagnetic ions might have over  $Pb^{2+}$ , but this advantage may not be enough to overcome the problems such a diamagnetic ion might have--inefficient quenching and weak complexation.

One diamagnetic ion is  $Cd^{2+}$ . Results from fluorescence experiments with  $Cd^{2+}$  would be useful because there is so much data from  $Cd^{2+}$ -FA titrations with the ion-selective electrode for comparison (Chapter 2). However, at pH 7.5, there is virtually no SFA fluorescence quenching by  $Cd^{2+}$ , even up to  $C_{Cd}/C_{SFA} = 15$ . The results from this experiment are listed in Table 19. Ion-selective electrode experiments show that an easily measurable amount of  $Cd^{2+}$  is bound to SFA at pH 7 or 8 (Chapter 2), so the fluorescence experiment is not useful for analysis of  $Cd^{2+}$ -FA complexation.

#### Co<sup>2+</sup> and Ni<sup>2+</sup> quenching

Two metal ions for which we have no ion-selective electrodes are  $Co^{2+}$  and  $Ni^{2+}$ . They are paramagnetic ions and on the basis of stability constants for complexes that they form with model ligands such as salicylic acid (Martell and Smith, 1977), they should form moderately strong complexes with fulvic acid. The paramagnetism and expected

complexation strength of these ions indicate that fluorescence analysis of the complexation should work.

A comparison of SFA fluorescence without metal ion and SFA fluorescence with either  $\text{Co}^{2+}$  or  $\text{Ni}^{2+}$  as a function of pH appears in Figure 26. This figure shows results of experiments that are similar to earlier ones done for  $\text{Cu}^{2+}$  and SFA (Figure 20) and for  $\text{Cu}^{2+}$  and WFA (Figure 21). The figures are similar in that fluorescence diminishes in the presence of any of these metal ions as the pH is raised.  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  cause nearly the same amount of quenching, and somewhat less than that caused by  $\text{Cu}^{2+}$ . It is not surprising that  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  cause the same response in the presence of SFA; their ionic radii and hydrolysis constants are very similar.

If quenching of a fluorophore by a bound  $\text{Co}^{2+}$  or bound  $\text{Ni}^{2+}$  ion is complete and if free  $\text{Co}^{2+}$  or free  $\text{Ni}^{2+}$  causes no quenching, then the relatively small amount of quenching caused by  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  indicates that they bind to SFA less strongly than does  $\text{Cu}^{2+}$ .

Figure 27 shows pH 4.0 and pH 6.0 titrations of  $\text{Co}^{2+}$  and SFA; Figure 28 shows similar titrations of  $\text{Ni}^{2+}$  and SFA. The data again show that  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  respond similarly to each other in the presence of SFA. These figures also show the increased quenching and presumably increased complexation at pH 6.0 compared to that at pH 4.0.

It remained to be seen whether the assumptions of complete quenching when bound and no quenching when not

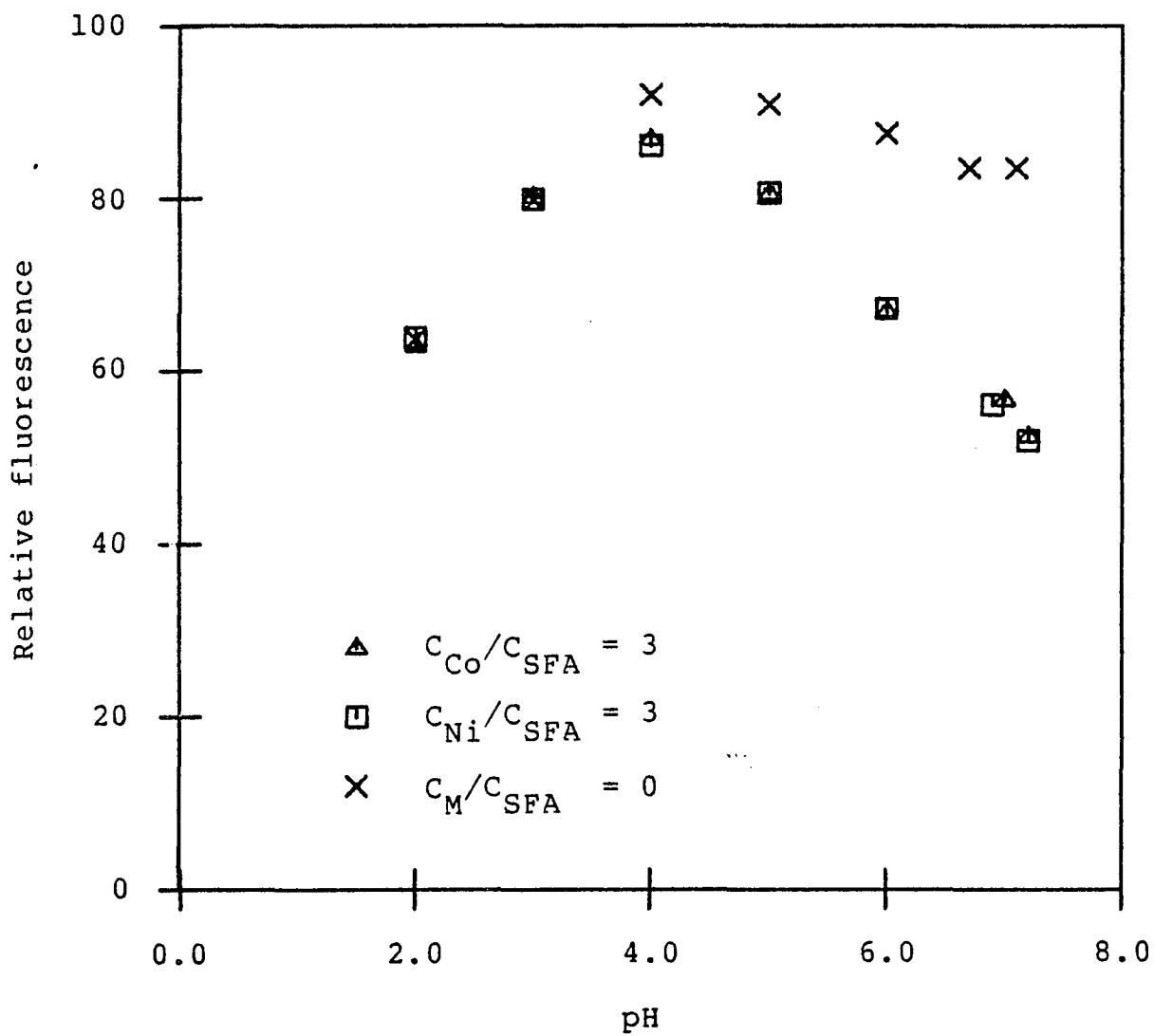


Figure 26. Fluorescence of  $5 \times 10^{-5}$  M solutions of soil-derived fulvic acid (SFA) without metal ion and in the presence of either  $Ni^{2+}$  or  $Co^{2+}$ .  $I = 0.1$  M and  $T = 23^{\circ}C$ .

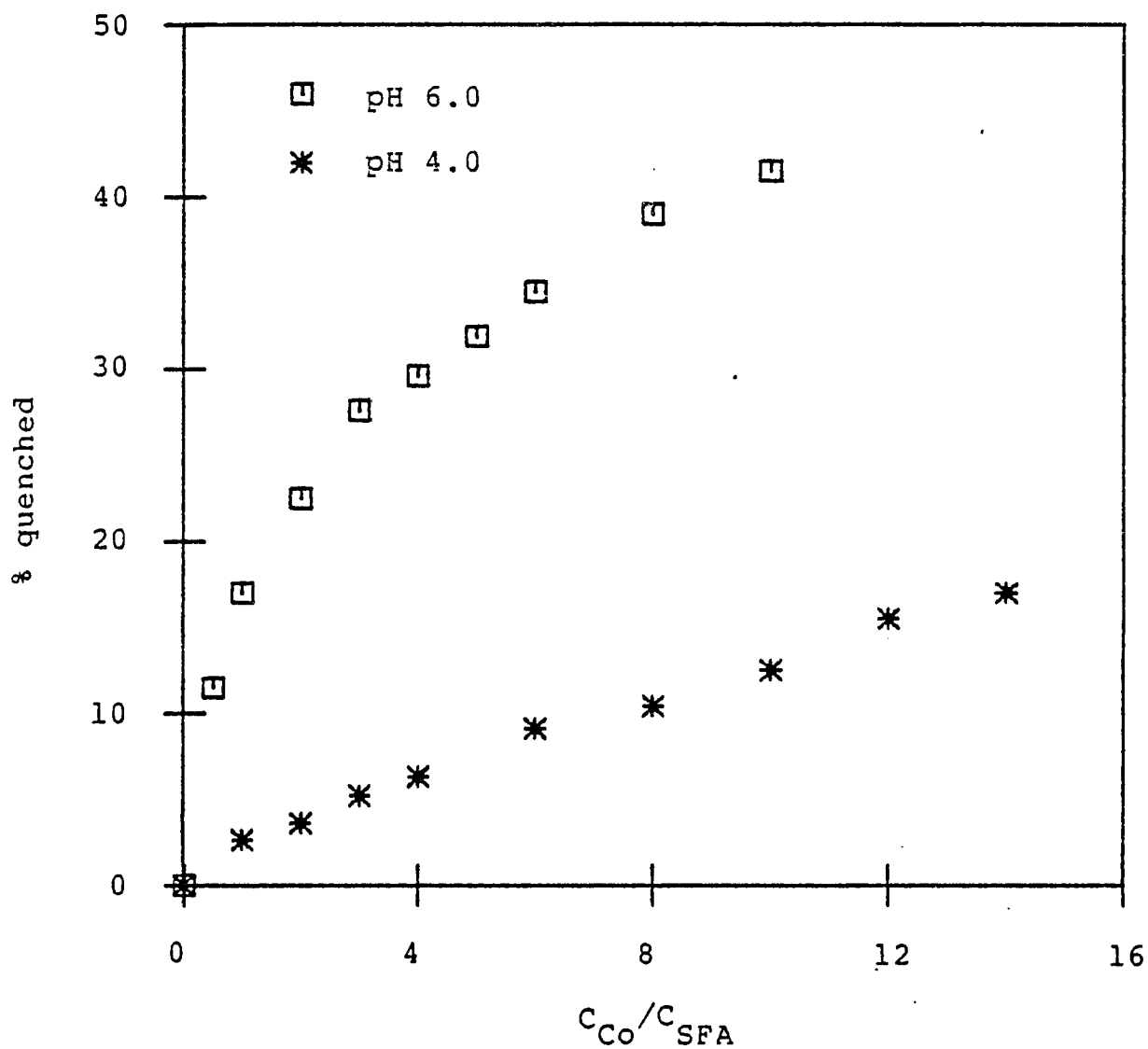


Figure 27. Fluorescence titrations at pH 4.0 and 6.0 for  $Co^{2+}$  and soil-derived fulvic acid (SFA).  $[SFA] = 5 \times 10^{-5}$  M,  $I = 0.1$  M, and  $T = 23^{\circ}C$ .

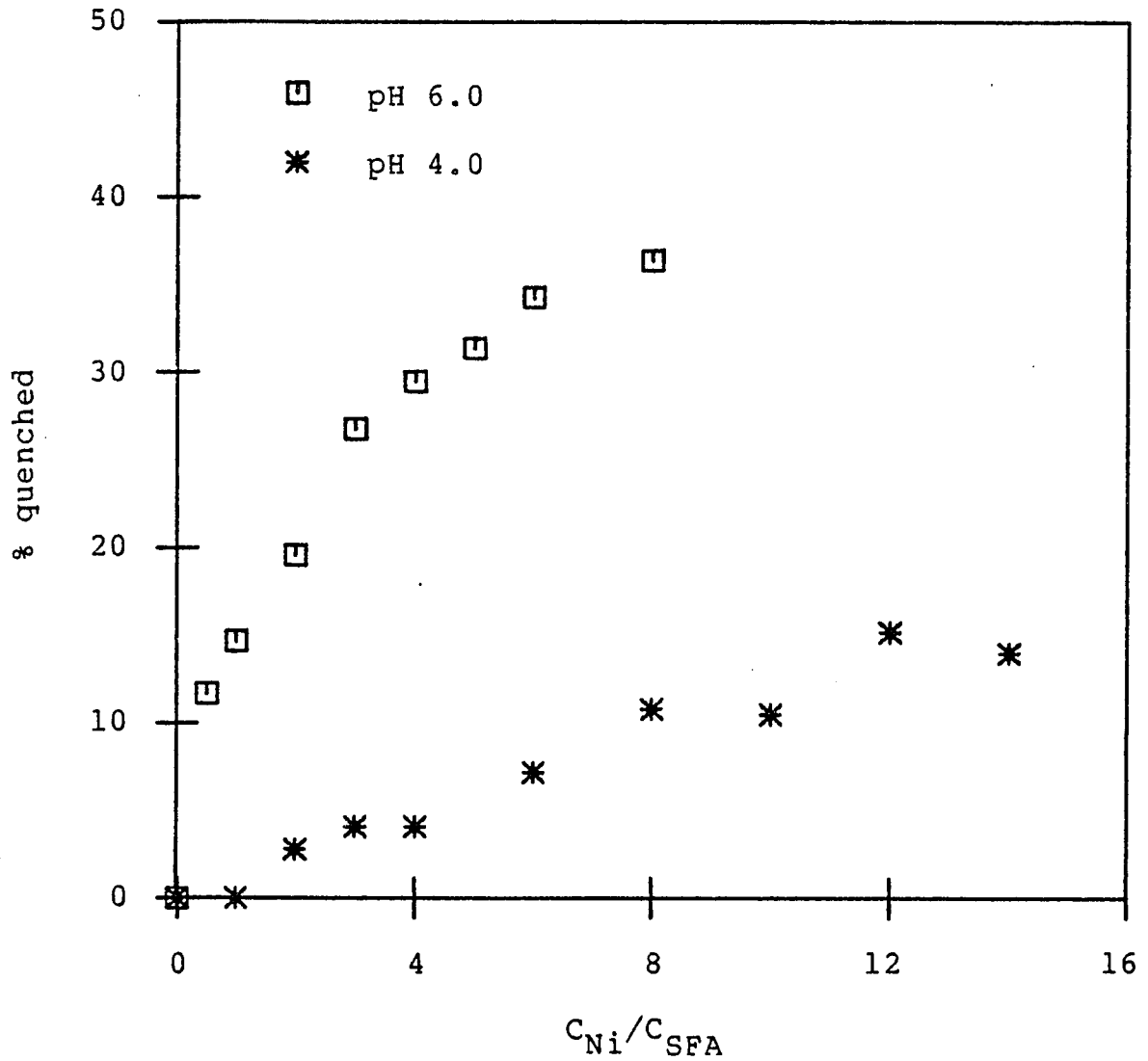


Figure 28. Fluorescence titrations at pH 4.0 and 6.0 for  $Ni^{2+}$  and soil-derived fulvic acid (SFA).  $[SFA] = 5 \times 10^{-5}$  M,  $I = 0.1$  M, and  $T = 23^{\circ}C$ .



bound are true for  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$ . I initially tried to verify the assumptions with salicylic acid ( $\text{H}_2\text{Sal}$ ), but as the association constants in Table 16 show, it is difficult to form appreciable amounts of Co-Sal or Ni-Sal unless the pH is very high or there is a tremendous excess of metal ion. The two derivatives of salicylic acid, 5-nitro- and 3,5-dinitrosalicylic acid have lower proton affinities: the nitro group(s) pull electron density out of the aromatic ring, causing the phenol proton to be more acidic. The nitro groups do not cause as much of a drop in metal ion affinity (Table 16), so more complex will form with these nitro derivatives of salicylic acid than with salicylic acid itself. Unfortunately, the nitro group(s) also markedly reduce the fluorescence intensity of these ligands; the fluorescence of either nitro derivative has less than a hundredth the intensity of the emission from salicylic acid. The emission weakness pushes the fluorescence spectrophotometer to the limit of its sensitivity.

Another problem with the nitro or dinitrosalicylic acid is that the emission wavelength is 50-60 nm from the excitation wavelength. This is the position of a Raman scattering band for water (Parker, 1968). The water Raman peak is about one-third the height of the emission from a metal-free  $1.5 \times 10^{-4}$  M 3,5-dinitrosalicylic acid sample. The Raman peak reduces the usefulness of ligands that are already marginally useful.

Another problem particularly associated with the

5-nitrosalicylic acid is that  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  complexes with this ligand absorb strongly at the emission wavelength. The low levels of emission, the interfering Raman band, and the absorption of complexes at the emission wavelength all work against using 5-nitrosalicylic acid or 3,5-dinitrosalicylic acid as compounds to check  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  quenching properties. These results do not rule out the use of other model compounds. However, such a model compound would have to meet several requirements:

(1) It should fluoresce strongly enough, certainly more strongly than the nitro derivatives of salicylic acid.

(2) It should have high enough published or measurable stability constants with metal ions of interest to yield a substantial amount of complexation at pH values of interest.

(3) It should be a diprotic ligand with  $\text{pK}_{a1} = 2-4$  and  $\text{pK}_{a2} = 8-10$ , values which are typical of fulvic acid (Burch et al., 1978).

(4) It should have a visible spectrum that does not change upon complexation with metal ions.

Salicylic acid remained a possible ligand to check the ability of  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  to quench fluorescence. To obtain a measureable amount of complex, the pH had to be high (near 8.0) and the concentration of  $\text{Co}^{2+}$  or  $\text{Ni}^{2+}$  had to be large. The samples contained mole ratios of metal ion to SFA of 35, 73, 109, and 145. It took nearly a week to adjust the pH to near 8.0 because carbon dioxide kept dissolving after each addition of base, resulting in a continual drift downward in

pH. The percentage quenched for these samples at high pH and their hydrogen-ion concentrations appear in Table 20. Also appearing in that table are the results of quenching experiments at pH 5.0 and the percentages of salicylic acid bound at high pH, calculated with the stability constants listed in Table 16. I did the pH 5.0 experiments because with such high concentrations of metal ion, there could be some collisional quenching. No measurable amount of binding occurs at pH 5.0 between salicylic acid and either  $\text{Co}^{2+}$  or  $\text{Ni}^{2+}$ , so any reduced fluorescence would be due to collisional quenching.

The results in Table 20 show that the percentage quenched at pH 8 almost exactly equals the percentage bound at that pH (as calculated from the stability constant) plus the percentage quenched at pH 5.0, the contribution from collisional quenching. Precipitates appeared in several samples, and the results for those samples were not usable.

This experiment shows that bound  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  completely quench the fluorescence of salicylic acid and that unbound  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  do not quench the fluorescence unless their concentrations are very high--much higher than would be used in complexation experiments with fulvic acid. Therefore, the quenching data shown in Figures 27 and 28 can be used for calculation of stability constants assuming that bound  $\text{Co}^{2+}$  or  $\text{Ni}^{2+}$  completely quenches fulvic acid fluorophores.

Although 1:1 and 1:2 (metal ion:ligand) complexes may

Table 20

Co<sup>2+</sup>- and Ni<sup>2+</sup>-salicylic acid fluorescence, pH 5 and 8<sup>a</sup>

$[M^{2+}]$ (M x 10 <sup>3</sup> )	$[H^+]^b$ (M x 10 <sup>8</sup> )	Quenched pH 5	Quenched pH 8	Calculated <sup>c</sup> % bound
-----Ni <sup>2+</sup> -----				
2.03	0.8414	1.5 %	9.9 %	7.9 %
4.07	1.047	2.6	15.2	12.1
6.10	1.175	5.6	precip.	15.6
8.14	1.318	7.6	precip.	18.0
-----Co <sup>2+</sup> -----				
2.03	1.318	2.9	5.3	3.1
4.07	1.230	6.7	13.5	6.5
6.10	1.380	9.4	18.1 <sup>d</sup>	8.5
8.14	1.698	12.0	precip.	9.1

<sup>a</sup> For all samples, [salicylic acid] is  $5.6 \times 10^{-5}$  M. The experiments had I = 0.11 M and T = 23°C.

<sup>b</sup> For high pH samples only.

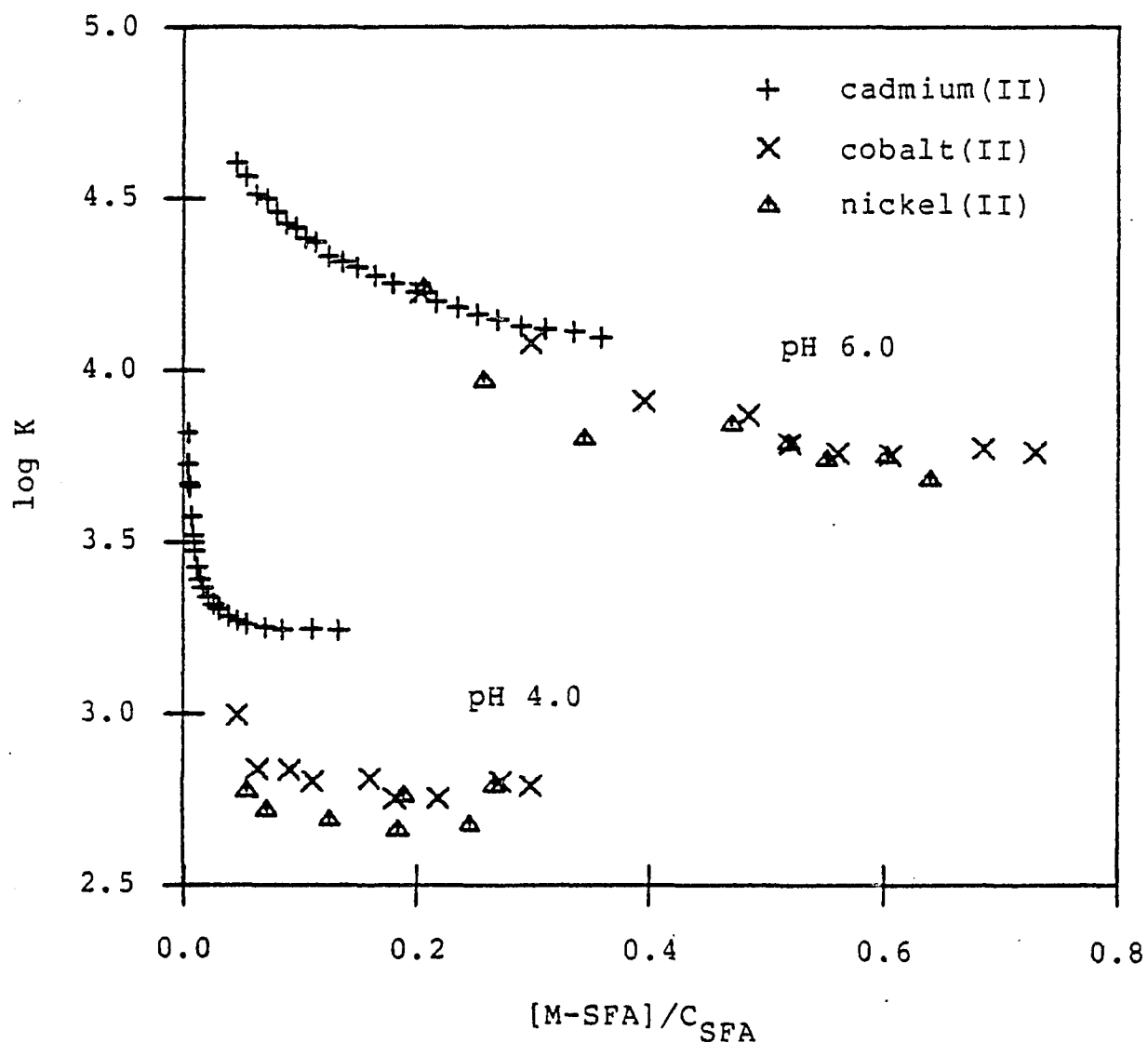
<sup>c</sup> For I = 0.15 M at 20°C. The stability constants are from Martell and Smith (1977).

<sup>d</sup> May be invalid due to slight precipitation.

form for  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  complexes with fulvic acid, just as fulvic acid appears to do with  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$ , the fluorescence data has very high  $C_M/C_{\text{SFA}}$ , so little 1:2 complexation would occur. These experimental conditions rule out use of the Buffle method which calculates stability constants for 1:1 and 1:2 complexes (Buffle et al., 1977). A 1:1 only model would appear to be more appropriate. An initial assumption will be that  $\bar{\nu}$  values for  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  are 1/57th the percentage of fluorescence quenching, just as they are for  $\text{Cu}^{2+}$ . Figure 29 shows the change in K, the 1:1 conditional stability constant for  $\text{Co}^{2+}$ -SFA,  $\text{Ni}^{2+}$ -SFA, and  $\text{Cd}^{2+}$ -SFA complexes as the value of  $[M\text{-SFA}]/C_{\text{SFA}}$  increases. The data included in the figure are for pH 4.0 and 6.0. The SFA concentration was  $5 \times 10^{-4}$  M for the  $\text{Cd}^{2+}$ -SFA ion-selective electrode titrations and  $5 \times 10^{-5}$  for the  $\text{Co}^{2+}$ -SFA and  $\text{Ni}^{2+}$ -SFA fluorescence experiments. Because of the SFA concentration effect for  $\text{Cd}^{2+}$ -SFA, the log K values for  $\text{Cd}^{2+}$ -SFA when  $[\text{SFA}] = 5 \times 10^{-5}$  M should be increased by 0.3 units for  $[M\text{-SFA}]/C_{\text{SFA}}$  above 0.2 (Saar and Weber, 1979).

The results in Figure 29 show that  $\text{Cd}^{2+}$ -SFA stability constants appear to be higher than the constants for  $\text{Co}^{2+}$ -SFA and  $\text{Ni}^{2+}$ -SFA. For either  $\text{Co}^{2+}$  or  $\text{Ni}^{2+}$  complexes,  $\log K = 2.8$  at pH 4.0 and  $\log K = 3.8$  at pH 6.0. These values are lower than those reported by Schnitzer and Hansen (1970) for  $\text{Ni}^{2+}$ -SFA:  $\log K = 3.1$  at pH 3.0 and  $\log K = 4.2$  at pH 5.0.

Even though the  $\text{Co}^{2+}$ -SFA and  $\text{Ni}^{2+}$ -SFA conditional



**Figure 29.** Comparison of stability constants (K) calculated for  $Cd^{2+}$ -SFA,  $Co^{2+}$ -SFA, and  $Ni^{2+}$ -SFA complexes at pH 4.0 and 6.0. Soil-derived fulvic acid (SFA) concentrations were  $5 \times 10^{-4}$  M for the  $Cd^{2+}$  titrations and  $5 \times 10^{-5}$  M for the  $Co^{2+}$  and  $Ni^{2+}$  titrations.  $I = 0.1$  M and  $T = 23-25^{\circ}C$ .

stability constants determined here are smaller than the stability constants for  $\text{Cd}^{2+}$ -SFA, the increase in  $K$  from pH 4.0 to 6.0 for  $\text{Co}^{2+}$ -SFA and  $\text{Ni}^{2+}$ -SFA is larger than is the increase over the same pH span for  $\text{Cd}^{2+}$ -SFA. The magnitude of change in  $K$  as a function of pH is proportional to the number of protons released for each complex formed. From this preliminary data, approximately 0.5 protons are released for each  $\text{Co}^{2+}$ -SFA or  $\text{Ni}^{2+}$ -SFA complex formed. This is close to 0.4, the value reported by Schnitzer and Skinner (1963) for  $\text{Ni}^{2+}$ -SFA complexation, and somewhat larger than the 0.28 protons released upon formation of each  $\text{Cd}^{2+}$ -SFA complex as reported in Chapter 2. A higher value for the number of protons released appears to indicate a closer type of binding--binding that involves more displacement of phenol protons in a salicylic-type site.

The experiments show that bound  $\text{Co}^{2+}$  or bound  $\text{Ni}^{2+}$  quenches salicylic acid fluorescence completely, but these metal ions may not quench fulvic acid fluorescence as well. A smaller conversion factor (than 57) between  $\bar{\nu}$  and percentage quenched would arise if  $\text{Co}^{2+}$  or  $\text{Ni}^{2+}$  quenching were not as efficient as  $\text{Cu}^{2+}$  quenching. Higher values of  $\bar{\nu}$  and  $K$  would result; such a change might raise the fluorescence-determined stability constants for  $\text{Co}^{2+}$ -SFA and  $\text{Ni}^{2+}$ -SFA to values larger than those for  $\text{Cd}^{2+}$ -SFA.

Even with these difficulties, fluorescence analysis of fulvic acid complexes with paramagnetic ions may be done. However, much work is still needed. It appears that

calibration of  $\bar{v}$  and percentage quenched for each metal ion to be tested will be necessary.



## CHAPTER 5

### EPILOGUE: METAL-ION COMPLEXATION BY FULVIC ACIDS

#### THE VARIABLES IN FULVIC ACID RESEARCH

If fulvic acid did not have important effects on metal ion solubility, speciation, and toxicity, it probably would not be the object of so much research, because many features of this research are unsatisfying. The various research groups obtain their humic materials from different soils and water bodies, and they use a variety of isolation procedures; some of the conditions are chemically harsh, and others are mild. A researcher, then, does not know how a given fulvic acid sample compares to one obtained elsewhere, possibly by different procedures. However, work is underway to aid comparisons between fulvic acid samples (Burch et al., 1978).

Aside from not knowing how universal one's starting material is, the data on metal complexation are hard to obtain, and the interpretation of that data is difficult.

So many variables affect fulvic acid complexation of metal ions that to specify them all may reduce the results to a case of mere special or local interest.

Several variables traditionally concern researchers in this area:

(1) The source of the fulvic acid: the location and type of soil or water body from which the organic matter has been extracted.

(2) The method with which organic matter was isolated.

(3) The ionic strength of the experiments.

(4) The pH at which the experiments were performed.

(5) The method of complexation analysis.

(6) The method of data manipulation and stability-constant calculation.

Two new variables of concern are presented in this dissertation:

(7) The concentration of the fulvic acid, at least for weakly bound metal ions such as  $\text{Cd}^{2+}$ .

(8) The early onset of precipitation for  $\text{Pb}^{2+}$  and the effect this solid formation has on removal of free metal ion.

This is a large number of variables to keep in mind. They also make it difficult to compare work in different groups. The following is a discussion of the way these variables affect the work described here and, hence, of the applicability of this work to work done elsewhere.

Sources of fulvic acid. Although the chemistry of

soils and water bodies may vary widely from one place to another, many properties of dissolved organic matter in general and fulvic acid in particular are common. Fulvic acid is a polyelectrolyte with many aromatic carbons and a large number of oxygen-containing functional groups. Research in this laboratory shows the similarity of fulvic acids derived from a soil and a freshwater body (Weber and Wilson, 1975) and the similarity of their behavior in the presence of  $\text{Cu}^{2+}$  (Bresnahan et al., 1978),  $\text{Cd}^{2+}$  (Chapter 2; Saar and Weber, 1979), or  $\text{Pb}^{2+}$  (Chapter 3; Saar and Weber, 1980a, 1980b). The conclusions arrived at in this dissertation can probably be applied to other fulvic acids derived from soils or freshwater bodies, at least in the temperate zone.

Probably more humic-matter research has been done in Schnitzer's group than in any other. Our fulvic acid derived from soils was isolated with the method developed in that group (Schnitzer and Skinner, 1968). The extraction method, therefore, will not prevent comparison with their work. There is relatively less information on extraction of organic matter from water. It remains to be seen if a "standard" method will evolve.

Ionic strength. A majority of the experiments reported in the literature (including those described in this dissertation) are done at an ionic strength of 0.1 M. Such a high ionic strength allows addition of reagents without appreciable alteration of the ionic strength or, therefore,

the the activity coefficients. An ionic strength of 0.1 M would simulate brackish water. Experiments described in Chapter 2 and elsewhere (Stevenson, 1977; Schnitzer and Hansen, 1970) show increased stability constants for metal ions when the ionic strength is reduced to 0.01 M. These results indicate the type of adjustment needed to extrapolate findings at 0.1 M ionic strength to freshwater systems.

Effect of pH. Fulvic acid researchers generally recognize the importance of pH on metal-ion complexation by fulvic acid. The type of data presentation, however, may vary. Only occasionally does a single paper study a large number of pH values to characterize the effect of pH (for example, Takamatsu and Yoshida, 1978). The  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  work discussed in this dissertation were done at four or more pH values. The trends are clear. As the pH is raised, the conditional stability constants rise slowly for  $\text{Cd}^{2+}$  and more quickly for  $\text{Pb}^{2+}$ , indicating that few protons are displaced during  $\text{Cd}^{2+}$ -FA complexation (0.2-0.3 per complex formed), and a larger number are displaced during  $\text{Pb}^{2+}$ -FA complexation (approximately one proton per complex formed). It is not clear whether the  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  bind to different sites that are originally protonated to a different degree or whether  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  bind to similar sites, but to different degrees of "closeness," so that different numbers of hydrogen ions are displaced. I do not believe there are sets of sites specific to each metal ion, so I favor the

second possibility of varying closeness of metal ion-fulvic acid association.

Method of analysis. Measurement of free metal ion concentration is the most common way to provide data for stability constant calculations. The various methods of metal-ion analysis, however, can give different values for free-metal ion concentration for an identical sample. Ion-selective electrodes and fluorescence, for example, do not disturb the equilibrium between metal ion and ligand. Anodic stripping voltammetry can disturb the equilibrium during the plating step when metal ion is removed from solution. Ideally, dialysis or ultrafiltration membranes could completely separate free and complexed metal ion. In reality the separation is not complete because some of the ligands in the fulvic acid mixture pass through membrane pores. Because of this, the distinction between free and bound metal ion becomes uncertain.

It is interesting to speculate, though, on whether free metal ion as measured by any of these techniques is related to the amount of metal ion in a natural system that is available to an organism or available to participate in a geochemical process such as adsorption onto sediments. It may be that a rigorous chemical separation of free and complexed metal ion is not the best type of analysis for determining metal-ion toxicity. Bioassay is so prevalent because, by definition, it measures the biological activity of metal ions in a system. Whether inorganic analysis can

do the same remains to be seen.

Work in progress is designed to compare the analysis of  $\text{Cd}^{2+}$ -SFA and  $\text{Cu}^{2+}$ -SFA complexes by ion-selective electrode and by anodic stripping voltammetry. If the organic-matter adsorption on the mercury electrode is not serious, then a kinetic correction for the dissociation of complexes during plating (Shuman and Cromer, 1979) should cause the results to be similar.

Fluorescence analysis appears to provide useful information for complexes including paramagnetic ions. The method analyzes "free" ligand, a different perspective from that provided by other methods. However, the method is difficult because individual samples must be prepared. A major improvement would be development of a flow spectrofluorescence titrator, in which metal ion and other reagents could be added to a solution circulating through the analysis cuvette. A fluorescence titration would then become as easy as an ion-selective electrode titration.

Data manipulation. There are many ways to calculate stability constants. The differences lie primarily in the stoichiometry assumed for the complex or complexes. The methods considered in this dissertation include the Scatchard method (Scatchard, 1949), the Buffle method (Buffle et al., 1977), and a simple calculation method for  $\text{Cd}^{2+}$  assuming 1:1 complexation. Although the Scatchard method may be used and a Scatchard-type plot generated for the data reported here, the results are unsatisfactory and

perhaps misleading. The mathematics of Scatchard analysis assumes that complexes have no more than one ligand. However, complexes with more than one fulvic acid molecule may exist, especially in the presence of strongly binding metal ions such as  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$ .

The Scatchard treatment is marginally applicable for another reason. Stability constants and numbers of sites are determined by drawing straight lines through points on a Scatchard plot and finding slopes and intercepts. Fulvic acid, however, does not have discrete classes of sites, but rather, a continuum of sites with varying strengths; the plots are curved in all places. It is not a trivial matter to decide which points in the curved plots should be included in calculation of the best-fit line.

The method developed by Buffle and coworkers calculates constants for 1:1 and 1:2 (metal ion:ligand) complexes. This calculation overcomes the problem of 1:2 complexes that the Scatchard treatment cannot handle, but it does not allow for 2:1 complexes the way the Scatchard treatment does. One alternative, that would cover 2:1, 1:1, and 1:2 complexes would be to use the Buffle method to calculate stability constants for 1:1 and 1:2 complexes when ligand is in excess and use the Buffle method again but with a reversal of the variables of ligand and metal ion in the equations, to allow calculation of 1:1 and 2:1 complexes when metal ion is in excess. Ideally, the 1:1 constants from each run of this method should give the same number. The method does not

apply to  $\text{Pb}^{2+}$  because excess metal ion causes precipitation. It might, however, work for  $\text{Cu}^{2+}$ , assuming that these three types of complexes form.

The 1:1 calculation scheme appeared to work quite well for the  $\text{Cd}^{2+}$ -FA data presented in Chapter 2. However, the stability constants vary smoothly with mole ratio of  $\text{Cd}^{2+}$  to FA (Figures 3 and 4). This variation signifies the same continuum of site strengths that the Scatchard treatment does, but the values for stability constants are more accessible in plots like those in Figures 3 or 4 than in a Scatchard plot. The key point to realize is that stability constants depend on the extent of metal loading, that is, on the mole ratio of total metal ion to total fulvic acid.

In the final analysis, does it really matter which calculation scheme is used or what assumptions are made? Table 21 may provide a partial answer to this question. Compared there are Buffle and Scatchard stability constant calculations for  $\text{Cu}^{2+}$ -SFA, Buffle calculation for  $\text{Pb}^{2+}$ -SFA, and Buffle and 1:1 calculations for  $\text{Cd}^{2+}$ -SFA. For  $\text{Cu}^{2+}$ -SFA and  $\text{Cd}^{2+}$ -SFA, for which calculations are done two ways, the results are quite similar. It appears that it does not matter what calculation scheme is used. One possible reason is that none of these calculation models is particularly good, and that they all fall short of some ideal in different ways, but to similar extents.

Effect of fulvic acid concentration. The work in Chapter 2 should alert researchers that complexation



TABLE 21

Conditional stability constants calculated by various methods for complexes between metal ions and soil-derived fulvic acid

pH	<u>Log K</u>		
	copper (II)	lead(II)	cadmium (II)
4.0	<u>Scatchard</u> <sup>a</sup>	<u>Buffle</u>	<u>1:1</u>
	strong: 5.60 weak: 3.95	4.0	3.23
	<u>Buffle</u>		<u>Buffle</u>
	4.00		3.00
5.0	<u>Scatchard</u>	<u>Buffle</u>	<u>1:1</u>
	strong: 6.00 weak: 4.08	4.9	3.80
	<u>Buffle</u>		<u>Buffle</u>
	4.60		3.48
6.0	<u>Scatchard</u>	<u>Buffle</u>	<u>1:1</u>
	strong: 6.30 weak: 3.78	6.3	4.08
	<u>Buffle</u>		<u>Buffle</u>
	5.95		3.60
7.0			<u>1:1</u>
			4.32
			<u>Buffle</u>
			4.00
8.0			<u>1:1</u>
			4.63
			<u>Buffle</u>
			4.4

<sup>a</sup> Scatchard results from Bresnahan et al., 1978.

experiments done at widely varying concentrations of fulvic acid might not be strictly comparable, at least for weakly bound metal ions like  $\text{Cd}^{2+}$ . It would be gratifying to see concentration-dependent results for metal ions such as  $\text{Mn}^{2+}$  or  $\text{Ca}^{2+}$ .

Early onset of precipitation. The formation of precipitates is an especially serious problem with  $\text{Pb}^{2+}$ -FA complexes, but may arise for complexes with other metal ions as well. Solids would be especially likely to form in soils and sludges where metal ion and organic matter concentrations are high. Further studies should focus on learning why  $\text{Pb}^{2+}$  is so efficient at dragging fulvic acid out of solution.

#### FLUORESCENCE IN FULVIC ACID RESEARCH

The use of fluorescence as a tool to measure complexation between metal ions and natural organic matter provides a new perspective on this perverse mixture of ligands that we call fulvic acid. Our understanding of fulvic acid chemistry has advanced slowly, perhaps because the models we use are designed for simpler systems. Even so, the information now or soon to be available is useful and may be detailed enough to provide the information needed on the role of fulvic acid in metal speciation.

APPENDIX

```

C   PROGRAM NAME:  BUF4.FOR, 8/24/79
C   THIS PROGRAM OPTIMIZES B1 AND B2 IN EQUATION 14 OF
C   BUFFLE ET AL., ANAL. CHEM. 49, 216 (1977).
C
C
COMMON /AREA/XLT(50),FRMET(50),TMET(50),B2
COMMON /AREB/B1,TFUN,NN
COMMON /AREC/KA,ZZ,NAA
COMMON /ARED/XX(500)
COMMON /AREE/NM,NE,NF,NG
COMMON /AREF/YB1,YB2,NH
DOUBLE PRECISION FINP, FOUT, B1, B2
10  CONTINUE
    NM = 0
    NE = 0
    NF = 0
    NG = 0
    NH = 0
    DO 12 J = 1,500
    XX(J) = 0.0
12  CONTINUE
    DO 15 J = 1,5
15  CONTINUE
    WRITE(5,20)
20  FORMAT(' INPUT FILE NAME')
    READ(5,25)FINP
25  FORMAT(A10)
    WRITE(5,30)
30  FORMAT(' OUTPUT FILE NAME')
    READ(5,25)FOUT
    OPEN(UNIT=1,ACCESS='SEQIN',FILE=FINP)
    OPEN(UNIT=20,ACCESS='SEQOUT',FILE=FOUT)
    WRITE(5,40)
40  FORMAT(' HOW MANY LINES TO BE IGNORED?')
    READ(5,*)K
    DO 60 J=1,K
    READ(1,25)D
60  CONTINUE
    WRITE(5,80)
80  FORMAT(' HOW MANY DATA POINTS?')
    READ(5,*)KA
    DO 120 J=1,KA
    READ(1,100)XLT(J),FRMET(J),TMET(J)
100 FORMAT(26X,2E11.4,11X,E11.4)
120 CONTINUE
C   WRITE(5,130)
C130 FORMAT(' WHAT % SHOULD EACH ITERATION CHANGE BY?')
C   READ(5,*)Z
    Z = 8.0
    ZZ = Z/100.0
C   WRITE(5,150)
C150 FORMAT(' ENTER INITIAL VALUE OF B1')
C   READ(5,*)BI1
    BI1 = 1000.
C   WRITE(5,160)

```

```

C160  FORMAT(' HOW MANY STARTING STEPS?')
C      READ(5,*) JE
      JE = 7
      JX = 4*JE + ((JE-1)**2)/2 + (JE-1)/2
      WRITE(20,170)FINP,K,KA,Z,JE,B11
170   FORMAT(1H1,////////,X,A10,'          PB(FA) AND PB(FA)2
1     ANALYSIS',////////,
1     ' LINES SKIPPED:      ',I2,4X,'      DATA POINTS:      ',
2     I2,/, ' INITIAL % CHANGE:      ',F3.0,4X,'STARTING'
3     'STEPS:      ',I3,/, ' INITIAL B1:      ',E11.4)
      JD = JE - 1
600   DO 700 J = 0,JD
      B1 = B11*(4**J)
      B2 = B11*(4**J)
      YB1=B1
      YB2=B2
      CALL ST
      JQ = JD + 3 - J
      DO 700 JJ = 1,JQ
      B2 = B11*(4**J)*(4**JJ)
      B1 = B11*(4**J)
      YB1=B1
      YB2=B2
      CALL ST
700   CONTINUE
      WRITE(20,710)
710   FORMAT(//,'          B1          B2          FUNCTION',
1     ' STARTING B1  STARTING B2  ITERATIONS',/)
      WRITE(20,715)
715   FORMAT(/,'  -----',
1     '-----')
720   FORMAT(/,2E12.4,E14.6,2E12.4,5X,F5.0)
      JY = JX-NH-NM-1
      IF(NAA.GT.10) GO TO 725
      GO TO 728
725   CONTINUE
      NAA = 10
728   DO 770 JU = 1,NAA
      DO 750 J = 1,JY
      IF(J.GT.1) GO TO 730
      BF = XX(4)
      IN = J-1
730   CONTINUE
      KQ = 4 + 6*J
      CF = XX(KQ)
      IF(CF.GT.BF) GO TO 750
      IN = J
      BF = CF
750   CONTINUE
      LQ = 1 + 6*IN
      LR = 2 + 6*IN
      LS = 3 + 6*IN
      LT = 4 + 6*IN
      LU = 5 + 6*IN
      LV = 6 + 6*IN

```

```

WRITE(20,720)XX(LR),XX(LS),XX(LT),XX(LU),XX(LV),
1  XX(LQ)
XX(LT) = 1000.
770  CONTINUE
WRITE(20,715)
WRITE(20,830)NM,NG,NE,NF,NH
830  FORMAT(///,' REPETITION LIMIT REACHED: ',I5,/,
1  4X,' SUBROUTINE ST CALLED: ',I6,/,
2  4X,' SUBROUTINE SS CALLED: ',I6,/,
3  4X,' FUNCTION LOOP CALLED: ',I6,/,
4  6X,' B1 VALUES < 100: ',I6)
WRITE(5,850)
850  FORMAT(' TYPE 1 TO RUN AGAIN')
READ(5,*)NI
IF(NI.EQ.1) GO TO 10
STOP
END

```

C  
C

```

SUBROUTINE SS
COMMON /AREA/XLT(50),FRMET(50),TMET(50),B2
COMMON /AREB/B1,TFUN,NN
COMMON /AREC/KA,ZZ,NAA
COMMON /ARED/XX(500)
COMMON /AREE/NM,NE,NF,NG
COMMON /AREF/YB1,YB2,NH
NE = NE + 1
NN = NN + 1
IF(NN.LT.200) GO TO 170
NM = NM + 1
GO TO 200
170  CONTINUE
TFUN = 0.0
DO 180 J=1,KA
NF = NF + 1
A = TMET(J)/FRMET(J)
Y = (XLT(J)/TMET(J))*A/(A-1.0)
PART1 = (B1/(2.0*B2))*((1.0+(4.0*B2/(B1**2.0)))*
1  (A-1.0))**0.5-1.0)
PART2 = (1.0/TMET(J) - B1/A)*A/(A-1.0)
FUNC = (1.0/Y*(2.0 + PART1*PART2) - 1.0)**2.0
TFUN = TFUN + FUNC
180  CONTINUE
IF(TFUN.GE.0.0) GO TO 200
TFUN = -1.0*TFUN
200  CONTINUE
RETURN
END

```

C  
C

```

SUBROUTINE ST
COMMON /AREA/XLT(50),FRMET(50),TMET(50),B2
COMMON /AREB/B1,TFUN,NN
COMMON /AREC/KA,ZZ,NAA
COMMON /ARED/XX(500)

```

```

COMMON /AREE/NM,NE,NF,NG
COMMON /AREF/YB1,YB2,NH
NG = NG + 1
WRITE(5,220)NG,NF
220  FORMAT(I3,I7)
      XNC = 1.0
      NN = 0
      NA = 1
      CALL SS
230  OFUN = TFUN
      BB1 = B1
      BB2 = B2
C     BB1 IS THE B1 VALUE ASSOCIATED WITH THE LOWEST
C     FUNCTION VALUE; SIMILARLY, BB2 IS THE BEST B2 VALUE.
240  CONTINUE
      NB = 1
250  B1 = B1*(1.0 + ZZ/XNC)
      NA = NA + 1
      CALL SS
      IF(NN.EQ.200) GO TO 500
      IF(TFUN.GT.OFUN) GO TO 270
      GO TO 230
270  B1 = B1/(1.0 + ZZ/XNC)
      IF(NA.GT.2) GO TO 300
280  B1 = B1*(1.0 - ZZ/XNC)
      CALL SS
      IF(NN.EQ.200) GO TO 500
      IF(TFUN.GT.OFUN) GO TO 290
      OFUN = TFUN
      BB1 = B1
      BB2 = B2
      GO TO 280
290  B1 = B1/(1.0 - ZZ/XNC)
300  B2 = B2*(1.0 + ZZ/XNC)
      NB = NB + 1
      CALL SS
      IF(NN.EQ.200) GO TO 500
      IF(TFUN.GT.OFUN) GO TO 340
      OFUN = TFUN
      BB1 = B1
      BB2 = B2
      GO TO 300
340  B2 = B2/(1.0 + ZZ/XNC)
      IF(NB.GT.2) GO TO 400
350  B2 = B2*(1.0 - ZZ/XNC)
      CALL SS
      IF(NN.EQ.200) GO TO 500
      IF(TFUN.GT.OFUN) GO TO 390
      OFUN = TFUN
      BB1 = B1
      BB2 = B2
      GO TO 350
390  B2 = B2/(1.0 - ZZ/XNC)
400  CONTINUE
      XNC = XNC + 2.0

```

```
IF(XNC.GT.10.0) GO TO 420
NA = 1
GO TO 240
420 CONTINUE
IF(BB1.GT.100.0) GO TO 430
NH = NH + 1
GO TO 500
430 CONTINUE
NAA = NG-NM-NH
LL = 6*NAA-5
L = 6*NAA-4
M = 6*NAA-3
N = 6*NAA-2
NO = 6*NAA-1
NP = 6*NAA-0
XX(L) = BB1
XX(M) = BB2
XX(N) = OFUN
XX(NO) = YB1
XX(NP) = YB2
XX(LL) = NN
500 RETURN
END
```



L7132.B5 PB(FA) AND PB(FA)2 ANALYSIS

LINES SKIPPED: 16 DATA POINTS: 8  
 INITIAL & CHANGE: 8. STARTING STEPS: 7  
 INITIAL B1: 0.1000E+04

B1	B2	FUNCTION	STARTING B1	STARTING B2	ITERATIONS
0.6203E+05	0.2831E+09	0.192239E-01	0.2560E+06	0.2621E+09	38.
0.6301E+05	0.2735E+09	0.192340E-01	0.1024E+07	0.2621E+09	63.
0.6308E+05	0.2725E+09	0.192410E-01	0.1000E+04	0.2621E+09	76.
0.6314E+05	0.2725E+09	0.192487E-01	0.4000E+04	0.2621E+09	58.
0.6327E+05	0.2712E+09	0.192656E-01	0.6400E+05	0.2621E+09	24.
0.6335E+05	0.2691E+09	0.192782E-01	0.4096E+07	0.2621E+09	71.
0.6337E+05	0.2699E+09	0.192803E-01	0.1600E+05	0.2621E+09	40.
0.6642E+05	0.2382E+09	0.206277E-01	0.1000E+04	0.6554E+08	133.
0.6723E+05	0.2301E+09	0.212731E-01	0.4096E+07	0.6554E+08	125.
0.6753E+05	0.2278E+09	0.215457E-01	0.4000E+04	0.6554E+08	118.

REPETITION LIMIT REACHED: 4  
 SUBROUTINE ST CALLED: 49  
 SUBROUTINE SS CALLED: 5982  
 FUNCTION LOOP CALLED: 47824  
 B1 VALUES < 100: 0

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TABLE 19

Cd<sup>2+</sup>-SFA fluorescence quenching at pH 7.5-8.0<sup>a</sup>

$\text{Cd}^{2+}$ (M x 10 <sup>4</sup> )	$C_{\text{Cd}}/C_{\text{SFA}}$	pH	% quenched <sup>b</sup>
0.0	0.0	7.50	0.0
0.246	0.5	7.50	1.4
0.492	1.0	7.50	-0.9 <sup>c</sup>
0.983	2.0	7.87	3.2
1.97	4.0	7.51	-0.5 <sup>c</sup>
2.95	6.0	7.60	2.3
4.42	9.0	7.41	0.5
5.90	12.0	7.85	1.9
7.37	15.0	7.56	1.9

<sup>a</sup> SFA concentration is  $4.9 \times 10^{-5}$  M. The experiments were performed in 0.1 M KNO<sub>3</sub> at 23°C.

<sup>b</sup> The uncertainty in the % quenched values is ±2%.

<sup>c</sup> Negative values indicate that the fluorescence intensity is greater for SFA with metal ion than for the reference sample that has no metal ion.