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THE DETERMINATION OF STABILITY
CONSTANTS AND COMPLEXING
CAPACITIES OF NATURAL ORGANIC
MATTER FOR COPPER (2+), COBALT (2+)
AND MANGANESE (2+) BY
FLUORESCENCE QUENCHING
TITRATION

DAVID K. RYAN

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THE DETERMINATION OF STABILITY CONSTANTS
AND COMPLEXING CAPACITIES OF NATURAL ORGANIC MATTER FOR
 Cu^{2+} , Co^{2+} AND Mn^{2+} BY FLUORESCENCE QUENCHING TITRATION

BY

David K. Ryan
B.S., Le Moyne College, 1977

A DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
in
Chemistry

May, 1983

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To My Family

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ABSTRACT

THE DETERMINATION OF STABILITY CONSTANTS AND COMPLEXING CAPACITIES OF NATURAL ORGANIC MATTER FOR Cu^{2+} , Co^{2+} AND Mn^{2+} BY FLUORESCENCE QUENCHING TITRATION

by

David K. Ryan

University of New Hampshire, May, 1983

The purpose of this study is to develop methodologies for the investigation of the metal ion binding properties of natural humic substances. Humic materials are prevalent in soil and water and their important metal ion detoxification and transport properties are the major justification for this work. The primary emphasis is the determination of the complexing capacity of a sample for a particular metal ion under fixed conditions.

The initial part of the investigation involves the application of chelating ion exchange to remove and quantify humic-chelated trace metal ions from water samples. To achieve this goal, amine, dithiocarbamate and 8-hydroxyquinoline chelating agents immobilized on porous glass beads are compared to Chelex 100 in column operation. In the presence of humic materials, modeled with isolated soil-derived fulvic acid (SFA), the removal of metal ions with Chelex is significantly hindered while the stronger immobilized chelates still perform adequately. All of the chelates tested removed SFA from solutions as well as metal ions making them unsuitable for

preparing natural water samples for complexing capacity measurements.

A fluorescence titration technique is also described which can be used to determine complexing capacities (C_L) and 1:1 conditional stability constants (K) of fluorescing ligands with paramagnetic metal ions at the micromolar level. An equation is derived allowing calculation of the amount of ligand bound from fluorescence quenching curves. Further analysis of the data and fitting to a theoretical equation by a nonlinear regression computer program results in the best fit K and C_L . Fluorescence results for SFA with Cu^{2+} at pH 5, 6 and 7 compare well with results for the same material using different techniques. Titrations of the model compound L-tyrosine prove the validity of the technique. Rayleigh scattering data collected simultaneously with fluorescence indicates precipitate formation during complexing capacity titrations. The combined fluorescence and scattering measurements result in a powerful method that can distinguish between solution phase complexation and processes that may be occurring at the solid-solution interface.

The natural fluorescence of humic substances in both marine and freshwaters is also quenched upon complexation to Cu^{2+} ion. The fluorescence technique is therefore applied to several samples to determine stability constants and complexing capacities. The results indicate that this method directly measures organic matter complexation only, and C_L values are unaffected by hydrolysis. Scattering and fluorescence trends

were similar for all samples except an estuarine sample which demonstrated a high degree of scattering with the addition of Cu^{2+} . Pore water obtained from several estuarine sediment cores contained a high organic matter content but a low C_L value, probably due to competition from other ions. A multiple correlation study of several titration parameters for the freshwater samples showed a statistically significant trend between UV absorbance and C_L values.

The binding of Cu^{2+} to soil-derive fulvic acid (SFA) measured by fluorescence quenching titration is much stronger than Co^{2+} and Mn^{2+} which are similar. The conditional stability constants from curve fitting at pH 6 are 1.1×10^5 for Cu^{2+} , 5.1×10^3 for Co^{2+} and 4.2×10^3 for Mn^{2+} . Co^{2+} and Mn^{2+} not only tend to bind more weakly, but also bind to fewer sites. Scattering experiments demonstrate that Cu^{2+} is more effective at precipitating and aggregating SFA than Co^{2+} and Mn^{2+} .

CHAPTER 1

METAL ION SPECIATION IN NATURAL WATERS: AN OVERVIEW

INTRODUCTION

The transport, toxicity and removal of trace metal ions in aquatic systems is intimately related to their speciation (Sposito, 1981; Neely, 1980). The term speciation, as it is used here, relates to the chemical state or form in which a metal ion might be found. It may be present in one or more oxidation states and simply hydrated, complexed to various ligands or adsorbed on a surface. Under certain conditions naturally occurring humic and fulvic acids can play a major role in determining the forms of certain trace elements present in natural waters (Florence and Batley, 1980; Mantoura and Riley, 1975). The ability to predict speciation of various metal ions in a given system relies heavily on data for organic matter complexes as well as the relatively well understood behavior of inorganic ligands. In order to obtain reliable information on the extremely complex trace metal-humic material interactions, existing data must be supported and confirmed using alternate methods. This is especially true for the challenging analytical problems that natural systems provide.

In order to better understand metal ion complexation by humic materials many different types of studies are needed. The complexities of this natural system also require that

data from different experimental methods be compared to obtain unambiguous results.

The objective of this study is to develop an appropriate methodology for investigating trace metal-organic matter speciation in natural waters. The reasons for doing this are to obtain data to support or contradict existing data from a new experimental perspective, provide additional information from combining two or more techniques, and address some unanswered questions. No one technique or procedure can be expected to elucidate all the intricacies of a difficult problem. At best any new methodology may strengthen weak areas in existing knowledge and explore the fringes of what is known. There will, of course, be limitations to any new procedure even though it may be a significant improvement over what has preceded.

Complexing Capacity

The general approach to speciation used here is to determine the capacity of organic ligands in a sample to complex added metal ion. It is also desired to measure the extent to which naturally present trace metals are complexed by organic matter. The term complexing capacity describes this, and the value determined is for a given metal ion (i.e. Cu^{2+}). In natural systems where trace metals are already present it is necessary to define some additional terms to better describe the system. Truitt (1980) has

developed a system of nomenclature for this purpose which is outlined here with only minor modifications.

The metals present in a sample will be occupying some binding sites on organic matter which will be referred to as the natural complexing level (NC_L). The NC_L is with respect to a particular metal ion but, can be determined in a sample for any metal ions for which it can be detected. For example some values of NC_L for a typical river sample might be 1×10^{-8} M for Cu^{2+} , 1×10^{-9} M for Pb^{2+} and 1×10^{-9} M for Co^{2+} . These concentrations represent the amount of the metal ion that is complexed. The total concentration of each metal ion in the sample may be higher or it may be about equal to the NC_L value. For strongly bound metals like Pb^{2+} and Cu^{2+} it will probably be about equal because nearly all the metal ion is bound. For a weak binding metal ion like Co^{2+} the total concentration will probably be somewhat higher than the NC_L indicating a significant amount of free Co^{2+} .

When metal ion is added to a sample, it will be bound by unoccupied sites on the natural organic matter. It will also compete for binding sites with trace metals already present. If the metal ion binds very strongly to organic compounds in the sample it may effectively displace the labile trace metals that are complexed. This process can lead to the release of very toxic metal ions in a water system even if it becomes polluted with an ion of relatively

low toxicity. Other weakly binding ions may not compete effectively with many naturally complexed metals. Certain metal complexes of organic matter (probably a small fraction of the total) may be inert and therefore unaffected by large amounts of another metal ion.

The goal of this research is to develop a reliable method to determine the potential complexing capacity of natural organic matter in aquatic systems. It is also desired to effect a removal of naturally present metal ions, leaving a sample with no metal and only unbound ligand. In this way the total complexing capacity of the sample could be measured and compared with its C_L . From this information it is possible to get an idea of how well an ion competes with other ions and to what extent it may cause the release of existing complexed toxic metal ions.

The ability of a water system to bind metal pollutants has several important uses. It is generally agreed that uncomplexed or "free" metal ions are more toxic than complexed metals (Zamada and Sunda, 1982; Crecelius et al., 1982). However, there are no fixed guidelines or standards that indicate a limit of free metal ion concentrations at a certain total metal ion concentration. The data generated by any study therefore, must be sufficiently complete so that a variety of types of information can be extracted from it. For example, for a given metal ion it may be desirable to know the amount of the metal ion that can be added to a

water system to produce 50% of the metal ion free and 50% bound to organic matter. However, it might also be desired to know the total metal level that gives rise to a fixed free metal concentration for the metal ion of interest. Going above this free metal concentration may cause toxic effects. On the other hand it could be important to know what metal ion level will saturate available organic binding sites so that further added metal ion will remain free. It is this latter parameter that is specifically determined here and is designated the complexing capacity or total ligand concentration (C_L) with respect to a particular metal ion. The other two criteria describing the overall speciation of a metal ion can also be calculated from the data reported. By using the C_L value and the conditional stability constant (K) very good estimates can be made defining the position of the equilibrium at any total metal ion concentration. Studies aimed at computer modeling of speciation patterns in complex systems such as rivers or estuaries can also use this data. The metal ion-organic matter K value is input with the desired concentration level and the effect of natural organic ligands as well as inorganic ligands on the trace metal speciation can be calculated. Therefore even though the use of a simple 1:1 stability constant is not the ideal model to describe the actual chemistry, it turns out to be a very practical way to define the properties of the overall system.

General Properties of Humic Materials

The vast majority of organic compounds found in natural waters are humic materials. Humic materials are polymeric compounds formed through the process of humification or decomposition of plant and animal matter (Hartenstein, 1981). Humification occurs aerobically and is the relatively rapid first step in the fossilization of organic matter which may eventually become coal. The humic substances formed are mixtures of compounds with aromatic and aliphatic backbones and a wide diversity of bond types and functional groups. This makes them nearly impossible to characterize in definitive chemical terms. They do not have a specific structure, molecular weight, or ultraviolet (UV) absorbance spectrum, etc. They are also difficult to separate from lignin precursors and non humics such as carbohydrates and amino acids.

The humic material used as a model in the experiments described here is soil derived fulvic acid (SFA). Fulvic acid is the fraction of natural organic matter that is soluble in both acid and base. On the basis of its solubility it can be separated from humins (acid and base insoluble) and humic acid (base soluble) organic matter.

Fulvic acid consists of polyfunctional acids, as do the other fractions of humic material, but it generally contains more carbonyl and phenolic groups per molecule. The molecular weight range encountered for fulvic acid

(500-10,000 daltons) is generally smaller than for other humics. One very important property of fulvic acid is its metal chelating ability. It is generally thought to be a result of salicylate and phthalate functions.

Metal complexation by fulvic acid will be discussed in much greater detail in the following chapters. Further information on fulvic acid and humic materials in general can be found in several books (Schnitzer and Khan, 1972; Gjessing, 1976; Konanova, 1960) and reviews (Reuter and Perdue, 1977; Jackson, Jonasson and Skippen, 1978).

Method Development

Several factors should be considered when undertaking the development of an analytical method. These include: the end result desired, the types of samples to be analysed, the possible modes of use for the method, the skill level of the probable users, and the required precision and accuracy. The path to attaining these goals should be divided into steps with intermediate goals each of which will be attacked separately as part of the overall plan. Trying to solve the entire problem with one ingenious experiment only leads to difficulties.

There are three major subdivisions of this project which are described in the next three chapters. The first aspect was to attempt the removal and measurement of naturally present metal ions from a water sample without

otherwise altering it. This would presumably leave an ideal matrix for adding back metals and studying the binding properties of the organic matter without interference from the natural metal ions. Quantification of the metals removed would give information on the natural levels of bound metal. The second step was to develop a method for measuring complexing ability and stability constant relationships for metal ions and organic matter. SFA was used as model organic matter with Cu^{2+} as the ion of interest. Thirdly the method was applied to real environmental samples from several sources to evaluate its actual utility. Cupric ion was again used as a probe ion for this work. An additional stage in this work described in Chapter 5 is the extension of the method to Co^{2+} and Mn^{2+} ions and the comparison of their binding properties to SFA with those of Cu^{2+} .

Additional Considerations

Research on complex systems such as humic materials in natural aquatic environments requires a realistic and knowledgeable appreciation of all the variables of the system that are known or suspected to be important. It is very possible that humic substances from water will be slightly different from those obtained from soil. Geographic and climatic conditions might also affect the nature and composition of the material. The method by which material was isolated or samples collected can also have a direct

bearing on the results. Alteration of the organic matter is likely when harsh reagents are used. The way the sample is stored, the degree of exposure to sunlight, even the way in which solutions of isolated material are prepared can affect the properties of the resulting solution and thus the data collected.

If the solution is filtered through a microporous filter membrane some material will be lost. It is always observed that when solutions of SFA are filtered the filter membrane has a noticeable brown film remaining. The size and amount of the material lost will depend on the porosity of the membrane.

The concentration at which solutions are prepared may also be a subtly important variable. Two solutions of the same concentration, one prepared by serial dilution of a concentrated solution the other by dissolving powdered material directly, may be different. There are definite solubility limits for SFA as a whole but there may also be narrower solubility ranges for certain component compounds in the SFA mixture. Concentration has even been shown to be an important variable in metal complexation studies with Cd^{2+} (Saar and Weber, 1979). The age of samples is important because there always exists the possibility of degradation, especially by microbes.

All of the above mentioned considerations are important, and to a greater or lesser degree affect the results of experiments with SFA. Since there are no set protocols to follow, it becomes necessary to be completely consistent from experiment to experiment. Although this may seem obvious the consistency must be extended to the point of adopting procedures not normally deemed necessary.

When working with natural water samples as opposed to laboratory prepared SFA solutions, additional considerations must be made. Natural systems although sometimes considered to be in a state of equilibrium, are not really. They are constantly changing due to energy input from the sun, wind, and biological activity, and chemical and physical (particulate) input from the air, sediments, and plant and animal life.

The process of sampling, enclosing a small portion of the natural system in a container, cuts it off from input and output allowing chemical forms to come to equilibrium with particulate matter and the walls of the container. The equilibrium once achieved in the water sample may be very different from the chemical state of the water in the natural system and any analogies drawn between them may be considered technically invalid (Robertson, 1976).

With these considerations in mind it is easy to justify filtration of samples through 0.4 μm membranes as soon as possible after sampling to remove particulates that may

cause chemical changes. It may also be prudent to equilibriate sample containers with the natural water to be sampled immediately prior to sampling in order to satisfy active sites on the container walls (except in the case of acidification). Also storage of samples at 4 °C is recommended to slow down chemical and biological processes. Although many papers have been published on the storage of natural water samples, most advocate the use of various agents as preservatives. In speciation experiments this cannot be done.

CHAPTER 2

COMPARISON OF CHELATING AGENTS IMMOBILIZED ON GLASS WITH CHELEX 100 FOR TRACE METAL REMOVAL AND PRECONCENTRATION

INTRODUCTION

In an attempt to achieve the goal of removing trace metals from natural water samples, chelating ion exchange separations were investigated. This method was chosen because it has been reported to be capable of quantitative or complete sequestering of trace metals (Riley and Taylor, 1968; Abdullah and Royle, 1972). In addition ion exchangers are fairly easy to use, requiring no elaborate or expensive equipment, and there are several types available for different applications.

Chelating ion exchangers are generally insoluble substances such as organic polymer resins or silica based supports with strong chelating groups attached. When they come in contact with a solution containing metal ions these materials remove the metal ions because the chelating functional groups are solvated to some extent and they behave similar to ligands in solution. However, they are bound to a support and thus extract the metal ions from solution onto the solid phase. In theory chelating ion exchangers are ideally suited for the task described previously. They should remove trace metal ions without altering a solution in any other way.

The use of exchangers in a column mode of operation has several attractive features. As sample moves down a column it continually comes in contact with fresh chelating agent and can come to equilibrium or at least reach a steady state removal rate if the flow rate is properly adjusted. Thus this mode of operation is inherently more efficient than batch methods. Column systems can handle large volumes easily without much sample manipulation and are amenable to automation.

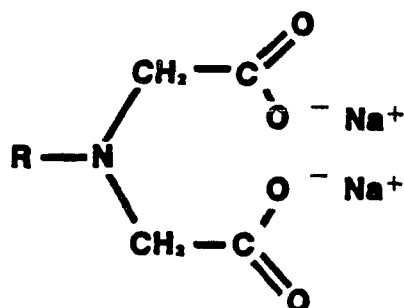
An added advantage of using chelating ion exchangers to remove metal ions is that they can also aid in the quantification of the total levels of various metal ions in the sample.

Preconcentration. Analysis of trace or ultratrace levels of metal ions in natural waters is often coupled with some sort of preconcentration step to bring the analytes up to levels that are easily and reliably measured. One commonly used method is to pass a large volume of sample through a column of a cation exchange resin which will retain the trace metals. The metals can then be recovered by eluting with acid into a much smaller volume thus effecting a preconcentration equal to the ratio of the volume of sample to the volume of acid eluate. This is typically done prior to conventional atomic absorption (AA) analysis since flame AA does not have low enough detection limits for natural levels of many important trace metals.

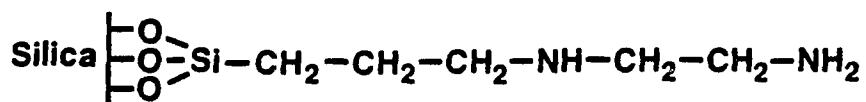
One chelating resin that has seen widespread use in this type of application is Chelex 100. Figure 1 shows the structure of Chelex which has iminodiacetic acid functional groups. These groups have a fairly high affinity for transition metal ions, and also an affinity for alkaline earth ions. The "R" in Figure 1 represents the styrene divinylbenzene co-polymer support to which the functional groups are anchored (Bio-Rad, 1978).

Although Chelex is used for trace metal preconcentration, work by several different groups has shown that Chelex is ineffective in giving quantitative separations of many metal ions from water samples with appreciable amounts of naturally occurring fulvic and humic acids (Florence and Batley, 1976; Figura and McDuffie, 1977). This natural organic matter binds metals and competes with the Chelex. In addition some metal complexes of natural organic matter may be excluded from the pores of the resin because of their size preventing direct exchange with most of the functional groups. This tends to further lower the efficiency of an already difficult separation.

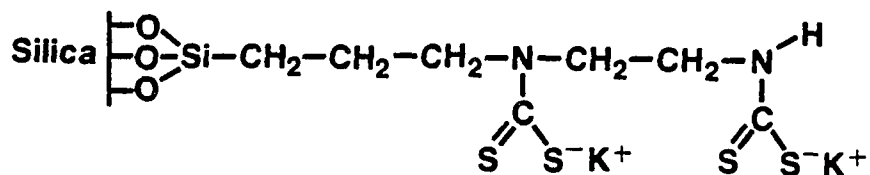
To achieve quantitative separations of trace metals from media containing these natural complexing agents certain chelating agents immobilized on glass were investigated. These materials would also allow preconcentration of these ions prior to analysis. The chelating agents are covalently anchored to a rigid glass



Chelex 100



Diamine



DTC

Figure 1. Structure of Chelex 100 (Bio-Rad Laboratories, 1978), silica immobilized ethylenediamine (diamine) and bis(dithiocarbamate) (DTC). R represents the styrene divinylbenzene copolymer support.

support and offer a high degree of specificity for metal ions over alkali metal and alkaline earth ions and increased mechanical strength for work at high pressures and rapid flow rates. Two of these immobilized chelates are shown in Figure 1, the N-propylethylenediamine (diamine) and the bis(dithiocarbamate) (DTC). The diamine is readily made via a silylation of a reactive silane with the surface OH groups of porous glass beads or silica gel. The anchored diamine is a somewhat weak binder of metal ions but can be converted to a very strong chelator, the DTC, by reaction with CS_2 in basic media. The DTC has a very high affinity for transition metals but is plagued by instability toward acid (Halls, 1969).

Figure 2 gives the structure of an immobilized chelate with 8-hydroxyquinoline functional groups (8-HQ). As it is shown here the immobilized diamine is used as the starting material which is coupled through a diazonium linkage to 8-hydroxyquinoline (Ryan and Weber, 1979). Previously reported work with 8-HQ made use of a single amine group in the synthesis (Hill, 1973; Sugawara, Weetall and Schucker, 1974; Jezorek and Freiser, 1979). By using a diamine the surface concentration of chelating groups is potentially doubled. This procedure has recently been adopted by others (Fulcher et al., 1981).

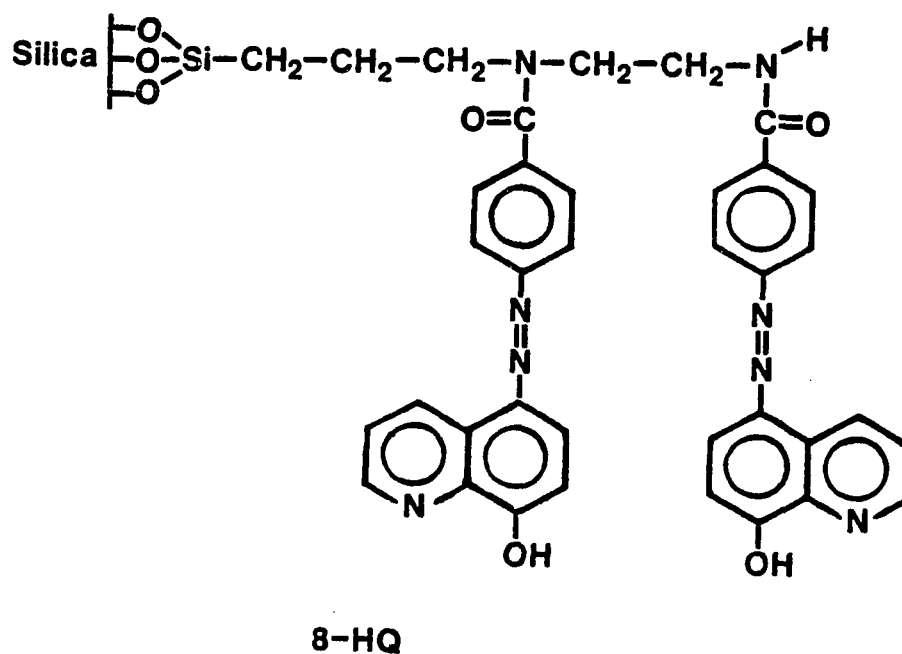


Figure 2. Proposed structure for the 8-hydroxyquinoline immobilized chelate (8-HQ) prepared from the diamine.

LITERATURE

Methods of immobilization. There has been a great deal of work reported on various schemes and specific procedures for immobilizing reagents on insoluble solid, inert supports. Much of this has been directed at improving ion exchange separations and producing novel chelating ion exchangers. However, work in the areas of immobilized catalysts, immobilized enzymes, chemically modified electrodes, adhesive coatings and high pressure liquid chromatography (HPLC) all have contributed.

The method of immobilization used in this study is silylation. In a report concerning a wide variety of reactions of silanes with surfaces Arkles (1977) describes the silylation reaction between a reactive silane, and the surface silanol groups of glass. There are four steps in the overall reaction. Initially the labile groups (chloro, methoxy, or ethoxy) undergo hydrolysis either with traces of water in non-aqueous solvents or in aqueous solution. This is followed by condensation to oligomers which then hydrogen bond to the surface silanol groups. The final step is the curing process where stable siloxane bonds are formed with the loss of water at 80 to 120 °C.

Publications concerned with the technology of improving bonded stationary phases for HPLC contain information applicable to immobilized chelating agents. Grushka (1974) and others (Grushka and Kikta, 1977; Unger et al., 1976)

have reviewed the silylation of microporous silica and its usefulness in preparing bonded phases for HPLC.

Probably the most common method of attaching chelating agents to solid supports is via adsorption. One procedure uses Apiezon L coated glass beads (Miyazaki and Okubo, 1976; Okubo et al., 1979). Apiezon L is a gas chromatography stationary phase made up of a mixture of hydrocarbons that allows the hydrophobic adsorption or partitioning of ligands on the glass support. Ligands such as dibenzoylmethane, benzoyltrifluoroacetone, 8-hydroxyquinoline and others have been immobilized in this way. The best results were obtained with beta-diketones due to their lower solubility in the aqueous mobile phase. Column experiments with these materials gave only fair results. Even though the immobilized ligand may quantitatively bind any metal present, about 2% of the metal elutes from the column due to the solubility of the metal complexes in the aqueous mobile phase.

Terada and coworkers (1977; 1980) adsorbed chelating agents directly on silica gel from dioxane solutions and then dried the material. The 2-mercaptobenzathiazole immobilized ligand was used to retain cadmium, copper, lead and zinc from freshwater and seawater. A p-dimethylaminobenzylidenerhodanine chelate on silica gel concentrated silver, gold and palladium from seawater.

An interesting alternative to chelating agents adsorbed

or covalently bound to a support is the use of a "chelating agent-loaded anion exchanger" (Lee, Lee and Lee, 1978). This approach uses chelating agents with an added anionic functional group such as 7-iodo-8-hydroxyquinoline-5-sulfonic acid, chromotropic acid and 5-sulfosalicylic acid. These are loaded on an anion exchanger, Dowex 1-X8, and used for selective separation of metal ions. By varying pH and the chelate used quantitative separations of Fe^{3+} from Zn^{2+} , Pb^{2+} and Cu^{2+} are achieved as well as Cu^{2+} from Mg^{2+} , Ni^{2+} and Pb^{2+} and Cr(VI) from Pb^{2+} , Zn^{2+} and Ni^{2+} . One drawback to this system is that the anions present in the metal ion solution may tend to exchange with the chelates on the resin reducing the capacity of the loaded resin and leaching chelating agent and metal complexes into solution. The extent to which this occurs is dependent on the anions present and their selectivity for the resin. Due to the anionic nature of natural organic material and the diversity of other anions present in natural waters, especially seawater, this technique may find limited use in natural systems.

One very common method of producing insoluble chelating ion exchange material is to incorporate ligands in polymer resins. Although there are many examples of this in the literature they will not be discussed in depth here. Of particular interest however, are Chelex 100, the resin based 8-hydroxyquinoline (Hoek and Reedijk, 1980; Vernon and Eccles, 1973; Parrish, 1982) and the polydithiocarbamate

chelates (Barnes and Genna, 1979).

Another procedure that has seen some application is the bonding of various groups to cellulose (Smits and Van Grieken, 1978; Bauman et al., 1967). Certain modified starch and cellulose materials are commercially available.

Chelates bound via silylation. Recently a great deal of work has been reported on the immobilization of chelates on microporous glass beads or silica gel via silylation. The chelating functional groups most commonly employed for this are amines and diamines. The reasons for their popularity are:

1. The immobilization is easy using either an aqueous room temperature preparation (Leyden et al., 1975a; 1975b; 1975c; Hercules et al., 1973) or a non-aqueous one in refluxing toluene (Fulcher et al., 1981) or acetone (Hill, 1973).

2. Their low cost and availability due to applications in adhesive coatings.

3. Their well-understood metal complexation since non-immobilized ethylenediamine and N-propylethylenediamine complexes have been extensively studied (Smith and Martell, 1975).

4. They are used as starting materials for other chelate systems (Leyden et al., 1975a; Fulcher et al., 1981) and in chemically modified electrodes (Moses et al., 1978; White and Murray, 1979).

5. They can be used for binding anions as well as cations (Leyden et al., 1976b; 1978).

Much of the early work on the use of silica immobilized amines for preconcentrating trace metals was done by Leyden and coworkers (1975a; 1975b; 1976a; 1976b; 1976c). Their primary application was for sample preparation and concentration prior to x-ray fluorescence analysis. Metal ions such as Hg^{2+} , Cu^{2+} , Zn^{2+} and Cd^{2+} were found to be 99% removed by the diamine chelate (Figure 1) in the pH 7 to 8 range (Leyden and Luttrell, 1975a). However, most of the work was done on simple aqueous metal ion solutions and only a couple of examples were given of real samples that had not been prepared in the laboratory. Furthermore no verification was given for these real samples using a total mass balance or a referee method.

Leydens group (1976a; 1975b) and Hercules et al. (1973) also reported the preparation and use of dithiocarbamate immobilized chelates prepared from the amine and diamine functional groups. Dithiocarbamates form very stable complexes with transition metals and can, therefore, out perform amines. However they are not stable toward acid attack (Halls, 1969). At low pH it is attacked by protons at the "lone pair electrons" on nitrogen, driving the CS_2 group off. Once complexed to a metal ion the electronic structure of the DTC is extremely stable and is no longer susceptible to acid attack. Both of these reagents have

been used by Leyden and coworkers (1975a, 1976a) for the preconcentration of metals prior to X-ray fluorescence analysis.

The preparation of 8-hydroxyquinoline immobilized on silica was first reported by Hill (1973). He found the material to be quite stable below pH 9 and capable of removing Cu^{2+} from solutions of high ionic strength. The Cu^{2+} was easily recovered from the chelate with 1 M HCl. Sugawara, Weetall and Schucker (1974) also reported the bonding of 8-hydroxyquinoline via silylation reaction. They carried out some column experiments with metal ion solutions including the determination of the capacity of the immobilized chelate and recovery studies of the chelate in solutions of various first row transition metals. The stability of the chelate was also studied as a function of pH and found to be good in acid and neutral solutions.

The 8-HQ chelate has been shown to compete successfully with the thiocyanate ligand for trace Cu^{2+} , Pb^{2+} , Cd^{2+} and Zn^{2+} (Moorhead and Davis, 1974). These workers found that passage through a short column of 8-HQ was the only practical way in which they could remove trace metals from their 6.0 M NaSCN electrolyte. By passing the solution through a column of 8-HQ twice at pH 9 they were able to remove the metals to the sub-part-per-billion level where they did not interfere with the anodic stripping voltametry (ASV) of gallium. Others have used 8-HQ for

preconcentration of metals from natural water samples (Guedes da Mota and Griepink, 1977; Sturgeon et al., 1981). Guedes da Mota and Griepink (1977) used an automated system to compare columns of an ion exchange resin with 8-hydroxyquinoline immobilized on a glass support. They found that for removing Cu^{2+} from neat solutions and natural water samples the immobilized chelate accomplished the separation rapidly. However, there was no apparent difference in efficiency of separation between the immobilized chelate and the ion exchanger. One aspect the authors did not consider is the clear advantage of the 8-HQ in selectivity for transition metals. Ion exchange resins with high capacities generally retain large amounts of Ca^{2+} and Mg^{2+} from water samples in addition to metal ions. These ions often cause problems in the subsequent analyses making 8-HQ the material of choice.

An excellent paper on the use of 8-HQ in the analysis of seawater samples has recently appeared (Sturgeon et al., 1981). These workers passed large volumes of seawater through columns of 8-HQ in order to preconcentrate the divalent ions Cd, Cu, Pb, Ni and Zn. The results obtained agreed quite well with the accepted values for these samples determined by other methods.

Jezorek and Freiser (1979) reported a novel application of the immobilized 8-HQ. They separated mixtures of metal ions such as Cd^{2+} , Pb^{2+} and Zn^{2+} using the chelate as a

packing for high pressure liquid chromatography. Metal ions were loaded on the column and sequentially eluted using gradient elution with acid over a small pH range. They found that 8-HQ on Porasil B chromatographic silica (Waters Associates) prepared by the method of Hill (1973) was superior to commercially available 8-HQ on Controlled Pore Glass (CPG, Corning). The surface area of the Porasil B is two to three times greater thus giving more than twice as many $\mu\text{equiv/g}$ of bonded 8-HQ. The stability of the chelate to 1 M acid was found to be very good.

One difficulty with the routine use of 8-HQ is its difficult preparation. There are five steps in the synthesis of this material and the procedure generally takes several days. However, prior to the final step in the reaction sequence an immobilized diazonium salt is prepared that has several uses. Recently some of the synthetic aspects of preparing this chelate have been reviewed (Fulcher et al., 1981). Procedures are outlined that presumably give the highest surface coverage for 8-HQ. Since the amine and diamine moieties are used as starting materials for 8-HQ their synthesis is also covered. An important aspect of the procedure is the preparation and treatment of the diazonium intermediate product. Its poor stability and high reactivity warrant careful handling. Bauman and coworkers (1967) demonstrated the versatility of the diazonium linkage for immobilizing a variety of chelating agents. They used carboxymethylcellulose as a

support to couple dithizone, 8-hydroxyquinoline, cupferron and other ligands. The immobilized dithizone was characterized and used to preconcentrate trace metals from seawater. The results seem to indicate complete recovery of metals. However, the metal analysis was only semi-quantitative. This chelate was found to give optimum results between pH 5 and 8 and exhibited diagnostically useful color changes upon coordination of a metal ion.

Other considerations. In most cases the behavior of the immobilized materials are completely analogous to the unbound reagent. However, it must be expected that differences between immobilized species and their solution analogs will be important. In some instances surfaces used for immobilization impart some special properties. Interactions between bound amines and the surface, especially surface silanol groups, may deactivate the material through hydrogen bonding (Leyden et al., 1978). Much more work is needed, however, to prove this. Surface spectroscopy and a variety of other studies have been used to elucidate this problem (Grime and Sexton, 1982; Moses et al., 1978; White and Murray, 1979). A detailed knowledge of these interactions should lead to a more rational approach to synthesis and applications.

EXPERIMENTAL

Materials. For most of the experiments described here Porasil B spherical porous silica beads (Waters Associates)

were used as a support for immobilizing chelating agents. This support is designed for use as a stationary phase in gas chromatography and has a surface area of 125-250 m²/g and an average pore diameter of 100-200 Å. The size of the beads are 80-100 mesh or 125-177 um in diameter. Initial experiments employed CPG (Pierce Chemical Co.) of 200-400 mesh with 40 Å pores and about the same surface area. These silica particles were found to be irregular in shape. Silica gel was also used as a support in preliminary experiments to perfect the synthetic techniques of immobilization.

The silica supports were prepared for synthesis with a cleaning procedure which was designed to remove inorganic and organic contaminants and fully activate the surface. A slurry of the glass beads was mixed with each of the following solutions: distilled water, 1 M HCl, distilled water, 1 M NH₄OH and finally distilled water. Each washing step consisted of stirring the slurry for 15 min with a motor driven paddle type stirrer, allowing the beads to settle and decanting the solution. Each distilled water washing step was followed by drying the silica on a watch glass at 180 °C. Care was taken in every step to avoid undue agitation or mechanical force that would cause breakage of beads.

Immobilization of the diamine by silylation. Two procedures were used for the silylation reaction. The

aqueous preparation which was normally used employed a mixture of 9.0 mL of glacial acetic acid and 36 mL of distilled water. Exactly 5.0 mL of 3-(2-aminoethylamino) propyltrimethoxysilane (Corning Z-6020, Petrarch Systems) was added slowly with stirring (Leyden et al., 1975a; 1976a). As much as 35 cc of cleaned and dried silica was added to this mixture, stirred for 10 min and filtered through a medium glass frit. Some reactions were carried out under a reduced pressure of 40 mm of Hg to remove air trapped in the pores of the glass beads.

Another method of preparing the immobilized diamine was to reflux approximately 10 cc of silica in 45 mL of dry toluene to which 5 mL of Z-6020 silane had been added (Weetall, 1970; Fulcher et al., 1981). The reaction was allowed to go overnight before filtering and washing with solvent. The final step in both procedures was curing at 80 °C overnight. In a few instances the Z-6020 silane was vacuum distilled (95 °C at approximately 2 Torr) before use to remove polymeric species (White and Murray, 1979).

Dithiocarbamates. The monodithiocarbamate of the ethylenediamine immobilized chelate was prepared according to Hercules et al. (1973). The reaction mixture was a 100 mL solution of absolute ethanol, 0.1 M in KOH and 1.5 M in CS₂. Five grams of the ethylenediamine chelate was added and the mixture was refluxed for 4 hrs. The reaction mixture was filtered, the chelate was washed with solvent

and then oven dried at 110 °C.

The bis(dithiocarbamate) derivative of the ethylenediamine immobilized chelate was synthesized according to the procedure of Leyden and Luttrell (1975a). The reaction mixture consisted of 5.0 g of the chelate, 25 mL of water, 11 mL of 0.25 M NaOH, and 11 mL of 2-propanol. To this, 20 mL of CS₂ was added with stirring for 15 min. The mixture was then filtered by suction through Whatman filter paper, rinsed with 100 mL of water, and dried in the oven at 110 °C for 30 min.

8-Hydroxyquinoline. An 8-hydroxyquinoline immobilized chelate was prepared by the method of Hill (1973) with certain modifications. The immobilized diamine was used as the starting material for this preparation and was reacted with p-nitrobenzoyl chloride in freshly distilled CHCl₃. Distilled triethylamine was added to neutralize HCl produced in the reaction and to help solubilize the acid chloride. In addition a few drops of 2,4,6 trimethylpyridine was usually added as a catalyst (Moses et al., 1978). Typical concentrations of the reagents are 0.27 M p-nitrobenzoyl chloride and 0.36 M triethylamine with 10 to 15 g of the diamine. The reaction was conducted at 50 °C for about 48 hrs. The product, nitrobenzoylated silica, was first filtered off, washed with chloroform and allowed to air dry. The immobilized nitro group was reduced to the amine by reacting with 100 mL of a 5% (w/v) solution of sodium

dithionite in distilled water. This aminophenyl derivative was diazotized with 100 mL of 2% (w/v) NaNO_2 in 1% (v/v) acetic acid at 5 °C for 35 min. The silica was then filtered, washed with cold distilled water and added to 50 mL of a 2% (w/v) solution of 8-hydroxyquinoline in absolute ethanol. The final product, immobilized 8-hydroxyquinoline, was filtered from the reaction mixture and washed consecutively with ethanol, distilled water, 1 M HNO_3 and again with distilled water.

Preliminary metal binding tests. All of the immobilized chelates prepared were initially tested for metal binding ability in a non-quantitative manner. A solution of approximately 1000 ppm Cu^{2+} was prepared and a 10 mL aliquot was mixed with 10 ML of pH 6.0 succinate buffer. Approximately 100 mg of the immobilized chelate was added to this, stirred for 10 min, and filtered. The filtered chelates were visually examined for color change due to complexation of Cu^{2+} . Filtration and acid washing was used to remove Cu^{2+} followed by a repeat of the complexation procedure.

Copper (II) titrations of 8-HQ. An accurately weighed portion of the 8-HQ chelate (0.3 to 0.4 g) was titrated with Cu^{2+} at pH 5 in duplicate. The titration was conducted under N_2 in 0.1 M KNO_3 and was monitored with a copper ion selective electrode (ISE) and a pH electrode. The results were analyzed using a Scatchard plot (Scatchard, 1949) to

determine the endpoint and stability constant.

Column methods. Chelating agents immobilized on Porasil B and CPG as well as the chelating ion exchange resin Chelex 100 (Bio-Rad Laboratories 100-200 mesh) were used in 0.7 cm i.d. borosilicate glass columns with polypropylene end caps and a 35 μ m polypropylene frit (Bio-Rad Econo Columns). Solution reservoirs were 1 L polyethylene wash bottles and 3-way valves were polypropylene with Teflon stopcocks. Tygon tubing made all connections. Column flow rates were maintained with a peristaltic pump (Sigmamotor, model TGS) and timing was done with a Gra Lab Universal Timer (model 171). All reagents were AR grade except nitric acid which was Alfa "ultrapure HNO_3 ".

Immobilized chelating agents and Chelex 100 were slurry packed into columns and washed with distilled water. Chelex 100 was cleaned of trace metals and converted to the calcium form following the method of Figura and Mc Duffie (1977). The 8-HQ and diamine chelates were also washed with 1 M ultrapure HNO_3 and along with the DTC were buffered to a pH of 6.00 ± 0.05 with 100 to 200 mL of dilute citrate buffer of 0.01 M ionic strength (Perrin, 1963). Buffered sample solutions (pH 6.00 ± 0.05 and $I = 0.01$) containing Cu^{2+} (25 to 100 $\mu\text{g/L}$) or Cu^{2+} and SFA (25 to 100 mg/L) were then passed through the columns at known flow rates and the effluent collected in volumetric flasks. All solutions containing

both metal ion and SFA were allowed to equilibrate overnight prior to use. The columns were then eluted with 1 M ultrapure HNO_3 which was collected in 25 or 50 mL volumetrics.

Buffer, acid and sample solutions as well as column effluents were analyzed prior to use by differential pulse ASV using Princeton Applied Research instruments models 174A, 315 and a modified 303 (see Appendix). Solutions containing SFA were acidified to 0.1 M with ultrapure HNO_3 and subjected to ultraviolet irradiation in quartz cells overnight prior to analysis to destroy organic matter (Batley and Farrar, 1978). Acid eluents were analysed by AA.

Adsorption of organic matter on chelates. In preliminary studies with the chelating agents and natural water samples it was observed that a significant amount of organic matter was adsorbed to the immobilized chelate. To study this phenomenon further as a function of chelating functional group and support medium a series of columns were prepared with 1 cc each of untreated CPG, untreated silica gel, and ethylenediamine on silica gel. Through each of these columns was passed 50 mL of 20 mg/L SFA at pH 6.0. The ultraviolet absorbance of these solutions before and after column elution was measured at 260 nm on a Cary 14 spectrometer to quantify adsorption.

In the column experiments with Cu^{2+} , the absorbance of

the column effluents was compared to portions of the sample solutions as an indication of adsorption of SFA on the columns. The background absorbance of the citrate buffer used in all the experiments was also obtained.

Iron removal from natural water. Water samples were collected from the Barrington Swamp and Drew Brook Pond in October 1979 and immediately filtered through 0.4 μ m filters. The sampling procedures and chemical characterization are similar to those described in Chapter 4 and were reported elsewhere (Truitt, 1980; Truitt and Weber, 1981b). These samples were used to evaluate the performance of the immobilized chelates for removing iron from natural waters.

Accurately weighed amounts of the DTC and 8-HQ chelates were placed in polyethylene vials. To each vial was added 10.0 mL of either Drew Brook Pond or Barrington swamp water samples. The vials were capped and shaken several times over a forty eight hour period. The immobilized chelates were allowed to settle to the bottom of the vials and the residual iron in solution was determined by atomic absorption. The pH and UV absorbance of the samples was also determined after equilibration. The data was subjected to Analysis of Variance (ANOVA) to determine statistical significance of the trends.

RESULTS AND DISCUSSION

Synthesis and preliminary tests of immobilized chelates. The structures of the immobilized diamine and DTC are shown in Figure 1 (Leyden et al., 1976a). The monodithiocarbamate is very similar to the DTC but reportedly contains only the terminal dithiocarbamate group (Hercules et al., 1973; Leyden et al., 1976a) because the reaction conditions are too mild to convert the secondary amine of the diamine. All three of these materials had an off-white or yellowish color after drying. Once Cu^{2+} is bound at pH 6 the diamine becomes blue in color bordering on purple depending on the extent of Cu^{2+} loading. The immobilized bis(dithiocarbamate)- Cu^{2+} complex was dark brown and the monodithiocarbamate- Cu^{2+} complex was green-brown. All of the immobilized complexes were easily distinguishable by their color. This initial test was used as a qualitative determination of metal binding and was carried out with each batch of chelate prepared as an indication of the success or failure of the reactions.

The 8-HQ chelate (Figure 2) is deep red in color and does not undergo a noticeable color change when complexed to Cu^{2+} . During the reaction sequence used to prepare the 8-HQ the material took on useful color changes at a few of the steps. The reaction of the immobilized diamine with acid chloride (p-nitrobenzoyl chloride) did not produce a color change. This was unfortunate because this step has a great

potential for problems. It is well known that acid chlorides react with water to produce HCl and the corresponding carboxylic acid. Aromatic acid chlorides are less reactive in this sense but it is still very likely that moisture in the air can significantly degrade an old bottle of p-nitrobenzoyl chloride. This problem did come up in the course of these experiments and caused several reactions to fail. It was only after new reagent was obtained that the reaction succeeded.

The conversion of the aromatic nitro group to the amine gave a yellow product. This was significantly different from the off-white color of the immobilized nitrobenzoyl group. The diazonium salt prepared from the aromatic amine was orange in color. This material was quite reactive so it was kept at 0 °C and reacted with 8-hydroxyquinoline immediately. This produced the deep red 8-HQ product that was desired.

The diamine and 8-HQ chelates readily released their bound Cu^{2+} when exposed to dilute acid. The blue diamine- Cu^{2+} complex readily lost its color even when exposed to 0.02 M HNO_3 . The bis(dithiocarbamate)- Cu^{2+} complex could not be broken up readily with acid. The brown complex did not lose its color even after treatment with 4 M HNO_3 . Reagent 15.9 M HNO_3 only served to fade the color.

Potentiometric titration of 8-HQ. The titration of the 8-HQ immobilized chelate with Cu^{2+} was conducted exactly as

if the ligand was in solution. A titration curve obtained by monitoring free Cu^{2+} with a potentiometric ion selective electrode is shown in Figure 3. This curve is very similar to one for solution phase complexation. The endpoint for 1:1 complexation calculated by the second derivative method is $1.3 \times 10^{-3} \text{ M } \text{Cu}^{2+}$, corresponding to a capacity of 9.5×10^{-5} moles Cu^{2+} /g of chelate. This is significantly higher than the early results reported for this chelate (Hill, 1973; Jezorek and Freiser, 1979; Sugawara et al., 1974; Moorhead and Davis, 1974). The reason for this higher value is that the diamine was used as the starting material for the 8-HQ. Previous reports mentioned above used a mono-functional amine starting material. The 8-HQ could potentially have twice the capacity. In actuality this is not realized because certain steps in the reaction sequence may not go 100% to completion. A recent report (Fulcher et al., 1981) gave rather high capacity values using the diamine resulting from efforts to optimize each step in the synthesis.

Titration results were also analyzed by Scatchard analysis (Scatchard, 1949), yielding a conditional stability constant of 6.2×10^7 for pH 5 and an n value of 1.1. The n value indicates stoichiometry and was expected to be close to unity since a 1:1 assumption had already been made in calculating the endpoint. The conditional stability constant is significantly higher than the literature value for 8-hydroxyquinoline (2.8×10^7 for pH 5) calculated from

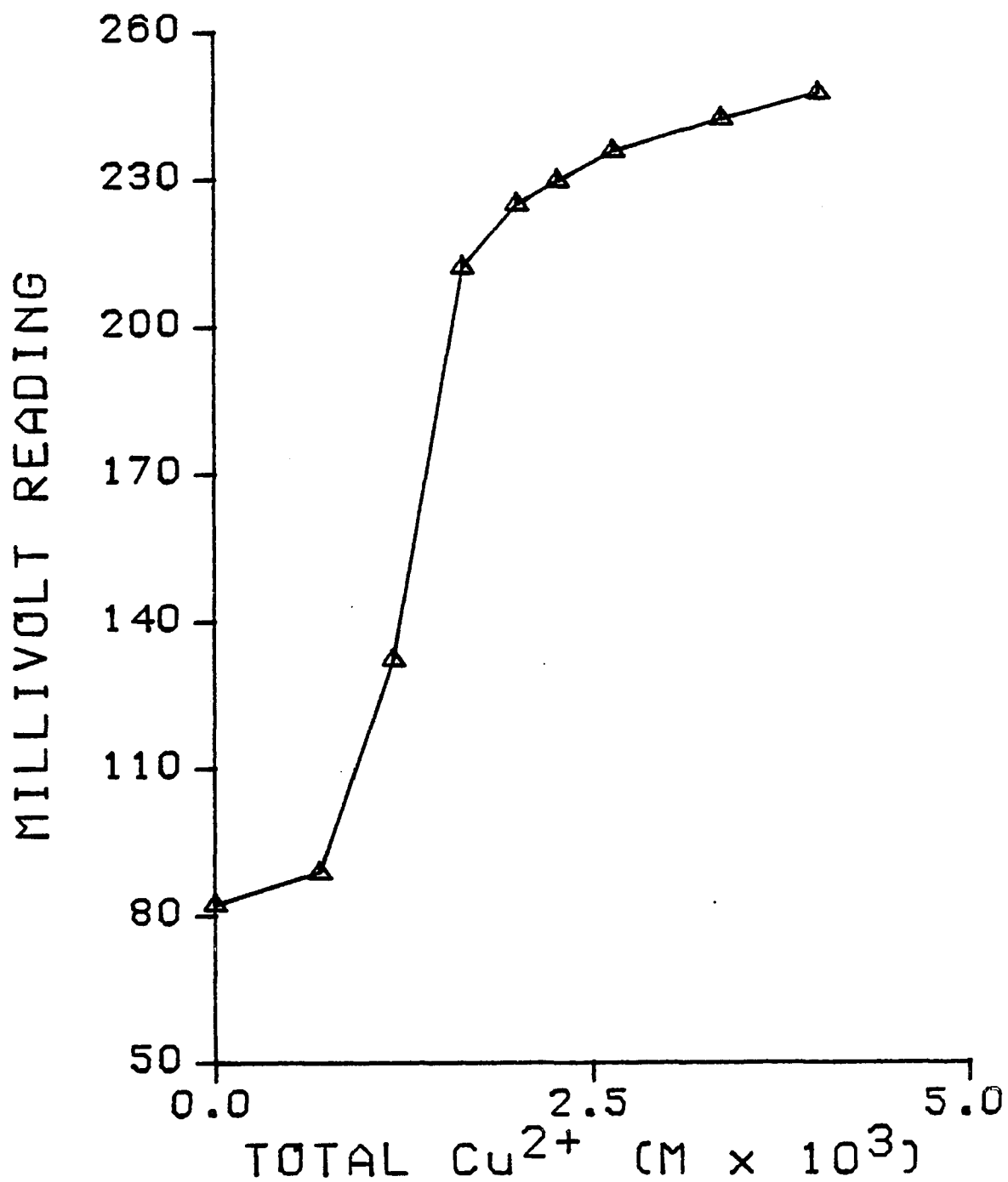


Figure 3. Potentiometric titration curve of total Cu^{2+} vs. mV measured at a Cu^{2+} ion selective electrode for immobilized 8-hydroxyquinoline (8-HQ) at pH 5.

the thermodynamic K value and the acid dissociation constant (Martell and Smith, 1977). One possible reason for this is that 8-HQ is chemically different from non-immobilized 8-hydroxyquinoline. The azo linkage used for 8-HQ may increase the stability of the Cu^{2+} complex by donating some electron density. Obvious evidence for altered electronic configuration is the red color of 8-HQ compared to the colorless 8-hydroxyquinoline.

One problem was encountered in titrating the immobilized chelate. Very long equilibration times were needed in order to get a constant millivolt reading from the Cu^{2+} electrode. This was found to be due to slow uptake of the metal ion by 8-HQ in the batch mode. Even with vigorous stirring of the glass beads with N_2 Cu^{2+} ion must diffuse through an unstirred static layer around each bead of chelate. Slow diffusion of Cu^{2+} in and out of the static layer made it necessary to allow several hours equilibration time between each addition of Cu^{2+} thereby extending the titration over a period of days. No difficulty was encountered with electrode stability over this time period. The pH electrode was removed from the titration cell and recalibrated regularly and the ISE was calibrated before and after the titration. Both calibrations showed very good Nernstian response.

Column Results. Column experiments were conducted using the system diagrammed in Figure 4. A typical run for

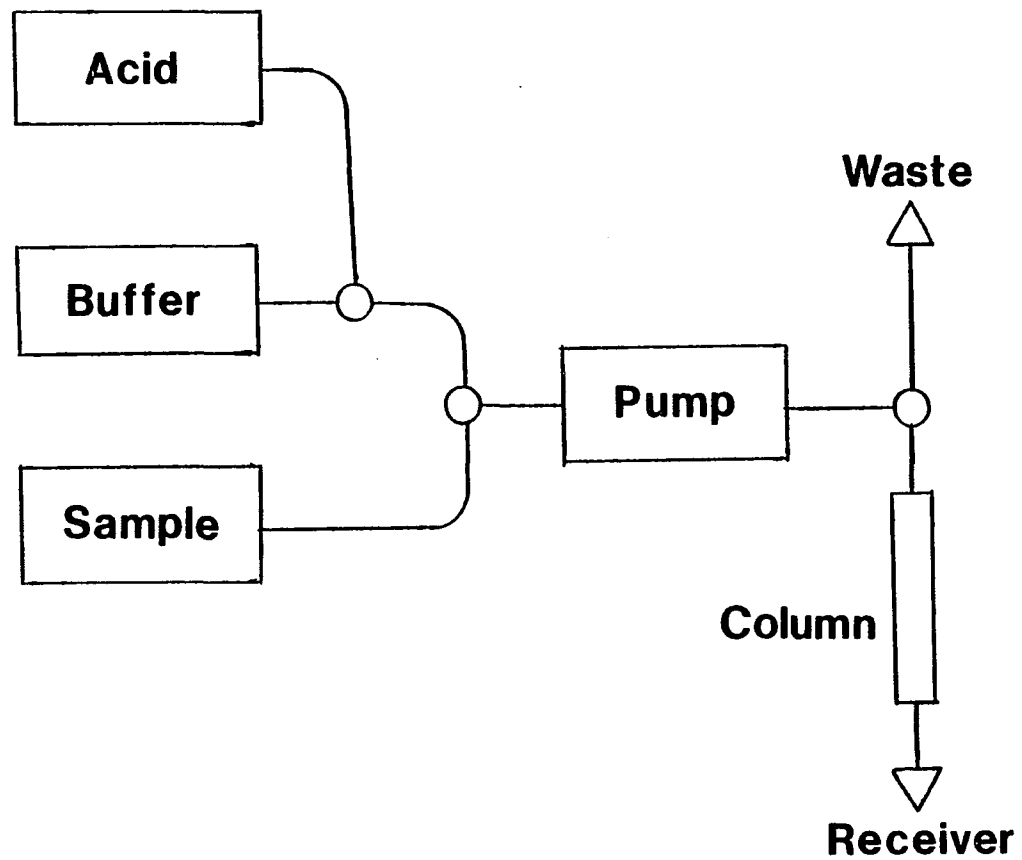


Figure 4. Block diagram of the system used for column experiments with immobilized chelating agents. Acid was 1 M ultrapure HNO_3 and buffer was pH 6.00 succinate, 0.01 M ionic strength.

immobilized chelates would involve first passing 1 M ultrapure HNO_3 through the system to waste to remove trace metals. The column of chelate was also acid washed except in the case of DTC. Next 100 to 200 mL of buffer was passed through the system to flush out the acid and bring the column up to pH 6.00. During these preliminary steps the flow rate of the column was roughly adjusted for the experiment using the clock and a graduated cylinder. Sample solution was then pumped through, first to waste in order to equilibrate the system materials with sample, then through the column. The timer was initiated simultaneously with this latter step and collection of column effluent was begun. The void volume of buffer ahead of the sample in the column was collected with the first fraction of column effluent. When the entire sample had passed through the column (typically 1 L) the timer and flow through the column were stopped. Effluent was analyzed by ASV for any Cu^{2+} that passed through the column. Acid was pumped through the system to waste to clean out any adsorbed sample and then was passed through the column to elute the Cu^{2+} . The acid eluent was collected in a volumetric flask for AA analysis. The void volume of sample solution in the column ahead of the acid was collected with the acid eluent accounting for the previously collected void volume of buffer. Once the initial aliquot of acid effluent was collected an additional 10 mL is collected and analyzed by ASV to determine if any residual Cu^{2+} was leaching from the column. Following this

the column was eluted with buffer and was ready for another run.

The use of this system as described allows reagents to be easily applied to the column and was extremely well adapted to avoid contamination problems often encountered at ultratrace metal ion levels. A similar procedure was used for Chelex 100, eluting first with acid to remove trace metals, then with 2 M KOH to convert to the potassium form, followed by $\text{Ca}(\text{NO}_3)_2$ to produce the calcium form. Distilled water or buffer wash followed each step. Chelex is difficult to buffer due to its large surface area and volume inside pores of the resin beads. The pH of the sodium form is about 11 (Bio-Rad, 1978). Thus even though samples are at pH 6.00 the actual pH of the sample in the column is higher. Therefore, Chelex was operating in its optimum range for trace metal recovery ($\text{pH} > 6.5$).

Table 1 shows results of Chelex 100 experiments with Cu^{2+} solutions with 25 mg/L of SFA. Removal of Cu^{2+} was incomplete even when a fairly long column of Chelex (30 cm) was used. These results agree with those of Figura and McDuffie (1979) and Pakalns and Batley (1978). Chelex could not give quantitative results from waters containing natural ligands. Others have also reported that Chelex in column operation only removes a certain labile fraction of metal ion in equilibrium with natural organic matter (Stolzberg and Rosin, 1977; Nygaard and Hill, 1979).

Table 1. Chelex separations of Cu^{2+} from 25 mg/L soil fulvic acid (SFA) solutions at pH 6.00.

<u>Column Height</u>	<u>Flow Rate (mL/min)</u>	<u>Cu^{2+} Given (ug/L)</u>	<u>Column Effluent (ug/L)</u>	<u>% Cu^{2+} Removed</u>
10cm	3	100	25.0	75.0
30cm	2	27.8	10.6	61.9

Table 2. Column separations of Cu^{2+} at pH 6.00 with immobilized ethylenediamine (diamine).

<u>Amt. (g)</u>	<u>Flow Rate (mL/min)</u>	<u>Sample Soln</u>		<u>Column Effluent (ug/l)</u>	<u>% Cu^{2+} Removed</u>
		<u>Cu^{2+} (ug/l)</u>	<u>SFA^a (mg/L)</u>		
0.5	10	223.2	0	1.51	99.3
1.0	10	90.3	0	1.77	98.0
0.4	10	100	25	23.0	77.0
1.0	4.3	100	100	15.4	84.6

a SFA is a soil fulvic acid

The results obtained for the immobilized diamine are shown in Table 2 and are similar to those for Chelex-100. With no SFA essentially all the Cu^{2+} was removed from solution. However in the presents of SFA incomplete recovery was observed. The diamine is not an extremely strong ligand, therefore, these results are not surprising. In addition it was observed that SFA adsorbed to the chelate possibly reducing its efficiency (see below).

The DTC gave better results in some respects and these are summarized in Table 3. Quantitative removal of Cu^{2+} was acheived from solutions as high as 100 mg/L in SFA. A problem encountered with the DTC was that the Cu^{2+} could not be eluted from the column. The Cu^{2+} -dithiocarbamate complex was so stable that even 15.9 M HNO_3 does not completely remove the metal. In addition columns of the DTC were observed to adsorb SFA.

More promising results were found for the 8-HQ chelate and are shown in Table 4. Cu^{2+} removal was complete from solutions containing only metal ion. Solutions 25 mg/L in SFA pose a more difficult problem and only 37% of the Cu^{2+} was removed under the same conditions as for free metal ion. However, going to longer columns and slower flow rates gives much better results; 91 to 94% removal.

Changing the length of the column and the flow rate affects the amount of time that each increment of the eluting solution spends in contact with the immobilized

Table 3. Column separations of Cu^{2+} with the immobilized bis(dithiocarbamate) (DTC) at pH 6.00.

Amt. (g)	Flow Rate (mL/min)	Sample Soln		Column Effluent (ug/l)	% Cu ²⁺ Removed
		Cu ²⁺ (ug/l)	SFA ^a (mg/L)		
0.5	3.6	100	0	1.6	98.4
1.0	4	100	100	1.3	98.7
4.0	10	11.0	25	0.3	97.6

^a SFA is soil fulvic acid.

Table 4. Column separations of Cu^{2+} with the 8-hydroxy-quinoline immobilized chelate (8-HQ) at pH 6.00.

Amt. (g)	Flow Rate (mL/min)	Sample Soln		Column Effluent (ug/l)	% Cu ²⁺ Removed
		Cu ²⁺ (ug/l)	SFA (mg/L)		
0.5	10	197.8	0	5.3	97.4
0.5	10	198.4	0	0.6	99.7
0.5	10	11.2	25	---	37.1
1.7	5	24.8	25	1.5	93.9
2.7	5	26.8	25	2.4	91.0
0.5	10	10.5	0	---	100.0
0.5	30	10.5	0	---	93.9
0.5	30	11.2	25	---	30.4

^a SFA is soil fulvic acid.

functional groups. This can be described by the following equation:

$$T_c = \text{Void Volume (mL)} / \text{Flow Rate (mL/min)}$$

where T_c was the "contact time" in min and the void volume is the volume of solution in the column at any given time (Figura and McDuffie, 1979). The void volume for any particular column packing will vary linearly with column length or column cross sectional area. The contact time was an important variable in this type of system where an immobilized ligand is competing with a soluble ligand.

SFA loss to columns. Preliminary experiments with some of the immobilized chelates and the untreated supports demonstrated that the amine functional groups caused loss of SFA. The data in Table 5 shows that untreated supports had little or no effect on the SFA passing through the column as measured by UV absorbance. The DTC also passed all the SFA. However, the diamine removed practically all the SFA and the monodithiocarbamate removes more than half. This is probably due to simple charge attraction between protonated amine groups and anionic SFA. The monodithiocarbamate has a secondary amine group that removes some SFA but not as much as the diamine. The DTC has no nitrogens that can be protonated at pH 6 so it does not remove SFA.

It was observed that when solutions containing both SFA

Table 5. Adsorption of soil fulvic acid (SFA) on chelates and untreated supports at pH 6.00.

<u>Immobilized Chelate</u>	<u>Support</u>	<u>% SFA Passing through Column</u>
---	untreated CPG ^a	97.7
monodithiocarbamate	CPG	46.9
---	untreated silica gel	101.1
diamine ^b	silica gel	2.7
DTCC ^c	silica gel	103.4

a CPG is Controlled Pore Glass (Corning)

b Diamine is immobilized ethylenediamine

c DTC is immobilized bis(dithiocarbamate).

Table 6. Loss of soil fulvic acid (SFA) to chelate columns

<u>Chelate^a</u>	<u>% Loss of SFA</u>	<u>% Cu²⁺ Removed</u>
8-HQ	5.1	37.1
8-HQ	59.2	85.1
8-HQ	70.9	93.9
DTC	74.9	90.0
DTC	93.3	97.6
Chelex	12.3	61.9

^a 8-HQ is immobilized 8-hydroxyquinoline;
DTC is immobilized bis(dithiocarbamate);
Chelex is Chelex 100 (Bio-Rad Laboratories).

and Cu^{2+} were passed through the columns of the chelates a loss of color occurred even for the DTC. Table 6 shows the percent loss of color for different experiments with 8-HQ, DTC and Chelex 100. Also included are the data for percent Cu^{2+} removal. As can be seen from Table 6 for a given chelate, as the percent Cu^{2+} removal increases, the percent loss of color increases. It is, therefore, believed that the adsorption of SFA on the column occurs due to the formation of a mixed ligand complex of the Cu^{2+} with the immobilized chelate and SFA. This phenomenon prevented use of the immobilized chelate columns for removing metals prior to complexing capacity measurements.

Iron removal from natural water samples. Experiments to evaluate the effectiveness of immobilized chelates for removing iron from natural water samples were statistically designed. ANOVA was used to determine the effect of chelate, amount of chelate and water sample on the removal of iron, the change in pH, and UV absorbance of the samples. Table 7 contains the variables, experimental design, and the average responses of duplicates. Effects and F ratios are given in Table 8.

ANOVA results indicate that percent iron removal varies significantly (95% confidence level) with differences in water samples, chelates and amount of chelate. In all cases 8-HQ removed significantly more iron than the DTC. The values for 8-HQ ranged from approximately 76% to 90% (Table

Table 7. Experimental design and results for removal of iron from natural water samples with immobilized chelates.

Factor	Levels	
	+	-
A = Water sample	Barrington Swamp	Drew Brook Pond
B = Chelate	bis(dithiocarbamate) (DTC)	8-hydroxyquinoline (8-HQ)
C = Amt. of chelate	75 mg	300 mg

							Responses		
A	B	C	AB	AC	BC	ABC	% Iron Removed	Change in pH	% Original Color (UV absorbance, 260 nm)
+	+	+	+	+	+	+	31.7	2.9	397
-	+	+	-	-	+	-	20.7	2.4	397
+	-	+	-	+	-	-	100	-1.8	120
-	-	+	+	-	-	+	93.9	-2.2	116
+	+	-	+	-	-	-	27.7	2.0	216
-	+	-	-	+	-	+	9.3	1.8	235
+	-	-	-	-	+	+	87.4	1.6	52
-	-	-	+	+	+	-	76.4	1.8	62

Table 8. Variable effects and F ratios for treatment of natural water samples with immobilized chelates.

<u>Factor</u>	<u>d.f.</u>	<u>% Iron Removal</u>		<u>pH Change</u>		<u>% UV Absorbance Change</u>	
		<u>Effect</u>	<u>F Ratio</u>	<u>Effect</u>	<u>F Ratio</u>	<u>Effect</u>	<u>F Ratio</u>
A	1	+11.63	<u>16.06</u>	+0.11	3.53	-3.13	1.35
B	1	-33.54	<u>534.69</u>	+1.21	<u>92.38</u>	+111.88	<u>1673.30</u>
C	1	+5.69	<u>15.38</u>	-0.74	0.20	+58.13	<u>451.00</u>
AB	1	+1.54	1.12	+0.06	0.83	-1.63	0.33
AC	1	<u>-1.54</u>	1.12	+0.11	0.61	+4.13	2.19
BC	1	<u>-1.84</u>	1.61	+1.11	5.38	+27.63	<u>101.40</u>
ABC	1	-0.31	0.05	<u>-0.04</u>	1.15	+0.63	0.06

^a Factors A, B and C as in Table 7.

^b Degrees of freedom.

^c Underlined values indicate 95% confidence level significance. Critical F value for 95% confidence is 5.32 (1,8 d.f.).

7). The value of 90% removal of iron is a conservative estimate based on the detection limit for AA analysis. The detection limit was determined by the method of Grant and Skogerboe (1971) to be 40 ug/L Fe. The larger amount of both chelates was more effective than the smaller amount, and the removal of iron from the Barrington Swamp sample was more complete than from the Drew Brook Pond sample.

As expected larger amounts of both chelates removed more iron. It may be possible to further increase the amount of the chelate and attain complete or near complete iron removal. However, a certain portion of the iron may be inert to complexation by the immobilized reagents.

The ANOVA results indicate that there was a significant difference between the two samples in the extent to which iron could be sequestered. The two water samples, however, are very similar in amount of organic matter as measured by dissolved organic carbon (DOC) and UV absorbance at 260 nm (color). The DOC values were 12.0 and 11.8 mg C/L for the Drew Brook Pond and Barrington Swamp, respectively. Color values were equivalent to 16.5 and 17.0 mg/L of SFA respectively. The difference in the results for iron removal may be due to the composition of the organic matter in these bodies of water which can vary considerably depending on many factors (Reid et al., 1980). These two samples were also similar in iron content. The Barrington Swamp sample contained 404 ug/L Fe and the Drew Brook Pond

had 335 ug/L Fe.

The change in pH of the water samples after equilibration with the immobilized chelates was found to be dependent upon the chelate used and the interaction between chelate and amount of chelate. 8-HQ was found to reduce the pH in all cases and the DTC increased pH. Also the larger amounts of the chelates had more effect on pH than the smaller quantities. No difference was observed between the effects on different water samples even though the pH for the Barrington Swamp was 5.4 while the Drew Brook Pond had a pH of 6.0.

The direction of pH change for each chelate is not surprising in view of the procedures involved in preparing these materials. The DTC is prepared in basic solution followed by rinsing with distilled water. Any residual base not completely washed out in the rinse step would increase the pH of the water sample as is observed. The 8-HQ chelate is washed with 1 M HCl as a final step in the synthesis and releases a proton upon complexation of metal ion. Therefore this chelate causes a reduction in pH of samples. This problem could be minimized very easily by buffering the immobilized chelates before use.

The UV absorbance of the samples at 260 nm was monitored before and after equilibration with chelates. This was done primarily to determine if any of the organic matter present was adsorbed on the immobilized reagents.

The results are reported in Table 7 as "% of original color". It was observed, however, that in the majority of the experiments the absorbance increased. The ANOVA results showed that type of chelate, amount of chelate and the interaction of the two are the significant factors at the 95% confidence level. The DTC increased the absorbance the most while 8-HQ caused an increase at its high level and a 40 to 50% decrease at its low level. The cause of increased absorbance is probably the loss of functional groups from the immobilized chelates to the solution. During the 48 hr equilibration time for the experiment a small fraction of the bonds may hydrolyse and release reagent into the column effluent. Decreased color for the low level of 8-HQ is probably mainly due to adsorption of organic matter on to the chelate. However, there is probably some leaching in this case as well. These two phenomena have an opposite effect on the UV absorbance of the solution and it is not possible to quantify the extent of each with the available data. It is obvious that both are occurring and have a serious affect on the results.

CONCLUSIONS

Although it was possible to obtain complete removal of copper and iron from samples containing natural ligands, the further use of immobilized chelates was not feasible for this project. Not only was it necessary to remove trace metals from natural water samples but it was also desired to

leave unperturbed the original amount of natural ligands. From the studies described in this chapter it was concluded that immobilized chelates, used under the various conditions employed here, could not achieve this result. Samples that came in contact with immobilized chelates lost large amounts of organic ligands via adsorption or possibly through the formation of mixed complexes with trace metals and immobilized ligands. Other potential problems included the possible leaching of chelating groups from the immobilized reagents and alteration of sample pH.

The immobilized chelates are, however, very useful for analytical applications such as removing or preconcentrating trace metals. Further studies will focus on the determination of a samples potential complexing level and not its natural complexing level.

CHAPTER 3

FLUORESCENCE QUENCHING TITRATION TECHNIQUE FOR DETERMINATION OF COMPLEXING CAPACITIES AND STABILITY CONSTANTS OF FULVIC ACID

INTRODUCTION

The metal ion binding properties of naturally occurring organic matter have been studied using a variety of techniques including ASV (Florence, 1977; O'Shea and Mancy, 1978; Shuman and Cromer, 1979; Bhat et al., 1981), ISE (Ramamoorthy and Kushner, 1975; Buffle et al., 1977; Saar and Weber, 1980a; 1980b; 1980c), including hydrogen ion (Gamble et al., 1980; Stevenson, 1977), ion exchange (Figura and Mc Duffie, 1979; 1980), equilibrium dialysis (Truitt and Weber, 1981a; Rainville and Weber, 1981), ultrafiltration and others (Gachter et al., 1978; Hanck and Dillard, 1977a). All of these methods have one thing in common. In order to determine the extent of organic matter complexation a separation or direct measurement of "free" metal ion is performed and subtracted from the total metal ion concentration. This indirect manner of determining the extent of binding of natural organic ligands is prone to error from several sources. Metal ions may be complexed by inorganic species in the sample and adsorbed to colloidal particulates that are initially present, or that form as a result of coagulation of the organic matter (Saar and Weber, 1980b; 1980c; Pagenkopf and Whitworth, 1981; Sholkovitz and

Eckert, 1976). When the organic ligand is in excess the free metal ion concentration is extremely low and difficult to measure. Operating the measurement technique near its detection limit will give unreliable data and cause error in the calculation of bound ligand. When metal ion is in excess, on the other hand, an error can be introduced when taking the small difference of two large numbers. Free and total metal ion concentrations may be orders of magnitude higher than the micromolar and submicromolar ligand concentrations encountered in natural systems.

A more direct method of studying humic material binding is to measure a property of the organic matter itself that changes with metal ion complexation. The natural fluorescence of humic materials is such a property (Saar and Weber, 1980a; Schnitzer and Ghosh, 1981). Titrating a sample with metal ion provides a means by which the capacity of the ligand and its stability constant can be determined.

A major difference between fluorescence and the techniques mentioned above is that fluorescence measures unbound ligand. The signal is reduced or quenched when the ligand binds to a paramagnetic metal ion. Titrations performed with fluorescence as the means of detection directly demonstrate the effect of binding on the fulvic acid ligand instead of relying on a difference measurement involving metal ion.

This chapter describes a quantitative method for the determination of 1:1 conditional stability constants (K) and complexing capacities (C_L) of fluorescing ligands with a metal ion using a titration procedure. An equation is derived that relates measured fluorescence intensities to the fraction of the ligand bound. Incorporation of this equation into a 1:1 conditional stability constant expression results in an equation that describes the entire titration curve of total metal ion vs. fluorescence intensity. Computer curve fitting by non-linear regression allows determination of K and C_L values. The inherent sensitivity of molecular fluorescence combined with the power of curve fitting allows work at natural water concentrations of organic matter. The results of Cu^{2+} -SFA, Cu^{2+} -salicylic acid and Cu^{2+} -tyrosine titrations are analyzed.

Saar and Weber (1980a) outline the general fluorescence properties of SFA used in this work and show close correlations for binding studies of Cu^{2+} and SFA measured by fluorescence and ISE. In addition the fluorescence quenching of salicylic acid (SA) with Cu^{2+} was demonstrated to be very similar in magnitude to the fraction of SA bound to Cu^{2+} as calculated from the published stability constants. Finally SFA fluorescence was studied vs. the concentrations of H^+ , Co^{2+} , Ni^{2+} , Mn^{2+} , Pb^{2+} and Cd^{2+} . The paramagnetic metal ions were found to quench more, while Pb^{2+} , even though it is known to bind strongly (Saar and

Weber, 1980b), only quenches to a small degree. Cadmium does not quench SFA fluorescence at all. Cline and Holland (1977) also studied the fluorescence quenching effect of Cu^{2+} and Co^{2+} on humic material. Again Cu^{2+} was found to quench more than Co^{2+} .

The changes that occur in the fluorescence excitation spectrum of fulvic acid upon complexation to Cu^{2+} and Fe^{3+} have been described by Schnitzer and Ghosh (1981). They determined in a qualitative sense the quenching of the excitation band at 360 nm with emission measured at 520 nm. A second peak at 465 nm was only slightly affected by complexation of either metal ion at pH 4 and 6. One important point brought out by this study is the effect of ash content on the fluorescence of isolated SFA. Samples with high inorganic content (10-12% ash) showed much lower fluorescence at 360 nm compared to 465 nm. A possible interpretation is that naturally present Fe^{3+} is bound to the SFA, quenching its fluorescence. A similar effect was reported by Levesque (1972). He noted that isolated humic fractions with high iron levels showed little fluorescence.

Part of the theoretical approach employed here to describe the fluorescence quenching titrations is analogous to Shuman and Woodward's (1973) treatment of ASV data. Starting from the simple 1:1 complex model they derived an equation that relates total metal ion in equilibrium with a ligand to ASV stripping currents via K and the ligand

concentration. They proved the validity of the theory with the Cd-EDTA system and further discussed some of its limitations with regard to kinetic contributions to the ASV current. Shuman and Woodward (1977) extended the theory to include other complex stoichiometries and applied it to natural water samples. Their experimental results indicate that assumption of a 1:1 stoichiometry for Cu^{2+} and natural organic matter chelates best describes their data.

A review of the literature indicates that the fluorescence properties of salicylic acid have been thoroughly studied. Thommes and Leininger (1958) developed a fluorometric analysis for salicylic acid in solutions containing the meta and para as well as ortho isomer. Analysis is based on the fact that salicylic acid fluorescence arises from the singly ionized form which predominates from pH 5.5 to 13, while the meta isomer only fluoresces as the dianion. They report that the para isomer does not fluoresce at any pH. Measurements at pH 5.5 correspond to salicylic acid, but at pH 12 both SA and its meta isomer are measured. Linear calibration curves are obtained up to 12 ppm of either compound. They also suggest that the first ionization constant of SA can be determined using fluorescence because the protonated form does not fluoresce while the singly ionized form does. They determined a value of 3.17 for the pKa.

Kovi et al (1972) have extensively investigated the spectroscopic properties of SA. They give a value of 3.0 for the ground state pKa determined by absorptiometric titration and further state that the excited state pKa cannot be determined by fluorescence because the equilibrium is too slow to occur in the lifetime of the excited state species. This indicates that the value obtained by fluorescence may be the ground state value but leaves open the possibility that it could be shifted somewhat toward the higher value that might be predicted for the excited state pKa. Some evidence for this is that the values reported by Kovi et al. (1972) and Thommes and Leininger (1958) are both higher than the accepted pKa value of 2.8 from potentiometric studies (Martell and Smith, 1977). It is however quite certain that SA exhibits intramolecular hydrogen bonding and intramolecular proton transfer (phototautomerism) upon excitation of the unionized and singly ionized species (see below).

THEORY

The simple 1:1 equilibrium between a metal ion and a ligand can be expressed in terms of the conditional stability constant at a given pH

$$K = [ML]/([M][L]) \quad (1)$$

where K is the conditional stability constant, [ML] is the

concentration of complex, $[M]$ is the uncomplexed metal ion concentration and $[L]$ is the total concentration of uncomplexed ligand (charges omitted). Mass balances on metal ion and ligand can be written

$$C_M = [M] + [ML] \quad (2)$$

$$C_L = [L] + [ML] \quad (3)$$

defining C_M as the total metal ion concentration and C_L as the total ligand concentration or titration endpoint in terms of molar metal concentration.

The quenching of a ligand's fluorescence by complexation with a metal ion can be described in terms of the individual fluorescence of each species present. The overall measured fluorescence intensity (I) is therefore equal to the sum of the fluorescence from the free ligand (I_L) and the bound ligand (I_{ML}) such that

$$I = I_L + I_{ML} \quad (4)$$

The individual fluorescence intensities are related to the concentration of the particular species by their fluorescence efficiencies

$$I_L = Q_L [L] \quad (5)$$

$$I_{ML} = Q_{ML}[ML] \quad (6)$$

where Q_L and Q_{ML} are the fluorescence efficiencies of the free and bound ligand respectively. Substituting Equations 5 and 6 into 4 we get

$$I = Q_L[L] + Q_{ML}[ML] \quad (7)$$

In the titration experiment where metal ion is added to ligand we can define two extremes as follows. When no metal has been added $[ML] = 0$ and therefore $I_{ML} = 0$ and $C_L = [L]$, Equations 4 and 7 become

$$I = I_L = Q_L C_L \quad (8)$$

At the end of the titration when metal ion is in large excess all L is bound, $[L] = 0$ and therefore $I_L = 0$ and $C_L = [ML]$, then

$$I = I_{ML} = Q_{ML} C_L \quad (9)$$

Substituting Equation 3 into 7 and rearranging an expression is obtained in terms of bound ligand

$$[ML] = (I - (Q_L C_L)) / (Q_{ML} - Q_L) \quad (10)$$

Substituting in Equation 8 and 9 gives

$$[\text{ML}]/C_L = (I - I_L)/(I_{\text{ML}} - I_L) \quad (11)$$

The quantity $[\text{ML}]/C_L$ is the fraction of the total ligand bound expressed in terms of the measured fluorescence intensity (I), the fluorescence intensity at the start of the titration (I_L), and the fluorescence intensity when all the ligand is bound as complex (I_{ML}). From Equation 11 the fraction of ligand bound can be calculated for each experimental point in the titration of a fluorescing ligand with metal ion (Seitz, 1981).

We can further derive an expression that is a function of total metal titrant added in terms of the stability constant. From equations 1 and 3

$$[\text{ML}]/C_L = (K[\text{M}])/(K[\text{M}] + 1) \quad (12)$$

Designating the fraction bound as a single variable x_{ML} such that $x_{\text{ML}} = [\text{ML}]/C_L$, expressions for the metal ion and complex can be written

$$[\text{ML}] = x_{\text{ML}}C_L \quad (13)$$

$$[\text{M}] = C_M - (x_{\text{ML}}C_L) \quad (14)$$

Substituting into Equation 12 we get

$$x_{ML} = (K (C_M - x_{ML}C_L)) / (K(C_M - x_{ML}C_L) + 1) \quad (15)$$

Equation 15 defines the entire titration curve of total metal ion versus fraction of ligand bound calculated from fluorescence intensities in terms of the stability constant and the molar ligand concentration. This equation can be cast in the form of a quadratic in x_{ML}

$$x_{ML} = (1/(2KC_L)) \left[(KC_L + KC_M + 1) - \sqrt{(KC_L + KC_M + 1)^2 - 4K^2C_LC_M} \right] \quad (16)$$

Substituting in Equation 11 for x_{ML} and setting I_L equal to 100 results in a solution for measured fluorescence intensity (I)

$$I = ((I_{ML} - 100)/(2KC_L)) \left[(KC_L + KC_M + 1) - \sqrt{(KC_L + KC_M + 1)^2 - 4K^2C_LC_M} \right] + 100 \quad (17)$$

The I_{ML} is a limiting value below which the fluorescence will not decrease due to the addition of metal ion. Using the experimental data in the form of fluorescence intensity versus total metal ion added (C_M) this equation can be solved by non-linear regression analysis for K and C_L (Shuman and Cromer, 1979; Bhat et al., 1981).

EXPERIMENTAL

Data Treatment. The non-linear regression analysis was performed by a program called NONREG which is part of the University of North Carolina Chapel Hill group of statistical programs. The program uses simplex optimization to estimate unknown parameters by least squares (Nelder and Mead, 1965; Ryan et al., 1980). For a given set of data points the curve fitting procedure gave the best fit values of K , C_L and I_{ML} from Equation 17. Replicate experiments were analyzed both individually and by pooling experimental points.

Apparatus. A Perkin Elmer model 204 spectrofluorometer was used to obtain fluorescence and Rayleigh scattering data. It was equipped with two grating monochromators, each with fixed 10 nm bandpass, a 150 W Xenon arc source and an R212 photomultiplier tube. The instrument was used in conjunction with a flow through system consisting of a 10 mm flow through quartz cuvette (Precision Cells, type 57H), a temperature jacketed titration cell and top (Princeton Applied Research, models K64 and K66), a peristaltic pump (Sigmamotor model T8) and Teflon and Tygon tubing. Inserted in the holes in the top of the cell were a combination pH electrode (Corning, model 476050), coupled to an Orion model 701A specific ion meter, a solution inlet and outlet tube and, an N_2 purge tube. The system was operated under anaerobic conditions for all experiments.

A P.M. Tamson constant temperature water bath (type T9) maintained the titration cell at 25 ± 0.1 °C. Solutions were pumped from the titration cell via the peristaltic pump and entered the spectrometer through a port on the side. Tygon was used in the pump head only, all other tubing was Teflon. Reagent additions were made with a Gilmont ultra-precision micrometer buret (model S3200A) and a Gilson model P200 variable microliter pipet. Absorbance measurements were made with a Cary 219 spectrophotometer. All solutions were filtered through 0.4 μ m membranes using a Nuclepore polycarbonate filtration apparatus described previously (Truitt and Weber, 1979).

Reagents. The isolation and characterization of SFA (Weber and Wilson, 1975; Wilson and Weber, 1977; 1979), its metal ion binding (Saar and Weber, 1979; 1980a; 1980b; 1980c; Bhat et al., 1981; Bresnahan et al., 1978; Truitt and Weber, 1981a; Rainville and Weber, 1982), and fluorescence properties (Saar and Weber, 1980a; Seitz, 1981) were described in earlier publications. The sample has only 0.8% ash, and low metal content which should have negligible affect on the fluorescence (Schnitzer and Ghosh, 1981). All SFA experiments were conducted at 10.0 mg/L and an ionic strength of 0.1 M in KNO_3 medium. Tyrosine solutions were made approximately 36 μ M and 0.1 M in KClO_4 to avoid interference of the fluorescence wavelengths by the absorbance of nitrate. Copper ion standards were prepared from reagent grade nitrate or perchlorate salts in 1×10^{-4}

M acid and standardized by EDTA titration (Flaschka, 1964). L-tyrosine was purchased from Eastman.

Procedures. Solutions of SFA and tyrosine were made up in the appropriate electrolyte and filtered. The tyrosine solutions were assayed by monitoring the UV absorbance of the ionization band at 240.5 nm in 0.01 M KOH. Either 25.0 or 50.0 mL of the sample was pipeted into the titration cell and deaerated with N_2 . The fluorescence titration was conducted as follows:

(1) The pH was adjusted to the desired value ± 0.02 units with KOH and/or HNO_3 .

(2) The sample was then circulated through the cuvette and back to the titration cell for several minutes.

(3) Fluorescence and scattering were observed.

(4) Flow was then reversed to return all of the sample to the titration cell.

(5) Cu^{2+} titrant was added and the process repeated. Approximately 15 min elapsed between successive additions of titrant. Adequate stirring was maintained in the titration cell with a magnetic stir bar as well as with N_2 bubbling. The fluorescence of a buffered portion of the sample was also measured in a separate cuvette to detect any fluctuations in source intensity and allow correction if necessary. The fluorescence excitation and emission wavelengths were 350 nm and 445 nm for SFA. For tyrosine 277 nm and 296 nm were used. Scattering experiments were done with both emission and excitation monochromators set to

the same wavelength, either 400 nm or 500 nm. Fluorescence measurements made after scattering doubled over its initial value were discarded. All measurements were made with the sample solution flowing through the system at a rate of approximately 15 mL/min. Any increased noise due to turbulence was minimal.

RESULTS AND DISCUSSION

Model studies. Figure 5 gives examples of hypothetical quenching curves for the titration of a fluorescing ligand with a paramagnetic metal ion. These curves are typical of classic spectrophotometric titrations. Each line is a plot of Equation 17 with different values of K such that the product KC_L is 100, 10 and 1 for curves A through C, respectively. The C_L value is 1×10^{-5} and the I_{ML} value is 10 for all curves. The endpoint of curve A is readily visible and could be determined graphically because of the large value of KC_L . As KC_L decreases however the curve becomes broader and the endpoint less sharp. It would be very difficult to accurately determine the endpoint for curve C without the aid of curve fitting. If the experimental data can be described by a theoretical equation with one or more unknown constants, non-linear regression analysis can be used to determine values for the constants (Shuman and Cromer, 1979; Bhat et al., 1981). A third constant in Equation 17 is the limiting or residual fluorescence value (I_{ML}). In theory the I_{ML} value could be

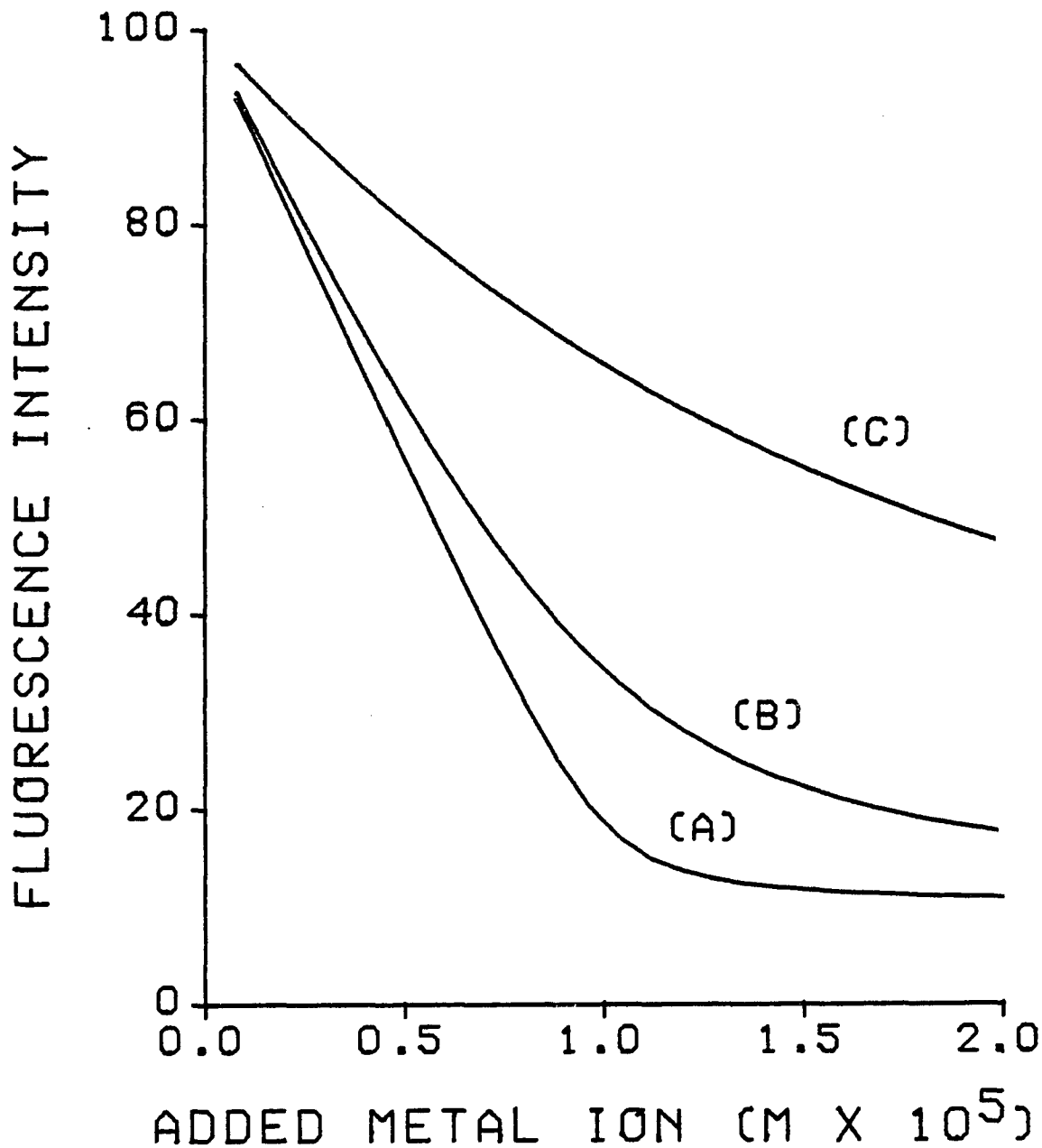


Figure 5. Hypothetical fluorescence quenching curves for the titration of a fluorescing ligand with a paramagnetic metal ion, $C_L = 1 \times 10^{-5} \text{ M}$, $I_{ML} = 10\%$: (A) $K = 1 \times 10^7$; (B) $K = 1 \times 10^6$, (C) $K = 1 \times 10^5$.

determined directly from a plot of the raw data. Figure 5 curve A shows that the I_{ML} value is 10 however this is not obvious for curves B and C. There are some experimental difficulties in carrying the titrations far enough to get a good number for I_{ML} . For SA a large excess of metal must be added bringing the Cu^{2+} concentration to about $1 \times 10^{-2} \text{ M}$ where precipitation of the neutral Cu-SA complex occurs and where hydrolysis becomes a problem at pH 6. For SFA precipitation of the Cu-SFA complex occurs at high metal to SFA ratios (Saar and Weber, 1980c). Precipitation causes scattering and may invalidate fluorescence measurements in this region. For these reasons and several others that will be discussed later, the I_{ML} value was fitted along with K and C_L rather than being experimentally determined.

Titration of two model compounds L-tyrosine and salicylic acid with Cu^{2+} were used to test the proposed theory and the ability of the curve fitting program. Figure 6 is a plot of the data points for one tyrosine titration and the best fit line obtained using the NONREG program. The average values determined for the constants for triplicate experiments are given in Table 9 and the data shown in Figure 7. The predicted value for C_L is quite close to the experimental concentration used. The average K value obtained also agrees well with the first conditional stability constant at pH 6 of 5.89×10^4 calculated from the literature values for the thermodynamic stability constant and acid dissociation constants (Martell and Smith,

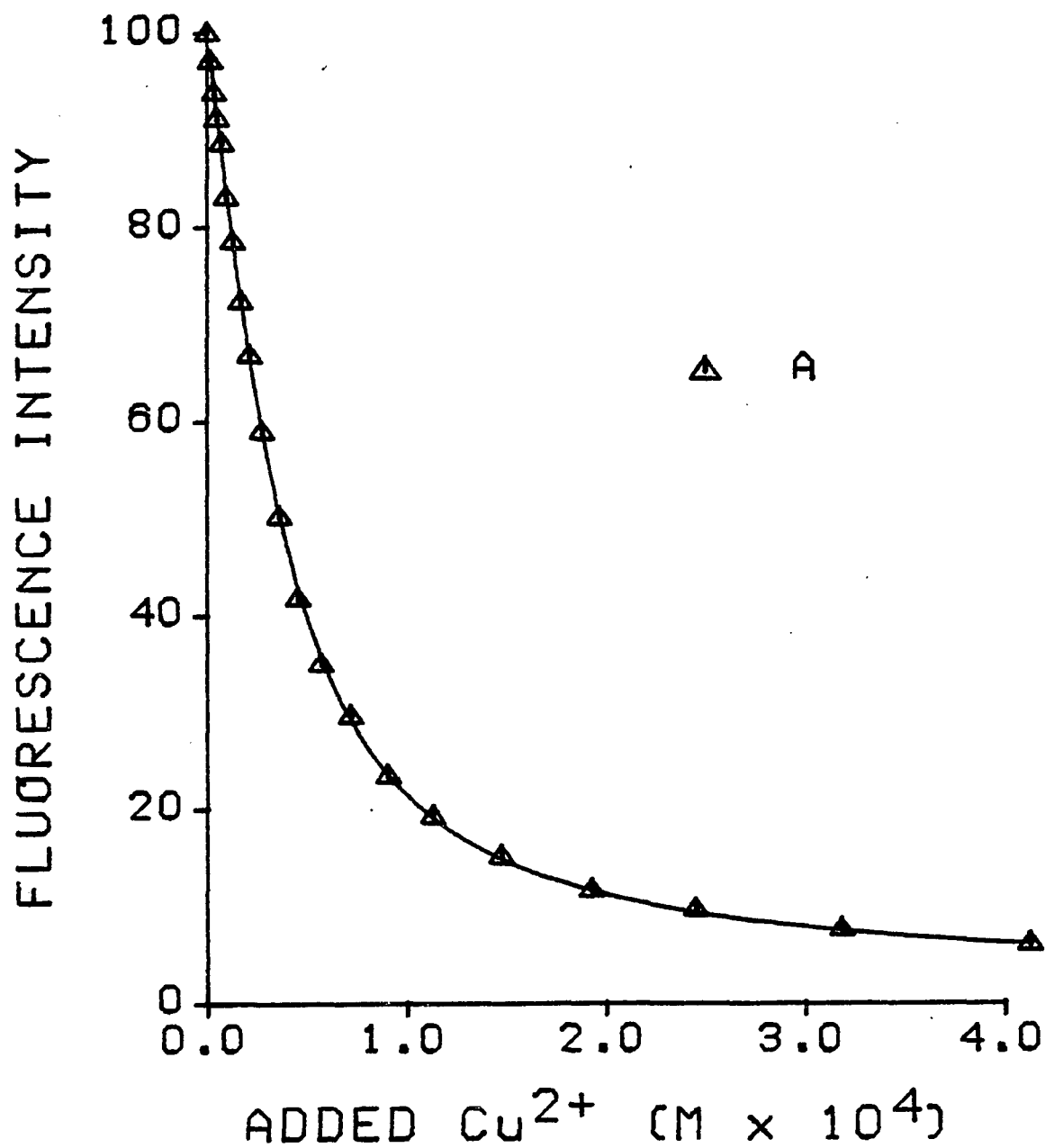


Figure 6. Fluorescence quenching curve for 3.57×10^{-5} M L-tyrosine in 0.1 M KClO₄ titrated with Cu²⁺ at pH 6.00 and 25 °C: \triangle Cu²⁺-tyrosine. Line is NONREG fitted curve.

Table 9. Average NONREG fitted parameters for fluorescence titrations with of soil fulvic acid (SFA) with Cu^{2+} a,b.

Sample	pH	Experimental Concentration	C_L , μM (s.d.)	$K(\times 10^{-5})(\text{s.d.})$	$I_{ML}(\text{s.d.})$
L-tyrosine	6.00	35.7 μM	33.0 (± 2.1)	0.58 (± 0.11)	2.6 (± 1.7)
SFAC	5.00	10.0 mg/L	22.1	0.48	40.2
SFA	6.00	10.0 mg/L	19.7 (± 6.1)	1.08 (± 0.08)	22.4 (± 1.4)
SFA	7.00	10.0 mg/L	19.6 (± 3.6)	2.84 (± 0.81)	20.7 (± 3.5)

a 0.1 M ionic strength at $25 \pm 0.1^\circ\text{C}$.

b C_L is the complexing capacity;

K is the conditioned stability constant for the stated pH;

I_{ML} is the limiting fluorescence intensity;

S.d. is the standard deviation of three replicates.

c One experiment only at this pH.

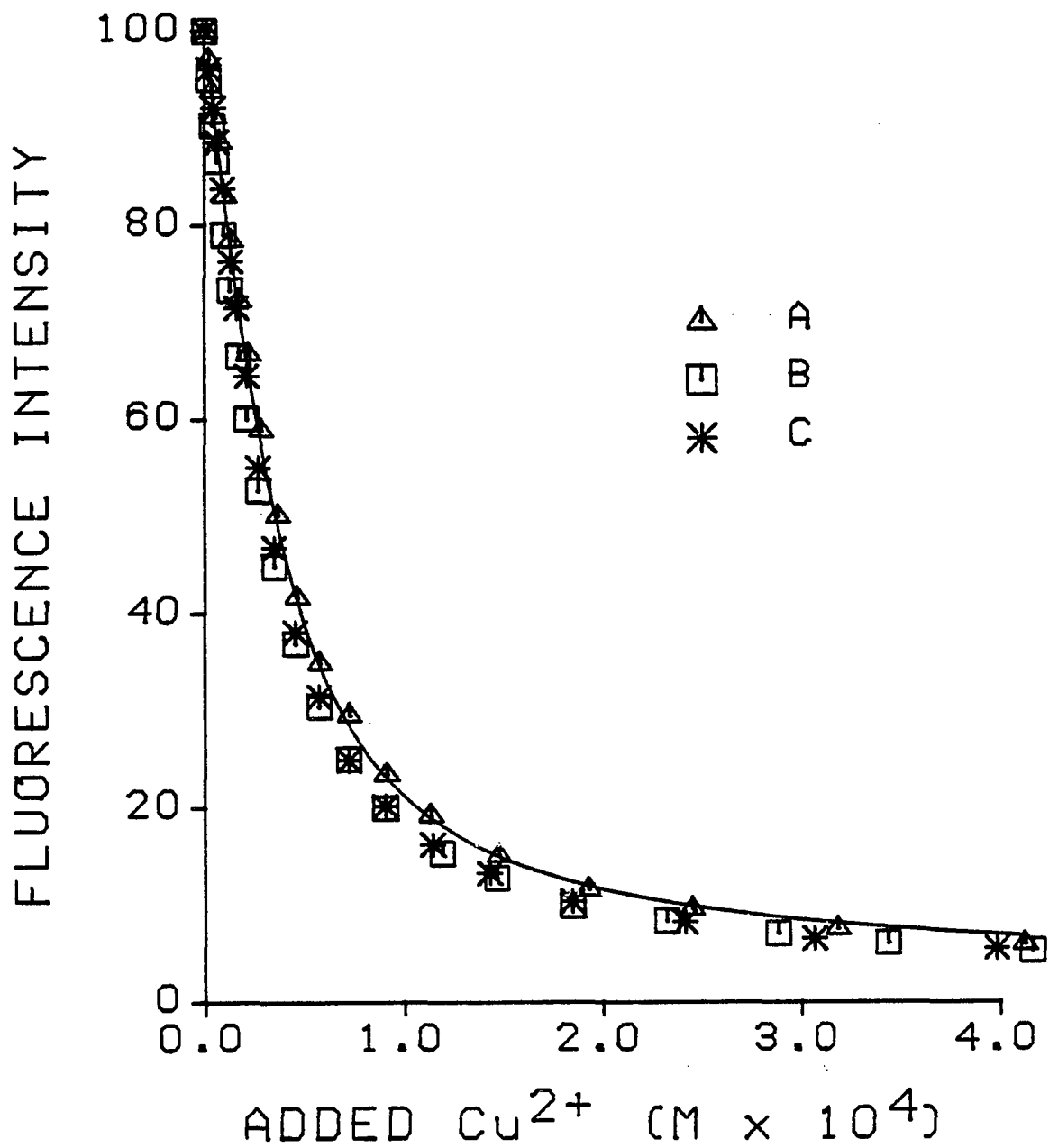


Figure 7. 3.57×10^{-5} M L-tyrosine in 0.1 M KClO_4 titrated with Cu^{2+} at pH 6.00 and 25°C . Line is NONREG curve using average constants from Table 9.

1974). The I_{ML} value is very low at 2.6% and can be considered essentially zero with error indicating that the complex is considerably lower in fluorescence efficiency than the free ligand.

Salicylic acid titrations with Cu^{2+} gave smooth but non reproducible curves. Figure 8 shows data for three titrations of 1.5×10^{-4} M SA at pH 6 corrected for dilution. Figure 8 also includes the predicted curve for Cu^{2+} -SA using equation 11 with the experimental concentration and the conditional stability constant calculated from literature values (Martell and Smith, 1977). The C_L values determined by curve fitting in general compare very poorly to the known values. The K values, although somewhat better are not in good agreement with the theoretical.

Initially it was thought that the binding of SA with Cu^{2+} at pH 6 was too weak to give good results. The first conditional stability constant of 1660 was relatively low and combined with the micromolar ligand concentration gave broad curves as in Figure 5 curve C. This may not be readily analyzed by curve fitting. Several experiments were conducted under different conditions to give higher values of KC_L . The ligand concentration was increased, the pH was increased to give a larger conditional K and the Fe^{3+} -SA system was studied as well. All gave the same poor results. The reason for the failure of these experiments may be

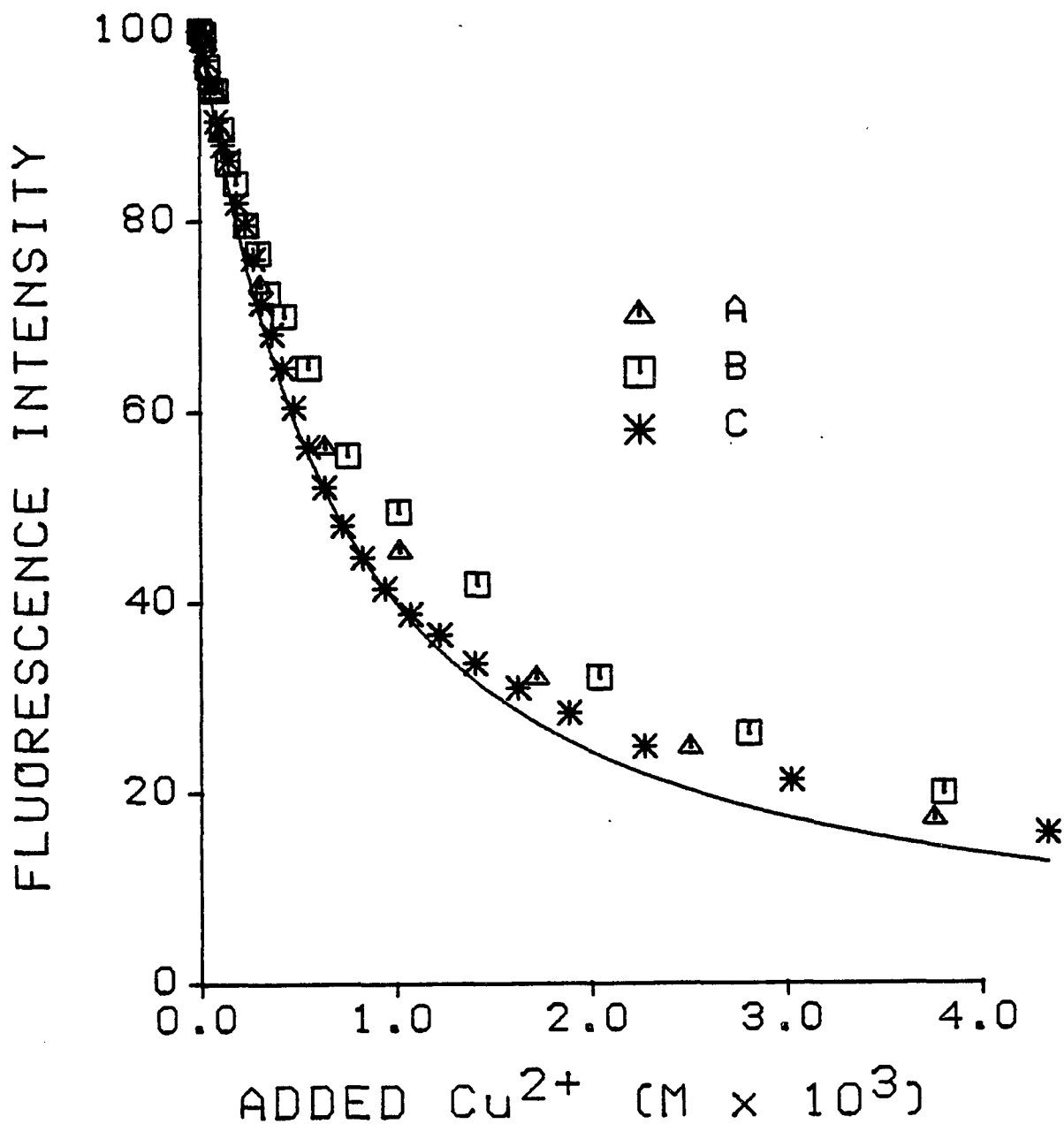


Figure 8. TRiplicate fluorescence quenching curves for 1.5×10^{-4} M salicylic acid in 0.1 M KNO_3 titrated with Cu^{2+} at pH 6.00 and 25 °C. Line is predicted curve using Equation 17 with experimental concentration and conditional stability constant from literature.

related to the intramolecular proton transfer discussed earlier. However, other possibilities do exist such as the shifting of the spectral maxima for the complex compared to the free ligand.

Assumptions for SFA experiments. Extension of this technique to the fulvic acid ligand mixture requires certain assumptions which must be considered when analyzing the final results. These assumptions are warranted on the basis of the widely accepted view that fulvic acid is a mixture of ligands with varying degrees of similarity. These assumptions are:

1. The material in the sample that fluoresces is representative of the bulk of the material.
2. The bulk material acts as one type of ligand with average properties represented by K and C_L .
3. Under the conditions used in these titrations, with metal in excess, primarily one-to-one complexes are formed.

There are several limitations inherent in this treatment with these assumptions. For example, the isolated fulvic acid used in this study reportedly contains only about one percent fluorescing material (Seitz, 1981). On the basis of this, a simplified model can be used to describe the binding and fluorescing properties of the sample. Four types of molecules can be defined. These include material that:

- a. fluoresces and binds metal ions and therefore undergoes fluorescence quenching
- b. fluoresces but does not complex
- c. does not fluoresce but complexes
- d. does not fluoresce or complex.

The relative amounts of these classes of molecules are unknown. Types a and b are accounted for in the theoretical treatment and type d is not of concern in this study. Type c is not accounted for and may cause an error in the results if it is present in significant amount. If the type c material has a similar stability constant to type a it will have little effect on the results. However if the stability constant for type c is considerably higher or lower than type a there will be an error in the calculated stability constant and complexing capacity.

Describing all of the material in a fulvic acid sample by one K value is certainly not ideal but it is necessary in this treatment in order to obtain the complexing capacity. Defining a differential equilibrium function as proposed by Gamble et al. (1980) may be more appropriate but is not amenable to the curve fitting analysis essential at this low concentration of SFA.

When titrating a ligand with metal ion, the ratio of moles of metal ion to moles of ligand increases throughout. When metal ion is in excess it becomes less likely, as the titration proceeds, that any complexes with more than one ligand per metal will occur. It is possible, however, that complexes with two or more metals per ligand will form (Pagenkopf and Whitworth, 1981). It is not clear whether binding more than one metal ion will have any effect on the fluorescence. For tyrosine when all the ligand is bound to one metal ion the residual fluorescence value (I_{ML}) drops practically to zero (Table 9). Binding another metal ion, although not likely, would have little or no effect on the fluorescence. It may therefore be inferred that binding a second or third metal ion to fulvic acid may not affect the fluorescence. Since the K and C_L values are determined from the fluorescence intensity it may be that a one-to-one model is appropriate and the K and C_L values obtained apply only to binding the first metal ion and are unaffected by multiple binding.

SFA results. Figure 9 gives the results of Cu^{2+} titrations of 10 mg/L SFA at pH 5, 6 and 7 in 0.1 M KNO_3 . At least three replicates are shown for each pH. The curves show the expected trends with a higher degree of quenching as pH increases due to increased binding. The curve for pH 7 shows the sharpest break with broader curves for pH 6 and 5. The NONREG results are given in Table 9. These are averaged results for three experiments at each pH except for

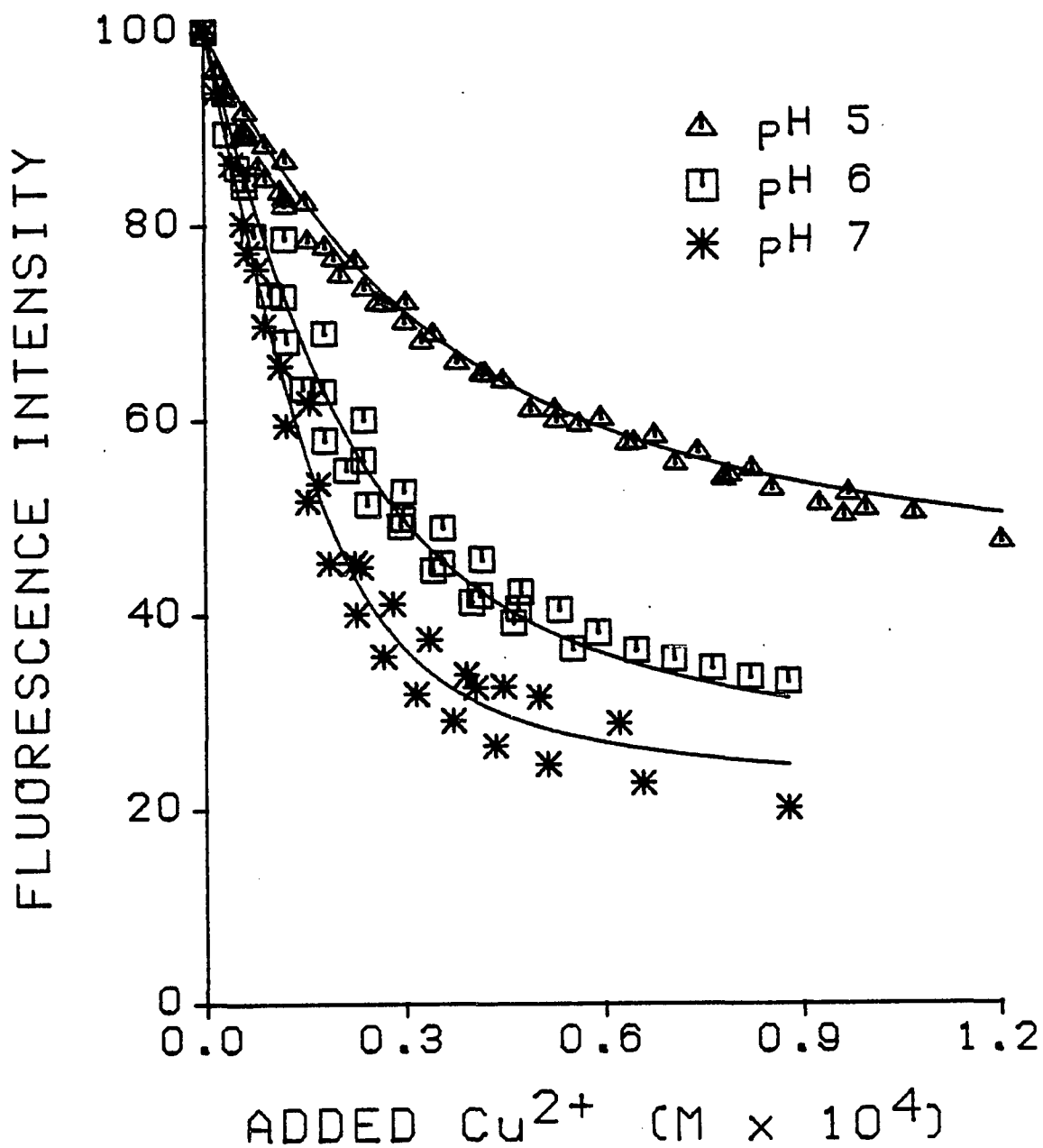


Figure 9. Fluorescence quenching curves for 10 mg/L SFA with Cu^{2+} in 0.1 M KNO_3 at 25 °C: \triangle pH 5, four replicates; \square pH 6, three replicates; $*$ pH 7, three replicates. Lines are fitted curves for averaged NONreg results listed in Table 9.

pH 5 which is discussed below. The complexing capacities for pH 6 and 7 are about 20 μM and the K value about two to three fold greater for pH 7. It is also noted that the final fluorescence value (I_{ML}) is approximately 21 for pH 7 and 22 for pH 6, as shown in Figure 9. This is markedly different from the essentially zero values of the tyrosine experiments and can be interpreted in two ways. First, fluorescence efficiency of the complex may be about one-fifth that of the free ligand giving rise to the significant I_{ML} values after all the ligand is bound. Second, as mentioned above, the residual fluorescence may be due to material in the sample that does not bind (type b material). The fluorescence of the binding material is then thought to be completely or almost completely quenched as in the case of tyrosine.

The experiments conducted at pH 5 did not give reasonable results when analyzed by NONREG. Results for four experiments were highly scattered and often gave negative C_L values. The reason for this is probably the very weak binding at pH 5 resulting in a low value for KC_L . Even though the curves were highly reproducible at this pH (Figure 9) they could not be analyzed well by curve fitting. The only experiment at pH 5 that did not have negative results gave K and C_L values that follow the trends for pH 6 and 7 (Table 9). The I_{ML} value however was 40.2% which is higher than those at pH 6 and 7 and may be erroneous. The NONREG curve for this one experiment is shown with the pH 5

results in Figure 9 for comparison.

Comparison of the fluorescence C_L values with those obtained by other techniques for this SFA at 10 mg/L and under very similar conditions is given in Table 10. ASV and ISE were used to determine free metal ion in the presence of complex while dialysis separates free metal ion from complex and ligand prior to analysis by atomic absorption (Bhat et al., 1981; Truitt and Weber, 1981a; Rainville and Weber, 1982). Two types of dialysis experiments are listed. The first is a thirty day dialysis of a single solution which is titrated with Cu^{2+} (Truitt and Weber, 1981a). The second is the result of two day dialysis experiments of about fifteen solutions containing different amounts of metal ion corresponding to the points in the titration (Rainville and Weber, 1982).

The fluorescence values fit in very well at pH 6; however there is a large difference at pH 7. C_L values obtained from ISE and dialysis titrations are much higher at this pH. In addition the values at pH 5 are generally lower than the single fluorescence value. This brings out a significant difference between the complexing capacities measured by fluorescence and those measured by other techniques, that is, the fluorescence values do not increase with pH. Complexing capacities should be independent of pH for a particular sample, yet many workers have observed the trend of increasing complexing capacities and offered

Table 10. Cu^{2+} complexing capacities (μM) with standard deviations for 10.0 mg/L soil fulvic acid (SFA) in 0.1 M KNO_3 .

<u>Technique</u> ^a	<u>pH 5</u>	<u>pH 6</u>	<u>pH 7</u>	<u>Source</u>
Fluorescence	(22.1) ^b	19.7 \pm 6.1	19.6 \pm 3.6	This work
ASV	5.2 ^c	18.2	--	Bhat et al. (1981)
ISE	17	--	--	Bhat et al. (1981)
ISE ^d	22.7 \pm 0.8	26.4 \pm 3.0	43.7 \pm 3.7	Truitt (1980)
Dialysis	16.2 \pm 3.1	24.1 \pm 7.2	28.7 \pm 12.4	Truitt and Weber (1981a)
Dialysis	10.8 \pm 1.6	20.3 \pm 3.0	14.2 \pm 14.2	Rainville and Weber (1982)

a ISE is Cu^{2+} ion selection electrode;

ASV is anodic stripping voltammetry.

b Less reliable value; see Bhat et al. (1981).

c One experiment only.

d 0.01 M KNO_3 .

various explanations (Shuman and Cromer, 1979; Bhat et al., 1981; Truitt and Weber, 1981a; Rainville and Weber, 1982). It is proposed here that precipitating fulvate complexes adsorb free metal removing it from solution. For techniques that measure free metal this is interpreted as further complexation. A more detailed discussion is given in the following sections.

Rayleigh scattering measurements. Metal ions can precipitate fulvic acid under certain conditions (Saar and Weber, 1980b; 1980c; Pagenkopf and Whitworth, 1981). This phenomenon has been generally ignored in the context of complexing capacity titrations even though precipitation is very likely near the end of the titrations. Figure 10 gives the results of Rayleigh scattering measurements made during titrations of SFA with copper at pH 5, 6 and 7. As metal ion is added and binds to SFA, scattering increases. Although the increase is gradual at first it eventually undergoes a rapid increase and then seems to level off. These trends are similar to those observed by Pagenkopf and Whitworth (1981) for humic acid with Cu^{2+} at pH 7. Some additional observations described here, however, lead to slightly different interpretations. Triplicate measurements at pH 7 with both monochromators set at either 400 nm or 500 nm demonstrate continued increase in scattering beyond the leveling off region. Obviously precipitation does not cease in this region and centrifugation of the solutions confirms this. This leveling off of the curve or rather the dramatic

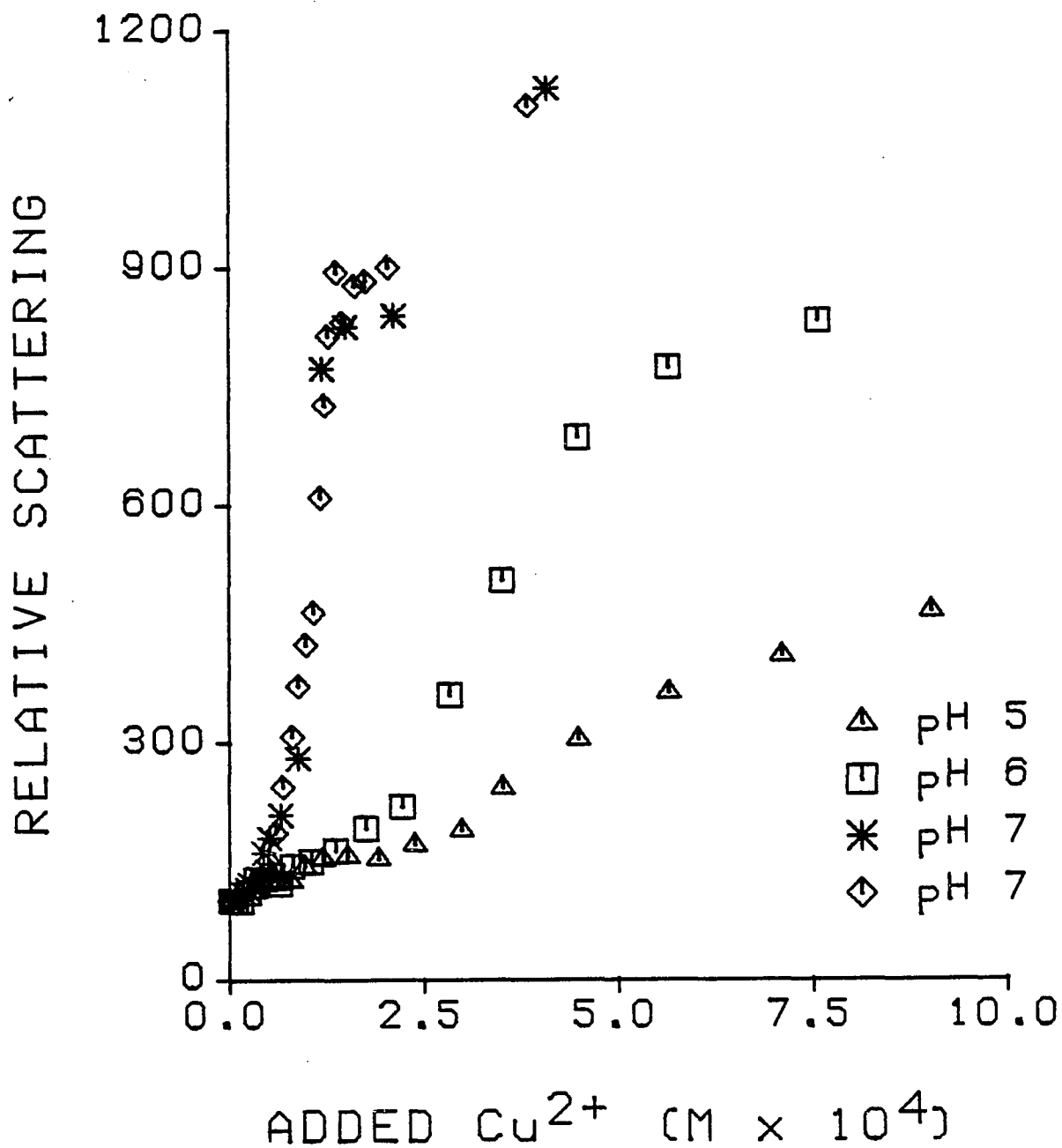


Figure 10. Rayleigh scattering at 400 nm for 10 mg/L SFA with Cu^{2+} in 0.1 M KNO_3 at 25 °C.

decrease in its slope is probably due to the formation of larger-sized aggregates. This increase in particle size due to aggregation does not, however, cause "large particle scattering" at the wavelength used. This is evident from the fact that curves obtained simultaneously at 400 nm and 500 nm obey the relationship

$$\text{scattering intensity} = \text{constant}/(\text{wavelength})^4$$

which is expected for Rayleigh scattering (Parker, 1968). The following simplified mechanism for the formation of these large aggregates is proposed. As metal binds to fulvic acid its negative charge is neutralized (Pagenkopf and Whitworth, 1981). Once this occurs the hydrophobic part of the molecule, containing possibly aliphatic chains and aromatic rings (Liao et al., 1982), dominates its water solubility and it comes out of solution. Scattering should increase linearly with the number of particles as in standard nephelometry. As more of these particles form some of them may come together due to a lipophilic or hydrophobic attraction forming larger aggregates. However these larger aggregates scatter less efficiently than smaller ones due to their size in relation to the wavelength of light.

Effect of precipitation on complexing capacities. Once insoluble solids form in the sample its properties may no longer be controlled by solution chemistry. Interactions between ions in solutions and solid surfaces may become the dominant process. One possible result could be the removal

of metal from solution by adsorption onto the surface of particles (Saar and Weber, 1981b; 1981c). As mentioned before all of the previous work on complexing capacities has relied on determining free metal ion in equilibrium with complex and ligand. Any process that removes free metal ion from solution will be interpreted as complexation.

Figure 10 demonstrates that precipitation occurs more readily at higher pH. Complexing capacity titrations for different pH values covering approximately the same range will have much higher amounts of particulate matter at pH 7 compared with pH 6 and may therefore exhibit a higher apparent complexing capacity due to adsorption (Table 10). In the case of fluorescence titrations, however, scattering was measured simultaneously and none of the fluorescence data was used after the scattering became double its initial value (Figure 10). The complexing capacities determined by fluorescence are the same for pH 5, 6 and 7 within experimental error (Table 10) and this is what is expected on the basis of ionic equilibrium consideration alone.

CONCLUSIONS

Fluorescence spectroscopy has the required sensitivity for work at natural concentration levels. The fact that fluorescence differentiates free from bound ligand makes it an excellent complement to these other complexing capacity techniques which measure free metal ion. The results described here are similar to those for ASV, ISE and

equilibrium dialysis. In some respects fluorescence has advantages over these other techniques. It is fairly rapid and there are no problems with adsorption of SFA on an electrode as in ASV (Bhat et al., 1981). Kinetic corrections are also not a problem (Shuman and Cromer, 1979; Bhat et al., 1981). Fluorescence does not have the problems associated with separation techniques like dialysis and ultrafiltration such as completeness of separation or adsorption of metal ions on the membrane. It does not shift equilibria either like some of the ion exchange or gel filtration techniques. The high sensitivity of fluorescence makes it easily adaptable to natural levels of species of interest and, it does not require compromising sample integrity with the use of supporting electrolyte or buffer. The method does not rely on the difference measurement of bound metal ion which can lead to erroneous conclusions due to binding by inorganic species. Solution scattering can also be measured with the same instrumentation giving valuable information on precipitate formation. The usual goal of complexing capacity determinations is the nature of solution equilibria. By carefully considering both fluorescence and scattering, complexation can be distinguished from adsorption of metal ions on particulates. This gives a more complete picture of processes related to metal ion binding for this complex system.

The fact that the fluorescence technique measures directly a property of the ligand, SFA, may make it the technique of choice in correlation studies of the organic matter. The fluorescence technique with the data treatment described and curve fitting analysis may find use in other areas such as metal ion binding studies of proteins (Chen, 1976).

CHAPTER 4

COPPER (II) COMPLEXING CAPACITIES OF NATURAL WATERS BY FLUORESCENCE QUENCHING

INTRODUCTION

A logical extension of the fluorescence quenching technique described in Chapter 3 is its application to "real" environmental samples (Ryan and Weber, 1982b). In order for a method to be useful it must have the desired accuracy, precision, sensitivity and detection limit as well as freedom from interferences when applied to the system of interest. The true test of the fluorescence method therefore is the determination of complexing capacities (C_L) for natural water samples.

Initially it must be demonstrated that natural water organic matter has analogous fluorescence properties to SFA. It can not be assumed that the isolation procedure has left the material chemically unaltered or that it behaves identically to the organic matter in the natural system from which it was taken. Also the fluorescence must be quenched upon complexation to the metal ion of interest and the degree of quenching must be proportional to the extent of binding. From the literature considerable similarity does exist in the fluorescence spectra of aquatic humic substances from a variety of sources. Table 11 lists fluorescence excitation and emission maxima reported by many

Table 11. Fluorescence spectral maxima reported for water samples and isolated humic materials

<u>Sample</u>	<u>Excitation Max (nm)</u>	<u>Emission Max (nm)</u>	<u>Reference</u>
Various waters (UK)	340-350	400-460	Smart et al. (1976)
"Colored waters" (USA)	360-370	450-460	Ghassemi and Christman (1968)
Rivers and swamps (USA)	350 ^a	430-450 ^a	Zepp and Schlotzhauer (1981)
Brooks (USA)	344-355	432-450	Larson and Rockwell (1980)
Lake (USA)	339-403	426-490	Hall and Lee (1974)
Rivers (USA)	365	460	Willey and Atkinson (1982)
Creek (USA)	375	465	Larson (1978)
Freedlot runoff (Canada)	360	425-465	Lakshman (1975)
Lake (USA)	335	470	Wetzel and Otsuki (1974)
Lake (Netherlands)	365	470	DeHaan (1972)
Moor (Germany)	365	470	Muller-Wegener (1977)
Baltic Sea	325	420	Lindquist et al. (1978)
"Colored waters" (USA)	365 ^b	490	Black and Christman (1963)
Estuary (Netherlands)	350 ^b	420-440	Laane and Koole (1982)
"Colored waters" (USA)	361	460	Christman and Ghassemi (1966)
Rivers (USA)	365	460	Willey and Atkinson (1982)

Table 11. (Continued)
 Fluorescence maxima reported for isolated humic materials in water

<u>Sample</u>	<u>Excitation Max (nm)</u>	<u>Emission Max (nm)</u>	<u>Reference</u>
Marine sediment overlying water	350 ^b	433 ^a	Ewald (1976)
Fulvic acid (soil)	350 ^a	450 ^a	Zepp and Schlotzhauer (1981)
Humic acid (commercial)	350 ^a	430-460 ^a	Zepp and Schlotzhauer (1981)
Leaf extract	400-427	473-510	Hall and Lee (1974)
Fulvic acid	468	515-520	Levesque (1972)
Fulvic acid (soil)	360, 465 ^c	520 ^d	Schnitzer and Ghosh (1981)
Fulvic acid (water)	350	445-450	Saar and Weber (1980a)
Humic acid	340	460	Willey and Atkinson (1982)
Humic acid (water)	315 ^b	420-430	Almgren et al. (1975)
Humic acid (soil)	365 ^b	500-540	Seal et al. (1964)

a Corrected spectra.

b Fixed excitation wavelength.

c Two fairly resolved excitation peaks were reported, the largest at 360 nm.

d Fixed emission wavelength

workers for natural waters. Also in Table 11 are the maxima for many isolated humic and fulvic acids. In general excitation maxima cover the range from 340 nm to 375 nm with emission occurring from 400 nm to 520 nm. This gives only a qualitative view however, since exact maxima are a function of instrumental parameters for uncorrected spectra. Also for complex mixtures, such as aquatic humics, emission maxima may shift with changes in excitation wavelength (Parker, 1968; Saar and Weber, 1980a). The effect of pH on fluorescence peaks is not known either.

Ewald (1976) published corrected spectra of marine fulvic acid from pore water and overlying-sediment waters. His approach was to correct spectra not only for instrumental parameters but for Rayleigh, Raman and Tyndall scattering as well. Rayleigh and Raman corrections are commonly done but the correction for Tyndall scattering is very difficult. Tyndall scattering arises from the large molecule nature of fulvic acid and can only be corrected for using a molecule of similar size that does not fluoresce. For this Ewald (1976) chose polyvinyl alcohol with a molecular weight of 72000 daltons. Since this compound has no real similarity to fulvic acid, the value of such measurements is in question. It may be perfectly acceptable and far simpler to leave the Tyndall scattering component present in the spectra.

The quenching of natural water fluorescence has not been studied extensively. Cline and Holland (1977) demonstrated reduction of the absorbance corrected fluorescence caused by Co^{2+} and Cu^{2+} for a pore water sample from a freshwater lake. Very recently fluorescence quenching curves for both humic acid and river water with Fe^{3+} were demonstrated (Willey and Atkinson, 1982). Others have studied fluorescence quenching of isolated humic material (Saar and Weber, 1980a; Schnitzer and Ghosh, 1981; Banerjee and Mukherjee, 1972). Levesque (1972) noted that organic matter fractions with high Fe content fluoresced relatively little.

In this chapter the fluorescence properties of some natural water samples are described. Spectral characteristics are measured, Cu^{2+} complexing capacities and 1:1 conditional stability products are determined using the fluorescence titration technique. In order to obtain a realistic idea of some of the analytical characteristics of the fluorescence method detailed earlier, several types of water samples were collected. Triplicate titrations were done on separate days for each sample to determine the precision of the method. Six natural waters, including both freshwater and marine samples were used. These samples were expected to cover a range of C_L values from high to very low and to include any commonly encountered interferences. The presence of trace metals, which occupy humic material binding sites, and inorganic ligands, which compete with

organic chelates, may cause error in measured binding capacities. Colloidal size particles that pass through the 0.4 μm filter used can also bind or adsorb metal ions.

Some additional considerations are also addressed in this study. Commonly measured water characteristics for the samples are correlated with each other and the titration results in order to show statistically significant trends. If complexing capacities can be measured by fluorescence quenching it would be quite useful to be able to relate other more easily measured parameters to the C_L values. In this way it may be possible to use existing water quality data to estimate the binding or metal ion buffering ability of a water system.

THEORY

Use of a conventional stability constant to describe the binding ability of the complex mixture of ligands making up humic matter is not the most desirable approach (Gamble et al, 1980; MacCarthy and Smith, 1979; 1978). MacCarthy and Smith (1978; 1979) have addressed this problem and proposed a stability function or product (S_n) to describe the binding of a metal ion to N ligands in a multiligand mixture. For 1:1 binding this product takes the form

$$S_1 = \frac{\sum_{i=1}^N [M(L_i)]}{[M] \sum_{i=1}^N [L_i]} \quad (18)$$

where N is the number of ligand species present in the mixture; (L_i) and $[M(L_i)]$ are the equilibrium concentrations of the i th ligand and its metal complex, respectively, and $[M]$ is the free metal ion concentration.

Modifying Equation 1 (Chapter 3) to be consistent with the stability product approach results in

$$\frac{\sum_{i=1}^N [M(L_i)]}{C_L} = \frac{I_L - I}{I_L - I_{ML}} \quad (19)$$

where

$$C_L = \sum_{i=1}^N [L_i] + \sum_{i=1}^N [M(L_i)] = \sum_{i=1}^N ([L_i] + [M(L_i)]) \quad (20)$$

$$I_L = \sum_{i=1}^N I_{L_i} \quad (21)$$

$$I_{ML} = \sum_{i=1}^N I_{M(L_i)} \quad (22)$$

$$I = I_L + I_{ML} = \sum_{i=1}^N (I_{L_i} + I_{M(L_i)}) \quad (23)$$

Equation 20 gives the fraction of the overall mixture bound to the metal ion in terms of fluorescence intensities. Combining Equation 19, 20 and 21 in the manner described

previously (Chapter 3), we derive Equation 24 that relates the measured fluorescence intensity, a function of the total metal ion added, to the stability product and C_L .

$$I = ((I_{ML} - 100)/(2S_1C_L)) \left[(S_1C_L + S_1C_M + 1) - \sqrt{(S_1C_L + S_1C_M + 1)^2 - 4S_1^2C_LC_M} \right] + 100 \quad (24)$$

Equation 24 is identical to the previously derived Equation 17 in Chapter 3 except that S_1 replaces K . Therefore the fluorescence method allows determination of average stability products as well as C_L values for complex mixtures of ligands (MacCarthy and Smith, 1979).

It is not clear from this treatment whether or not the determined S_1 value is in the CLASP-1 region (limiting region) defined by MacCarthy and Smith (1979). Thus, it is not known if S_1 has a constant value or will vary with small changes in the composition of the sample under study. This means, therefore, that the stability products calculated here cannot be used for predictive or comparative purposes as they are not necessarily constant and may vary with C_M as well as C_L . In this study their use is necessary in the data treatment as a means of calculating complexing capacities (C_L) which are constant for the particular sample. Although the concept of S_1 is important to the interpretation of the results, its calculation in this data treatment is no different than for K . Therefore, in order

to be consistent with the previous work, K will be used in further discussions of the stability product.

EXPERIMENTAL

The experiments with natural water samples were carried out in the same manner as described in Chapter 3 for SFA. Some additions follow.

Apparatus. An Instrumentation Laboratory atomic absorption spectrometer (model 951) was used with background correction to determine iron concentrations. Water samples were filtered through 0.4 μm membranes using a Nucleopore 142 mm Teflon and Plexiglass filter support with Tygon tubing and the peristaltic pump. All materials that came in contact with samples or solutions were thoroughly acid washed with 10% HNO_3 (Laxen and Harrison, 1981).

Natural Water Samples. A variety of types of water samples were taken in southeastern New Hampshire. Freshwater systems included the Oyster River, Lamprey River, Portsmouth Reservoir and Barrington Swamp. In addition, marine samples were collected from the Great Bay Estuary (Jackson's Landing at low tide), and pore water from several gravity cores taken in the Great Bay. No special precautions were taken to keep the pore water anaerobic.

All the bulk water samples were collected in 10 L acid-washed polyethylene bottles and immediately taken to the laboratory and put on ice. Filtration was begun and

aliquots taken for pH measurements. After filtration both filtered and unfiltered portions of the samples were stored at 4 °C prior to characterization. The pore water was filtered through 0.4 um filters using a 47 mm support (Truitt and Weber, 1979). Extreme care was taken throughout to avoid contamination of the samples with metal ions.

Procedures. Several characteristic parameters of the water samples were measured according to procedures in "Standard Methods for the Examination of Water and Wastewater" (1975). Procedures were the same as described by Truitt and Weber (1981b) with the following exceptions. Color measurements (UV absorbances) were made at 250 nm with samples adjusted to 0.2 M in H_2SO_4 (Reid et al., 1980). Absorbances were referenced to our well characterized SFA analyzed in the same way (Truitt and Weber, 1981b). In addition the fluorescence of acidified samples was also measured and referred to SFA fluorescence. Excitation and emission wavelengths were 350 nm and 430 nm, respectively.

Fluorescence quenching titrations were conducted in the flow through system on 50.0 or 25.0 mL aliquots. Samples were deaerated by purging with moist N_2 in order to facilitate pH measurement and interpretation of C_L values. Fluorescence excitation and emission spectra were scanned and wavelengths of maximum intensity determined. The fluorescence-quenching titrations with Cu^{2+} ion were conducted as described previously (Chapter 3). Due to the

low ionic strength of the samples, precise pH measurement was difficult. Samples were titrated at their natural pH value ± 0.05 units. The fluorescence of a buffered portion of the sample was also measured in a separate cuvette to detect any fluctuations in source intensity and allow correction if necessary. All measurements were made at the excitation and emission wavelengths that produced the maximum signal. Scattering experiments were done with both emission and excitation monochromators set to 400 nm.

Data Treatment. The fluorescence quenching titrations were conducted in at least triplicate for all but the pore water which was done in duplicate due to limited sample. For a given set of experimental data the curve fitting procedure gives the best fit values of K , C_L and I_{ML} from Equation 24. In order to determine if replicate titrations for the same water sample have the same variance it was necessary to subject them to Bartlett's Test (Volk, 1980). Duplicate pore water experiments were analyzed by an F test (Volk, 1980). Variances are calculated by dividing the residual sum of squares by the appropriate degrees of freedom. The residual sum of squares is the parameter the NONREG program uses to determine the best fit of Equation 24 to the data. The degrees of freedom is the number of data points for the titration minus the number of constants in the fitted equation, which is three for Equation 24. Bartlett's Test determines if the variance of the titrations are different and whether, therefore, they form a

homogeneous group. The F test also measures homogeneity of variances where only two cases are involved. At the 0.05 probability level the variances of replicate titrations for the pore water and all the freshwater samples except Barrington Swamp do not show significant differences. Therefore the fitted parameters derived for titrations of a given sample can be averaged.

Titration parameters and the various water sample characteristics measured were analyzed in a multiple correlation study to detect trends. The correlation coefficients were checked for significance at the 90% confidence level.

RESULTS AND DISCUSSION

General Water Sample Characteristics. The water samples studied cover a broad range of types including a freshwater lake (Portsmouth Reservoir), two rivers (Lamprey and Oyster Rivers), a swamp (Barrington Swamp), an estuary (Great Bay) and marine sediment interstitial water (pore water). The first three samples are from local drinking water supplies. Table 12 lists the various measured parameters of the samples. In general the freshwaters fall in the expected pH range and are low in hardness ions, and low in alkalinity with the exception of the Oyster River. The marine samples are very high in Mg^{2+} , Ca^{2+} and alkalinity as expected. The additional alkalinity may be due mainly to borate for the Great Bay, however the

Table 12. Average natural water sample characteristics.

<u>Characteristics^a</u>	<u>Portsmouth Reservoir</u>	<u>Lamprey River</u>	<u>Oyster River</u>	<u>Barrington Swamp</u>	<u>Pore Water</u>	<u>Great Bay Estuary</u>
pH	6.0	6.0	6.7	5.8	8.0	8.0
Hardness (mg/L as CaCO ₃)	13.1	17.5	47.9	10.3	3900	4860
Alkalinity (mg/L as CaCO ₃)	4.3	4.0	20.2	3.8	3140	86.4
Conductance (umho/cm)	67.3	92.5	174.	71.7	--	39,400
Color (mg/L as SFA)	10.2	20.3	11.6	16.6	46.0	3.2
Fluorescence (mg/L as SFA)	29.7	43.5	32.7	29.3	170.	6.9
DOC (mgC/L as KHP)	7.2	11.5	8.6	8.3	40.8	4.3
Total Iron (uM)	1.0	3.8	1.4	4.1	0.2	0.2
C _L (uM) ^b	6.6(2.9)	17.1(1.2)	4.1(1.5)	14.6(2.7)	0.8(0.4)	--
K (M ⁻¹ x10 ⁻⁴) ^b	6.7(1.4)	5.3(0.3)	8.2(0.7)	6.1(0.4)	25.8(6.9)	--
I _{ML} (%) ^b	34.7(2.0)	20.0(0.5)	29.2(1.3)	20.0(1.0)	81.5(1.9)	--

^a KHP is potassium hydrogen phthalate; SFA is soil-derived fulvic acid; Color is UV absorbance at 250 nm; DOC is dissolved organic carbon; C_L is complexing capacity; K is the conditioned stability constant at the stated pH; I_{ML} is the limiting fluorescence intensity.

^b Values in parentheses are standard deviations.

extremely high value for the pore water is probably caused by borate, carbonate and other inorganic anions as well as organic compounds. Freshwater conductivities are low while that for the Great Bay is very high and can be used to estimate the salinity for the sample (Grashoff, 1976). The small quantity of pore water prevented measurement of its conductivity with available equipment.

Measurements of color (UV absorbance at 250 nm), fluorescence and dissolved organic carbon (DOC) give independent indications of the amount of organic material in the samples. The color and fluorescence data are reported in terms of the isolated SFA and estimate relative humic material content. The appropriateness of these optical measurements will be considered later. In general it is obvious from all three parameters (Table 12) that the pore water is the highest in organic matter while the Great Bay is the lowest. Of the freshwater samples the Lamprey River is undoubtedly the highest with the other samples showing little difference. The higher organic matter content of the Lamprey River may be due to a short period of rain a few days prior to sampling. Soil runoff and groundwater upwelling can cause an increase in organic matter entering the river from the surrounding land (Reuter and Perdue, 1977).

The fluorescence spectra of the samples were very similar to our isolated SFA (Saar and Weber, 1980a) and to those reported by others (Zepp and Schlotzhauer, 1981; see also Table 11). Table 13 gives the excitation and emission maxima for the various samples. There is very little variation in these values from sample to sample and the differences are not considered significant in comparison to the bandwidth of the optical system. Compared with the data in Table 11 the variation is very small for these samples taken from quite different bodies of water. The implication may be that the organic matter in a specific geographic region is quite similar and shows low variability in spectroscopic properties. However it is more likely that the differences reported by others (Table 11) are mainly due to the instrumental factors mentioned.

The Great Bay estuary sample exhibited some spectral differences from the other samples. In addition to the maxima listed in Table 13 there was an additional peak in both the excitation and emission spectra. With the emission monochromator set at 430 nm there was a peak at 374 nm in the excitation spectrum as well as the maximum at 350 nm. Scanning the emission with excitation at 350 nm gave rise to peaks at 398 nm and 430 nm. When the emission monochromator was set to 398 nm and the excitation scanned, only the 350 nm excitation peak was found.

Table 13. Fluorescence excitation and emission maxima for water samples^a.

<u>Sample</u>	<u>pH</u>	<u>Excitation Maxima (nm)</u>	<u>Emission Maxima (nm)</u>
Oyster River	6.7	350	430
Barrington Swamp	5.8	352	435
Portsmouth Reservoir	6.0	347	430
Lamprey River	6.0	350	432
Pore Water	8.0	350	428
Great Bay ^b	8.0	350	430

^a See experimental section for instrument specifications.

^b See text for discussion of minor bands.

Smart et al. (1976) found evidence for two emission peaks in freshwater samples excited at 350 nm. The two peaks however, appeared at 410-420 nm and 440-450 nm and were of almost equal intensity making up the major band. Zepp and Schlotzhauer (1981) studied several natural waters and found emission maxima ranged from 430 to 470 nm with shoulders at 400-420 nm and 500-520 nm. The additional peaks in the Great Bay spectra were only slightly less intense than the maxima and may be indicative of some fundamental differences in the marine organic matter compared to that from freshwater sources (Stuermer and Payne, 1976). However, the overall intensity of the fluorescence spectrum of the Great Bay sample was lower than the other samples by a least a factor of four. The additional peaks may be due to a trace component of the organic matter only measurable at the higher instrument sensitivities required for the sample. This trace component might also be present in the other samples, especially the pore water, but may not be discernable by being under the large and rather broad major peak.

Freshwater Cu²⁺ Titrations. Fluorescence quenching titrations of the water samples with Cu²⁺ ion proceeded much as the previous experiments with SFA (Chapter 3; Ryan and Weber, 1982a). Figures 11-14 show titration curves for the Oyster River, Lamprey River, Portsmouth Reservoir, and Barrington Swamp. Three replicates were conducted for each sample. The overall results for all the samples look quite

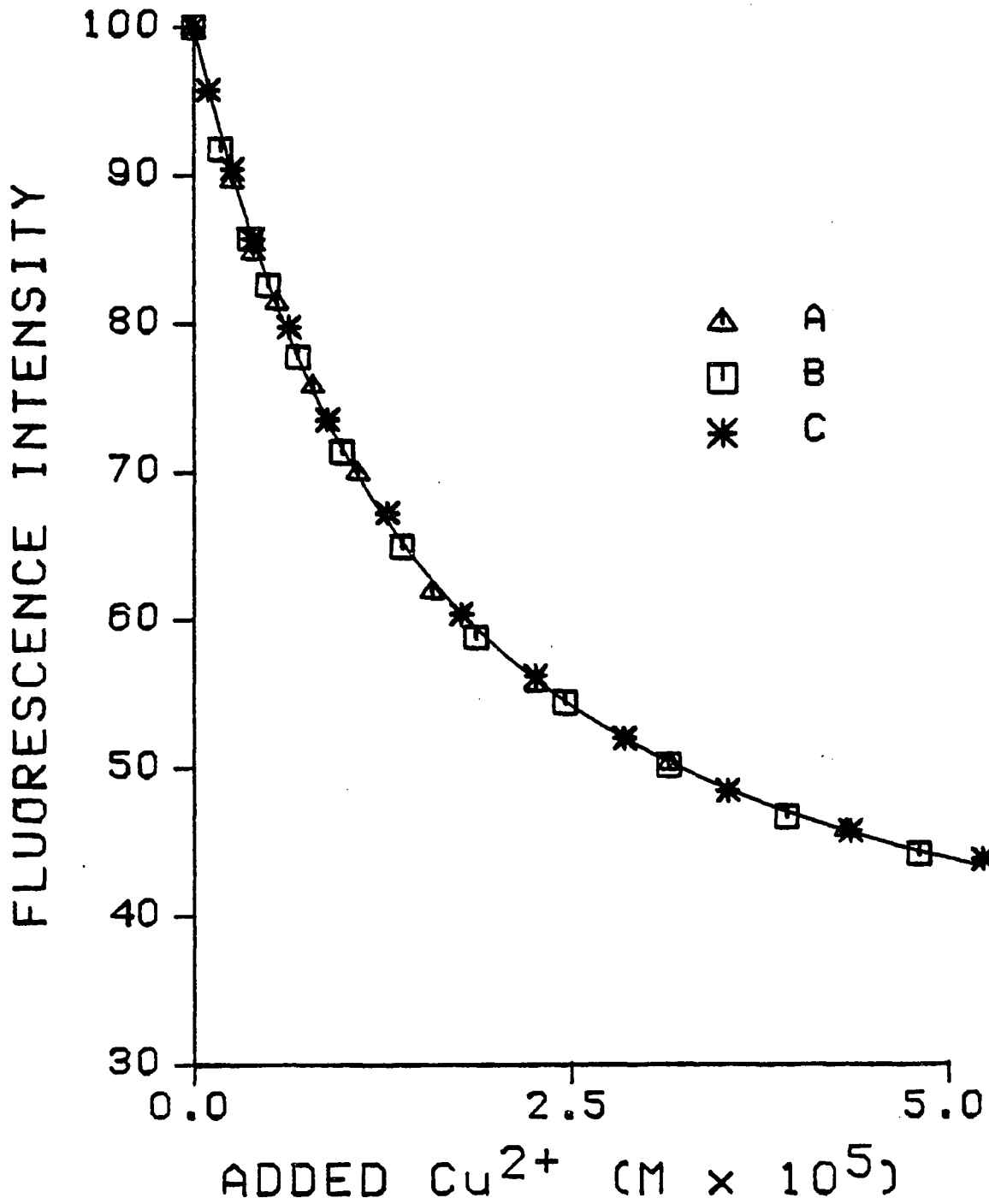


Figure 11. Triplicate fluorescence quenching curves for the Oyster River sample titrated with Cu²⁺ at pH 6.7.

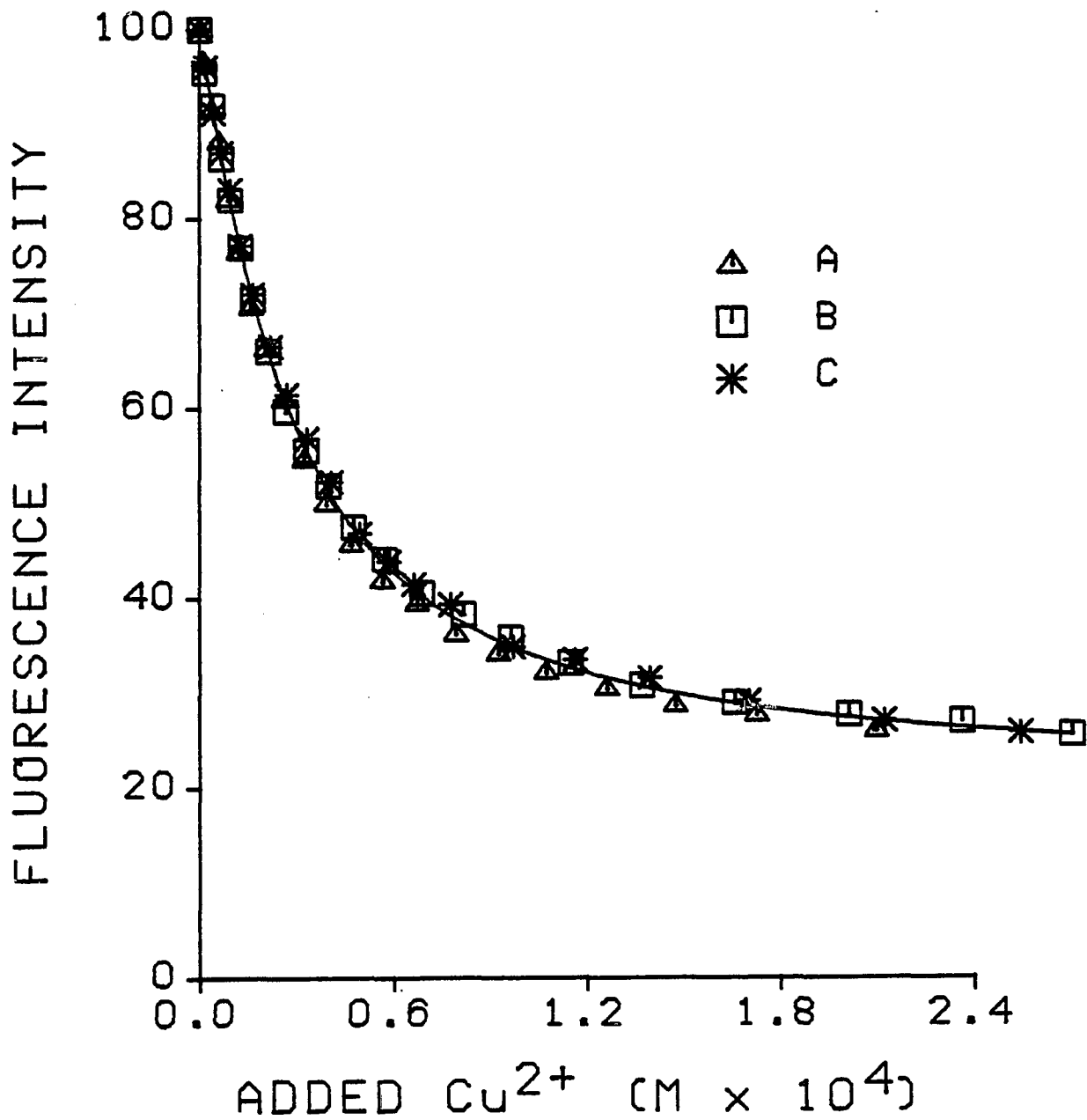


Figure 12. Triplicate fluorescence quenching curves for the Lamprey River sample titrated with Cu^{2+} at pH 6.0.

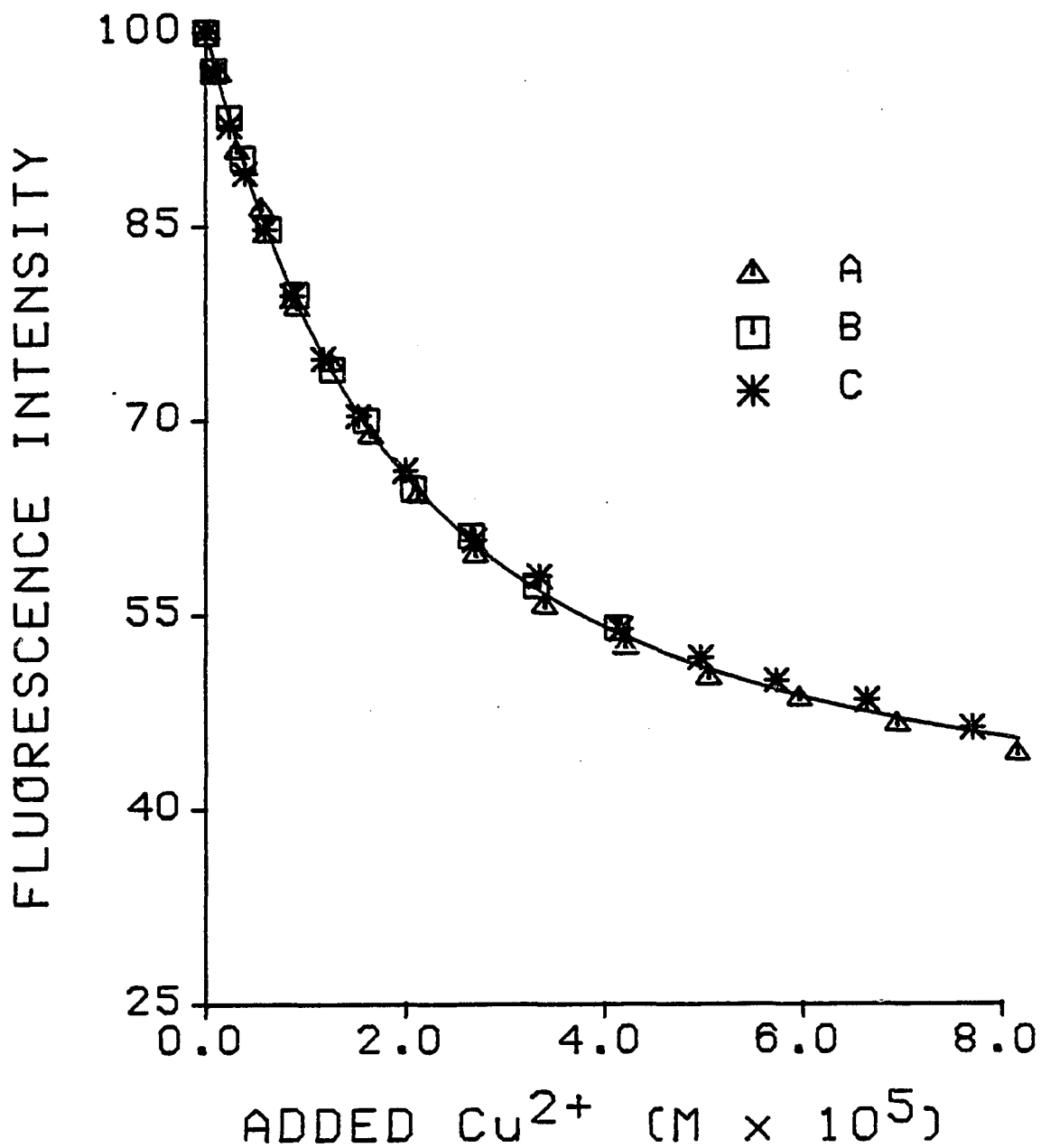


Figure 13. Triplicate fluorescence quenching curves for the Portsmouth Reservoir sample titrated with Cu^{2+} at pH 6.0.

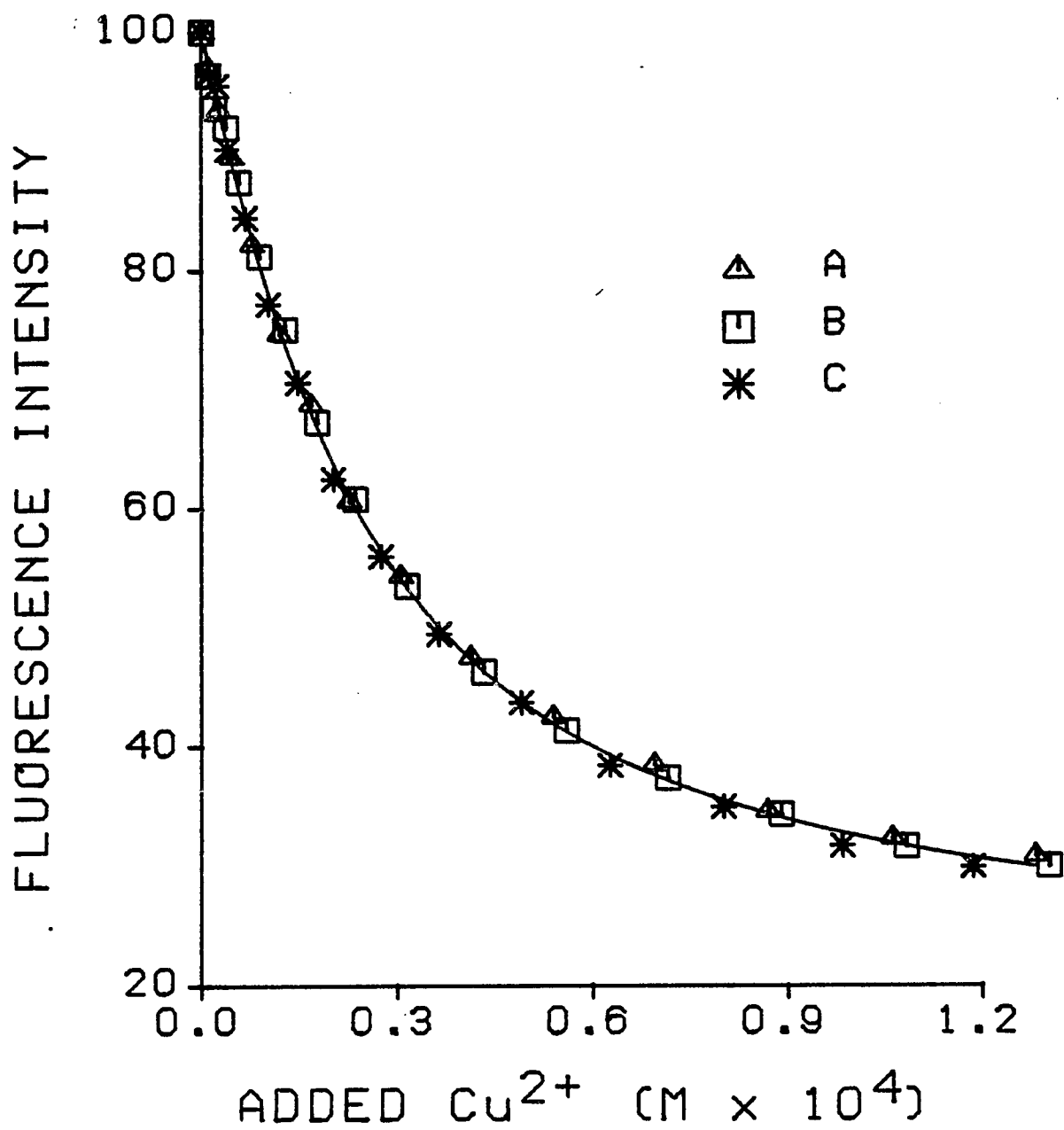


Figure 14. Triplicate fluorescence quenching curves for the Barrington Swamp sample titrated with Cu^{2+} at pH 5.8.

similar. The reproducibility of replicate curves appears to be quite good; better than titrations of SFA with Cu^{2+} (Chapter 3). The reason for this may be the superior homogeneity of the bulk water samples compared to the SFA solutions. SFA experiments were conducted on solutions made fresh each day from powdered material that was probably heterogeneous, because solids are almost impossible to homogenize.

The lines through the points in Figures 11-14 are the average best fit curves from the NONREG program using Equation 24. The titration parameters (C_L , K , and I_{ML}), averaged for the three runs, are listed in Table 12 along with the results for the other samples. The Great Bay sample did not give analyzable results as the NONREG program was unable to converge on the best fit parameters (see below).

Analysis of the data in Table 12 requires consideration of the compositions of the samples. The C_L values represent the potential complexing ability of the sample for Cu^{2+} ion (Truitt and Weber, 1980b). The samples contain appreciable amounts of metals such as iron (Table 12) which expend some of the ligands' total complexing capacity. Cu^{2+} added to these samples will complex unoccupied binding sites on the organic matter, and will probably displace weakly bound metal ions such as Zn^{2+} , Cd^{2+} , Ca^{2+} , and Mg^{2+} . More strongly binding ions such as Al^{3+} , Fe^{3+} and Pb^{2+} may

compete successfully with Cu^{2+} for sites. Near the end of the titration when Cu^{2+} is in large excess, Al^{3+} and Fe^{3+} may be the only metal ions capable of still competing.

Marine Samples. Titrations of the Great Bay sample with Cu^{2+} resulted in some significant differences from freshwater samples. In order to better characterize this sample's behavior, both emission wavelengths were monitored (398 and 430 nm) with excitation at 350 nm. Quenching occurred at both wavelengths but was considerably greater at 430 nm. Rayleigh scattering became significant very early in the titration in a region where fluorescence quenching with each successive addition of Cu^{2+} was still relatively large. For this reason we titrated the sample beyond the region where scattering was double the Cu^{2+} -free value. Figure 15 shows results for three titrations of the Great Bay sample and Figure 16 shows the scattering observed during titration B. Curves A and B in Figure 15 exhibit a marked increase in the measured signal after going through a minimum. This behavior was not expected on the basis previous results with humic material fluorescence (Sarr and Weber, 1980a; Ryan and Weber, 1982a; Chapter 3). In addition, our mathematical model (Equation 24) is invalid for this situation. Careful comparison of Figures 16 and 15 shows that Rayleigh scattering is about four times its initial value in the region of the titration where the fluorescence signal goes through a minimum and then begins to increase. We suspected that the increase in signal after

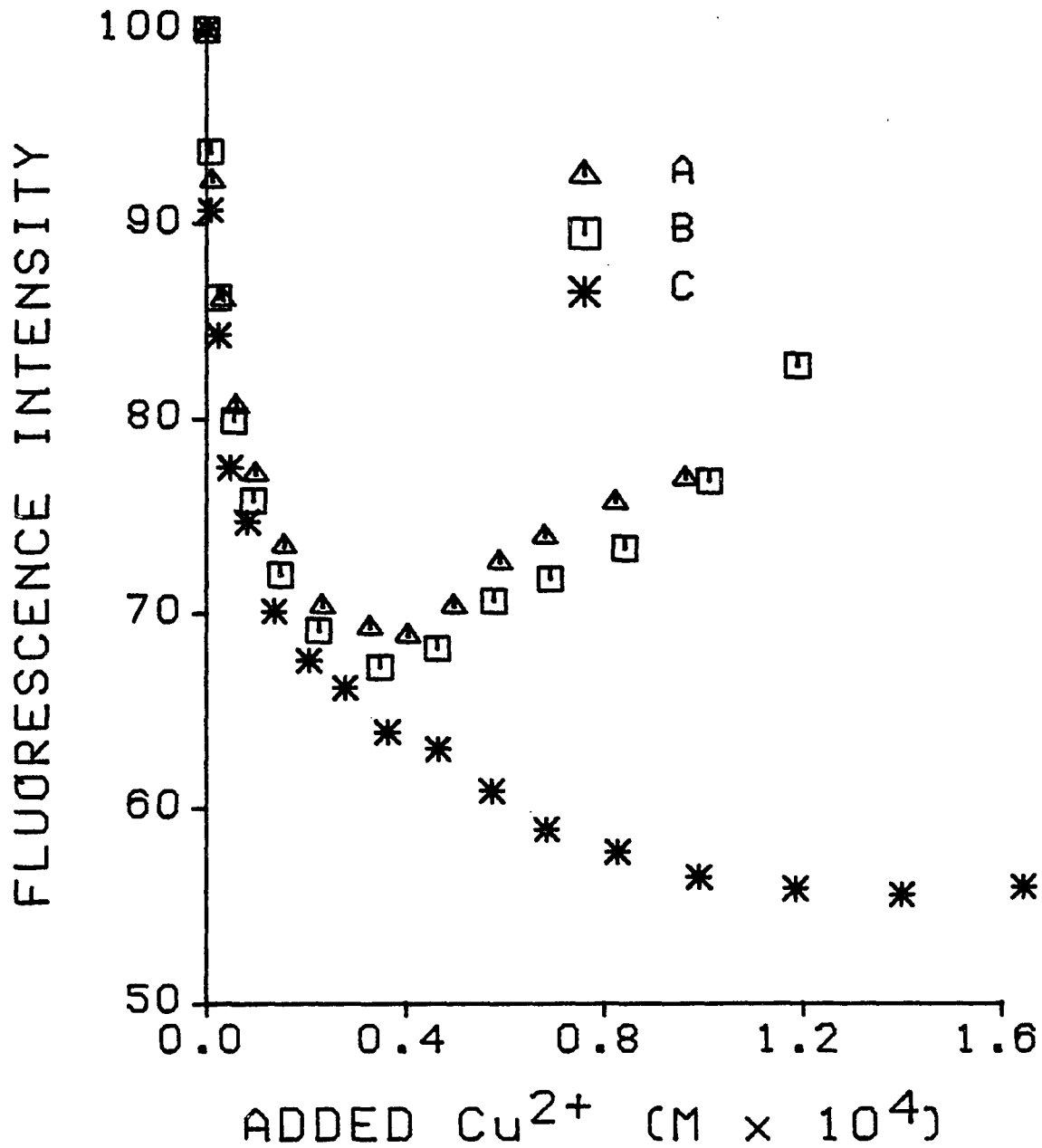


Figure 15. Triplicate fluorescence quenching curves for the Great Bay sample titrated with Cu^{2+} at pH 8.0: A, no filter, B, no filter, C, cut off filter used,

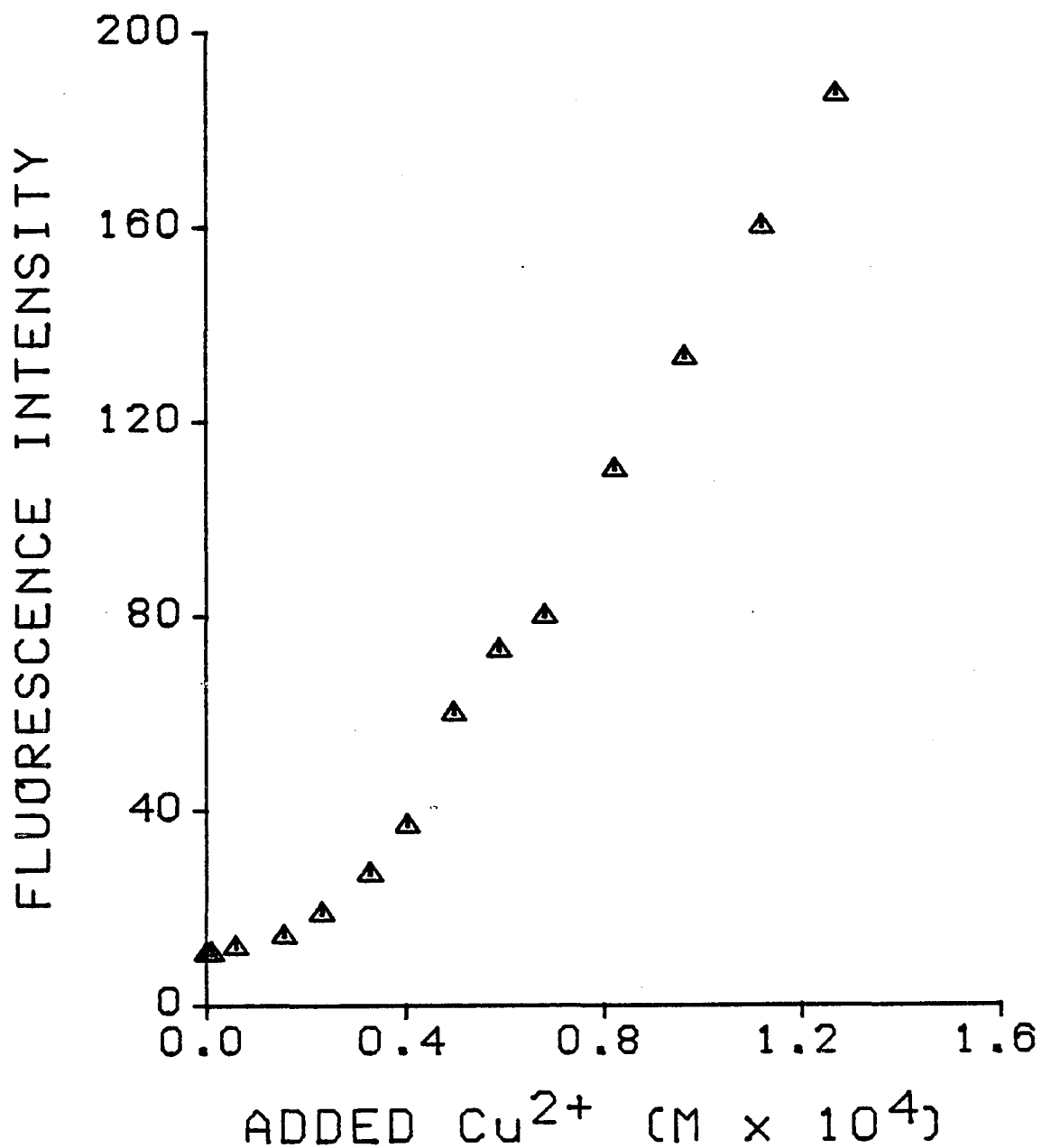


Figure 16. Rayleigh scattering at 400 nm measured simultaneously with fluorescence titration B of the Great Bay sample with Cu^{2+} (Figure 15).

3×10^{-5} M total Cu^{2+} was due to scattered radiation not rejected by the emission monochromator. To prove this and hopefully eliminate this interference, we conducted a third titration (C) using a 400 nm cut-off filter placed between the sample and the emission monochromator. The results shown in Curve C of Figure 15 demonstrate that the cut-off filter successfully blocked the scattered radiation and gave the expected curve. However, the NONREG program did not give reasonable parameters for any of the replicate titrations conducted with the filter.

There are several possible reasons for the distinctly different behavior of the Great Bay estuarine sample compared to the others. The organic matter concentration, as measured by DOC, color and fluorescence, is extremely low indicating a very small complexing capacity that may be unmeasurable by this technique. The composition of this material may also be significantly different. Its origin may be predominantly from marine organisms compared to the terrestrial origin of freshwater organic matter (Stuermer and Payne, 1976). High concentrations of Ca^{2+} and Mg^{2+} in the Great Bay sample probably compete with Cu^{2+} for the relatively few binding sites available. In addition, the high salt content and high pH aid flocculation and precipitation of humics as Cu^{2+} is added (Pagenkopf and Whitworth, 1981). This causes the observed early onset of scattering.

The pore water sample (Figure 17) had a very low C_L value, yet its organic matter concentration is the highest by far of all the samples as measured by solution color, fluorescence, and DOC. The low C_L value made titration results more difficult to obtain and gave poorer reproducibility (Table 12).

The most likely reason for its low C_L value and high organic carbon content is the presence of non-binding organic matter. It has been shown that marine sediment organic matter contains anywhere from 30-70% humic substances (Nissenbaum and Kaplan, 1972) and much of this may be associated with naturally present trace metals. Another interesting parameter for this sample is its unusually high I_{ML} value (Table 12). The I_{ML} value is the lower limit of the sample's fluorescence when all the available ligand is bound and its fluorescence quenched. This limiting fluorescence could be due to material in the sample that fluoresces but does not bind and is therefore not quenched (Chapter 3). In these samples it may also be due to fluorescing complexes of strongly-binding diamagnetic metal ions such as Pb^{2+} and Al^{3+} which do not quench the fluorescence of humic materials as effectively as paramagnetic metal ions (Saar and Weber, 1980a). The I_{ML} values for all the other samples were in the range of 20 to 35%. This is similar to the results obtained for Cu^{2+} -SFA (Chapter 3), and is probably due to the lower trace metal loading of these samples. The high I_{ML} value for the pore

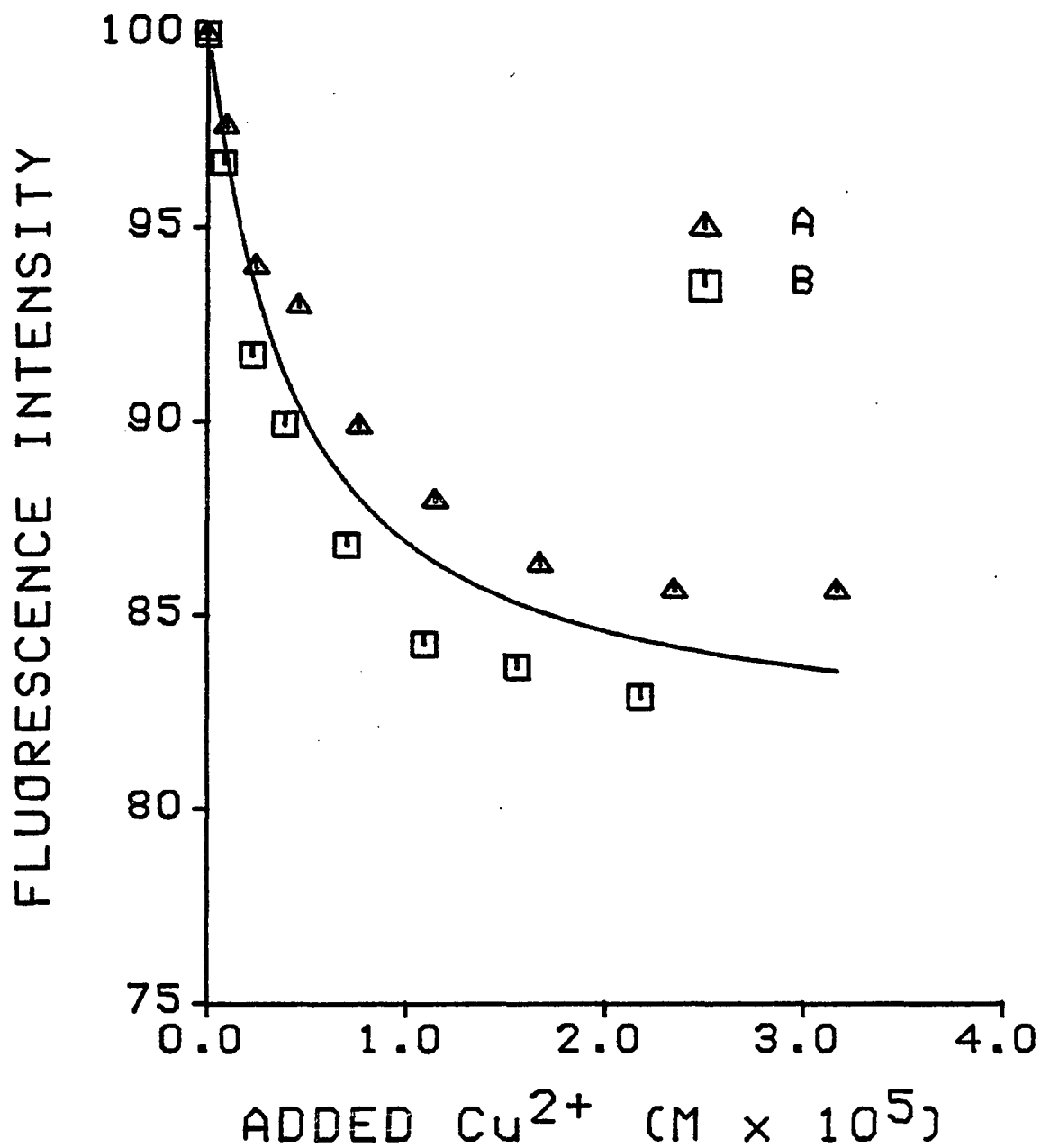


Figure 17. Duplicate fluorescence quenching curves for the titration of the pore water sample with Cu^{2+} at pH 8.0.

water also tends to magnify the results in Figure 17 by expanding the y-axis scale. This has the effect of increasing the disparity between the two curves reported.

Experiments carried out above pH 6 should probably be corrected for hydrolysis of Cu^{2+} . This is done using the "side reaction coefficient" (Ringbom and Wanninen, 1979) which is a measure of the extent of reactions other than the principal one for the species of interest. For hydrolysis

$$\alpha = 1 + [\text{OH}^-] \beta_1 + [\text{OH}^-]^2 \beta_2 \quad (25)$$

where β_1 and β_2 are the overall formation constants for the mono- and dihydroxy Cu(II) species and $[\text{OH}^-]$ is the molar concentration of hydroxide ion. For our titrations, conducted at fixed pH values, α is a constant that, when carried through the derivation of Equation 12 (Chapter 3) gives the following equation

$$\frac{[\text{CuL}]}{C_L} = \frac{\alpha K [\text{Cu}^{2+}]}{\alpha K [\text{Cu}^{2+}] + 1} \quad (26)$$

Thus when corrected for hydrolysis of Cu^{2+} Equation 17 would have an αK wherever K appears. This demonstrates that the C_L values (Table 12) obtained from the fluorescence technique are not affected by hydrolysis. Only the K values in Table 12 would be divided by α to make the correction. This was not done because significant differences exist in

literature values of β_1 and β_2 from different sources (Baes and Mesmer, 1976; Lindsay, 1979; Ringbom and Wanninen, 1979). For this reason, and the fact that the ionic strength of the samples is not known, the hydrolysis correction is only an approximation.

Correlations. Each of the measured water sample characteristics listed in Table 12 was correlated with the other characteristics in a multiple correlation study. Table 14 lists the correlation coefficients for the four freshwater samples only. The underlined coefficients are statistically significant at the 90% confidence level. Among the significant correlations are those of pH, alkalinity, hardness and conductance, which all correlate with each other. This behavior is predicted since these parameters measure the inorganic species in the samples. High alkalinity is expected to give high pH and possibly increased conductivity. The presence of significant levels of the hardness ions, Ca^{2+} and Mg^{2+} , would be expected to contribute to the conductance. The origin of these ions from carbonate minerals gives rise to their correlations with alkalinity. Other correlations that are of importance include significant correlations between color, C_L , and iron concentration ($[\text{Fe}]$). The color in these samples probably comes from two sources, organic matter and certain Fe(III) species. High organic matter concentrations presumably give rise to increased C_L values. The correlation between $[\text{Fe}]$ and C_L may indicate that much of the binding organic matter

Table 14. Correlations of natural water characteristics^a

	Hard- ness	Alka- linity	Conduc- tance	Color	Fluor- escence	DOC	[Fe]	C _L	K	I _{ML}
pH	<u>0.989</u>	<u>0.976</u>	<u>0.967</u>	-0.461	0.017	-0.041	-0.595	-0.724	0.857	0.402
Hard- ness		<u>0.986</u>	<u>0.993</u>	0.361	0.049	0.028	-0.476	0.641	0.830	0.271
Alka- linity			<u>0.973</u>	-0.457	-0.113	-0.118	-0.510	0.709	<u>0.901</u>	0.319
Conduc- tance				-0.258	0.111	0.113	-0.368	-0.552	0.778	0.158
Color					0.730	0.860	<u>0.918</u>	<u>0.946</u>	-0.778	<u>-0.930</u>
Fluor- escence						<u>0.963</u>	0.400	0.553	-0.500	0.553
DOC							0.601	0.678	-0.553	-0.695
[Fe]								<u>0.936</u>	-0.625	<u>0.975</u>
C _L									<u>-0.914</u>	-0.875
K										0.604

^a Color is UV absorbance at 250 nm; DOC is dissolved organic carbon; [Fe] is total iron concentration; C_L is complexing capacity; K is the conditioned stability product; I_{ML} is the limiting fluorescence intensity. Underlined correlation coefficients are significant at the 90% confidence level.

is associated with Fe as discussed by Giesy et al. (1978) and Florence and Batley (1980). The general notion supported by various experimental evidence is that Fe colloids that are small enough to pass through the membrane filter are stabilized by organic matter. It is contrary to the idea that Fe is occupying binding sites on the organic matter since this would reduce the potential binding ability of the sample and give smaller C_L values with increased [Fe].

Dissolved organic carbon (DOC) correlated significantly with sample fluorescence. Smart et al. (1976) found DOC measurements gave statistically significant correlations with fluorescence for natural water samples. Others (Brun and Milburn, 1977) have used this as the basis of a method to analyze humic materials. However, Ghassemi and Christman (1968) found fluorescence to DOC ratios were not constant. Neither fluorescence nor DOC showed significant trends with color or C_L value. These results are similar to those of Truitt and Weber (1981b) for DOC, color and Cu^{2+} - C_L values obtained from dialysis titrations of natural waters. The C_L values represent the potential binding ability of the sample but its correlation with parameters that measure organic matter content can be affected by many factors. The most important of these are probably trace metal loading of the sample (Truitt and Weber, 1981b), the presence of inorganic ligands, and the variable amount of complexing organic matter making up the total organic composition of samples

from different sources (Langford et al., 1979). The non-significant correlation between DOC and color that we observed is contrary to a previous report (Reid et al., 1980) using the same analytical methodology for measuring color. This may be due to limited sampling. However, Stewart and Wetzel (1981) concluded that DOC, absorbance and fluorescence were not linearly related in either isolated organic matter or the natural material found in fifty-five lakes. One reason for the absence of a significant correlation between the two optical parameters in both their study (Stewart and Wetzel, 1981) and ours is that color was measured at 250 nm while fluorescence was excited at 350 nm. Greater sensitivity is achieved under these conditions but the data would probably agree better if 350 nm were used for both. The lack of consistent results in the literature demonstrating relationships between DOC, fluorescence, color and C_L values (Truitt and Weber, 1981b; Shuman and Woodward, 1977) indicates that they do not measure the same properties. Therefore, simple generalizations are impossible.

Certain correlations were unexplainable on the basis of present knowledge of the natural water systems under study. In these cases statistical significance may be coincidental due to the limited number of samples used. These include the correlation of alkalinity and K , and the negative correlation of color and I_{ML} . Since all the samples were thoroughly purged with N_2 before and during fluorescence

titrations and acid added to keep them at their original pH, it is difficult to explain relationships between any titration parameters and alkalinity. The complexing capacity titrations should indicate the behavior of the organic matter alone in the freshwater samples. The results for the pore water sample may be influenced significantly by borate ion, silicates and other inorganic species.

The negative correlation of K and C_L cannot be explained chemically, but may be an artifact of the data treatment. From Equation 17 it is obvious that if K gets smaller C_L increases and vice versa for a given set of data. This interrelationship may give rise to the correlation. The data treatment in this paper is a rather simple one from a theoretical standpoint and has several limitations when applied to this complex system. It is proposed here as a first attempt to describe fluorescence data.

The same correlations discussed above and listed in Table 14 were made for all the data including the two marine samples. This gave four degrees of freedom (six cases) for most parameters but only three degrees of freedom for some, due to incomplete data for the Great Bay sample. Generally the same parameters were significant at the 90% confidence level with a few additions. The additional significant correlations were felt to be somewhat artificial due to the distinct differences between freshwater and marine samples. For example, the relatively high alkalinities of the marine

samples and the high K value for the pore water caused these two parameters to correlate with nearly every other property. This effect is not considered to have any chemical significance and was therefore ignored.

CONCLUSIONS

The results described here demonstrate that the fluorescence quenching technique allows determination of Cu^{2+} complexing capacities of natural water samples at the micromolar and submicromolar level. The work described in Chapter 3 indicates that these values are consistent with those measured by other techniques. The water samples offer a significantly greater challenge than laboratory prepared solutions of isolated SFA. The natural samples are more complex with respect to their inorganic constituents, and trace metals block and/or compete for binding sites. The natural waters had lower C_L values than our 10 mg/L SFA, however, the fluorescence method is extremely sensitive and can be applied even into the submicromolar C_L range.

The experiments described here were done under N_2 in order to make pH control easier. This, however, is not a requirement. Neither CO_2 nor O_2 interfere with the fluorescence (Saar and Weber, 1980a). It is not necessary to alter the sample in any way, as in adding an electrolyte for example. Filtering is not critical if proper means are used to reject scattered radiation.

CHAPTER 5

COMPARISON OF Mn^{2+} , Co^{2+} and Cu^{2+} BINDING TO FULVIC ACID AS MEASURED BY FLUORESCENCE QUENCHING

INTRODUCTION

Among the environmentally important metal ions there are several, besides Cu^{2+} , that are paramagnetic and thus potential candidates for SFA fluorescence binding studies. These include Co^{2+} , Mn^{2+} , Fe^{2+} , Fe^{3+} and Ni^{2+} . Heavy metal ions can also cause quenching as demonstrated for Pb^{2+} (Saar and Weber, 1980a). In this chapter SFA fluorescence quenching by Co^{2+} , Mn^{2+} and Cu^{2+} is analyzed. The natural fluorescence of soil-derived fulvic acid (SFA) is used as a probe to compare the complexation of Mn^{2+} , Co^{2+} , and Cu^{2+} and demonstrate the effect of pH. Much of the data reported here was collected by Carl P. Thompson (Ryan, Thompson and Weber, 1983). It is used to demonstrate the applicability of the data treatment described to these metal ions. Most experiments were repeated to give additional data points and for verification. Other experiments were performed to elucidate the mechanism of Cu^{2+} -SFA precipitate formation.

The mobility of Co^{2+} has been studied in both freshwater and marine environments by Mahara and Kudo (1981). Using radiotracer experiments they found that sediments would be the predominant sink for Co^{2+} in freshwater systems between pH 4.5 and 8. However, in marine environments the Co^{2+} was

considerably more mobile. In excess of 80% of the Co^{2+} remained in the water column for an extended period of time. Both pH and the presence or absence of O_2 were important variables. The study did not specifically address the role of humic materials in explaining the observed behavior. It is probable that humic materials in sediments are important in determining the form and thus the mobility of cobalt.

This work and other studies on Co^{2+} in the environment are extremely important because cobalt-60 is a typical radionuclide produced by nuclear power plants and disposed of at low levels in coastal waters (Mahara and Kudo, 1981). In addition, cobalt in vitamin B-12 is essential for life giving a possible pathway for cobalt-60 to be assimilated by living organisms.

Until recently very little work has been done describing the effect of metal complexation on the fluorescence of humic materials. The quenching ability of Cu^{2+} and Fe^{2+} on soil humic and fulvic acids was found to be greater than that of Co^{2+} and Ni^{2+} by Banerjee and Mukherjee (1972). The work of Cline and Holland (1977) on Cu^{2+} and Co^{2+} with pore water was probably the most extensive up to the very recent study by Saar and Weber (1980a). Mn^{2+} has all but been ignored.

One area that is severely lacking is the development of quantitative models to describe the fluorescence quenching behavior of various metals on humic materials. The literature abounds with methods for describing complexation in terms of

the various forms of the metal ion measured (Sposito, 1981; Perdue, 1982; MacCarthy and Smith, 1978; 1979; Hunston, 1975). However, similar results can be derived from fluorescence quenching when it is related to the fraction of this natural ligand that is bound (Ryan and Weber, 1982a). This type of information is completely analogous to Scatchard's (1949) $\bar{\nu}$ ($[\text{bound metal ion}]/([\text{total ligand}])$) which is usually calculated from free and total metal ion concentrations (Saar and Weber, 1980a; 1980b; 1980c). The results reported here have implications towards the nature and strength of binding of Mn^{2+} , Co^{2+} and Cu^{2+} to humic matter in natural waters. In addition, Rayleigh scattering experiments conducted simultaneously with fluorescence give valuable solubility information about SFA-metal ion complexes. Scattering polarization measurements are used to determine if aggregation of precipitate particles is significant.

EXPERIMENTAL

The apparatus and procedures used for fluorescence quenching titrations have been described in detail in Chapter 3 and 4 and elsewhere (Ryan and Weber, 1982a; 1982b). All solutions used in this study were 10 mg/L SFA in 0.1 M KNO_3 and filtered through 0.4 μm filters. In all experiments the SFA solutions were titrated at constant pH with 1000 mg/L Mn^{2+} , Co^{2+} or Cu^{2+} atomic absorption standards (Fischer, 50-M-81, 50-c-193, and 50-C-194). The fluorescence excitation wavelength was 350 nm with emission measured at 445 nm.

Rayleigh scattering measurements were made with both excitation and emission monochromators set at 500 nm. Scattering polarization experiments were done at 400 nm with a SLM Instruments model 8000/8000S spectrofluorometer with data acquisition system. No wavelength filters were used between the sample and detector.

RESULTS AND DISCUSSION

Binding of a paramagnetic metal ion to SFA causes its fluorescence to be quenched (Ryan and Weber, 1982a; Saar and Weber, 1980a). The degree of quenching is related to two factors; the quenching ability of the metal ion and the extent of complexation. Figure 18 shows the decrease in fluorescence intensity of 10 mg/L SFA in 0.1 M KNO_3 as Mn^{2+} is added at pH 5, 6, 7 and 8. At least duplicate titrations were conducted for each pH and the reproducibility is fairly good. A trend of increased fluorescence quenching with increasing pH is clearly evident. This same trend is demonstrated for Co^{2+} in Figure 19. These results are directly comparable to the Cu^{2+} results in Chapter 3 (Ryan and Weber, 1982a) done under identical conditions and analogous to initial studies with these systems (Saar and Weber, 1980a). This data can be explained by noting that as pH increases conditional stability constants for weak acid ligands increase (Ringbom and Wanninen, 1979). At higher pH there is less competition from protons for potential metal ion binding sites. For a fixed ratio of total metal ion concentration (C_M) to total ligand

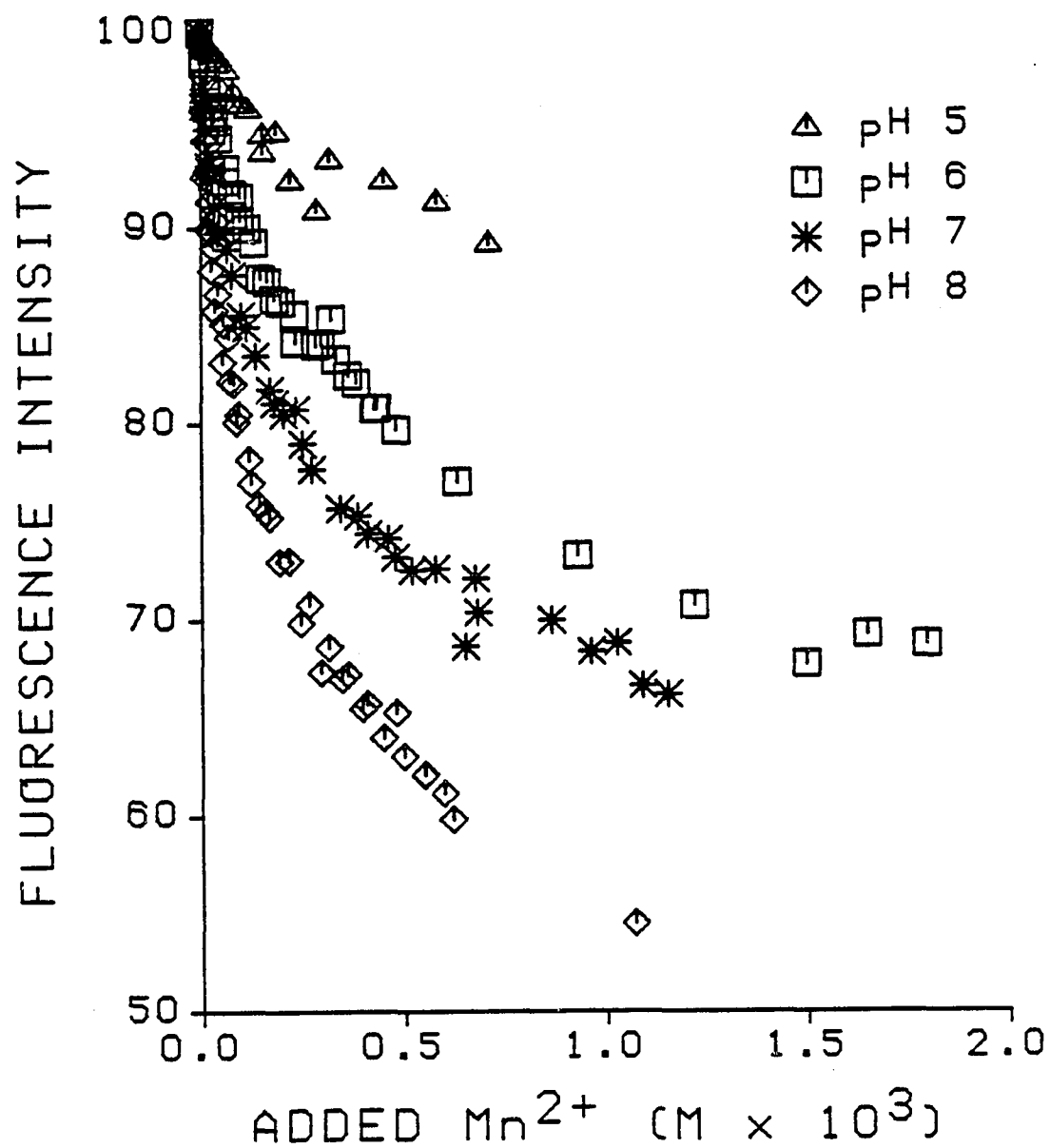


Figure 18. fluorescence quenching curves for 10 mg/L soil fulvic acid (SFA) with Mn^{2+} in 0.1 M KNO_3 at four pH values. Duplicate titrations are shown for pH 5 and 8, triplicates for pH 6 and 7.

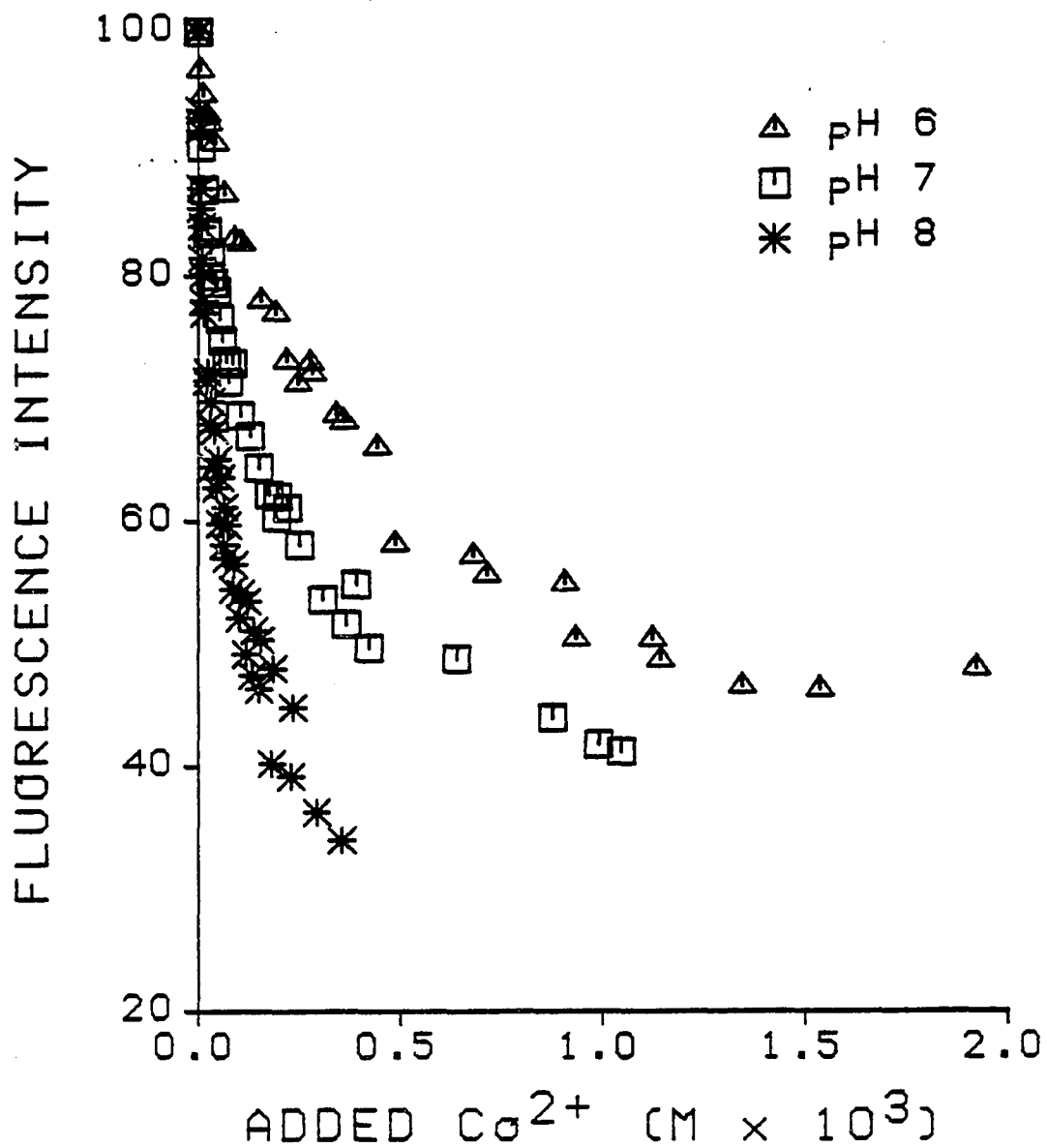


Figure 19. Fluorescence quenching curves for Co^{2+} under the same conditions as in Figure 18. Duplicate titrations are shown for pH 8, triplicates for pH 6 and 7.

concentration (C_L) the fraction of the ligand complexed will be greater at higher pH. The quenching ability of a given metal ion is assumed to be constant as pH is varied and the overall quenching effect is related to the extent of complexation. Changes in SFA fluorescence with pH in the absence of metal ion have been shown to be small in the pH range of these experiments (Saar and Weber, 1980a). Therefore the trends observed in Figures 18 and 19 show the effect of pH on the conditional stability constant.

For convenience in making comparisons the initial fluorescence intensity in the absence of metal ion (I_L) is set to 100%. The data was not corrected for hydrolysis of the metal ions. The general effect of hydrolysis, as discussed in detail in Chapter 4, is to reduce the amount of metal ion available to complex with SFA and quench its fluorescence. The fraction of metal ion hydrolysed is constant at constant pH and is therefore easily accounted for.

The relationship between the measured fluorescence intensity and complexation is described mathematically by Equation 11 (Chapter 3). The right side of this equation describes fluorescence quenching as the ratio of the decrease in the initial fluorescence intensity observed at any point in the titration to the difference between the maximum and minimum values. It should be noted here that $[ML]/C_L$ is the fraction of ligand bound to the metal ion regardless of the complex stoichiometry.

Equation 11 allows construction of binding curves (total added metal ion vs. fraction of SFA bound) from fluorescence titration data. Figures 20 (pH 6) and 21 (pH 7) show comparisons of this type for Mn^{2+} , Co^{2+} and Cu^{2+} . Triplicate curves for each metal ion are given at both pH values. Cu^{2+} binds very strongly at both pH values, and is at or near complete saturation of the ligand at about $1 \times 10^{-4} \text{ M}$ Cu^{2+} added. The curves for Mn^{2+} and Co^{2+} are quite similar to each other, but show much weaker binding than Cu^{2+} . In both Figures 20 and 21 the curves for Mn^{2+} fall just below that for Co^{2+} indicating that the conditional stability constant (K) for Mn^{2+} -SFA is slightly lower. Curve fitting analysis described previously (Chapter 3 and 4) substantiates this to some extent. Pooled replicate experiments at pH 6 gave K values of 4.2×10^3 for Mn^{2+} and 5.1×10^3 for Co^{2+} . The C_L values were $1.5 \times 10^{-6} \text{ M}$ for both. These compare with a K of 1.1×10^5 and C_L of $2.0 \times 10^{-5} \text{ M}$ for Cu^{2+} at pH 6 (Chapter 3). Experiments with Cu^{2+} and Mn^{2+} at pH 7 did not converge on meaningful results for the constants. The fitted C_L values were negative. The most likely reason for this is that the pH 7 experiments were not carried far enough for curve fitting to be effective. From Figure 21 it is evident that the Mn^{2+} and Co^{2+} curves do not quite level off at the end as they did for pH 6 (Figure 20).

Various K values for complexation of these metal ions with different organic ligands also indicate that Cu^{2+} binds much more strongly than Co^{2+} and Mn^{2+} whose binding strengths

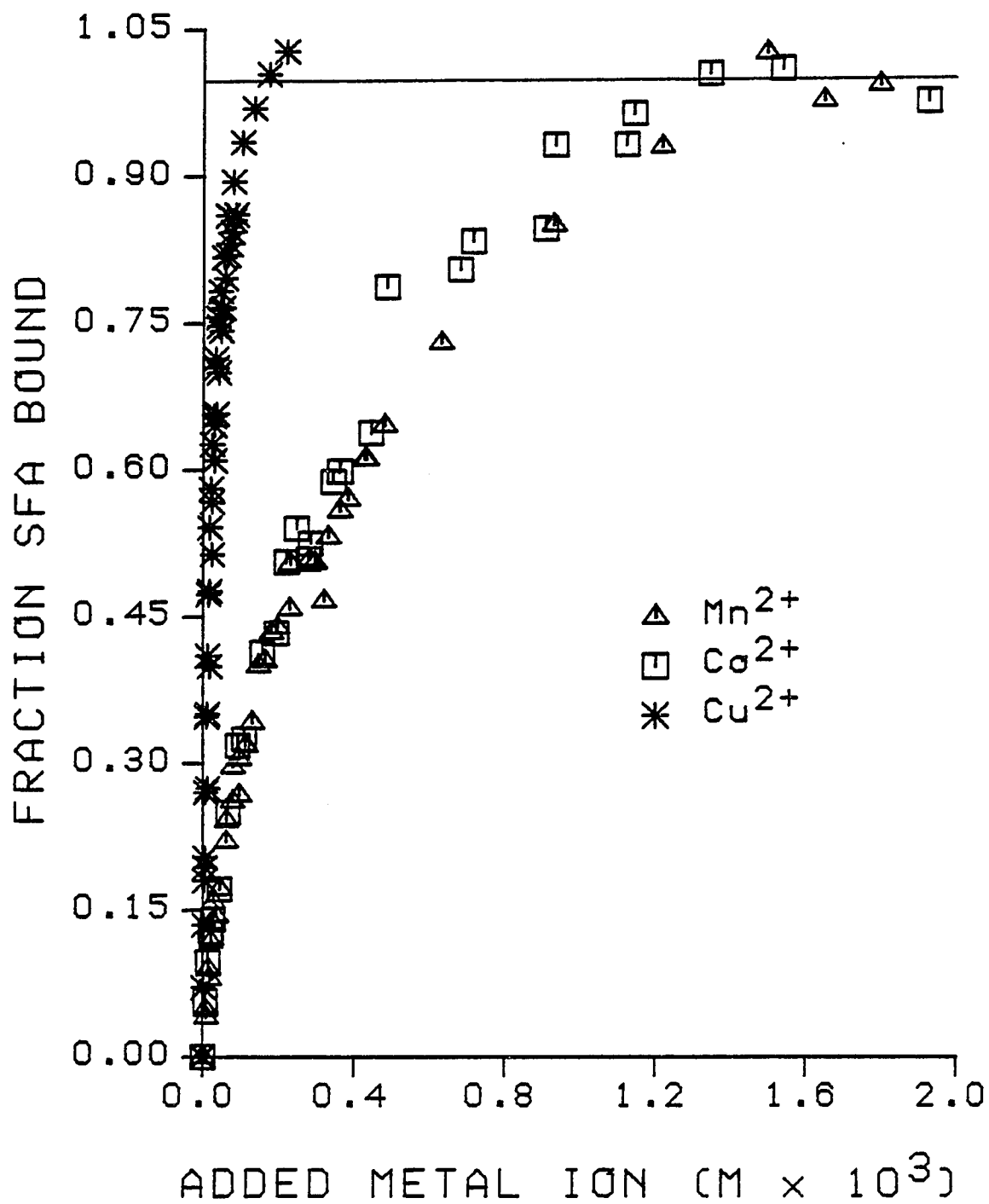


Figure 20. Binding curves of total added metal ion concentration vs. fraction of soil fulvic acid bound $[ML]/C_L$ for Mn^{2+} , Co^{2+} and Cu^{2+} at pH 6. Conditions same as Figures 18 and 19. Line is theoretical maximum.

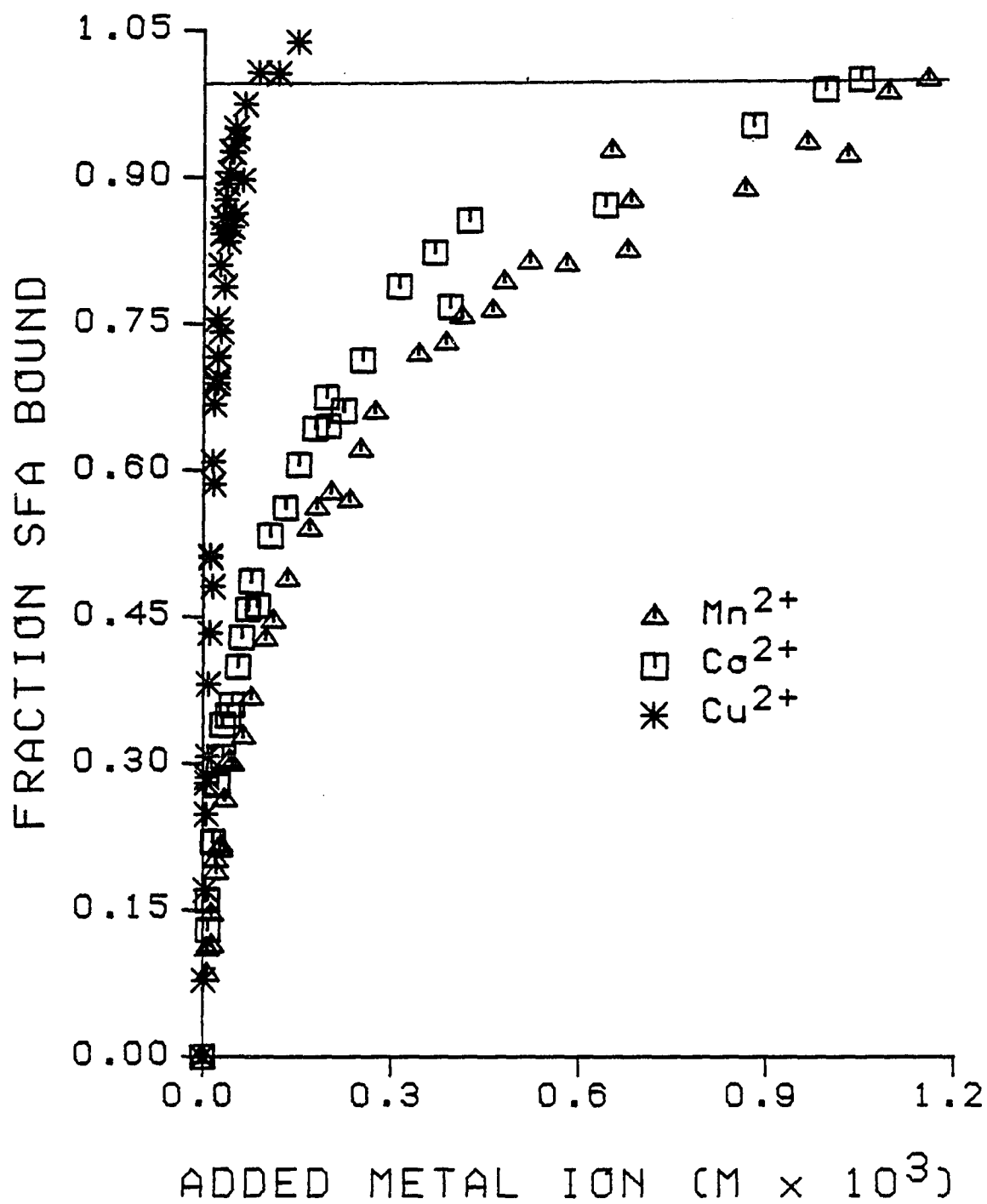


Figure 21. Binding curve for pH 7, all other conditions the same as Figure 20.

are similar. For example salicylic acid forms complexes of these metal ions with log K values of 5.9 for Mn^{2+} , 6.7 for Co^{2+} and 10.6 for Cu^{2+} at 25 °C and 0.1 M ionic strength (Martell and Smith, 1977). This trend is predominantly rationalized on the basis of "effective nuclear charge" (Huheey, 1978) however, Co^{2+} should have a slight advantage in binding strength over Mn^{2+} due to ligand field stabilization energy upon complex formation. The binding of these metal ions to fulvic acid has been demonstrated to follow the relationships expected from inorganic considerations. The large amount of literature on Cu^{2+} -fulvic acid complexation demonstrates that Cu^{2+} is among the strongest binding of the divalent cations (Mantoura et al., 1978; Holtzclaw et al., 1978). Co^{2+} and Mn^{2+} are generally considered to be much weaker than Cu^{2+} and often indistinguishable in binding strength.

Langford and coworkers (1978) found a difference in the type of binding of Mn^{2+} and Cu^{2+} to fulvic acid using paramagnetic relaxation NMR in water. Their results indicate that Mn^{2+} binds to SFA with its hydration shell intact. This outer sphere type of binding is opposite to the behavior of Cu^{2+} which loses its associated water molecules and shows inner sphere behavior. Although their work was done at pH 2 and 4 the interpretation can probably be extended to the higher pH values used in this study due to the very low hydrolysis constant of $10^{-10.5}$ for Mn^{2+} (Baes and Mesmer, 1976). The outer sphere binding of Mn^{2+} indicates that it is

not bound as tightly or strongly to SFA as Cu^{2+} , and the attraction may be primarily electrostatic in nature.

The strength of metal ion-SFA binding can also affect the shapes of the relatively large molecules of SFA. Strong inner sphere complexation may cause conformational changes making available binding sites that the weaker outer sphere complexation of Mn^{2+} can not induce. In the absence of metal ions the shapes of SFA molecules are probably mainly controlled by hydrogen bonding and electrostatic forces. In the pH range of these studies (5-8) the carboxyl groups of SFA are predominantly ionized and phenolic groups are partially or incompletely ionized. The repulsion between ionized carboxyls and possible hydrogen bonding between ionized carboxyls and unionized phenolic groups probably controls the shape of SFA molecules. A flat molecule may be folded over and held in a particular configuration in this way.

The same functional groups that determine conformation are also involved in binding metal ions. An ion such as Mn^{2+} that binds weakly is not effective at competing with protons for weakly acidic phenolic sites. It can therefore, only bind to certain carboxyls. In addition many sites may be occluded in a microenvironment internal to a folded molecule of SFA. The strong binding of Cu^{2+} , however, can probably break up the majority of the existing hydrogen bonds by forming a very stable coordination complex with several sites. Bresnahan et al., (1978) previously observed a large increase in Cu^{2+} -SFA

binding between pH 5 and 6. The Cu^{2+} binding may alter the shape of the molecule and give it a new configuration. This new conformation is controlled by the Cu^{2+} ion and could consist of the molecule being fully or partially wrapped around one or more metal ion center. This type of conformational change may expose sites that were internal to the molecule, thus making them available for binding to additional Cu^{2+} ions. Mn^{2+} cannot induce the conformational changes so the internal sites remain unavailable giving lower binding capacities for this metal ion. All of the same arguments described here for Mn^{2+} may apply equally well for Co^{2+} .

The discussion of the type of binding of Mn^{2+} and Cu^{2+} to SFA has direct implications to their fluorescence quenching ability. At least three possible quenching mechanisms may be operable in the systems under study. For paramagnetic ions quenching is presumed to be due to an increased rate of internal conversion caused by perturbation of the molecular orbitals of the ligand and formation of new energy levels with d electrons of the metal ion (Parker, 1968). There is very little quantitative or predictive theory describing the effect of such processes on the fluorescence, but it is not likely that different metal ions will have the same effect. For example Co^{2+} with three unpaired electron will probably not behave the same as Mn^{2+} with five unpaired electrons.

Two other possible quenching mechanisms are the heavy atom effect and Forster long range quenching. Even though first row transition elements are not strictly heavy atoms their incorporation in a complex can increase the rate of intersystem crossing and reduce the ligands fluorescence at the expense of phosphorescence (Parker, 1968). Finally, if the binding site in a molecule and its fluorophore are separated by a distance of 30-80 Å quenching may be due to Forster energy transfer (Forster, 1965). This process relies on the spectral overlap integrals of a donor's fluorescence and acceptor's absorbance as well as the fluorescence quantum efficiency. In the absence of conclusive structural and spectral information concerning the binding sites and fluorophores of fulvic acid it is impossible to determine the exact mechanism of quenching. In addition the combined effect of the three quenching mechanisms described cannot be separated into its component parts. However, any of these processes will probably contribute to the quenching to a greater or lesser extent depending on the distance of the metal center from the binding site. Mn^{2+} is insulated from the binding site by its hydration sphere thus it is probably farther from the fluorophore and may not perturb the orbitals of SFA to such a large degree. Therefore its quenching ability as well as its binding ability may be impaired. Co^{2+} may also behave in the same manner as Mn^{2+} as evidenced by similar binding ability.

A direct comparison of the relative fluorescence quenching effects of Mn^{2+} , Co^{2+} , and Cu^{2+} on SFA can be made using I_{ML} values. Table 15 lists the experimentally determined values for Co^{2+} and Mn^{2+} from this work and those determined from curve fitting for Cu^{2+} (Chapter 3). These values represent the limit of quenching ability since they are the residual fluorescence, reported as percent of total, when $[\text{ML}]/C_L$ (Equation 11) is unity. Some generalizations can be made from this data. Copper ion quenches to the greatest extent. Cu^{2+} binds to more sites than either Co^{2+} or Mn^{2+} and this is the reason for its greater quenching ability. However, these other ions are not capable of binding to the extent that Cu^{2+} does and therefore cannot quench as much. At pH 6 and 7 Cu^{2+} quenches nearly 80% of SFA fluorescence while Co^{2+} is less than half as effective. Mn^{2+} is the poorest quencher by far. It is also evident from Table 15 that I_{ML} values decrease with increasing pH. The additional value for Cu^{2+} at pH 5 also supports this. The reason for the observed trend is probably related to the variation of SFA fluorescence with pH (Saar and Weber, 1980a). It must be noted that the values in Table 15 are reported as percentage of the maximum value at the particular pH. All fluorescence intensities in the absence of metal ion are normalized to 100% as discussed above.

Another marked difference was noted for Co^{2+} and Mn^{2+} compared with Cu^{2+} in titrations of SFA. Previous studies have shown that Cu^{2+} causes an increase in solution scattering

Table 15. Limiting Fluorescence Intensity (I_{ML}) at various pH values^a.

<u>Metal ion</u>	<u>pH 5</u>	<u>pH 6</u>	<u>pH 7</u>
Mn ²⁺	-	68.6	66.2
Co ²⁺	-	46.9	41.2
Cu ²⁺ ^b	40.2 ^c	22.4	20.7

^a Expressed as percent of initial fluorescence intensity at indicated pH.

^b Determined by curve fitting.

^c Less reliable value (see Chapter 3).

and eventually effects the precipitation of fulvic acid (Saar and Weber, 1980c; Chapter 3; Underdown et al., 1981) and natural water organic matter (Cline and Holland, 1977; Ryan and Weber, 1982b) while Co^{2+} and Mn^{2+} do not. In these titrations the added Co^{2+} and Mn^{2+} was about five to ten times as much as Cu^{2+} titrations. No scattering increase was observed for Mn^{2+} and a relatively small increase was noted for Co^{2+} compared to Cu^{2+} . Similar results were obtained by Sholkovitz and Copeland (1981) for the coagulation of river organic matter by Cu^{2+} , Co^{2+} and Mn^{2+} . They found Co^{2+} gave no measurable precipitation and Mn^{2+} very little while Cu^{2+} and Fe^{3+} were extremely effective. Their conclusion is that cation-induced coagulation is less for Mn^{2+} and Co^{2+} because of their weaker association to humic materials. In this study since Co^{2+} and Mn^{2+} did not cause a great deal of scattering it was possible to conduct the titrations well into the region where fluorescence leveled off. This allowed direct experimental determination of I_{ML} values which was not possible for Cu^{2+} (Chapter 3).

In Chapter 3 it was proposed that Cu^{2+} induces aggregation of SFA as well as precipitation. Figure 22 curve A is a Rayleigh scattering titration curve for 10 ppm SFA in 0.1 M KNO_3 with Cu^{2+} at pH 7. As Cu^{2+} is added, the number of precipitate particles formed increases and scattering goes up. Of particular interest in Figure 22 is the region of curve A above 1.5×10^{-4} M added Cu^{2+} where the slope decreases defining a break in the curve. Previous work using

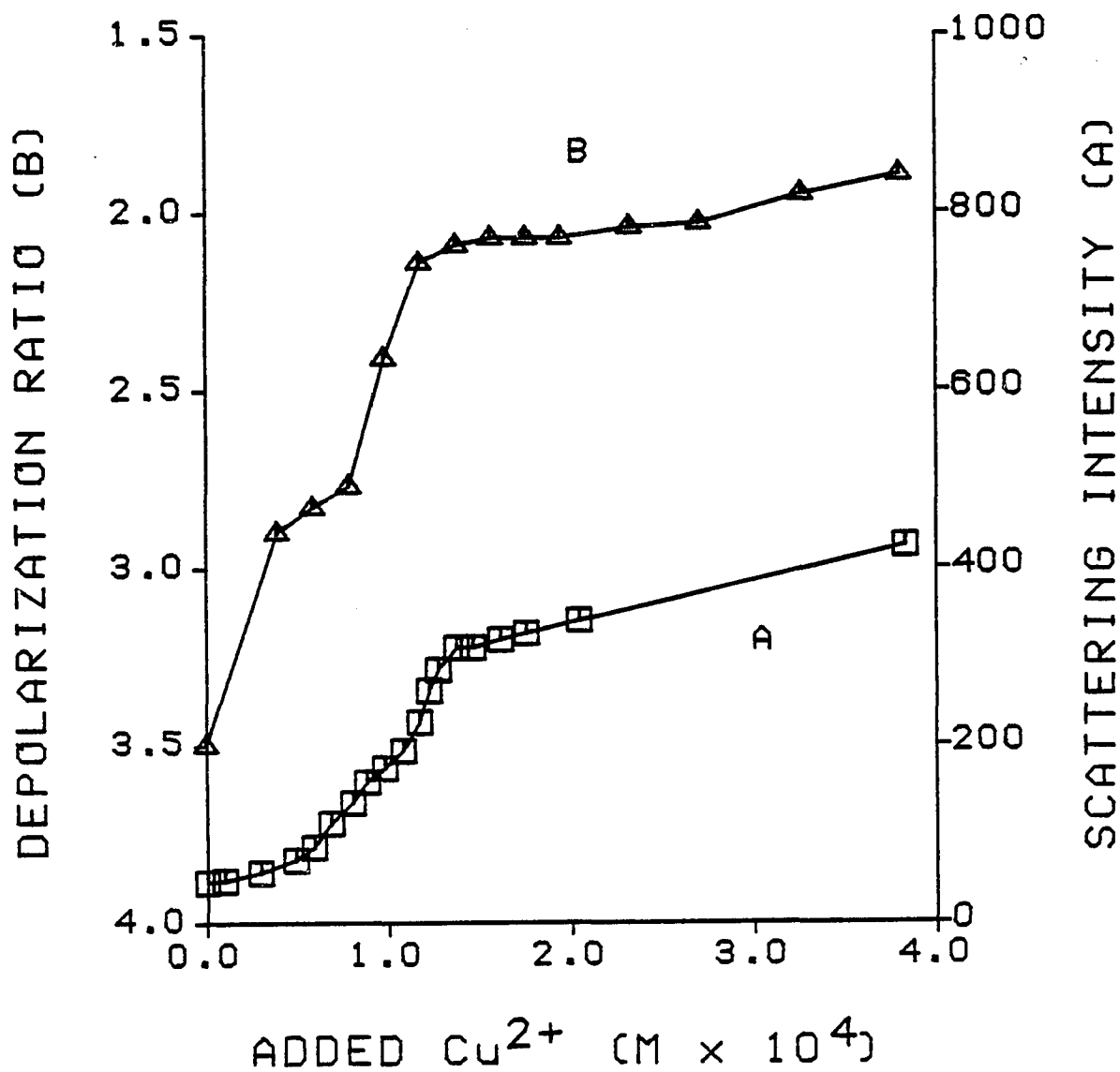


Figure 22. Total added Cu^{2+} concentration vs. Rayleigh scattering intensity (arbitrary units) measured at 500 nm (curve A, right axis) and depolarization ratio (I_{\perp}/I_{\parallel}) measured at 400 nm (curve B, left axis) for 10 mg/L SFA in 0.1 M KNO_3 .

centrifugation as well as scattering showed that precipitation did not cease in this region possibly indicating that aggregation was taking place (Chapter 3). The formation of large aggregates may explain why precipitation occurs with a simultaneous loss in sensitivity of scattering. The larger aggregates are less efficient at scattering light of a given wavelength than smaller particles (Olsen, 1975). Thus, once aggregation becomes significant above $1.5 \times 10^{-4} \text{ M Cu}^{2+}$ the rate at which scattering increases is less. To support this a scattering polarization experiment was conducted. Using a plane polarized source, the intensity of Rayleigh scattering for both parallel (I_{\parallel}) and perpendicular (I_{\perp}) components was measured. The scattering polarization of a Cu^{2+} -SFA solution also changes as Cu^{2+} is added and binds to SFA. From Figure 22 curve B it is evident that the depolarization ratio ($p = I_{\perp} / I_{\parallel}$) decreases rapidly at first and then seems to level off, decreasing slowly after that. This behavior is inversely related to the Rayleigh scattering in curve A.

The decrease in the depolarization ratio indicates that the scattering particles are becoming more isotropic (Gutfreund, 1974). That is to say the initial Cu^{2+} -SFA particles formed are probably somewhat anisotropic (i.e. pancake shaped) (Lapen and Seitz, 1982). They may become more isotropic due to aggregate formation. The aggregates may more closely approach a spherical shape giving rise to isotropic scattering and lower depolarization ratios. Although the evidence presented does not conclusively prove the proposed

theory of aggregate formation, it is presented as a

possible explanation. This phenomenon also explains why the Rayleigh scattering curve undergoes a "break" or drastic change in slope at the same total Cu^{2+} concentrations as the depolarization curve. Scattering of unpolarized light measured at 90° may have some extra components of polarization due to anisotropy (Gutfreund, 1974). As aggregates form and the scattering particles become more isotropic, the added component due to anisotropic scattering becomes less. Thus the scattering does not increase as rapidly once the polarization has leveled off. The effect of anisotropy on scattering can be written

$$I_s = I_R + I_a$$

where I_s is the measured scattering intensity, I_R the Rayleigh component and I_a the anisotropic component. This effect may be rather large due to the size of p . An equation has been derived to calculate the additional scattering due to anisotropy (I_a) from polarization data (Gutfreund, 1974). For SFA with no Cu^{2+} added this is 1.08. At the end of the titration it is 1.03. Thus corrections of scattering intensity for anisotropy may be important for SFA and have bearing on molecular weight calculated from light scattering (Underdown et al., 1981).

CONCLUSIONS

The binding of Cu^{2+} to SFA is much stronger than that of Co^{2+} and Mn^{2+} which are similar. The conditional stability constants from curve fitting at pH 6 are 1.1×10^5 for Cu^{2+} (Chapter 3), 5.1×10^3 for Co^{2+} and 4.2×10^3 for Mn^{2+} . The slight advantage in strength for Co^{2+} over Mn^{2+} is also demonstrated by the binding curves at pH 6 and 7. These two metal ions not only tend to bind more weakly but also bind to fewer sites giving C_L values of about $1.5 \times 10^{-6} \text{ M}$ compared to $2.0 \times 10^{-5} \text{ M}$ for Cu^{2+} at pH 6 (Chapter 3). This lower binding capacity may be due to outer sphere complexation that does not allow access to some sites complexed by inner sphere binding Cu^{2+} . Cu^{2+} is also effective at precipitating and aggregating SFA while Co^{2+} and Mn^{2+} are not. Cu^{2+} ion probably neutralizes the negative charges on SFA molecules allowing larger hydrophobic aggregates to form and precipitate.

CHAPTER 6
COMMENTS ON CALCULATION METHODS FOR STABILITY CONSTANTS
AND COMPLEXING CAPACITIES OF METAL ION-NATURAL
ORGANIC MATTER SYSTEMS

INTRODUCTION

The procedures involved in calculating a stability constant and complexing capacity using data from metal ion titration of a ligand are generally straight forward. The complexing capacity or C_L (total ligand concentration) is merely an endpoint for the titration. Endpoints are often determined graphically and may then be further defined by a calculation technique. Stability constants are simple ratios and can be determined if at least three independent parameters defining the system are known. These are usually the total molar metal ion concentration, free metal ion concentration and the complex stoichiometry. Other parameters may be substituted for these such as free and total ligand concentrations.

For the titration of a solution of a simple ligand this information is often readily obtainable. The total amount of metal ion added is usually known and the solution may be monitored for the concentration of free metal ion, free ligand or complex. The complex stoichiometry may be known or inferred from structural information and if not at least the molecular weight of the ligand is known.

An endpoint can be determined by simply plotting the data or using a more sophisticated technique such as the second derivative method or a Gran plot (Rossotti and Rossotti, 1965).

When the ligand system under study is poorly defined several complications are apparent. If the stoichiometry of the metal complex (or complexes) is not known calculations become more involved. This is often the case with metal binding proteins. Once the protein is isolated frequently the next step is to determine its molecular weight. Scatchard (1949) binding studies can then be performed to determine the binding constant (or constants) and number of binding sites per molecule.

Metal binding studies of natural organic matter have added factors of difficulty. These mixtures of ligands are so complex that separation procedures seldom reduce their complexity. For these systems molecular weights have little meaning and several stoichiometries may exist in one sample. In addition the mixture of ligands tends to give broad curves with no discernable break or endpoint. When a sample of a body of water is titrated with metal ion typically nothing is known about the ligands present, their composition, concentration, metal binding strength or stoichiometry. This can be a difficult analytical challenge.

Knowing only the amount of metal ion added and, possibly, the free metal ion concentration, the system must be defined. Often a stoichiometry must be assumed. Several methods have

been used to calculate stability constants and complexing capacities from this information. Some of these are geared towards specific techniques others are more general in applicability. None of them are ideal for describing metal ion complexation by natural organic matter. In this chapter several of these calculation techniques will be briefly described and compared with each other and the curve fitting technique described in the preceding chapters for fluorescence data.

Endpoint Methods

Endpoints for compleximetric titrations have been calculated by several methods for decades. Extrapolation of graphical data is probably the most common technique employed and is even incorporated in many sophisticated treatments. One form of this method involves plotting instrument response vs. total metal ion concentration and determining the endpoint by taking the x-intercept (total metal ion axis) of a regression line through the upper most points (Truitt and Weber, 1981a; 1981b; Rainville and Weber, 1982). In many cases the lower data points before the endpoint are also fitted with a straight line and the endpoint determined by dropping a vertical line from the intersection of the upper and lower lines (Bhat et al., 1981; Shuman and Woodward, 1977). For spectrophotometric studies which often measure ligand or complex, the method described above is used as well as the classical Job's method of continuous variation or the Mole (Slope) Ratio Method (Olsen, 1975).

Potentiometric titrations are typically analyzed by taking the first or second derivatives of the millivolt response. This converts the standard sigmoidal shaped curve into a form that clearly shows the endpoint as a peak or a plot that goes negative at the endpoint. Curve fitting methods have also been used for endpoint determination but they are often only automated versions of graphical treatments (McCullough and Meites, 1975).

Probably the best endpoint method available is the Gran method (Rossotti and Rossotti, 1965). It is extremely sensitive because in essence it exaggerates the break in a titration curve allowing it to be easily and accurately determined. These features allow its routine application even to fairly weak metal-ligand associations.

Metal ion complexation models for natural organic matter.

Several different models have been proposed to quantitatively describe the metal ion binding properties of natural organic matter. All of these models can be classified as one of the two general types (Perdue and Lytle, 1982). Discrete complexation models are by far the most common and are characterized by assumptions of stepwise complex formation in the classical sense with all ligands in the mixture lumped together.

Simple 1:1 models, such as the one described in Chapter 3, are extensively used for describing metal ion-humic material interactions. The principle drawback with assuming one metal ion and one ligand per complex is that it is

admittedly an oversimplification. Multiple stoichiometries are very likely at either extreme of a titration. In addition it has been demonstrated that stability constants calculated under such conditions are not really constant (Gamble et al., 1980; MacCarthy and Smith, 1978; 1979).

Expanding the approach to include two types of complexes has been reported by several groups (Bresnahan et al., 1978; Buffle et al., 1977). These models predict stepwise complexation of a metal ion by two ligands. Often this improves the fit of the model to the experimental data but is not much of an improvement over 1:1 models because it does not adequately describe the complexity of the system.

The Scatchard treatment (Scatchard, 1949) is also included as a discrete type of calculation. Its widespread use is probably a consequence of its easy application to titration data and its ability to extract information from a poorly defined system. A Scatchard plot of a protein titration will give stability constant and stoichiometry data when only the molecular weight of the protein is known. However, the use of this data treatment for more complex systems is a misapplication that has been discussed (Scheinberg, 1982; Perdue and Lytle, 1982). The usual result of Scatchard analysis of metal ion-humic material data is a continuous curve instead of a straight line or two straight line segments as predicted for the 1:2 case.

An improved method of examining titration data of natural systems has recently been published simultaneously by two

separate authors (Ruzic, 1982; Van den Berg, 1982a). The technique is still based on 1:1 complexation but both authors have considered the effect of 1:2 and 2:1 complexation as well as complexation by two ligands of different types. The most attractive feature of the procedure is that it gives linear plots when the data is represented as free metal ion concentration vs. the ratio of free over bound metal ion concentration. Van den Berg's (1982a) treatment is probably the more complete of the two and he has also provided experimental data for Cu^{2+} titrations of seawater in a companion paper (Van den Berg, 1982b). The presentation of the theory, however, is complicated somewhat by the need to include the equilibria involved with the weak ion exchanger MnO_2 that is used in the experimental procedure (Van den Berg, 1982a).

An important aspect of trace metal-humic material chemistry is brought out in addition to the new theoretical perspective (Van den Berg, 1982a). In most natural waters trace metal ions are at very low concentrations compared to the natural ligands. The metal ions that are complexed are bound to the strongest sites available in the ligand mixture. As metal ion is added in a titration strong ligand sites are used up and weak ones are titrated. Measurement of a stability constant gives a value that is some type of average of all the ligands present. If it is desired to estimate the stability constant of the strongest binding material for a situation where ligand is in excess, then the

titration experiment must be conducted accordingly. That is, only a small range of metal ion should be covered instead of a large range that extends into the region where metal is in excess. A linear data treatment is essential to extract the information in this case because there is no break in the curve. Van den Berg (1982a; 1982b) has taken advantage of this. In addition this procedure probably limits the data as closely as possible to conditions where the stability constants measured are true constants as described by MacCarthy and Smith (1978; 1979).

In Chapter 4 the multiligand model proposed by MacCarthy and Smith (1978; 1979) was briefly discussed. In essence these workers propose that metal binding by mixtures of several ligands can be described by a stability product that is very similar to a stability constant. The stability product is calculated by summing the concentrations of the various ligand species in the mixture and inserting them in what amounts to a standard stability constant expression. For example the sum of the concentrations of metal complexes is the numerator and the denominator is the product of free metal ion concentration and the sum of free ligand concentrations.

The reaction of each ligand with one metal ion gives a 1:1 stability product (S_1). Higher order complexes are also considered in this theory giving S_2 , S_3 , etc. These are overall stability products for the reaction of two or more ligands with a metal ion.

The most important aspect of this theory is the clear demonstration that stability products (or stability constants) applied to multiligand systems are not true constants. This fact has also been pointed out by Gamble et al. (1980). Constant values are obtained only in certain upper and lower regions of total metal concentration (MacCarthy and Smith, 1978; 1979).

One last type of discrete data treatment not discussed above is based on adsorption isotherms. Models such as the Langmuir or Freundlich isotherms are often used to describe metal ion binding in natural systems (Sanders, 1980). There is little difference between these models and the 1:1 treatments. Adsorption coefficients can be loosely equated with stability constants. Often extra terms or factors are included in adsorption equations to improve the empirical fit of the equations to the data. In certain forms these treatments are completely analogous to the Scatchard treatment (Scatchard, 1949). Ruzic (1982) has also included a discussion of adsorption isotherms in his linear 1:1 model showing these similarities.

An alternate concept of metal ion binding to humic materials is to view the ligand mixture as containing a large number of sites of different strengths that vary continuously between limits. This type of "continuous model" seems to agree with what is known about humic materials and what is generally accepted (Perdue and Lytle, 1982). Humic polymers contain phenolic and carboxylic acid groups with some portion

present as salicylic acid and phthalic acid type binding sites. The carbons adjacent to or in the general vicinity of these groups are most likely substituted with a wide range of carbon containing moieties. This will modify the binding strength of the various sites by different amounts. Therefore the ligands in this complex mixture are probably similar but vary in binding strength from weak to strong, with intermediate strengths covering the entire range.

One way of modeling these metal ion-ligand associations mathematically is to assume a Gaussian distribution around a certain binding strength. Perdue and Lytle (1982) have developed this approach and used curve fitting to determine a mean log K value for a ligand mixture titrated with a metal ion and, the standard distribution of log K around the mean. A comparison of the Gaussian model with the Scatchard treatment for a metal ion titration of humic material demonstrates that the Gaussian model gives a better fit to the experimental data. However, a significant difference between the Gaussian model and the experimental results did exist at low metal ion to ligand ratios (Perdue and Lytle, 1982). This behavior may be due to an inherent limitation of the Gaussian treatment. If there are two predominant groupings of binding strengths the Gaussian model will give poor results. The mathematics, curve fitting and computer implementation of this model are difficult enough to make it impractical to consider attempting the determination of more than one average log K per data set. Another limitation of the

treatment is that it gives a weight-average (or in this case strength-average) log K as opposed to a number-average log K which is more desirable.

One other continuous multiligand model has been applied to humic substances with reasonable success. This model is the "affinity spectrum" technique first described by Hunston (1975) and more recently applied by others (Thakur et al., 1980; Shuman et al., 1982). The major advantage of this technique is that it allows transformation of free vs. bound metal ion concentration data into a "spectrum" of log K vs. number of sites with that log K value (i.e. intensity). Several major K values in a mixture can be elucidated with quantitative information about their prevalence. There is no assumption of 1:1 binding per se and presumably K values for 1:2, 1:1 and any other types of complex will be measured if they are significant. This has been demonstrated to some extent for the three dissociation constants of H_3PO_4 (Shuman et al., 1982).

Conceptually the affinity spectrum model is very attractive because it provides a picture of humic material complexation that is intuitively satisfying. Several groupings of ligand strengths may exist with certain ones predominating and others barely observable. Water samples from one geographic region may show similar profiles ("spectra") that are markedly different from samples from other areas. Samples collected from the same body of water at different times or under different trace metal loadings

may show the presence or absence of high affinity ligands. The amount of information available from this approach is significantly greater than from other data treatments.

There are, of course, some disadvantages or difficulties encountered with the affinity spectrum. Initially there is some confusion associated with going from a fairly simple idea to a reasonably complex and conceptually unclear mathematical treatment. However, the procedure can be conducted by hand as well as by computer. It has been clearly pointed out by Hunston (1975; Thakur et al., 1980) that the resolution of the affinity spectrum is at best no better than one log K unit. In addition it is necessary to have a great deal of data on either side of the K to be measured in order to get a good spectrum. This requires an experimental technique with a wide dynamic range (typically greater than three orders of magnitude) and preferably a low detection limit. Ion selective electrodes, commonly used for studying metal ion-humic material binding, are probably a poor choice. It is also necessary to determine the endpoint of a titration by some other method prior to applying the affinity spectrum calculations.

Some of these drawbacks can be demonstrated by an example. In order to apply the affinity spectrum technique to the fluorescence data presented in Chapter 3 it is first necessary to treat the data with the curve fitting technique also described in Chapter 3. This is essential to obtain free metal ion and bound metal ion concentrations needed for

the affinity spectrum. But, using the curve fitting model imposes a 1:1 stoichiometry on the data as well as all the other assumptions inherent in that data treatment. Therefore some information may be lost before being able to take full advantage of the affinity spectrum.

When the tyrosine data from Chapter 3 was analyzed with the affinity spectrum technique the log K values obtained ranged from 4.4 to 5.1. This neatly brackets the theoretical log K value of 4.77 (Smith and Martell, 1974) but is rather imprecise compared to the 4.76 ± 0.01 results from curve fitting of the same triplicate titrations.

Finally, some problems may be encountered when interpreting affinity spectra due to artifacts. The spectra for the tyrosine titrations were quite rough and contained a couple of spurious peaks and two satellite peaks, one on either side of the expected peak in each spectrum. These additional features in the spectrum may be caused to some extent by this initial crude attempt at performing the various steps in producing the affinity spectrum. However, Hunston (1975; Thakur et al., 1980) has stated that artifacts do occur and has provided criterion for discounting them.

CONCLUSIONS

There has been a definite trend in the recent literature on humic-metal ion chemistry to emphasize the improvement of data treatments. Those discussed above include the most widely used and newer ones that show the most promise. In the past a large amount of data had been generated without a

clear indication of how it should be interpreted. There is still room for a great deal of work on more sophisticated and realistic data treatments. Statistical interpretations are also needed to better understand the variance associated with calculated constants (Stolzberg, 1981).

In future studies, broader based approaches are probably warranted. For example, mathematical models that can predict solution phase complexation as well as adsorption phenomena might better approximate natural systems. Experimental techniques such as fluorescence binding studies should also be improved and refined to give more reliable data. Laboratory experiments should also be conducted under conditions that are as close as possible to natural waters instead of convenient conditions or those that conform to the data treatment.

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APPENDIX

2

APPENDIX

MODIFICATION OF THE STATIC MERCURY DROP ELECTRODE

INTRODUCTION

Voltammetry is a powerful technique and combined with a dropping mercury electrode (DME) or hanging mercury drop electrode (HMDE) has seen widespread use. However, both electrodes can often be difficult to operate. The DME is cumbersome and requires careful, labor intensive operation. HMDE's also have several limitations despite improvements that have been suggested (Bellamy, 1980; Bonelli et al., 1979).

The more recently introduced static mercury drop electrode (SMDE) (Princeton Applied Research, model 303) combines the DME and HMDE in one easy-to-use module (Peterson, 1978). This design overcomes many of the practical drawbacks of the two electrodes and allows operation with either electrode by the flip of a switch. Bond and Jones (1980) discussed the advantages of the SMDE used as a DME for polarography. It is also superior to the conventional HMDE. The mechanical plunger delivers drops (small, medium, or large) of highly reproducible sizes. The large, easily refilled reservoir of the SMDE eliminates frequent electrode capillary manipulations and the associated problems (Bonelli et al., 1979). In addition one can, if necessary, replace a capillary fairly easily without emptying the reservoir. The SMDE has its own

nitrogen purge timing mechanism, drop dislodger and dispenser. When interfaced with any of the PAR analysis control units, drop dislodging and purging are automatically initiated by the controller. This system will dispense a new drop automatically for each replicate scan, which is recommended for anodic stripping voltammetry (ASV) (Moorhead and Daub, 1977).

The SMDE was used for the ASV measurements discussed in Chapter 2, however the electrode unit had been modified for certain experiments (Bhat et al., 1981). As the SMDE is seeing increased use as an analytical tool (Holak, 1980; Bond et al., 1980; Bond and Anderson, 1981) the modifications described here should surely extend its applicability and improve its performance. The changes include a water-jacketed cell for constant temperature work, and an easily replacable Ag/AgCl reference electrode that can be used as a double junction reference. Other modifications allow the use of a pH electrode in the voltammetric cell, and reproducible stirring with nitrogen bubbling which is automatically controlled by the 315 controller. These alterations incorporate more versatility in the SMDE for varied research applications while retaining the convenient operational aspects.

MODIFICATIONS

Jacketed Cell. A constant temperature water jacket for the polarographic cell was constructed from standard 5.1 cm

O.D. borosilicate glass tubing and the SMDE cells supplied by the manufacturer (Figure 23). The jacket has an inside diameter of 4.8 cm, a height of 5.7 cm with the bottom sealed off, and had two hose nipples fitted into the side. A rubber gasket (4.4 cm O.D. and 0.24 cm thick) was used to seal the cell into the jacket. Alternatively, the cell could be cemented into the jacket. The jacketed cell is easily mounted on the SMDE by first removing the clip that secures the cup support arm and turning the arm upside down and refastening. Then the entire cup support is pulled outward and turned over. The cell fits snugly in place and can be connected via standard laboratory tubing to a constant temperature water bath. The constant temperature jacket described here is adaptable to either glass (PAR model G57) or polypropylene (model G116) SMDE cells. In routine application it is easy to use and places little restriction on the manipulation of the cell.

Other cell designs were also investigated and a similar type of jacketed cell of larger capacity was developed. This cell was cone shaped with a 4.4 cm diameter at the top and a 1.9 cm diameter at the bottom. It had a depth of 5.1 cm giving it a working volume of 20 mL compared with the 10 mL PAR cell. This larger capacity cell can be used with the same jacket described above and is useful in titration experiments where a larger volume is desirable (Bhat et al., 1981).

Stirring. There are provisions to connect a PAR

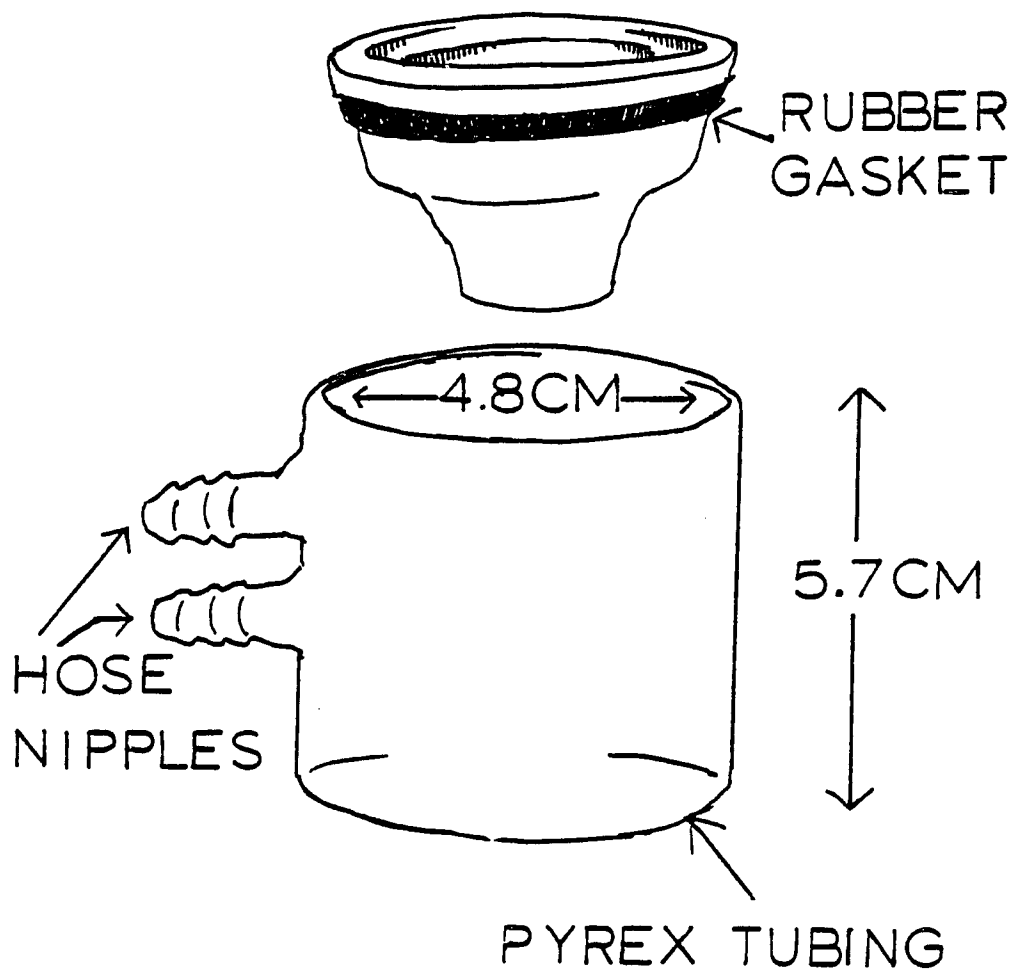


Figure 23. Constant temperature jacket for static mercury drop electrode (SMDE) cell.

stirrer at the rear of the model 303 SMDE, but this assembly is not compatible with any type of constant temperature cell. Alternatively, N_2 can be used to stir and deaerate the solution (Holak, 1980; Schonberger and Pickering, 1980). Since the SMDE has its own N_2 purging and blanketing system and purge timing mechanism, these must be bypassed and the purging system in the 315 Electroanalysis Controller used (this modification also applies to the PAR models 384 and 264). This is done by first connecting the " N_2 blanket" line from the 315 to the regular input on the 303. Then with the rear panel off the SMDE the tubing leading into the left side of the rear of the electrode support block is disconnected and a tube from the " N_2 purge" on the 315 is connected here.

In addition to these changes the purging mechanism must be connected to the auto stirring mechanism of the 315 so the purge can act as stirrer. This is done at the SMDE end of the cable connecting the 315 and 303. With the cable unplugged and the plastic outer covering of the plug removed, the wire to pin #8 is cut and resoldered to pin #24. The plastic covering can then be replaced and the unit reconnected. This wiring change causes N_2 purging/stirring of the solution at all times that the instrument is turned on except during the "equilibration" and "scan" steps of the 315 cycle. This can be manually overridden by the "stir" switch on the front panel of the 315 unit. When purging is off the cell is blanketed with N_2 .

A sensitive ball type flow-meter with needle valve should be placed in the N_2 line between the tank regulator and the input to the 315 controller to ensure reproducible stirring throughout an experiment. In addition, the purge tube in the polarographic cell should be extended to reach the bottom of the cell for efficient stirring and it should be directed away from the working electrode to prevent disturbance of the Hg drop.

Reference Electrode. The SMDE was fitted with an Ag/AgC reference electrode (Figure 24) prepared by the method of Sawyer (Sawyer and Roberts, 1974). The anodized silver wire was placed in a glass tube 4 mm O.D. x 6.4 cm with a 4 mm diameter Vycor frit sealed at the end with heat shrinkable Teflon (PAR, 1978). This tube was filled with approximately 0.5 mL of saturated KC, AgC filling solution supplied by PAR. The top of the tube was fitted with a small serum cap through which the silver wire protruded. To install the new reference electrode first the old reference electrode sleeve and silver wire were removed. Then the hole through which the silver wire had gone was enlarged to 0.48 to accommodate the new reference. It was first put through a small rubber stopper which enabled it to fit snugly into the indentation formerly filled with rubber cement, at the top of the electrode support block. Finally a small alligator clip was soldered to the end of the reference electrode lead wire coming out of the support block and was clipped to the silver wire of the reference.

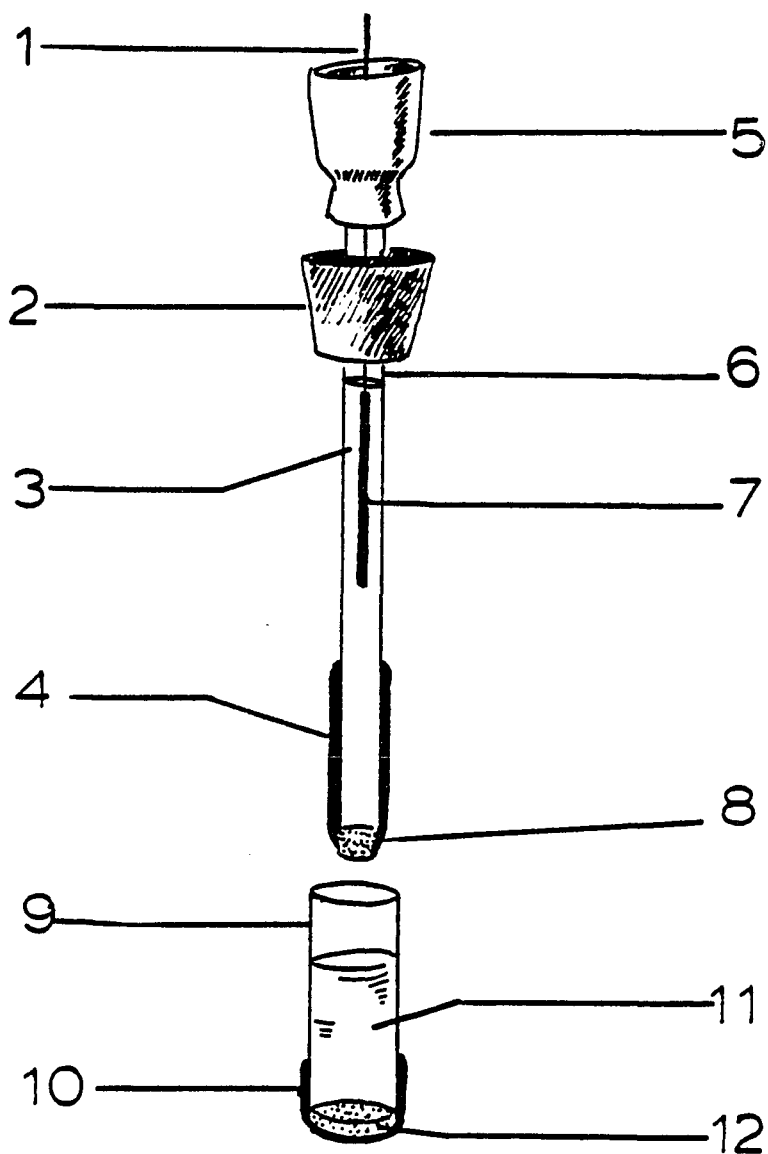


Figure 24. Ag/AgCl reference electrode and double Junction sleeve. (1) Ag wire; (2) rubber stopper; (3) saturated KCl. AgCl filling solution; (4) heat shrinkable Teflon; (5) serum cap; (6) Pvrex tubing, 4mm diameter; (7) AgCl coated wire; (8) Vycor frit, 4 mm diameter; (9) Pvrex tubing, 6 mm diameter; (10) heat shrinkable Teflon; (11) electrolyte; (12) Vycor frit, 6 mm diameter.

This reference electrode is much less expensive than the one supplied with the instrument and is easily prepared. Several can be kept on hand at all times. The electrode is easily removed for better access to the working and counter electrodes, and with the reference removed there is no need to keep water or electrolyte in the cell for storage. This can reduce problems with solution creeping up the SMDE capillary.

The reference electrode described above can also be easily adapted as a double junction type of reference electrode. This is done by preparing an outer sleeve from 6mm Pyrex tube approximately 3.8 cm in length and fitting it with a 6mm Vycor frit using the heat shrinkable Teflon (Figure 24). This double junction sleeve is filled with approximately 0.5 mL of the electrolyte used in the test solution and is fitted into the electrode support block from the bottom sliding it over the end of the reference electrode. The sleeve can be held into place in the hole provided for the built in reference electrode with a small rubber O-ring or a few winds of Teflon tape.

Incorporation of pH Electrode. A pH electrode (Sargent Welch combination electrode model S-30070-10) was incorporated in the model 303 by removing the support block from the unit, and drilling a 0.95 cm diameter hole in left side. It was drilled from the bottom through to the top at about a 20 degree angle from vertical to allow the electrode

to be inserted easily and to position the tip of the electrode near the center of the cell. The depth to which the electrode dips into the cell is easily controlled by inserting the electrode through a small stopper which is allowed to rest on top of the electrode support block. When the 303 is to be used without the pH electrode a small (0.95 cm diameter) Teflon stopper can be inserted into the hole.

CONCLUSIONS

The above modifications allow titrations to be conducted in the voltammetric cell at constant temperature and with pH monitoring (Bhat et al., 1981). The constant temperature jacket on the SMDE cell results in greater accuracy and precision of analysis since the hanging mercury drop is very sensitive to temperature changes (Baikerikar and Sathyanarayana, 1970). Accurate temperature control is essential in experiments involving complex equilibria and the measurement of kinetic parameters. The water jacketed cell also allows work at elevated or reduced temperature (Hanck and Dillard, 1977).

Stirring is necessary to ensure homogeneity in the voltammetric cell, and must be reproducible for techniques such as anodic and cathodic stripping voltammetry (Bond, 1980). Reproducibility of stirring with N_2 is very good when monitored with a flow meter. Twelve consecutive anodic stripping voltamograms of 4×10^{-7} M Cu^{2+} in 0.1 M HNO_3 gave a relative standard deviation (rsd) of 1.6%. This can be

compared with the 0.56% rsd for the reproducibility of the SMDE drop size (Peterson, 1978). As expected, a plot of anodic stripping current vs. concentration of added metal ion follows a linear relationship over a wide concentration range.