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Full Name of Author — Nom complet de l'auteur

STEPHEN LAI FOE LEE

Date of Birth — Date de naissance

31 DEC 1947

Country of Birth — Lieu de naissance

ISURMA

Permanent Address — Résidence fixe

1105 CAMELOT DRIVE
OTTAWA ONTARIO

Title of Thesis — Titre de la thèse

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Name of Supervisor — Nom du directeur de thèse

DR. H. LANGEFORD TH. D. CAMPBELL

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STUDIES OF THE BINDING FUNCTION FOR A SOIL ORGANIC
ACID WITH COPPER AND MAGNESIUM

by

STEPHEN L.F. LEE

A Thesis

Submitted to the School of Graduate Studies
in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

CARLETON UNIVERSITY

Ottawa, Ontario

April, 1982

The undersigned hereby recommends to the Faculty of Graduate Studies the acceptance of this thesis submitted by Stephen L.F. Lee, B.Sc., M.Sc., in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Supervisor

Supervisor

Chairman of Chemistry Department

External Examiner

TO MY FATHER AND THE MEMORY OF MY MOTHER

Abstract

Complexation is an important process in the metal ion speciation in natural aquatic and soil systems. The complexations of copper and magnesium with naturally occurring organic materials were studied in great detail. Fulvic acids, the soil derived acid-soluble fractions of humic substances were used as models for the laboratory examination of the nature of these interactions in pure aqueous systems.

Ion selective electrode potentiometry was used in the Cu-FA and Mg-FA binding studies. The experiments with Cu^{2+} and Mg^{2+} were approached at two levels; namely, stoichiometric and thermodynamic. At the stoichiometric level, the apparent number of binding sites or the "binding capacity" was determined. This was found to be a sensitive function of pH, ionic strength and the concentration of fulvic acid.

Since fulvic acid is known to be a mixture, the thermodynamics of binding of Cu^{2+} and Mg^{2+} by a mixture ligand -- fulvic acid, was studied by adopting a formalism given by Gamble and Schnitzer. The binding function K_4 obtained was observed to vary considerably as the binding sites became progressively occupied by metal ions. This variability of K_4 was the reason for the variation of stoichiometry with changing conditions.

Light scattering technique was used to monitor the particle size or aggregation of the fulvic acid solutions. It was found that

Mg^{2+} , like Cu^{2+} was able to induce aggregation in the fulvic molecules. No pH effect, however, was observed in the fulvic acid solutions of three pH levels- 6.0, 8.0, 10.0. It was postulated that Mg^{2+} was bound to ionized carboxylic acid groups of the fulvic acid molecules.

Stopped-flow kinetic method was also used to study the Mg-fulvic acid system. A fluorescent reagent, 8-hydroxyquinoline-5-sulfonic acid was found to be highly sensitive for the detection of Mg^{2+} ion as well as having characterizable kinetic behavior. The Mg-reagent complex has a λ_{ex} and λ_{em} of 380 nm and 500 nm respectively. Under pseudo first-order conditions, the pseudo first-order rate constant of $1.0 \times 10^3 s^{-1}$ at $25^\circ C$ was determined for the reaction between free hydrated Mg^{2+} and the reagent. The second-order rate constant was calculated to be $4.3 \times 10^5 M^{-1} s^{-1}$. The reagent was subsequently applied in the study of Mg-fulvic acid complex systems.

From the results of kinetic analysis, only one component in the Mg-fulvic acid system was identified and it was related to free Mg^{2+} ion. This was attributed to the lability of the Mg-fulvic acid complex. In other words, Mg-fulvic acid complex is labile on the time scale of the formation of Mg-oxine reagent complex.

From the kinetic studies and the values of the standard Gibbs free energies calculated, it was also postulated that Mg^{2+} ion is bound electrostatically to fulvic acid molecules as outer sphere complexes. In this respect, Mg^{2+} is similar to Mn^{2+} ion, but differs from Cu^{2+} and Fe^{3+} ions which are known to form inner sphere complexes with fulvic acid molecules. From the binding studies,

it appears that at low coverage, Cu^{2+} is strongly bound as inner sphere complex in a covalent type binding to fulvic acid. At high coverage, it is weakly bound and presumably electrostatically. The Mg^{2+} shows only weak outer sphere complexing with strength comparable to that of Cu^{2+} at high coverage.

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CHAPTER 1

INTRODUCTION

The main objective of this work was to study the interactions between metal ions and a well-characterized naturally occurring organic material called fulvic acid in aqueous solutions. Potentiometry with ion selective electrodes was employed for this study in the estimation of equilibrium functions and binding capacities together with related methods such as light scattering and stopped-flow kinetics.

Fulvic acids are the acid-soluble fractions of humic substances which are widely distributed in nature. These humic materials are derived from the chemical and biological degradation of plant materials and/or from synthetic activities of microbes. The importance of these humic substances arises from their ubiquitous presence in soil solutions and natural waters and from their strongly chelating properties. In this study, fulvic acids were used as models for the laboratory examination of the nature of interactions between dissolved organic substances and metal ions in natural waters.

The studies of these types of interactions originated from the environmental concern over metal toxicities in water resources. The discovery of mercury as the cause of Minamata disease in Japan was a major impact on the awareness of the dangers of metal pollutions. Several other heavy elements have also been found to accumulate up food chains and to be toxic to aquatic and terrestrial life. Although most health effects have been related to high exposures to specific trace

elements such as mercury, cadmium and copper, of equal concern is the possible chronic disease relationships to the uptake of more typical quantities from food, water and air. Over the past few years, many reports have shown statistical correlations between drinking water quality, water hardness, calcium concentration and cardiovascular disease (Adelman, 1973).

Such epidemiological investigations as mentioned above indicate the importance of, and the necessity to understand the nature, incidence, variability and controlling factors for metals in natural waters and soil solutions. These understandings are also significant to the art of maintaining and improving the fertility of soils, and in facilitating the treatment of municipal and industrial waters.

The chemical and physical states of metals are of importance and interest for understanding the controls on their concentrations and transportation in natural waters. Whether an element exists in or adsorbed on a particulate or a colloidal system, or whether it exists as a specific form (species) in a soluble state is vital to the understanding of their behaviour. The common procedure of measuring the total concentration of a particular metal in a water sample (e.g. by atomic absorption spectrophotometry) can be misleading because, depending on the chemical forms of the metal, a water with a high total metal concentration may in fact be less toxic than another water with a lower metal concentration. For example, several workers (Nielson and Wiium-Andersen, 1970; Bryan, 1971) have concluded that ionic copper is far more toxic towards aquatic organisms than complexed copper,

and that the more stable the copper complex, the lower is its toxicity.

Besides adsorption of metals on particulate and colloidal systems, the other important mechanism in the controlling, transportation and speciation of metals is complexation and chelation by inorganic and organic ligands present in natural waters. The formation of complex compounds may have a variety of effects. For example, the formation of insoluble complex compounds may remove metal ions from solution. Complexation may also solubilize metal ions from otherwise insoluble metal compounds, e.g. oxides. It also intervenes in many important chemical reactions such as changes in oxidation state of the metal, redox, decarboxylation, etc., which occur in natural waters (Manahan, 1975).

The important inorganic ligands present in natural water systems are hydroxide, chloride, sulfate, carbonate, sulfide and phosphate (Leckie and James, 1975). Bilinski et al. (1976) reported that carbonate-complexes are the dominant inorganic form of Pb and Cu in fresh water, but that Cd and Zn are not complexed. In oxygenated seawater, the hydroxo-complexes of Zn and Cu, the chloro-complexes of Cd, and the carbonate-complexes of Pb are the predominant inorganic moieties (Zirino and Yamamoto, 1972; Stumm and Branner, 1975). The bisulfide and polysulfide complexes dominate speciation of Cd, Pb, Zn and Cu in sulfidic marine waters (Gardiner, 1974).

Dissolved organic ligands such as humic substances tend to be present at much lower concentrations than some inorganic ligands. But they tend to bind some metals much more strongly than most inorganic

Ligands (Benjamin, 1978). For example, J. Gardiner (1974) showed that humic substances bind Cd(II) to a greater extent than any of the major inorganic ligands such as CO_3^{2-} , SO_4^{2-} , Cl^- and OH^- in natural waters, particularly where humic ligands are predominant. Fig. 1 illustrates some of the major reaction pathways for metal transport, speciation and deposition in natural waters.

Naturally occurring dissolved organic matter in waters include humic and non-humic substances. The latter includes polymers such as polypeptides and polysaccharides. Their concentrations remain low because they are easily degradable by the most common hydrolytic enzyme systems. The hydrolytic breakdown products are always found at low concentration because they are rapidly and biologically assimilated. Humic substances are therefore the main contributors to dissolved organic carbon (Reuter and Perdue, 1977).

In view of the importance of humic substances in natural waters as chelating ligands and pollutants, the experiments in this work were designed to study the metal-organic complexation in aqueous systems using a fulvic acid extracted from soil. (The difference between humic and fulvic acids (HA and FA) will be discussed fully in the next chapter). The use of soil fulvic acid is justified since the major fraction of dissolved organic matter in most natural waters closely resembles soil fulvic acids (Reuter and Perdue, 1977). The metals studied were Cu and Mg, both of which are important constituents in natural waters; the former being a micronutrient and a potential toxic pollutant and the latter, a major constituent in waters, usually

TRACE METAL TRANSPORT AND ACCUMULATION IN AQUATIC SYSTEMS^a

^aAfter Davis, J.A., III. (1978)

Fig.1. Major Reaction Pathways for Metal Transport and Deposition in Sediments in Natural Aquatic Systems

present in concentrations in the neighborhood of 10 mg/L (in fresh waters), and along with Ca ion, accounts for water hardness. The choice of Cu and Mg would also show the contrast in the complexation with fulvic acid as copper is a strongly binding element in the Irving-Williams series and magnesium, a weakly binding alkaline earth metal. The divalent metal ion series is as follows:

The thesis could be divided into two major sections. Section one (chapter 2-6) is concerned with Cu-FA and Mg-FA complexation studies by potentiometry with ion selective electrodes and Mg-FA complexation and aggregation studies by light scattering. Section two discusses the use of stopped-flow spectrofluorimetric fast kinetic method to study the speciation of Mg in the presence of FA polymers.

Section one begins with a discussion of the aqueous chemistry of copper (II) and magnesium, and a general review of fulvic acid (humic substances) chemistry. Various methods for studying the nature of metal-fulvic acid interactions are also presented (chapter 2). The experimental procedures appear in chapter 3, followed by all the experimental results and calculation of Cu-FA and Mg-FA potentiometric titrations in chapters 4 and 5. The Cu-FA potentiometric results are also used in conjunction with the Cu-FA light scattering results obtained by our research group (Underdown, 1982) in order to gain insight into the copper induced aggregation problem of fulvic acid (FA). Chapter 6 presents the results of Mg-FA light scattering studies. The significance and implications of the contrast between the Cu-FA and Mg-FA systems are also discussed.

Section two begins with the characterization of a fluorescent reagent ligand for magnesium for the stopped-flow spectrofluorimetric studies. The results for the fast kinetic study of Mg-FA system and their implications are presented. Finally, an attempt to study Mg speciation in the presence of Fe(III) polymers in aqueous solution by kinetic method is discussed.

CHAPTER 2
THE CHEMISTRY OF COPPER, MAGNESIUM AND
FULVIC ACID IN AQUATIC ENVIRONMENTS

As natural waters circulate in the water cycle, their compositions change continuously. These could range from highly saline marine and estuarine waters, to pristine oligotrophic freshwaters, from stagnant organic-rich bog and marsh waters, to high-sulfide interstitial water in some sediments. The amount and type of inorganic and organic ligands available for complexation of trace metals vary from one type of aquatic system to another. The forms or species of dissolved trace metals will be different in each of these waters since they are affected by the ligands and the electrochemical environment.

The speciation of dissolved metals is an important factor in their distribution and transportation in a natural water system. In order to study the nature of copper-FA and magnesium-FA interactions, it is essential that their general chemistry in aquatic systems be well understood.

Copper (II) in Natural Aquatic Systems

Heavy metals, such as copper are natural constituents of every compartment of the environment. They take part in biogeochemical reactions and are transported between compartments by natural processes. The rates of these are frequently altered greatly by human activity.

Copper, like many other trace elements, is a micronutrient, necessary for the survival of plants, man and animals (Table 1). However, at excess levels in localized areas, it can be a toxic pollutant (Amnerman, 1970; Underwood, 1970). The knowledge and understanding of its essential nature in the metabolic processes of plants, man and animals have increased recently. This, together with its common use in industrial and agricultural operations (copper was used as pesticide in the form of copper sulfate and paris green-copper acetoarsenite until recently (Lee, 1977) has led to the recognition of its widespread distribution in the ecosystem. Statistics for world production of copper indicate that the amount of copper entering the ecosystem annually has steadily increased and currently amounts to about 1,800,000 metric tons per year (NAS, 1977).

The earth's crust concentration of copper is about 55 ppm (Table 1). In igneous rocks copper is concentrated in the basalts. In the sedimentary cycle, copper is concentrated in the clay mineral fractions, particularly those rich in organic carbon and/or manganese oxides (Davis 1977). Particulate copper forms could also include copper oxides (cuprite, copper hydroxide), elemental copper, copper sulfides (covellite and chalcocite), and a hydroxy carbonate (malachite).

Upon weathering copper is likely to be more mobile under acid than alkaline conditions. Most pollution problems due to copper (II) are localized near sources of industrial wastes. It is probable that

Table 1

Copper Contents of an Average Man, Earth's Crust, and
Natural Waters and Soil

<u>Sample</u> ^a	<u>Concentration</u> (ppm)
Man	4 ^b
Earth's Crust	55
Sea Water	3×10^{-3}
Fresh Water	1×10^{-2}
Soil	20

^a All data are from Ochiai (1977) except for soil's which is obtained from Brooks (1977).

^b Out of a total of 80-120 mg copper in an average man, about 8 mg is in liver, 15 mg in heart, spleen, kidney, brain and blood. Copper is crucial to blood formation, skeleton, nerve action and many enzymes (body catalysts).

much of the copper (II) found in surface water is introduced by metallurgical wastes, electroplating wastes, and other industrial sources. Runoff from agricultural lands, mineralized areas, and urban areas can also contribute to the input of copper into surface waters (Davis and Jacknow, 1975).

Of the inorganic anions likely to be found in polluted freshwaters, carbonate, sulfide, phosphate, cyanide, and hydrolysis products are important in Cu(II) complex formation. In actual seawater, the carbonate and hydroxo-complexes of copper (II) are calculated to be the predominant soluble species, without taking into account of the possible influence of organic compounds (Zirino and Yamamoto, 1972, Stuman and Branner 1975).

According to the "hard and soft acids and bases" concept, Cu^{2+} is classified as an intermediate between hard and soft electron acceptors (Vogel, 1978). And as such it forms strong complexes with many organic compounds and ligands containing oxygen, nitrogen, and sulfur as electron donor atoms. These electron donor atoms (N, O, S) are of greatest interest in natural systems, and Irving and Williams (1948) found that, in general, Cu^{2+} formed stronger complexes with them than other divalent transition metal ions, eg. Ni^{2+} , Co^{2+} , Fe^{2+} , Cd^{2+} , Mn^{2+} and Mg^{2+} , an alkaline earth metal.

The existence of the types of copper (II) species in natural aqueous systems is controversial. Ramamoorthy and Kushner (1975) reported that a good deal of Cu^{2+} binding activity in river water

was particulate. Alexander and Corcoran (1967) and Slowey et al. (1969) found that copper (II) was present in seawater both in particulate form and complexed with organic compounds. Nissenbaum and Swaine (1976) reported that the polymeric organic material dissolved in interstitial water, contained most of the copper. Schnitzer and Skinner (1966) Gamble and Schnitzer (1973) have demonstrated the strong complexing capability and capacity of humic materials for copper (II) and other transition metal ions. The complexing of copper by soil humic substances may cause copper deficiency in plants. The importance of the humic materials in controlling the speciation and distribution of copper (II) in aquatic systems should be assessed with great care. This is possible only until additional fundamental data such as complexation by humic compounds under various conditions are available.

Magnesium (II) in Natural Aquatic Systems

Of the cations found in most natural water systems, magnesium generally has one of the highest concentrations. It is an important element and plays a key role in many geochemical processes. The importance of its presence in drinking water was also stressed by the study of Moss (1980) whose finding indicates a link between the incidence of stomach cancer and the consumption of soft water.

Magnesium compounds are found in great abundance, both as solid deposits and in solution in the surface waters of the earth (see Table 2). The most common magnesium-containing ores are the

Table 2

Magnesium Contents of an Average Man, Earth's Crust
and Natural Waters^a

<u>Sample</u>	<u>Concentration (ppm)</u>
Man ^b	4.0×10^2
Earth's crust	2.09×10^4
Sea Water	1.35×10^3
Fresh Water	10

^a Data obtained from Ochiai (1977).

^b An average man of 70 kg. Magnesium is vital to bone structure, nerve transmission, muscle action, protein metabolism, energy production and heart action.

carbonates, magnesite (MgCO_3) and dolomite ($\text{MgCO}_3 \cdot \text{CaCO}_3$). In waters, the most common sources of magnesium are carbonate minerals such as dolomite. The concentration of magnesium in natural water systems, like many other metal ions, is therefore controlled by the solubility of its carbonates.

Magnesium, like calcium, is an alkaline earth metal. But, their chemical properties differ appreciably. The ionic radius of magnesium, $0.65 \text{ \AA}$, is smaller than that of calcium ($0.99 \text{ \AA}$), resulting in a much more intense charge density of the Mg^{2+} ion, and stronger binding of the waters of hydration. Its interactions with ligands in waters, therefore may be different from those of Ca^{2+} and will be of interest to study.

In water, magnesium exists primarily as Mg^{2+} ion. Certain amounts of $\text{Mg}^{2+} \text{HCO}_3^-$ and $\text{Mg}^{2+} \text{SO}_4^{2-}$ ion pairs may be present at high concentrations of bicarbonate and sulfate, respectively (Garrels and Thompson, 1962). Incidentally, MgSO_4 is believed to be responsible for the "abnormal" absorption of acoustic energy during a sonar transmission in seawater (Horne, 1969). The concentration of the $\text{Mg}^{2+} \text{SO}_4^{2-}$ ion pairs is higher than $\text{Mg}^{2+} \text{HCO}_3^-$ ion pair, but the exact amount is still controversial. The value reported ranges from a low of 3% to a high of 53% (Horne, 1969). The experimental methods used for estimation varied from precipitation of Mg^{2+} in seawater as $\text{Mg}(\text{OH})_2$ to electrode measurements of Mg^{2+} ion in standard seawater (Thompson, 1966). These contradictory findings indicate one important point, that is - familiar physico - chemical concepts and techniques

which work well on dilute single electrolyte solutions can bring us to grief when we attempt to apply them to so complex a soup as seawater. Note that there are generally, two ways to study a natural process. One is to collect natural samples and try to correlate several system parameters with one another. The second is to study model systems in controlled laboratory experiments. Clearly, the trade-off between the two involves applicability to natural systems in the first case versus ease of interpretation and greater potential for basic scientific advance in the second. For the studies in this thesis, the latter approach was chosen.

Magnesium can also form complexes with F, B and Si which are usually present at concentrations larger than 1 mg per litre. Fluorine has a high electronegativity and can form relatively stable complex with Mg^{2+} (Stumm and Branner, 1975). Borate ($B(OH)_4^-$) and silicate $H_3SiO_4^-$ form weaker complexes with Mg^{2+} .

Information on the interaction between Mg^{2+} ion (and other alkaline earth ions) and organic matters in waters are not numerous. Stumm and Branner (1975) reported that about one-third of the organic complex forming donor groups were bound to cations, mostly to Ca and Mg from their model calculations. The interaction between humus and 18 elements in lake water was studied by Benes et al. (1976) and they reported that alkaline earth metals were associated with humus to a rather high degree. This finding was in agreement with those of Barsdate (1967) who states that at pH close to neutrality a very

substantial portion of alkali and alkaline earth metals should be present in lake waters as humates and not as free ions. Van Dijk (1971) reported that at pH 5, no large difference in bond strength for the divalent ions Ba, Ca, Mg, Mn, Co, Ni, Fe and Zn²⁺ (in this order only slightly increasing) and that there were indications that the bond between humic acids and alkaline earth ions was partly covalent and partly electrostatic. The stability constant of Mg²⁺-FA complex, at ionic strength 0.1 was found to be (log k) 1.9 to 2.2 for pH 3.0 and pH 5.0 by Schnitzer and Hansen (1970) using continuous variations method and ion exchange equilibrium method. In an effort to understand the effect of Ca²⁺ (a major cation in waters) competition on the complexation of Cu(II) by FA, an interesting calculation was done by Gamble and Schnitzer (1973). At a FA concentration of 1 mg L⁻¹ and at a (Ca)/(Cu) ratio of 80, they calculated that the FA chelating sites would be almost fully loaded with Ca²⁺, with very little Cu(II) complexation. Reuter and Perdue (1977) extended the calculations and reported that substantial quantities of Cu(II) could be complexed by FA even in the presence of a 40,000-fold excess of Ca²⁺. Buffle et al. (1980) concluded from their conditional stability constant of Ca-FA complex that the complex was outer sphere. Since there is substantial difference in the chemical properties of Ca and Mg, the effect of Mg²⁺ (competition) on the Cu(II)-FA complexation should be of interest to investigate. The nature of Mg-FA binding -

whether Mg^{2+} bound to FA as an outer or inner sphere complex as well as the capacity and strength of the binding under various conditions should also be studied.

Humic Substances in Natural Aquatic Systems

Life on earth is responsible for the occurrence of organic substances in soils and natural waters. These organic matters consist of a mixture of plant and animal products in various stages of decomposition, of substances synthesized biologically and/or chemically from the breakdown products and of microorganisms and small animals and their decomposing remains (Schnitzer and Khan, 1972). For simplification, organic matters are divided into two groups: (a) non-humic substances, and (b) humic substances.

Non-humic substances include compounds that exhibit still recognizable chemical characteristics. These are predominantly polypeptides and polysaccharides but also include common organic acids, amino acids, carbohydrates, tannins, resins and other low-molecular-weight organic substances. But these substances are easily degradable and their abundance is therefore very low.

On the other hand, humic substances are high molecular weight polyelectrolytic macromolecules and are degradation-resistant materials. On the average, they constitute up to 60-80% of organic matter (Reuter and Perdue, 1977). These humic substances are amorphous, brown or black, hydrophilic, acidic, polydisperse substances of molecular weights

ranging from several hundreds to tens of thousands (Schnitzer and Khan, 1972; Dubach and Mehta, 1963).

Humic substances are commonly fractionated into three general categories based on their solubility in alkali and acid: humic acids, fulvic acids, and humin. The definitions for these categories are purely operational in nature. Humic acids are those components of humus which are soluble in dilute alkaline solution but is precipitated by acidification of the alkaline extract, fulvic acids are soluble in both acid and base, and humin is insoluble in both acid and base. The classical method of fractionation is shown on Fig. 2. From analytical data published in the literature (Schnitzer and Khan, 1972), it appears that structurally the three humic fractions are similar, but that they differ in molecular weight, ultimate analysis and functional group content, with FA having a lower molecular weight, containing more oxygen but less carbon and nitrogen, and having a higher content of oxygen-containing functional groups (e.g. -COOH , -OH , C=O) per unit weight than the other two fractions (Table 3). Weber and Wilson (1975) also reported that the total acidity and carboxyl values are higher in FA than in HA, irrespective of their source. The chemical structure and properties of the humin fraction appear to be similar to those of HA (Schnitzer, 1978). The insolubility of humin seems to be due to it being firmly absorbed on or bonded to inorganic soil and sediment constituents (Kononova, 1966). The observed resistance to microbial degradation of humic materials appears to a significant extent also to be due to the formation of stable metal and/or clay-

Fig. 2. Fractionation Scheme of Humic Substances.

Table 3. Elemental Composition (%) and Major Oxygen-Containing Functional Groups (meq/g) in Humic Substances^a

	<u>Soil FA's</u>	<u>Water FA</u>	<u>Soil HA's</u>	<u>Soil Humins</u>
C	42.5-50.9 (50.1) *	46.2	53.8-60.4	55.4-56.3
H	3.3-5.9 (3.5) *	5.9	3.7-5.8	5.5-6.0
N	0.7-2.8 (0.7) *	2.6	1.6-4.1	4.6-5.1
S	0.1-1.7 (0.4) *		0.4-1.1	0.7-0.8
O	44.8-47.3 (45.3) *	45.3	31.9-36.8	31.8-33.8
Carboxyl	7.7-9.1		1.5-4.7	2.6-3.8
Phenolic OH	2.7-5.7		2.1-5.7	2.1-2.4
Alcoholic OH	3.4-4.9		0.2-3.5	
Carbonyl	1.1-3.1		0.9-5.2	4.8-5.7
Total acidity	11.8-14.2		5.7-10.2	5.0-5.9

^aData adapted from Schnitzer and Khan (1972).

*Figures in parentheses are values obtained for the FA used in this work using a Perkin-Elmer 240 Elemental Analyser

organic complexes. The contents of elements and functional groups could be modified noticeably by environmental and industrial pollution. In an interesting experiment using IR, elemental and metal analyses, Kerndorff and Schnitzer (1979) and Schnitzer and Kerndorff (1980) were able to distinguish between polluted and non-polluted soils and sediments collected from different regions by the differences in metal, element and functional group contents. They reported that polluted HA's and FA's contained more N, S, H and all metals but less O and COOH groups and were more aliphatic than nonpolluted samples. Unusually high N and S contents of humic materials were said to be the most valid indications of pollution. It was noted that humic substances formed under widely differing climatic and geographic conditions have essentially similar chemical structures and characteristics. It appears that the differences mentioned above are due to pollution and not to climatic and geographic differences (Schnitzer, 1977).

The synthesis (formation) of humic substances in the natural environment has been the subject of much speculation. There are four hypotheses put forward by Felbeck (1971) for the formation of these materials. They are:

(a) The plant alteration hypothesis. Fractions of plant tissues which are resistant to microbial degradation, such as lignified tissues, are altered only superficially in the soil to form humic substances. High-molecular weight HA's and humins are formed in the first stages of humification. These are subsequently degraded into FA's and ultimately to CO_2 and H_2O .

(b) The chemical polymerization hypothesis. Plant materials are degraded by microbes to small molecules which are then used by microbes as carbon and energy sources. The microbes synthesize phenols and amino acids, which are secreted into the surrounding environment where they are oxidized and polymerized to humic substances.

(c) The cell autolysis hypothesis. Humic substances are products of the autolysis of plant and microbial cells after their death. The resulting cellular debris (sugars, amino acids, phenols, and other aromatic compounds) condenses and polymerizes via free radicals.

(d) The microbial synthesis hypothesis. Microbes use plant tissue as carbon and energy sources to synthesize intercellularly high molecular weight humic substances. This is followed by extracellular microbial degradation to FA and ultimately to CO_2 and H_2O .

It is difficult at the present time, to decide which hypothesis is the most valid one. It is likely that all four processes occur simultaneously, although under certain conditions one or the other could predominate. What all four hypotheses suggest, however, is that the higher molecular weight humic materials are formed first and that these are then degraded, most likely oxidatively, into lower molecular weight materials. Thus the sequence of events appears to be from HA to FA.

Molecular weight measurements of humic substances have been attempted by using a wide variety of methods. These methods can be grouped into three classes: (a) those measuring number-average

($\bar{M}_n$) molecular weights (osmotic pressure, cryoscopic, diffusion, isothermal distillation); (b) those determining weight-average ($\bar{M}_w$) molecular weights (viscosity, gel filtration); and (c) those measuring z-average ($\bar{M}_z$) molecular weights (sedimentation). The reported molecular weights range from a few hundred for FA's to several millions for HA's. The disagreement in the reported values is considerable not only between different methods measuring the same type of molecular weight but also between different laboratories. This is not too surprising when differences in origin, extractants, degree of purification, etc. are taken into consideration. For homogeneous systems, $\bar{M}_n = \bar{M}_w = \bar{M}_z$, while for heterogeneous systems such as humic materials, $\bar{M}_n < \bar{M}_w < \bar{M}_z$ (Schnitzer and Khan (1972). Wilson and Weber (1977) reported the first dissociation-corrected number-average weight $\bar{M}_n$ corr of aquatic FA. The nearly identical values of $\bar{M}_n$ corr of soil and aquatic FA's found suggest that the humic materials in the soil and water degrade on the average to FA molecules of similar size.

From a wide variety of chemical degradation experiments, the major HA and FA degradation products are found to be phenolic and benzene carboxylic acids. Present indications are that humic substances are not single molecules but rather associations or aggregates of these phenolic and benzene carboxylic acids referred to as "building blocks" (Schnitzer, 1980). On the basis of this and other such as X-ray findings that FA consists of a broken network

of poorly condensed aromatic rings with appreciable numbers of disordered aliphatic chains around the edges of the aromatic rings; similar information from electron microscopy, which shows that FA has a sponge-like structure of variable thickness (100-300 Å), perforated by voids of widely differing sizes (200-1,000 Å in diameter), thus suggesting a relatively "open", flexible structure and from variability in molecular weight estimates, it becomes apparent that humic substances are not single molecules but rather associations of molecules held together by weak linkages such as hydrogen-bonding, van der Waal's forces and possibly π -bonding. This evidence led Schnitzer to propose a partial chemical structure of FA given in Fig. 3. This molecular arrangement may account for a significant part of the FA structure. Each of the compounds that make up the structure has been isolated from FA without and after chemical degradation. Bonding between "building blocks" is by H-bonds, which makes the structure flexible, permits the "building blocks" to aggregate and disperse reversibly, depending on pH, ionic strength etc., and also allows the FA to react with inorganic and organic soil constituents either via oxygen-containing functional groups on the large external and internal surfaces, or by trapping them in internal voids.

In higher-molecular-weight fractions, i.e., HA and humin, the "building blocks" appear to be more complex and more stable (Neyroud and Schnitzer, 1974). The increased stability of each building block may arise from more energetic linkages of FA type and/or from

Fig. 3. A Partial Structure of FA Proposed by Schnitzer

additional chemical bonding via C-O and C-C bonds. The structure for HA given by Fuchs (Fig. 4) does not account for all of the structural parameters present in the literature, Draganov's structure, however, meets many requirements (Fig. 4). The structural model that Wershaw et al. (1977) proposed for HA pictures HA as being made up of a hierarchy of structural elements with the lowest level consists of simple phenolic, quinoid and benzene carboxylic acid groups. These groups are linked together by covalent bonds into relatively small particles with particle weights on the order of a few thousand or less. Groups of similar particles are in turn linked together by weak covalent and noncovalent bonds into aggregates. Further research is needed to provide more specific and detailed information on the structural arrangement(s) of HA's and FA's.

Humic substances have been found to complex a large number of trace metals with possible mechanisms being ion-exchange, peptization, adsorption, chelation, and complex coagulation (Mortensen, 1963). Schnitzer and Skinner (1965) selectively blocked phenolic-OH, alcoholic-OH, and carboxylic groups and found uptake of Cu^{2+} , Fe^{3+} , Al^{3+} to be significantly diminished when the carbonyl and phenoxy groups were neutralized, both appearing to react simultaneously by a mechanism similar to that between Fe^{3+} and salicylic acid (Chaberek and Martell, 1959).

Fig. 4 . a. Structure of Humic Acid according to Fuchs.
b. Structure of Humic Acid according to Dragunov.

(n varying from 1-3)

Many other studies also support the importance of carboxyl groups in metal binding (Gamble et al., 1970; Rashid, 1971, Khanna and Stevenson, 1962). Rashid (1971) fractionated ocean-sediment-derived HA by gel filtration chromatography and found the organics were capable of binding up to 200 mg of metal per gram of HA, that divalent cations complexed 3-4 times more strongly than trivalent ones, and that low molecular weight fractions complexed more metal than higher weight fractions.

Before discussing metal-HA or metal-FA interactions any further, it is relevant to understand the acid-base equilibria and redox properties characteristic of fulvic acid. Gamble (1970, 1972) treats FA as a polyelectrolyte, and considers it as an irregular polymer of moderately low molecular weight with a number of chemically non-identical acidic functional groups, whose respective dissociation constants are a function of the overall degree of ionization of the FA polymer. According to this model, the acid dissociation functions for the ionization of individual functional groups can be calculated.

He reported two types of acidic groups in FA extracted from Prince Edward Island B horizon podzol soil. They are type I - ortho to phenolic-OH group, estimated from conductometric titration, corresponding to the first equivalence point of 3.30 meq/gm FA, and type II - other than ortho to phenolic-OH, estimated from the total-COOH groups evaluated by potentiometric titrations. With the aid of Gran's equivalence point calculation method for a weak acid with two end points, an equivalence point of 7.72 meq. total-COOH groups per gm of FA was computed. Type II acidic groups are then equal to $(7.72 - 3.30)$ 4.42 meq per gm of FA. Borggaard (1974) in a similar potentiometric study indicated the presence of 1.5-3.0 meq/gm of highly acidic ($pK_1 = 2.8-3.4$) carboxylic groups, 2.7-4.2 meq/gm of moderately acidic ($pK_2 = 4.9-5.1$) carboxylic groups, and 1.1-1.7 meq/gm of weakly acidic ($pK_3 = 9.4-9.7$) phenolic hydroxyl groups. The value of the latter for P.E. I. fulvic acid is 3.3 meq/gm. Thus the acid-base titrations of FA appear to be very useful in characterizing the acidic functional groups. These acidic groups are the potential binding sites for metal ions.

The chemical equilibrium between metal-ion and humic substances have been studied for many metals. Numerous techniques for the measurement of apparent stability constants have been used. Among these methods are potentiometric titration and ion-exchange methods. Table 4 summarizes a few of the metal-humic substances stability data available in the literature. As the data indicates, there is a great

Table 4

Metal-Humic Substances Stability Data from Literature

Metal	Ligand	Parameter	Data	Notes	Source
Cu(II)	lake water	$\log K_{int}^{Me}$	3.68		Wilson and Kinney (1977)
Zn(II)	organics organic		3.37		
Mn(II)			2.50		
Cu(II)	soil FA	Condition Stab. Const. K (pH 4.0/5.0/6.0)	$4 \times 10^5 / 10 \times 10^5 / 20 \times 10^5$	Cu^{2+} ion-selective electrode	Bresnahan, Grant, and Weber (1975)
Cu(II)	water FA	K (pH 4.0/5.0/6.0)	$3 \times 10^5 / 9 \times 10^5 / 13 \times 10^5$		
Cd(II)	soil FA	K (pH 4-8)	$1.7 \times 10^3 - 4 \times 10^3$	Cd^{2+} ion selective	Saar and Weber (1979)
Zn(II)	5 soil HA	apparent by K_{int}^{Me}	3.13 to 5.13	based on ion-exchange equilibrium at pH 6.5	Ardakani and Stevenson (1972)
Cu(II)	pond and river	$\log \beta_1^* / \log \beta_2^*$	0.8-1.80/1.10-2.90	potentiometric titration.	Buffle et al. (1977)
Pb(II)	water organic matter	$\beta_i^* = \frac{[MeLi][H]^{ix}}{[M_e][LH_x]}$	1.42/2.24	pH=6.0 to 6.8	
Cu(II)	3 soil HA's	$\log K_2$	7.22-7.38	potentiometric titration, average of values pH = 4 and 5	Stevenson 1977)
Pb(II)			7.00-7.03		3
Cd(II)			6.01-6.06		

Table 5 continues on next page

Table 4 continues:

Metal	Ligand	Parameter	Data	Notes	Source
Cu(II)	lake water/peat FA's	log K overall	8.42, 7.85	gel filtration technique at pH = 8, free metal down to $10^{-8}M$	Mantoura and Riley (1975)
Ni(II)			5.14, 4.98		
Zn(II)			5.14, 4.83		
Cu(II)	Soil FA's	log K overall	3.3/4.0	ion-exchange method	Schnitzer and Hansen (1970)
Pb(II)		pH = 3.0/5.0	2.7/4.0		
Ca(II)			2.7/3.3		
Cu(II)	Soil FA	weighted average equilibrium (K_d)	4.5-15	ion-exchange method 0.1 M KCL - pH 3.2-2.5	Gamble, Schnitzer and Hoffman (1970)

deal of variation. The reason for this is the considerable uncertainty about how to define the problem, which method to use etc. To overcome these problems, Gamble et al. (1970, 1973, 1980) have proposed a general formulation for metal-FA interactions.

(a) The weighted average and differential equilibrium function. The most important functional groups are assumed to be carboxyl and phenolic OH's (alcoholic OH's do not take part in metal-organic interactions in FA's, Schnitzer and Khan, 1970). Two types of bidentate chelating sites would be considered to be possible. These are the salicylic type and the dicarboxylic (phthalic acid) type. The salicylic type is expected to react preferentially with Cu^{2+} (Cu^{2+} ion is used because experimental work was done with it), forming more stable chelates. Both types are freed by a first stage of H^+ dissociation:

$$\bar{K}_L = M_L M_H / M_{LH}$$

where M designates the molar concentration of the species indicated by the subscript (L = deprotonated ligand, H = proton, LH = protonated ligand), and

$$\bar{K}_D = M_D^M M_H / M_{DH}$$

where D = dicarboxylate sites.

At least three classes of the dicarboxylic sites described formally by the Equation 2 must be considered as possible. These are:

(a) the ortho dicarboxylic structure;

(b) two carboxyls on the same polymer molecule, but not on the same ring; (c) two carboxyls on different polymer molecules. In the third case, this bridging of Cu(II) between two fulvic acid molecules may contribute to coagulation. Since it is not experimentally practical to investigate separately metal ion chelation by the two types of sites, it is useful to examine them together as in Equation

$$\bar{K}_4 = M_C^M M_H / M_{SH}^M M$$

where the subscript c denotes complex.

The bidentate chelating sites in Equation 3 are assumed to be a mixture of sites since FA probably has a sufficiently irregular structure so that no two of its functional groups may be assumed to be entirely chemical identical. By definition, the equilibrium function $\bar{K}_4$ is obtained by applying the law of mass action formally to a mixture of chelating sites. Experimentally, $\bar{K}_4$ is measured macroscopically on the whole mixture. Not only can it not be a thermodynamic equilibrium constant, apart from the matter of activity coefficients, it cannot be any kind of real constant. In fact it is a variable, a function of the composition of the chelation solution.

It is possible to deduce theoretically the general properties of this equilibrium function $\bar{K}_4$. This is done by relating $\bar{K}_4$ to the properties of the components of the mixture of chelation sites. The chelation equilibrium of the i th small component of FA could be considered,

$$K_{4i} = M_{Cu} M_H / M_{S_i H} M$$

Then the concentrations of total chelate and of total free sites are related to those of the components by the material (mass) balance equations,

$$M_C = \sum_{i=0}^n M_{C_i} \quad (5a)$$

$$M_{SH} = \sum_{i=0}^n M_{S_i H} \quad (5b)$$

Introducing these material balance relations for the mixture into the equation for $\bar{K}_4$ gives

$$\bar{K}_4 = \frac{\sum_{i=0}^n M_{H_i} M_{C_i}}{\sum_{i=0}^n M_{M_i} M_{S_iH}} = \frac{\sum_{i=0}^n \left(\frac{M_H}{M_M} \right) M_{C_i}}{\sum_{i=0}^n M_{S_iH}} \quad (6)$$

From equation 4, $\left(\frac{M_H}{M_M} \right) M_{C_i} = K_{4i} M_{S_iH}$ (7)

Substituting this into equation 6,

$$\bar{K}_4 = \frac{\sum_{i=0}^n K_{4i} M_{S_iH}}{\sum_{i=0}^n M_{S_iH}} \quad (8)$$

Equation 8 provides an important insight into the chemical meaning of $\bar{K}_4$ which is measured experimentally for the whole mixture. The following points should be noted:

- (1) $\bar{K}_4$ is directly related to the chelation equilibrium functions K_{4i} of all of the components.
- (2) $\bar{K}_4$ is seen to be a weighted average of the K_{4i} values of all of the compounds.
- (3) The factors M_{S_iH} are the statistical weighting factors of the average $\bar{K}_4$.
- (4) $\bar{K}_4$ cannot be regarded as a constant. No corresponding thermodynamic equilibrium constant exists to which it can be related by means of activity coefficients. Instead, it is a variable and is a function

Table 5

Bidentate Chelation of Metal Ions M(II) by FA:

Postulated General Case

Type of chelation site	Chelate		Chelation site, singly ionized		Chelation site, fully protonated	
	symbol	structure	symbol	structure	symbol	structure
salicylic	LM		LH ⁻		LH ₂	
dicarboxylic	DM		DH ⁻		DH ₂	
total, (salicylic & dicarboxylic),	SM	both of the above	SH ⁻	both of the above	SH ₂	both of the above

of the composition of the sample solution.

(5) The dependence of $\bar{K}_4$ on the composition of the sample solution provides a valuable means of characterizing the chemical properties of the system.

For the purpose of examining the properties of $\bar{K}_4$ and for practical calculations, equation 8 would be further transformed. The material balance for the total chelating sites is

$$C_S = M_C + M_{SH} + M_{SH_2} \quad (9)$$

mole fraction of chelated, free, and protonated chelation sites are,

$$x_C = M_C / C_S \quad (10a)$$

$$x_{SH} = M_{SH} / C_S \quad (10b)$$

$$x_{SH_2} = M_{SH_2} / C_S \quad (10c)$$

Therefore, $x_C + x_{SH} + x_{SH_2} = 1 \quad (11)$

Analogous relationships exist for the i th small component. In particular, equation 12 defines the mole fraction of free i th component chelation sites, in the whole mixture;

$$(\Delta x_{SH})_i = M_{S_iH} / C_S \quad (12)$$

Introducing C_S into Equation 8 recognizing that $\sum_{i=1}^n M_{S_iH} = M_{SH}$ gives

$$\bar{K}_4 = \frac{C_S}{M_{SH}} \sum_{i=0}^n K_{4i} \frac{M_{S_iH}}{C_S} \quad (13a)$$

$$\bar{K}_4 = \frac{1}{x_{SH}} \sum_{i=0}^n K_{4i} (\Delta x_{SH})_i \quad (13b)$$

Fulvic acid may also behave as a polyelectrolyte. Therefore, Bjerrum's electrostatic factor is introduced to account for its electrostatic binding of cations. Since each component of the mixture will generally behave differently, it has its own individual Bjerrum electrostatic factor. For the i th component, this is

$$K_{4i} = K_{4i}^0 e^{-(\Delta G_i^e/RT)} \quad (14)$$

$$\bar{K}_4 = \frac{1}{X_{SH}} \sum_{i=0}^n K_{4i}^0 e^{-\Delta G_i^e/RT} (\Delta X_{SH})_i \quad (15)$$

There are three reasons why $\bar{K}_4$ is expected to accurately approximate a continuous function of X_{SH} :

1. The coefficient $\frac{1}{X_{SH}}$,
2. Large number of individual K_{4i}^0 functions will be too similar for separate titration equivalence points to be observed experimentally.
3. The electrostatic Gibb's energy terms, ΔG_i^e will be increasing functions of the amount of electrostatic charge on the polymer molecules and aggregates. The existence of electric charge requires that carboxyl groups be free from both H^+ and Cu^{2+} . That is,

$$\Delta G_i^e = f(X_{SH}) \quad (16)$$

One therefore has the continuous function.

$$\bar{K}_4 = f(X_{SH}) \quad (17)$$

* K_{4i}^0 is an intrinsic term; not at standard state

Because $\bar{K}_4$ approximates a continuous function, the summation of equation 15 may be replaced by an integration:

$$\bar{K}_4 = \frac{1}{x_{SH}} \int_0^{x_{SH}} K_4 dx_{SH} = \frac{1}{x_{SH}} \int_0^{x_{SH}} K_4^0 e^{-\Delta G^e/RT} dx_{SH} \quad (18)$$

There are two important aspects of this equation. The first is the chemical meaning of K_4 . The other is the dependence of the weighted average function $\bar{K}_4$ on the composition of the sample solution.

Equation 18 is now further transformed so that these two aspects may be examined. Rearranging

$$\bar{K}_4 x_{SH} = \int_0^{x_{SH}} K_4 dx_{SH}$$

Differentiating this gives an equation for K_4 in terms of the experimental quantity $\bar{K}_4$:

$$K_4 = \frac{d(x_{SH} \bar{K}_4)}{dx_{SH}} \quad (19)$$

The multitudes of K_{4i}^0 and ΔG_i^e functions in the summation of equation 15 have been replaced by the corresponding continuous functions of equation 18. These variables, K_4^0 and ΔG^e , must both be functions of x_{SH} . One reason for this is that they both "scan" across the components of the mixture as x_{SH} changes in value. The other reason in the case of ΔG^e is that the electrostatic term generally increases with increasing ionization. The results are;

$$\Delta G^e = f(x_{SH}) \quad (20a)$$

$$K_4^0 = f(x_{SH}) \quad (20b)$$

$$K_4 = f(x_{SH}) \quad (20c)$$

It is clearly seen in equation 18 and 19 that K_4 is defined as a differential function,

$$K_4 = K_4^0 e^{-\Delta G^e/RT} \quad (21a)$$

$$K_4 = \frac{\delta M_C M_H}{\delta M_{SH} M} \quad (21b)$$

K_4 has both theoretical and practical importance and should be noted with care. In particular, its chemical meaning is that it is a differential equilibrium function defined by equation 21, for an infinitesimal portion of the mixture of chelating sites. The quantities δM_C and δM_{SH} are the equilibrium concentration of the chelated and free chelating sites of this infinitesimal portion of the mixture. The differential equilibrium function K_4 and the weighted average equilibrium function $\bar{K}_4$ therefore have fundamentally different chemical meanings. Their practical properties will also usually be quite different.

(b) Composition dependence of the equilibrium and state functions.

The second question about equation 18 will now be examined.

The first part about the effects of solution composition is that $\bar{K}_4$ can be expected to show some sort of dependence on FA concentration.

Saar and Weber (1979) have recently demonstrated experimentally a concentration dependence for the Cd^{2+} -FA equilibrium function. They suspect that conformational changes may be responsible but a specific mechanism is not required. Earlier, Burch, Langford and Gamble, 1979; Stevenson, 1976, have shown both theoretically and experimentally that the acid dissociation $\bar{K}$ of FA is concentration dependent. This is a parallel phenomenon. In addition to total FA concentration, the composition of the solution is described by the mole fractions,

$$x_{\text{SH}} = 1 - x_{\text{C}} - x_{\text{SH}_2} \quad (22a)$$

$$dx_{\text{SH}} = -dx_{\text{C}} - dx_{\text{SH}_2} \quad (22b)$$

It is now seen from this discussion and the equations 22 that equation 17 does not describe a state function. Instead, equation 23 shows that three independent variables are required for describing the total dependence of $\bar{K}_4$ on the composition of the solution:

$$\bar{K}_4 = \bar{f}_4(x_{\text{C}}, x_{\text{SH}_2}, C_{\text{S}}) \quad (23)$$

C_{S} is taken here as the most convenient means of describing the total-FA concentration.

The effects of concentration on $\bar{K}_4$ are sufficiently important. However, since it is outside the scope of the present presentation, constant concentration systems will be discussed. With this restriction, equation 22 are substituted into equation 19.

$$K_4 = \frac{d[(1-x_C-x_{SH_2})\bar{K}_4]}{-(dx_C+dx_{SH_2})} \quad (24)$$

$$d[(1-x_C-x_{SH_2})\bar{K}_4] = -K_4 dx_C - K_4 dx_{SH_2} \quad (25)$$

This is now integrated, remembering that $\bar{K}_4$ does not exist at $x_C = 0$;

$$(1-x_C-x_{SH_2})\bar{K}_4 = -\int_0^{x_C} K_4 dx_C - \int_0^{x_{SH_2}} K_4 dx_{SH_2} \quad (26)$$

Rearranging this,

$$\bar{K}_4 = -\frac{1}{(1-x_C-x_{SH_2})} \int_0^{x_C} K_4 dx_C - \frac{1}{(1-x_C-x_{SH_2})} \int_0^{x_{SH_2}} K_4 dx_{SH_2} \quad (27)$$

At constant temperature and FA concentration, equation 27 is the basic working equation describing the Cu^{2+} -FA chelation equilibrium. $\bar{K}_4$ is now seen to depend on two independent variables. The two variables used in equation 27 for specifying $\bar{K}_4$ were chosen not only for convenience but also because they are chemically the most interesting. It is now obvious that the integral of equation 18 is not a state function. That is, its numerical value will depend on the pathway by which $\bar{K}_4$ gets from 0 to x_{SH} .

By taking one of these variables (mole fractions) as an experimental constant, the system may be characterized by scanning across the mixture experimentally with the other. In principle, either x_C or x_{SH_2} may be taken as the parameter of a family of

experimental curves. The differential equilibrium function K_4 is more interesting than the weighted average, $\bar{K}_4$ for these purposes, first because it reveals chemical detail more distinctly. This is because the averaging process always tends to obscure detailed structure. Another important reason is that $\bar{K}_4$, being measured for a mixture, is not related in any simple or conventional way to a Gibbs standard energy. Unlike the concentration quotient for an equilibrium system of pure reagent species, it cannot be related to a thermodynamic equilibrium constant by means of activity coefficients. The differential equilibrium function K_4 has an important advantage in this respect. Because an infinitesimally small portion of a continuous mixture has the attributes of a single chemical species, K_4 may be related to a thermodynamic equilibrium constant in the conventional way. Therefore

$$K_4 = K_4 \frac{\gamma_C \gamma_H}{\gamma_{SH} \gamma_M} \quad (28a)$$

$$\Delta G^0 = -RT \ln K_4 = -RT \ln K_4 - RT \ln \frac{\gamma_C \gamma_H}{\gamma_{SH} \gamma_M} \quad (28b)$$

In most practical cases, there will be no activity coefficient data available. When this precludes the calculation of ΔG^0 , the closely related expression in equation 29 will be very useful:

* Since it is impossible to obtain standard state for FA and metal-FA complexes in our experiments, all ΔG^0 mentioned in the thesis are not standard thermodynamic functions. But by plotting $\Delta G^0 + RT \ln \Gamma_4$ in the figures, it must be made clear that it is empirical or a phenomenological approach which has practical utility in resolving the problem involving in the systems.

$$RT \ln K_4 = -(\Delta G^0 + RT \ln \Gamma_4) \quad (29a)$$

$$\Gamma_4 = \frac{\gamma_C \gamma_H}{\gamma_{SH} \gamma_M}$$

For dilute solutions such as soil solutions or fresh waters, Γ_4 is expected to differ little from unity.

Variable Loading of Chelation Sites with Cu (II) and Constant Mole Fraction. Protonation

With x_{SH_2} experimentally held constant, $x_{SH_2} = x'_{SH_2}$, then the general working equation 27 becomes -

$$\bar{K}_4 = \bar{f}_4(x_C, x'_{SH_2}) \quad (30a)$$

$$\bar{K}_4 = - \frac{1}{(1-x_C-x'_{SH_2})} \int_0^{x_C} K_4 dx_C$$

The constant x'_{SH_2} may now be used as the parameter of a family of experimental curves. Equation 30 is readily solved for K_4 .

$$\int_0^{x_C} K_4 dx_C = - (1-x_C-x'_{SH_2}) \bar{K}_4 \quad (31)$$

$$K_4 = \frac{-d[(1-x_C-x'_{SH_2}) \bar{K}_4]}{dx_C} \quad (32)$$

$$K_4 = f_4(x_C, x'_{SH_2}) \quad (33)$$

Assuming the activity coefficients to have relatively unimportant effects,

$$\Delta G^0 \approx \Delta G^0(x_C, x'_{SH_2}) \quad (34)$$

The mixture of chelation sites may therefore be characterized in detail with experimental scans of both K_4 and the expression of equation 29.

Variable Protonation of Chelation Sites Obtained with Very Small Constant Loading with Cu(II)

In most geochemical systems such as soil solutions of surface waters, Cu(II) is present only at the trace level. In such a case, the properties of the Cu(II)-FA system will be dominated by H^+ instead of by Cu(II). The relevant method of characterizing the system is therefore to scan across the mixture of sites with variable protonation, at constant trace level of Cu(II) loading. That is

$$x_C \approx \text{constant}, dx_C \approx 0 \quad (35)$$

$$x_C \ll x_{SH_2}; (1 - x_C - x_{SH_2}) \approx (1 - x_{SH_2}) \quad (36)$$

The working equation 27 then reduces to,

$$K_4 = \frac{1}{(1 - x_{SH_2})} \int_0^{x_{SH_2}} K_4 dx_{SH_2} \quad (37)$$

Because equation 37 now presents the problem in acid dissociation form, it is convenient to introduce α_1 , the degree of ionization for the carboxyl group in the salicylic type structure in Table 6. This requires the postulate that with trace Cu(II) loading, the

salicyclic type sites are preferentially occupied. From equation (1)

$$x_{SH} = (1 - x_C - x_{SH_2})$$

$$\approx (1 - x_{SH_2})$$

$$dx_{SH} = -dx_{SH_2} \quad (38)$$

Equation 30 now becomes,

$$\bar{K}_4 = \frac{1}{x_{SH}} \int_0^{x_{SH}} K_4 dx_{SH} \quad (39)$$

By definition,

$$x_{SH} = \frac{M_{LH}}{C_S}, \quad \alpha_1 = \frac{M_{LH}}{C_L} \quad (40)$$

Let $M_{SH} = B_{M_{LH}}$, where B is simply the fraction of ionized carboxyl groups belonging to the salicyclic type sites in Table 6.

Then, $M_{SH} = B_{CL} \alpha_1$

$$x_{SH} = \frac{M_{SH}}{C_S} = \frac{B_{CL}}{C_S} \alpha_1 \quad (41a)$$

$$dx_{SH} = \frac{B_{CL}}{C_S} d\alpha_1 \quad (41b)$$

Substituting into equation 39, gives

$$\bar{K}_4 = \frac{1}{\alpha_1} \int_0^{\alpha_1} K_4 d\alpha_1 \quad (42)$$

$$K_4 = \frac{d(\alpha_1 \bar{K}_4)}{d\alpha_1} \quad (43)$$

Equation 42 and 43 were first presented by Gamble, Schnitzer and Hoffman (1970) for the applied problem of trace Cu(II) in soil solutions. This geochemical system may be characterized simply by an experimental plot of

$$K_4 = f_4 (\alpha_1, x'_C) \quad (44)$$

where x'_C is the constant, low Cu(II) loading of the salicyclic type sites.

Gamble and et.al. (1970, 1980) indicated the disadvantage of ion exchange method for chelation measurements. They found that the method was not successful in fulfilling the experimental requirement of very low loading of the sites by Cu(II). The rest of the chapter will therefore, be devoted to a brief discussion of methods available for experimental purpose.

Various methods are available for the studies of metal speciation and chemical equilibria in natural and soil waters. The criteria of a successful analytical technique for these studies are:

1. The technique must be sensitive enough to detect metal ions at the concentrations present in natural waters.
2. The techniques must be free from interferences or the interferences be well defined, readily identifiable and amenable to correction.
3. The results obtained must be easily interpretable in terms of the possible metal species, such as free metal ion, organic complexes, and colloidal species containing the metal ion.

Among the more promising, sensitive and commonly used techniques are: dialysis, ultrafiltration, and ion exchange in conjunction with a detection instrument such as atomic absorption spectrophotometer, anodic stripping voltammetry, potentiometry with ion selective electrodes and multicomponent kinetic method.

Dialysis, ultrafiltration, and ion-exchange methods

These separation methods have been used by Benes and Steinnes (1974, 1975) to study a large number of elements in river and lake waters. Guy (1976) and Guy et al. (1976) used dialysis, ultrafiltration and ion exchange in combination with atomic absorption spectrophotometry to study metal-organic interactions. Slowey and Hood (1971) applied dialysis and solvent extraction to separate different forms of zinc in seawater and found that 70% of the dissolved zinc was dialysable. They concluded that the non-dialysable zinc was associated with organic matter. Rona et al. (1962) studied the chemical forms of zinc and manganese in sea-water, by using dialysis and ultrafiltration. They found that a high percentage of zinc and manganese exists in sea-water in the form of high molecular-weight complexes.

Since these separation methods cannot differentiate free metal ions (hydrated) from small metal complexes, they are not useful for chelation measurements and stability-constant calculations. There are also several experimental difficulties (besides time-consuming) associated with their use such as adsorption on the

membrane, container wall, and porous backing which will result in erroneous conclusion. Metal contamination by dialysis tubes is also a vexing problem (Florence and Batley), (1977). The detection device most commonly used for the final analyses is atomic absorption spectrophotometer (AAS). Although the sensitivities of an electro-thermal graphite furnace AAS are adequate for the levels of metals present in natural waters, the matrix interference problem should not be overlooked.

Direct physicochemical measurements

Differential pulse polarography (DPP) and differential pulse anodic stripping voltammetry (DPASV) have been used to study speciation and metal ion complexation in waters. Odier and Plichon (1971) studied the shift in polarographic half-wave potential with change in pH of natural waters. The shifts in the half-wave potential were related to the nature and stability of complex metal species present. Other workers have used the position, height and shape of anodic stripping voltammetric (ASV) peaks to make similar calculations on the chemical forms of copper, lead and zinc in natural waters (Matson, 1968 and Ernst et al. 1975).

The use of polarographic or ASV methods for measuring the binding of metals by organic ligands usually involves titration of the ligand with metal ion, or vice versa, under conditions held constant. If the redox potentials of the metal ions shift according

to the ion-to-ligand concentration ratio, the shifts may be used to determine stability constants and coordination numbers of the complexes (Wilson et al. 1980). Alternatively, reductions in the limiting currents of the metal ions in the presence of increasing amounts of ligand may be used to estimate stability constants.

While these shifts have been successfully used to determine metal-ligand stability constants in synthetic solutions, it is extremely doubtful whether this technique can be extended to trace heavy metals in natural waters. Besides having very low concentrations of the metal under study, the natural systems contain a wide variety of complexing agents. The presence of these complexing agents such as humic matter and other surfactants have caused great difficulty in DPP and DPASV measurements of free metal ion concentration (Bresnahan et al. 1978; Greter et al. 1979; Buffle et al. 1979).

Potentiometry with ion selective electrodes (ISE) offers another direct electrochemical technique to differentiate between chemical forms of metals in natural waters. It measures the ion activity in solution. If the activity is equated to the free metal ion concentration, then the ISE can be used to distinguish between free metal ions and complexed species. The usefulness of ISE for chemical speciation and chelation measurements has been widely accepted. Reliable data from ISE measurements have been accumulated by many research groups notably Weber's, Buffle's and Gamble's (Bresnahan et al. 1978, Saar and Weber, 1979, 1980;

Buffle et al 1977, 1980; Gamble et al. 1980).

The problem with ion selective electrodes is inadequate sensitivity. Its lowest limit of detection is about 10^{-7} M whereas the upper limit of metal concentrations in unpolluted waters is 10^{-7} M. The electrodes are also subject to interferences from sulphide and iron, for example. Adsorption of surfactants onto the membrane may also be a problem in natural waters although Buffle et al. (1977) did not find any adsorption of HA and FA in Cu, Pb and Cd ion selective electrodes.

Multicomponent Kinetic Method

This promising method developed in this and other laboratories makes use of the capability of kinetic methods to differentiate between closely related components of mixtures by measuring the difference of reaction rates of the components with a common reagent. Consequently, under suitable conditions, this method can be applied to determine simultaneously the concentrations of the various compounds (or species) present in a multicomponent system. The rate constant becomes a parameter of qualitative analysis (Margerum et al. 1965, 1969, Pausch and Margerum, 1969; Willis et al. 1970; Langford, 1973, 1975, Langford et al., 1975, 1976; Mak et al. 1977).

Consider the following reactions, where A, B...N are the reactive components of a mixture and R a common reagent present in large excess, i.e., $[R]_0 \gg [A]_0 + [B]_0 + \dots + [N]_0$. Assume that R reacts with A...N by pseudo first-order processes.

The concentration of the product p at any time t is:

$$[P]_t = [A]_0 (1 - e^{-K_A t}) + [B]_0 (1 - e^{-K_B t}) + \dots + [N]_0 (1 - e^{-K_N t})$$

where $[A]_0$, $[B]_0$, ..., $[N]_0$ are the initial concentrations of the species. Experimentally, the change of $[P]_t$ is normally monitored by observing parameters such as absorbance, conductivity, or fluorescence.

Using a stopped-flow fast kinetic method, Fe(III) species in the presence of complexing agents such as glutathione and fulvic acid have been identified and conditional stability constants calculated (Khan and Langford, 1976; Langford and Khan, 1975). Mak (1980) has successfully analysed different species of Al(III) in Al(III)-citrate mixture and Al(III)-FA mixture using a laboratory-built stopped-flow spectrofluorimeter which has a higher sensitivity.

The main advantage of this technique for speciation is that no prior separation is required. Sample volume can be as small as a few millilitres and the speed of analysis is fast. The main setback is that the rate constants of different components have to be well separated (an order of magnitude). The problem

of sensitivity can be solved by using fluorimetry as the monitoring mode. This can bring the detection limits of metal ions to a level close to or at natural waters.

It is evident from the brief discussion above that no one technique is perfect and that all results must be treated with caution. A more informative and comprehensive approach is to apply two of the techniques to each sample whenever this is possible.

In this work, two techniques were chosen for use - potentiometry with ion selective electrodes to study Cu(II)-FA and Mg(II)-FA complexations and stopped-flow spectrofluorimetric method to study Mg(II)-FA complexations. The reasons for choosing the former are, the measurements are rapid, non-destructive, no sample pretreatment necessary, inexpensive equipment setup and its capability to measure free ion activity. The reasons for selecting the stopped-flow kinetic method for Mg(II) complexation studies are the rapidity of measurements, high sensitivity, no pre-separation necessary and its availability in the laboratory.

CHAPTER 3

EXPERIMENTAL METHODS AND MATERIALS

This chapter describes the analytical and experimental techniques used in this study. Some experimental results from the characterization of the fulvic acid samples are also presented here.

REAGENTS AND MATERIALS

All commercial chemicals used in this research were Analytical Reagent Grade. Solutions were prepared in doubly-distilled deionized water, with the second distillation from alkaline potassium permanganate solution.

The glassware and storage containers used were pyrex glass and polyethylene (Nalgene) bottles, and were previously leached with 1:1 nitric acid overnight.

Copper stock solutions were prepared from cupric chloride anhydrous (Fisher Certified Reagent) and magnesium stock solutions were prepared from magnesium sulfate anhydrous (Baker Analyzed Reagent). Both salts were previously dried at 110° C overnight.

Fulvic acid was extracted from the Bh horizon of the Armadale soil, an imperfectly drained Podzol in Prince Edward Island, Canada. Extraction procedure will be discussed later in this chapter. Fulvic acid (FA) stock solutions were stored under N₂ gas using refrigeration. New

solutions were prepared every three or four days.

The fluorescent reagent - 8-Hydroxyquinoline-5-sulfonic acid (Aldrich Chemicals) was recrystallized from water many times and dried at 110° C (Pal and Ryan, 1969; Ryan and Pal, 1969). The solution was freshly prepared by dissolving 0.030 g in 250 mL of $\text{NaHCO}_3/\text{NaOH}$ pH 10 buffer solution (Weast, 1976). The pH 10 buffer solution was made up of 50 mL of 0.05 M NaHCO_3 and 10.7 mL of 0.1 M NaOH.

Atomic absorption analytical standard solutions were prepared from 99 + % pure metals or metal oxides in Ultrex nitric acid. Standard Na and K solutions were prepared from their analytical grade chloride salts.

For pH calibrations, AnaChemia standard buffers were used.

INSTRUMENTS AND APPARATUS

The measurements of pH were made either with an Orion model 701A Digital Ionalyzer meter using a Beckman # 41263 glass electrode and a Fisher calomel reference electrode (Cat. No. 13-639-52) or with a Fisher Accumet pH meter model 620 using a Fisher microprobe combination electrode (Cat. No. 13-639-92).

In potentiometric titrations, the potentials of the metal ion solutions were measured with the Orion model 701A Digital Ionalyzer meter using either a divalent cation-selective electrode (Orion model 92-32) for magnesium-fulvic acid chelation studies or a cupric ion-selective electrode (Orion model 94-29) for copper-fulvic acid chelation studies. The Fisher calomel electrode (Cat. No. 13-639-52) was used.

as a reference electrode for both studies.

The titration vessel was a 250 mL pyrex beaker stoppered with a rubber stopper through which the pH microprobe combination, ion-selective and the calomel reference electrodes were inserted. Three additional holes allowed the addition of metal ion solutions, the passage of N_2 gas and the insertion of the tip of the buret which delivered sodium hydroxide (carbonate-free) or nitric acid for pH adjustment.

For light scattering studies, a Brice-Phoenix 2000 series Universal Light Scattering Photometer (Phoenix Precision Inst. Co.) equipped with a 5 MW He-Ne Laser (Spectra Physics) providing radiation source at 633nm was employed (see Fig. 9 on page 74). The sample cell was a semi-octagonal quartz cell, thermostated at a temperature of $25.0 \pm 0.2^\circ \text{C}$. Signal was transmitted to a Fisher Recordall series 5000.

Kinetic experiments were done using a stopped-flow spectrofluorimeter fabricated in this laboratory (Mak, 1980) and was used with slight modification (see Fig. 11 on page 80). The stopped-flow cell measuring 2x2 mm cross section was custom fabricated in quartz by Hellma of West Germany (Mak, 1980). The reacting solutions could be maintained at a desired temperature by the thermostated water-bath. The fast kinetic signals could be stored in a minicomputer (Nova 1220, Data General, courtesy of Dr. B. Hollebome, Carleton University) or in a model 549 storage oscilloscope (Tecktronix Inc., Portland, Ore.) with hard copies obtained using a polaroid camera. The data stored in the minicomputer could be retrieved via paper tape and analyzed in the Carleton University mainframe.

Sigma-Honeywell computer by using library programs such as LFIT which fits data to a straight line by the method of least-squares or NONLINEAR which calculated non-linear regression coefficients.

All flameless graphite furnace atomic absorption analyses were done on a Perkin-Elmer model 603 equipped with a Heated Graphite Atomizer, model 76B, and a Deuterium Background Corrector. Pyrolytic-coated graphite tubes were used in the atomizer. A Jarrel-Ash atomic absorption spectrophotometer, model 82 5000 (Fisher Scientific Co., Waltham, Mass.) was used for flame emission analysis for Na and K. Acetylene and air were used as fuel and oxidant respectively.

EXPERIMENTAL PROCEDURES

EXTRACTION AND PURIFICATION OF FULVIC ACID

The ideal solvent or extractant for FA should dissolve practically all of the fulvic material without altering its physical and chemical properties. Of the large number of solvents that have been tested, dilute aqueous NaOH remains the most commonly used and quantitatively the most effective reagent for extracting humic substances from soils or sediments. Despite the criticisms that alkaline extraction might modify the humic material (Bremner, 1950; Dubach et al., 1964), many workers have presented evidence showing that the damaging effects might have been exaggerated (Forsyth, 1947; Schnitzer and Skinner, 1968).

The FA used throughout this research work was prepared in our

research group (Underdown, 1982) using the facilities of the Chemistry and Biology Research Institute of the Department of Agriculture, Ottawa, Canada, under the supervision of Dr. D.S. Gamble. The method of extraction and purification was modified from Schnitzer and Desjardins, 1962; Schnitzer and Skinner, 1963 and is given as follows;

An air dried soil sample of about 2 kg was weighed into 20 L of 0.5 N NaOH, mixed well and extracted overnight. The sand and clay were then removed by centrifugation. Two cation exchange columns namely; Roughing column (1 m x 8.5 cm diameter) and Finishing column (55 cm x 4.6 cm diameter) were prepared beforehand by filling them with Dowex 50W-X8 of 20 to 50 mesh size. The centrifuged liquid was then run through the Roughing column with H^+ form ion exchange resin, at a flow rate of about 1 drop per sec. This neutralizes all of the NaOH, and removes most of the Na^+ . If the effluent pH exceeded 7, then the column would require regeneration. The effluent from the Roughing column was put through the Finishing column at a flow rate of about 1 drop per sec. This procedure was repeated until the effluent from the Finishing column gave negative flame test for Na^+ . The ion exchanged solution was analyzed for total ash and other common metals by atomic absorption. The solution was then freeze dried after the metal concentrations were proven acceptable and the dried powder recovered, stored in an air tight container under N_2 in a desiccator.

ATOMIC ABSORPTION SPECTROPHOTOMETRIC ANALYSES

Trace metal contents of the fulvic acid were analyzed by a Perkin-Elmer flameless AA model 603 courtesy of Dr. C.L. Chakrabarti at Carleton University. The metal analyzed were Al, Cu, Mg, Fe, Pb, Cd and Zn. Flame emission was used for the Na and K measurements.

Sample solutions were delivered into the graphite tube via an eppendorf pipet fitted with disposable plastic tips. Volumes of 5 μ L or 10 μ L sample solutions were delivered. A calibration curve was first prepared for each metal from the standard solution. The metal ion content of the FA was then read off this calibration curve. Fig. 5 and Fig. 6 illustrate some typical calibration curves for Fe and Al by AA and Na and K by flame emission. The instrument settings for graphite furnace atomic absorption spectroscopy and flame emission spectroscopy are listed in Tables 6 and 7. The results of all the measurements are shown in Table 8. The levels of metal concentrations as listed were regarded as acceptable and was available for experimental work.

FLUORESCENCE STUDIES

A Perkin-Elmer model 204S Spectrofluorimeter was used for fluorescence measurements. The readings were taken either on the meter or recorded on a strip chart recorder (model hp. Mosely 71008). Fused quartz cuvettes of 10 mm pathlength were employed. The slit selector for both the excitation and emission radiations were 10 nm.

After a sample cell was inserted into the sample cell compartment,

Fig. 5. Calibration Curves for Fe and Al by AA.

Fig. 6 . Calibration Curves for Na and K by FE (Flame Emission).

Table 6

Instrument Settings for Graphite Furnace Atomic Absorption Spectroscopy *

Element	Drying Temp., C	Charring Temp., C	Atomization Temp., C	Wavelength used (nm)
Al	150	650	2700	309.3
Cu	150	350	2700	324.7
Mg	150	500	2700	285.2
Fe	150	650	2700	248.3
Pb	130	350	2100	283.3
Mn	150	550	2700	279.5
Cd	130	200	2100	228.8
Zn	130	350	2100	213.9

* The times for drying, charring and atomization were 30, 25 and 7 sec respectively.

Table 7

Instrument Setting for Flame Emission Spectroscopy

<u>Element</u>	<u>Wavelength</u> (nm)	<u>Fuel and</u> <u>Oxidant*</u>
Na	589.0	C ₂ H ₂ , air
K	766.5	C ₂ H ₂ , air

* Fuel and oxidant pressures were adjusted to give a clear blue flame.

Table 8

Results of metal ion concentrations from AA Analysis

<u>Element</u>	<u>Concentration^a</u> <u>(mmole/g FA)</u>
Al	1.9×10^{-2}
Cu	ND
Cd	ND
Fe	3.0×10^{-2}
K	$< 2.6 \times 10^{-3}$
Mg	3.7×10^{-3}
Mn	ND
Na	6.5×10^{-2}
Zn	$< 1.5 \times 10^{-3}$
Total ash	0.6 %

Total ash was obtained with Pt dishes

^a Experimental error in the above AA analysis is $\pm 3.5\%$

the optimum excitation and emission wavelengths were set to give the fluorescence intensity reading. If a scan of excitation spectrum was required, the emission wavelength was set at an optimum wavelength, and the excitation wavelengths were varied automatically usually from 200 nm to 600 nm. When an emission scan was required, the excitation wavelength was set at an optimum value, and the emission wavelengths were varied automatically from the set excitation wavelengths to the desired wavelength. The spectra were recorded on the strip chart recorder.

For fulvic acid, excitation and emission spectra were run for two different concentration of 100 mg/L and 10 mg/L.

Excitation and emission spectra for 8-Hydroxyquinoline-5-sulfonic acid and its complex with Mg(II) were also obtained for characterization of the fluorescing properties.

POTENTIOMETRIC TITRATIONS

Copper(II)-Fulvic Acid (FA) Titrations

A calibration curve for each set of titration experiment was obtained first by titrating Cu(II) standard solutions of 1.0×10^{-3} M into a 100 mL aliquot of doubly distilled deionized water. A Gibson P200 Pipetman variable micropipet or an eppendorf micropipet was used as a dispenser. The calibration curve usually covered a Cu(II) concentration range of 2.50×10^{-6} M to 6.50×10^{-4} M. The pH of the titrating solution was initially adjusted to and maintained at 6.00 ± 0.02 by adding

suitable amount of dilute NaOH or HCl solutions of known strength via a kimax microburet (5.00 mL capacity). Since the tip of the buret was connected to an eppendorf yellow pipet tip and immersed into the liquid, very small amounts of dilute acid or base could be added for fine pH adjustment. Corrections for dilution were made when necessary. In order to avoid over-dilution, after 10.00 mL of $1.0 \times 10^{-3} \text{ M}$ Cu(II) standard had been titrated, $1.0 \times 10^{-2} \text{ M}$ Cu(II) standard solution was then used. A stream of inert N_2 gas was bubbled through the solution during the titration and also half an hour before the titration. The solution was stirred continuously at a constant rate with a magnetic stirrer and maintained, with a thermostated water bath at $25.0 \pm 0.2^\circ \text{C}$. Both the pH and the potentials measured to $\pm 0.1 \text{ mV}$ were carefully monitored and recorded. For titrations of higher ionic strengths, KCl solution was used in preparing the solutions, so that all solutions involved in the titrations would have the same ionic strength.

The copper ion selective electrode (ISE) used was an Orion Cupric ISE model 94-29. This electrode is based on the easily fabricated, low resistance, selectively permeable cast-disk and pressed-pellet mixed $\text{CuS-Ag}_2\text{S}$ membrane. The Nernstian response is given by the equation

$$E = E_{\text{constant}} + \frac{0.05916}{2} \log [a_{\text{Cu}^{2+}}]$$

where $a_{\text{Cu}^{2+}}$ represents the activity of Cu^{2+} ion. It predicts a slope of 29.58 mV/decade at 25°C , and a constant intercept for a curve constructed by plotting electrode potential vs. Cu^{2+} ion concentration in a

pure, highly dilute aqueous solution. This curve is of analytical importance, as unknown concentrations can be estimated by referring measured voltages to this curve. The electrode shows a Nernstian response for Cu^{2+} over a region of metal concentration 10^{-7} M to 1.0 M. Fig. 7 shows the electrode response to changes in copper ion concentration of a typical titration range.

In the Cu(II) -FA titrations, two different concentrations of FA were used. They were 10 mg/L and 100 mg/L solutions and were prepared in doubly distilled deionized water from solid FA samples. After filtering through 0.40 μm polycarbonate unipore filter (Bio-rad), a 100 mL aliquot was taken in the titration beaker and purged with N_2 gas for at least half an hour. The titration was then carried out exactly the same way as for the calibration curve. To avoid cross-contamination, a different titration beaker was used for FA titrations. For higher ionic strengths, all solutions including the FA solutions were prepared in KCl of desired ionic strengths. The pH and potential were again carefully monitored and recorded. For proper response the ISE electrode required frequent polishing. Normally after the addition of Cu(II) standard solutions and subsequent pH adjustment, a time lapse of at least five minutes was required to allow the potential readings to stabilize as reported by Truitt and Weber (1981). The titration vessel and the exclusion of artificial light during the titrations were measures taken to avoid unnecessary degradation of humic materials. Wershaw and Pinckney (1977) have reported that humic acids in alkaline

Fig. 7. Electrode Response to Change in Cu^{2+} Concentration.

solutions are oxidized by both air and molecular oxygen. They have suggested that oxidation of phenolic groups and carbonyl groups is taking place along with ring opening and cleavage of other bonds. They have further found that visible radiation stimulates oxidative degradation of humic acids. Whether these degradations would take place in near neutral dilute fulvic acid is not certain.

A replicate of at least three runs were done for each titrations, in most cases. All the data were then averaged using the polynomial least squares program - TE04 (courtesy of Dr. D.S. Gamble, Agriculture Canada) which was modified to be used in the Carleton University mainframe computer. The TE04 program is a FORTRAN IV computer program which fits data to a polynomial by the method of least squares. It will also print out the first derivatives of the data (see appendix).

An attempt to titrate FA at pH 8 was not successful due to hydrolysis of copper ions. Instead, a set of titrations were done without controlling the pH, i.e., the titrations were done around 3.6, the pH of the 100 mg/L FA solutions.

Magnesium(II)-Fulvic Acid Titrations

The Mg(II)-FA titrations followed the same procedure as the Cu(II)-FA titrations.

For Mg(II)-FA titrations, only one FA concentration of 100 mg/L was used. The titrations were done at three different pH's, namely 6, 8 and 10. A $1.0 \times 10^{-2} M$ Mg(II) standard solution was used in the

calibration as well as the actual titrations. Because of the nature of the membrane, the potential readings of the Mg(II) ion selective electrode, ISE (divalent cation) were less stable than the solid state Cu^{2+} ISE, and it took longer time (typically about 15 minutes) to stabilize after each addition of titrant. Before the titrations, the Mg(II) ISE or the Mg electrode sensing module was soaked in Mg^{2+} ion solution at pH 10 overnight. This procedure was performed to condition the divalent cation electrode so that it would be sensitive to the Mg^{2+} ion. At the end of a titration with fulvic acid, it was found that by soaking the reference and pH combination electrodes in a high pH buffer (e.g. pH 10) for a short period of time, the potential or pH readings became more stable. This was attributed to the removal of adsorbed FA on the liquid membrane of the electrode.

The Mg^{2+} or divalent cation-selective electrode used in the Mg(II)-FA titrations is different from the cupric ISE used earlier. It is a type of liquid-membrane electrode consisting of two concentric cylindrical tubes constructed of inert plastic (Fig. 8). The inner tube holds the internal reference electrode and an aqueous electrolytic solution containing the ion of interest. The outer compartment contains a charged or neutral organic ion-exchanger dissolved in an organic solvent that wicks into a thin hydrophobic membrane; this now forms a thin organic-membrane phase separating two aqueous solutions. The membrane may be, for example, a cellulose-acetate Millipore filter. An ion-exchange equilibrium is set up at both the inner and outer surfaces

Fig. 8. Schematic Representation of The Construction of a Liquid-Membrane

ISE

of the membrane, and the difference in activity of the ion of interest in the inner electrolyte and outer test solutions give rise to the potential response of the electrode.

The selectivity and sensitivity of this type of electrode is determined primarily by the selectivity of the particular organic ion-exchanger for the ion of interest and, secondarily, by the organic solvent used to dissolve the exchanger. The extent of interference is related to the stability of the complex formed between the ion and the ion-exchanger in the membrane, and the mobility of the complexes within the membrane.

The ion-exchanger in a divalent cation ISE is phosphate diesters ($(RO)_2PO_2^-$ with R groups in the C_8 to C_{16} range) dissolved in n-decanol as solvent. The detection limit of the Orion 92-32 is reported to be 6×10^{-6} M (Vesely et al., 1978). Since the lowest concentration of Mg^{2+} in the titrations was about an order of magnitude higher than the detection limit, the titrations could be considered reliable.

LIGHT SCATTERING EXPERIMENTS

When a beam of light is directed at a colloidal solution or dispersion, the light may be absorbed, scattered or transmitted undisturbed through the sample. All materials are capable of scattering light (Tyndall effect) to some extent. The noticeable turbidity associated with many colloidal solutions is a consequence of light scattering.

The intensity, polarization and angular distribution of the light scattered from a colloidal solution depend on the size and shape of the scattering particles, the interactions between them, and the difference between the refractive indices of the particles and the medium. Light scattering measurements are therefore, of great value for estimating particle size, shape and interactions (Shaw, 1970; Stacey, 1956). Since humic materials are known to be moderately high molecular weight polymers, it will be of interest to study the interaction between fulvic acid and a metal ion such as Mg^{2+} , and any subsequent change of particle size or aggregation by light scattering.

Apparatus necessary for light scattering measurements were described earlier in the chapter (Fig. 9). The semi-octagonal cell (Cat. No. D-101) measured 40 x 40 mm, and was scrupulously cleaned, inside and outside with detergent solutions before use. It was then rinsed thoroughly with doubly distilled deionized water and finally with reagent grade acetone, covered with "Kimwipes" wipers and left to dry. For temperature control, the cell was wrapped with Tygon tubing and taped into place with masking tape leaving a gap for incident and scattered light beams. The Tygon tubing was then attached to a thermostatically controlled water bath outside set at $25.0 \pm 0.2^\circ C$. The fulvic acid, Mg^{2+} ion solution and dilute HCl or NaOH solutions were all filtered through 0.4 μm unipore filters. The importance of adequate clarification of solutions cannot be overemphasized. A poorly clarified solution would give a noisy, erratic and non-reproducible

A THE RADIATION SOURCE IS REPLACED BY A 5 MW He-Ne LASER IN THIS WORK

B A STRIP CHART RECORDER IS USED

FIG. 9 . A Brice Phoenix Universal light scattering photometer

signal. A convenient visual test is to shine the laser light through the solution. Floating particles are seen as bright specks when viewed in the direction perpendicular to the laser beam.

An aliquot of 100 mL FA (0.100 g/L) was transferred into the sample cell and flushed with N_2 for half an hour with the cover on. The pH was adjusted to the desired value with a syringe through one of the holes in the cover of the cell. The other two holes were for the insertion of N_2 gas and combination pH microelectrode which was connected to the Fisher Accumet digital pH meter.

After purging with N_2 for half an hour, the recorder was turned on and the angle of the photometer was set at 0° by adjusting with the shutter(s) closed. After 10 minutes, the N_2 flow was stopped. The baseline was recorded for about five minutes at 0° angle with the shutter remaining closed. The shutter was then opened (with appropriate filter in place). The pen of the recorder should deflect about half scale. A scan (for FA with no Mg^{2+} ion in) was made for about two minutes. These recordings of the baseline and blank (FA) were done for all angles measured, namely 45° , 90° and 135° .

After measurements were made for all the four angles, the shutter(s) was closed and the N_2 gas turned on. A small aliquot of 0.50 mL of filtered Mg^{2+} solution ($1.00 \times 10^{-2} M$) was added through a buret. The pH was readjusted to the original pH using filtered dilute NaOH or HCl solution.

A scan of approximately ten minutes was made with the shutter closed

and N_2 gas on. Then the N_2 gas was turned off and a scan of about five minutes was made with the shutter opened (and appropriate filters in place). These measurements were made at 0° , 45° , 90° and 135° for both baseline and sample. The procedure was repeated until 10 millilitres of Mg^{2+} (0.50 mL at a time) were added.

The signals measured from the baselines to the peaks could be used for calculations of turbidity, Rayleigh scattering ratios and dissymmetry ratios of the sample solutions.

STOPPED-FLOW KINETIC EXPERIMENTS

The stopped-flow apparatus used has incorporated features that were drawn from reported designs as well as novel elements.

In the conventional architecture of most stopped-flow apparatus, there are three main sections; (a) the driving mechanisms, (b) the mixer, and finally, (c) the stopping mechanism (Fig. 10). With the advent of light guides, a second generation design, the Canterbury type stopped-flow apparatus (Caldin et al., 1973) emerged. The great advantage of this approach is in the improvement of temperature control since the stopped-flow unit is a totally separated unit detached from the spectrometer and the mixing unit (of the stopped-flow) can be thus wholly immersed in a thermostated bath. In previous designs, overall thermostating was achieved by a combination of immersion of the reactant reservoirs into a thermostated bath and simultaneously circulating the thermostated liquid in tubing around the mixer. This method was less

FIGURE 10

Fig. 10. Conventional architecture of a stopped-flow apparatus.

1. Pneumatic or manual drive unit
2. Driving syringes
3. Reactant reservoirs
4. 3-way T-taps
5. Mixer (2 jets T-mixer or multi-jets tangential mixer)
6. Outlet for circulating thermostating fluid
7. Transmitted radiation (monitor mode- absorption)
8. Photomultiplier placed inline with excitation and transmitted radiation (absorption mode)
9. Photomultiplier placed 90° to excitation radiation (fluorescence)
10. Emission radiation (90° to excitation)
11. Thermostating fluid circulating tubing
12. Inlet for circulating thermostating fluid from an external bath
13. Incident excitation radiation
14. Excitation Source
15. Effluent exhaust
16. Stopping syringe
17. Oscilloscope display
18. Microswitch trigger (initiation of data acquisition coinciding with the stoppage of flow)
19. Micrometer (adjustment of the position of the microswitch)

desirable due to the length of time required for temperature equilibration. At the time of construction of our stopped-flow instrument, due to the unavailability of efficient UV light guides, its use was not implemented. But a good part of the thermostating feature of the Canterbury instrument was adapted (Mak, 1980). The features of the new design are detailed in Fig. 11.

In this present design, the original observation compartment of an Aminco-Bowman Spectrofluorimeter was removed and the stopped-flow unit with its own observation compartment was attached easily to the fluorimeter via four bolts fastened to the top of the excitation monochromator housing. There is no alteration to the original mechanical and optical configurations of the fluorimeter. The original electronics are modified in order to handle the fast rise time of the kinetic signal. For further information on the instrument, the thesis of Mak(1980) could be consulted.

In every kinetic run, the concentration of the reagent (i.e. 8-Hydroxyquinoline-5-sulfonic acid) was at least ten times in excess of the Mg^{2+} concentration so the pseudo first-order kinetics could be assumed. After some studies on the time dependence of the complex formation, it was established that an experimental infinity was adequately achieved after the reaction mixture was allowed to equilibrate for about 10 min.(Table 9). In each set of stopped-flow experiments, a minimum of at least five runs were recorded and processed for the purpose of data averaging. All the experiments were conducted at a temperature of

SIDE
VIEW

FIGURE 11.

ELECTRONIC

BLOCK DIAGRAM

Fig. 11. Stopped-flow Aminco-Bowman spectrofluorimeter

i. Stopped-flow apparatus;

- A. Threaded piston shaft
- B. Stainless steel cylinder (mounted on A)
- C. Microswitch trigger (mounted on the back side of the pneumatic drive casing)
- D. Compressed air inlet from the actuation unit
- E. Pneumatic Drive unit (borrowed from a Durrum stopped-flow drive assembly/part 16199)
- F. Compressed air outlet to the actuation unit
- G. Driving syringes (2 ml B-D)
- H. 3-way T-taps
- I. Reactant reservoirs
- J. Thermostating fluid reservoir
- K. Extra length of heat shrunken PVC tubing serving as thermostated reactant reservoirs
- L. Outlet for thermostating fluid from an external bath pumped into the thermostating fluid reservoir through (M)
- M. Inlet for thermostating fluid pumped in from an external bath
- N. Brass tubings encasing the heat shrunken PVC reactant delivery tubes/ vertical positioning of the cell holder (Q) can be achieved by sliding these brass tubings up and down (P)
- O. Overflow outlet for the thermostating fluid reservoir/back to the external bath via a peristaltic pump
- P. A rotating disc mounted on the bottom of the cell holder (Q)
- Q. T-mixer cell holder with a cavity at the top allowing thermostating fluid coming in from (M) to circulate around the top part of the T-mixer and exit via (L)
- R. Photomultiplier mounted 90° to the excitation radiation
- S. Emission radiation (fluorescence mode)
- T. Excitation radiation
- U. T-mixer (manufactured by Hellma of W. Germany)
- V. Excitation source (150W Xenon lamp)

- W.. A length of tapered heat shrunken PVC tubing acting as the exhaust for the reacted mixture
- X. Thermometer placed in the neighborhood of (L) for an accurate temperature display of the thermostating fluid reservoir
- Y. Excitation monochromator housing on which the stopped-flow apparatus is mounted

ii. Electronics

- 1. Preamplifier
- 2. Main amplifier
- 3. Differential voltmeter (DVM)
- 4. Analog-digital-converter(ADC) (manufactured by computer data conversion system, model analogic, AN5800)
- 5. Minicomputer manufactured by Data General (Nova 1220)
- 6. Teletype terminal
- 7. Paper tape puncher
- 8. Oscilloscope display
- 9. Analog control unit which includes an actuation unit for the pneumatic drive, a digital counter, and the triggering circuit for the ADC and oscilloscope

2

Table 9

Time Dependence Study of Fluorescence Intensity

<u>Time</u>		<u>Fluorescence Intensity</u> (Arbitrary Units)
		0.0
1.	min	20.6
3.	min	20.1
6.	min	20.4
9.	min	20.6 ^a

a No significant change in intensity was observed after 24 hr.

* $[\text{Mg}^{2+}]_{\text{eff}} = 1.5 \times 10^{-6} \text{ M}$

$[\text{REAGENT}]_{\text{eff}} = 5.0 \times 10^{-5} \text{ M}$

* Denotes the effective concentration of the species after mixing

25.0±0.2° C.

The following procedures were followed for all the kinetic experiments observed with the stopped-flow spectrometer. An experimentally prepared blank was first loaded into the observation cell through one of the reservoirs and syringes. The baseline emission was displayed on the oscilloscope and the digital voltmeter (DVM). The voltage suppression circuit in the main amplifier was adjusted to offset the voltage due to the blank. The blank was then displaced by an "experimental infinity" solution of Mg^{2+} - reagent (8-hydroxyquinoline-5-sulfonic acid) (HQSA) system. A combination of the gain circuits of the pre-amplifier and main amplifier were adjusted to just below 10 volts. The cycle was repeated until the electronics and the oscilloscope were conditioned so that the magnitude of the corrected signal was approximately 9.5 volts, i.e. about 0 volts for the blank and about 9.5 volts for the experimental infinity. An expansion to exactly 10 volts is not advisable for fear of saturating the ADC with the slightest drift of the electronics, e.g. thermal drift. The exact final magnitudes of the blank and infinity were recorded and stored as separate files in the computer or recorded on a polaroid film from the oscilloscope. This information was later retrieved and served as the value of an equilibrium recovery of the Mg species in comparison with the recovery value elucidated from the kinetic data. Besides the two amplifiers, namely the pre-amp and the main amp, it is also possible to modulate the signal by varying the high voltage supplied to the photomultiplier and the slit of the

excitation and/or emission windows.

The actual sample containing Mg^{2+} ion solution was then loaded into one of the reservoirs while the other was loaded with the fluorescent reagent in a buffer solution. On actuation, the two solutions are rapidly mixed in the cell (10ms). Simultaneously the microswitch is depressed at the stop of the drive piston which triggers the oscilloscope and the computer. A rise signal for at least 20 half-lives could be thus recorded. In the Mg-reagent system, λ excitation and λ emission were 380 nm and 500 nm respectively.

CHAPTER 4
STUDIES OF COPPER-FULVIC ACID EQUILIBRIUM
SYSTEM BY ION SELECTIVE
ELECTRODE POTENTIOMETRY

The studies of metal-organic systems normally focusses on two major aspects; one is the stoichiometric binding or complexing capacity and the other is the thermodynamic stability functions (e.g. - stability constant in simple cases). Several procedures have been proposed for the determination of binding capacity such as bioassays (Davey et al. 1973), anodic stripping voltammetry (Chau et al. 1974), ion selective electrodes (Ramamoorthy and Kushner, 1975a, 1975b) and dialysis titration (Truitt and Weber, 1981). For stability constant, there are ion exchange and continuous variation methods (Schnitzer and Hansen, 1970), ion selective electrodes (Bresnahan et al. 1978, Saar and Weber, 1979, Buffle et al. 1977, 1980, Gamble et al. 1980), anodic stripping voltammetry (Buffle and Greten, 1979), and kinetic method (Langford and Khan, 1975, 1976, Khan and Langford, 1976).

In this chapter, the binding capacity determinations and the results for Cu-FA complexation studies under various experimental conditions are presented. Because of the growing evidence that humic and fulvic acids are complex mixtures (Wershaw and Pinckney 1973, Schnitzer and Khan, 1978, Gamble et al. 1980), it was felt

that the metal-FA equilibria would best be studied utilizing the treatment of Gamble et al. (1973, 1980). The results of the calculations of equilibrium functions, the Gibbs standard energy change etc are therefore, also presented.

The method used in this work for the calculations of binding capacity and equilibrium functions were basically similar to those of Buffle (1977, 1980), Khan (1979) and Gamble et al. (1980). Detailed experimental procedure has been described in chapter 3. The results of a representative copper-fulvic acid potentiometric titration (with fulvic acid (FA) concentration = 100 mg/L, Mg^{2+} concentration = $10^{-3} M$ and at pH 6.00) are given in Table 10. The Nernstian plot ($\log [Cu^{2+}]_{total}$ vs electrode potential in millivolt) is shown in Fig. 12. The calibration curve (without FA) has a slope of 28.0 mV/decade and merges with the titration curve after about $10^{-3} M$ of total copper concentration is added. The linearity of the calibration curve could be extended down to a $[Cu^{2+}]$ of about $10^{-7} M$ with extreme care (measurements could be made for concentration as low as $10^{-8} M$ as suggested by the manufacturer but response is not linear). The region where the two curves merge is comparable to that part of a conventional titration curve past the equivalence point. The potentials are converted to concentrations from the calibration curve and these values correspond to free copper. The bound copper is obtained by difference (Table 11). The binding capacity is obtained from the plot of bound copper vs total copper (Fig. 13). A polynomial least square computer program TE0₄ (see Appendix) was used to give the best fitting curve. The binding

Table 10

The titration of Cu-FA (100 mg/L FA, pH 6.00, 10^{-3} M Mg^{2+})

Initial Volume = 100 mL

<u>mmole Cu(II)/g FA</u>	<u>Total [Cu(II)]</u> ($\times 10^5$ M)	<u>$-\log[Cu(II)]$</u>	<u>Potential (mV)</u>
0.25	2.48	4.606	48.7
0.50	4.94	4.806	67.8
0.75	7.40	4.131	78.1
1.00	9.84	4.007	87.6
1.25	12.3	3.911	95.8
1.50	16.7	3.833	102.2
1.75	17.1	3.768	108.7
2.00	19.5	3.711	114.1
2.50	24.2	3.616	123.5
3.00	28.9	3.539	130.4
3.50	33.6	3.474	135.5
6.00	38.2	3.419	141.1
4.50	42.7	3.370	145.2
5.00	47.2	3.326	148.0
5.50	51.7	3.287	151.3
6.00	56.1	3.251	153.4
6.50	60.5	3.218	155.4
7.00	64.8	3.188	157.3
7.50	69.1	3.160	159.0
8.00	73.4	3.134	160.5
8.50	77.6	3.110	161.8
9.00	81.8	3.087	163.0
9.50	85.9	3.066	164.0
10.00	90.1	3.045	165.0
11.00	99.0	3.005	166.8
12.00	107	2.967	169.5
13.00	116	2.933	170.1
14.00	126	2.901	171.5
16.00	143	2.844	173.8
18.00	161	2.794	175.6
20.00	178	2.749	177.4
25.00	222	2.655	180.8
30.00	264	2.578	183.2
35.00	307	2.513	185.0
40.00	349	2.458	186.5

Table 11

Estimated Free and Bound Copper, (II) (100 mg/L FA pH 6.00, 10^{-3} M Mg^{2+})

<u>mmole Cu(II)/g FA</u>	<u>-log Free [Cu(II)]</u>	<u>Free Cu(II)</u> ($\times 10^5$ M)	<u>Bound Cu(II)</u> ($\times 10^5$ M)
0.25	7.320	0.0048	2.48
0.50	6.650	0.0224	4.92
0.75	6.300	0.0501	7.35
1.00	5.970	0.107	9.73
1.25	5.680	0.209	12.1
1.50	5.450	0.355	14.3
1.75	5.225	0.596	16.5
2.00	5.030	0.933	18.5
2.50	4.700	2.00	22.2
3.00	4.450	3.55	25.4
3.50	4.270	5.37	28.2
4.00	4.080	8.32	29.8
4.50	3.930	11.8	31.0
5.00	3.830	14.8	32.4
5.50	3.710	19.5	32.2
6.00	3.640	22.9	33.2
6.50	3.570	26.9	33.6
7.00	3.505	31.3	33.6

Fig. 12. The Titration of Cu-FA (100 mg/L FA⁻, 10^{-3} M Mg²⁺, pH 6.00).

Upper Curve = Calibration Curve , lower curve = titration curve.

Fig. 13. Bound Copper as a Function of Total Copper (100 mg/L FA, 10^{-3} M Mg^{2+} , pH 6.00) .

capacity corresponds to the portion of the curve with zero slope. The value obtained for this particular titration is 3.6 mmole Cu(II) per g FA.

Using the same method, by titrating with copper, the binding capacities for several model ligands and natural waters are estimated and presented in Table 12. The Nernstian plots of EDTA and Ottawa River water are given in Figs. 14 and 15. The binding capacity of Ottawa River water was found to be very close to the value of $2.54 \times 10^{-5} M$ obtained by Ramamoorthy and Kushner (1975b) although the sampling sites were different. The value for humic acid was reasonable comparing to the values obtained for fulvic acid later in the chapter. As for Rideau River water, the binding capacity value obtained was not much different from Guy's (1976) value of $4.9 \times 10^{-5} M$.

The uncertainty in the free metal-ion concentration is $\pm 1.4\%$ based on a standard deviation calculation from replicate titrations. In the estimation of maximum bound copper or the binding capacity, the uncertainty is 4% also based on a standard deviation calculation of replicate runs. The effect of FA adsorption on the electrode was found to be negligible as reported by Buffle et al: (1977) and Saar and Weber (1979).

A potentiometric titration of a Cu-FA system at pH 6.00 with fulvic acid concentration of 100 mg/L and no background supporting electrolyte will now be discussed in more detail. All the calculations were based on a titration curve which was obtained from averaging

Table 12

Binding Capacity of Model Solutions

<u>Solution</u> ^a	<u>Binding Capacity</u> ($\pm 4\%$)
1.0×10^{-4} M EDTA	1.0×10^{-4} M
20 mg/L humic acid (HA)	1.6 mmole/g HA
Rideau River water	4.7×10^{-5} M
Ottawa River water	2.6×10^{-5} M

^a Humic acid was obtained from Aldrich Chemical Co. without further purification. Rideau River water and Ottawa River water were sampled near Carleton University and at Britannia Beach respectively.

Fig. 14. The Titration of Cu-EDTA (pH 6.00)

Fig. 15. The Titration of Cu-Ottawa River Water (pH 6.00).

Upper curve = calibration, lower curve = titration.

three sets of replicate runs. The results of this Cu-FA chelation equilibrium are presented in Table 13 and the Nernstian plot is given in Fig. 16. The binding capacity $3.9 \times 10^{-4} \pm 4\%$ M is obtained from Fig. 17 which then used together with the degree of ionization values from Gamble (1972) to calculate the mole fractions. The mole fractions, χ , are given in Table 14 for the purpose of describing the composition of the system. Together with temperature and fulvic acid concentration, any two of the three mole fractions will totally specify the sample composition. According to Equation 27 from chapter 2, the most useful choice for this purpose is the use of χ_C and χ_{SH_2} that is, the composition of the experimental sample is specified in terms of bidentate sites occupied, by Cu(II) and by two protons. H^+ is required operationally for experimental control only. Fig. 18 depicts the behaviour of the three mole fractions during the Cu(II) titration. χ_{SH} and χ_{SH_2} are seen to decrease differently with increasing χ_C .

With copper loadings from $\chi_C = 0.061$ to 0.664, the weighted average equilibrium function $\bar{K}_4$ values in Table 15 and Fig. 19 decrease by two orders of magnitude. The $\bar{K}_4$ values obtained here are in good agreement with the values calculated by Gamble, et al (1981) from data obtained on soil FA at the same pH and about the same FA concentrations by Bresnahan, Grant and Weber (1978), and Bresnahan (1977). They also agree reasonably well with values given by Gamble et al. (1970, 1973) although they were determined at different pH values. The dependence of $\bar{K}_4$ values on χ_C is predicted

Table 13

Titration of Cu-FA (Averaged data, 100 mg/L FA, pH 6.00, no supporting electrolyte).

Initial Volume = 100 mL

<u>mmole Cu(II)/g FA</u>	<u>Total Cu(II)</u> ($\times 10^5$ M)	<u>$-\log[\text{Cu(II)}]$</u>	<u>Potential</u> (mV)
0.25	2.37	4.625	14.1
0.50	4.72	4.326	37.0
0.75	7.05	4.152	52.5
1.00	9.35	4.029	64.9
1.25	11.6	3.934	75.5
1.50	13.9	3.857	84.8
1.75	16.1	3.792	93.2
2.00	18.4	3.736	100.9
2.50	22.8	3.643	114.7
3.00	27.1	3.567	126.7
3.50	31.4	3.504	135.0
4.00	35.6	3.449	141.2
4.50	39.7	3.401	145.9
5.00	43.8	3.358	150.0
5.50	47.9	3.320	153.4
6.00	51.9	3.285	156.2
6.50	55.9	3.253	158.9
7.00	59.9	3.223	161.1
7.50	63.8	3.195	163.1
8.00	67.7	3.170	164.7
8.50	71.5	3.145	166.1
9.00	75.3	3.123	167.3
9.50	79.1	3.102	168.2
10.00	82.8	3.082	139.5

Fig. 16. The Titration of Cu-FA (Averaged Data, 100 mg/L FA, pH6.00, no supporting electrolyte). Upper curve = calibration, lower curve = titration.

Fig. 17. Bound Cu as a Function of Total Cu. (100 mg/L FA, pH 6.00, no supporting electrolyte)

Table 14

Mole Fractions of Cu-FA titration (100 mg/L FA, pH 6.00, No supporting electrolyte).

<u>mmole Cu(II)/g FA</u>	<u>χ_c^*</u>	<u>χ_{SH}^*</u>	<u>$\chi_{SH_2}^*$</u>
0.25	0.061	0.892	0.047
0.50	0.121	0.836	0.043
0.75	0.181	0.779	0.40
1.00	0.239	0.723	0.037
1.25	0.297	0.667	0.036
1.50	0.356	0.610	0.033
1.75	0.410	0.562	0.088
2.00	0.469	0.505	0.086
2.50	0.574	0.405	0.021
3.00	0.664	0.318	0.018
3.50	0.744	0.244	0.013
4.00	0.808	0.183	0.009
4.50	0.862	0.132	0.007
5.00	0.905	0.090	0.005
5.50	0.933	0.063	0.003
6.00	0.951	0.046	0.002
6.50	0.962	0.037	0.002
7.00	0.974	0.024	0.001

* χ_c = mole fraction of chelated sites

χ_{SH} = mole fraction of singly ionized chelation sites

χ_{SH_2} = mole fraction of protonated chelation sites

Fig. 18. Mole Fractions of un-ionized, ionized, and complexed Chelation Sites. (100 mg/L FA, pH 6.00 , no supporting electrolyte).

Table 15

Weighted Average and Differential Equilibrium Functions
for Cu-FA system
(100 mg/L FA, pH 6.00, no supporting electrolyte)

mmole Cu(II)/gFA	Weighted Average, Equilibrium Function ($\bar{K}_4$)	Differential Equilibrium Function (K_4)
0.25	80.0±10%	$14.2 \times 10^2 \pm 20.5\%$
0.50	24.6	420
0.75	10.8	101
1.00	5.16	35.0
1.25	2.97	27.9
1.50	1.76	19.9
1.75	0.73	6.93
2.00	0.69	3.82
2.50	0.33	3.63
3.00	0.18	1.78

Fig. 19. The Weighted Averaged Equilibrium Function $\bar{K}_4$ as a Function of Total Cu(II) (100 mg/L FA)

by Equation 23 shown earlier in chapter 2.

The values of the differential equilibrium function K_4 were calculated using equation 19 from chapter 2. This type of calculation does not produce a state function for direct comparison of different samples, unlike the other type of calculation using Equations 35 and 36 which gives K_4 as a state function having constant χ_{SH_2} . K_4 values were readily calculated using the TE04 computer program by fitting $\chi_{SH} \bar{K}_4$ vs χ_{SH} data to a polynomial by the method of least squares. The TE04 Fortran IV program produced a print-out of the first derivative, that is, $d(\chi_{SH} \bar{K}_4)/d\chi_{SH}$. Fig. 20 illustrates a plot of $\chi_{SH} \bar{K}_4$ against χ_{SH} and Table 15 lists the values of K_4 obtained. Fig. 21 is a plot of K_4 as a function of total Cu(II). K_4 is found in this case about 10 times greater than $\bar{K}_4$. This is theoretically anticipated for low Cu(II) loadings and it also emphasizes that the difference between K_4 and $\bar{K}_4$ is not a trivial one. K_4 only, and not $\bar{K}_4$ may properly be used for the calculation of thermodynamic functions or for comparison with the stability constants of chelates having monomeric ligands. The standard Gibbs energy ΔG° thus calculated are plotted in Fig. 22 as a function of χ_c . With increasing Cu(II) loading, ΔG° values are seen to decrease systematically. This is to be expected, because in a mixture, the strongest chelate or complex will form first.

The Effect of Fulvic Acid Concentration

Many workers have noted that complexation of metals to FA increases as the pH of the solution increases (Cheam et al., 1974, Brady and

Fig. 20. A Plot of $\chi_{SH} \bar{K}_4$ vs χ_{SH} (100*mg/L FA, pH 6.00, NSE)

Fig. 21. Differential Equilibrium Function K_4 as a Function of Total Cu(II).
(100 mg/L FA, pH 6.00, NSE).

Fig. 22. Gibbs Standard Free Energies as a Function of χ_C for Cu-FA System

Pajenkopf, 1978). Some also show that heavy-metal binding by FA decreases as the ionic strength of the solution increases (Mantoura et al., 1978). The dependence of metal binding on FA concentration, however, is a variable that is largely overlooked.

In this work, the effect of FA concentration on Cu(II)-FA complexation, equilibrium system was studied. Two concentrations of FA relevant to conditions in natural waters, namely, 100 mg/L and 10.0 mg/L (Reuter and Perdue, 1977) were investigated. The results of 100 mg/L FA concentration have been reported earlier in this chapter. Two sets of experimental conditions for 10.0 mg/L FA were run. One of them was at pH 6.00 with no background electrolyte and another was also at pH 6.00 but with ionic strength μ of the solution = 0.1 M. The results of the first set will be discussed here because they were obtained under the same conditions as the 100 mg/L FA titrations presented earlier.

One of the Nernstian plots is given in Fig. 23 for run No. 4 which is typical of all the other 10.0 mg/L FA titrations. The titration curve is slightly different from 100 mg/L FA titration (Fig. 16) as the whole curve is shifted toward lower $\log[\text{Cu(III)}]$ values because of lower copper concentration used. Fig. 24 is a plot of averaged bound copper values vs Total copper. The binding capacity estimated from this plot is $2.9 \times 10^{-5} \pm 4\%$ M. The mole fractions calculated are plotted in Fig. 25. The shapes of the curves are similar to the mole fraction curves of 100 mg/L FA in Fig. 18. The decrease in X_{SH} and X_{SH_2} are seen to be different from increasing X_{C} , the former falls much faster than the latter. The equilibrium

Fig. 23. The Titration of Cu-FA (10.0 mg/L FA, pH 6.00, no supporting electrolyte)
Upper curve = calibration, lower = titration.

Fig. 24. Bound Cu as a Function of Total Cu(II) .
(10.0 mg/L FA, pH 6.00, no supporting electrolyte)

Fig. 25. Mole Fractions of Un-ionized, ionized, and Complexed Chelation Sites. (10.0 mg/L FA, pH 6.00, no supporting electrolyte)

functions $\bar{K}_4$ and K_4 were calculated and plotted against total copper (II) added in Figs. 26 and 27. Again K_4 values are seen to be higher than $\bar{K}_4$ values at low Cu(II) loadings. Table 16 gives a comparison of chelation equilibrium data obtained for the two FA concentrations. It is evident from these data that FA of higher concentration has a larger capacity and it binds more strongly than similar FA of lower concentration. Many workers have suggested that conformational changes (FA coils up to smaller apparent molecular size) accompany increase in FA concentration or the degree of aggregation is a function of FA concentration (Saar and Weber, 1979; Wershaw et al. 1977). Ghosh and Schnitzer (1980) also argue that HA and FA behave like rigid spherocolloids at high concentration and become flexible linear colloids at low concentrations on the basis of their viscosity experiment. The increase in binding capacity of higher FA concentration for Cu(II) can therefore, be explained by the fact that due to the increase in the degree of aggregation or coiling of the FA molecules, functional groups that are otherwise too distant to chelate a metal ion will approach close enough for chelation. This type of binding can occur within a single FA molecule or between different FA molecules. It is probably due to the latter type of binding (henceforth be called "Pseudo-chelation"*) that Sipos et al. (1972) reported that polyvalent

* The term "pseudo-chelation" is used for a complex of CuL_2 type (Here, L is a chelation site on a FA molecule) where the functional groups that form the chelation sites come from two separate molecules. Although customarily, chelation is used for bonding with groups of one molecule. The "pseudo-chelation" is suggested here because the interaction is believed to be stronger than between two typical monomeric ligands of CuL_2 .

Fig. 26. K_4 as a Function of Total Cu(II) Added (10.0 mg. /L FA).

Fig. 27. K_4 as a Function of Total Cu(II) Added . (10.0 mg/L FA, pH 6.00, no supporting electrolyte)

Table 16

A Comparison of Chelation Equilibrium Data Obtained
for Two FA Concentrations

FA Concentrations	Binding capacity ($\pm 4\%$)	Weighted Average Equilibrium Function ($\bar{K}_4$) $\pm 10\%$	Differential Equilibrium Function (K_4) $\pm 20\%$	$-(\Delta G + RT \ln \tau)$ (KJ/mole) $\pm 8\%$
100 mg/L	$3.9 \times 10^{-4} M$ (4.7 mmole/gFA)	80.0-0.18	1420-1.78	18.0-1.43
10.0 mg/L	$2.9 \times 10^{-5} M$ (3.3 mmole/gFA)	1.81-0.35	20.6-1.36	7.50-0.76

metal ions increase the size of the aggregates in solution and that the higher the concentration of metal ions, the larger the aggregates.

Wershaw and et al. (1977) also suggested that the degree of aggregation is a function of the metal ions present. It was shown in this

laboratory (Underdown, 1982) by the method of Rayleigh light scattering that aggregation of FA increases as a function of added copper (II).

Figs. 28 and 29 depict the increase in aggregation (R_{90} is proportional to the intensity of the scattered light) as a function of total added copper (II) for FA concentrations of 100 mg/L and 10.0 mg/L respectively from the light scattering studies.

The Effect of pH on Binding

The effect of pH on the binding of metal ions with humic substances have been studied by a few workers. Saar and Weber (1979) concluded from their studies of Cd(II) complexation with aquatic and soil fulvic acids that the stability constants increased with increasing pH. Whitworth and Pagenkopf (1979), Brady and Pagenkopf (1978), and Cheam and Gamble (1974) all reported similar results. Stevenson (1977), on the other hand, noticed that the changes in stability constants of Cu, Pb, Cd and Zn with humic acids were slight.

To investigate the effect of pH on complexation in more detail, two sets of experiments at two different pH values were carried out. The two different pH's were 3.50 and 6.00. An attempt to study this system at pH 8.00 was not successful because of hydrolysis. (Results for Mg^{2+} at pH = 8.00 appear below). The concentrations of FA were

Fig. 28. Rayleigh Scattering Ratio R_{90} as a Function of Total Cu(II) Added.
(100 mg/L FA, pH 6.00, NSE)

Fig. 29. Rayleigh Scattering Ratio R_{90} as a Function of Total Cu(II) Added.
(10.0 mg/L FA , pH 6.00, NSE)

100 mg/L in both cases. No background electrolyte was present in either of these runs.

Fig. 30 shows the change of bound copper (II) as a function of total Cu(II) at pH 3.50. These data were obtained by averaging three runs and the standard deviation was about 4%. The binding capacity calculated was 2.6 mmole Cu(II)/g FA or 2.4×10^{-4} M. The equilibrium functions calculated were plotted and shown in Fig. 31. As in previous experiments at pH 6.00, both $\bar{K}_4$ and K_4 decreased systematically with increased copper (II) added. Fig. 32 also shows the Gibbs standard free energy changes as a function of χ_c . All these indicate that the first increment of Cu(II) ion was bound by the site forming the strongest complex. A comparison of chelation equilibrium data of Cu-FA titrations at pH values 3.50 and 6.00 (data for pH 6.00 were calculated and discussed earlier) is shown in Table 17. It is obvious that FA at pH 6.00 binds copper more strongly and has a higher binding capacity than FA at pH 3.50. The degree of ionization of functional groups normally increases as the pH value increases. This has an effect on the size or aggregation of the FA molecules as the polyelectrolyte molecules begin to uncoil or small aggregates begin to move away from each other or disaggregate due to the accumulated negative charges. The latter phenomenon although slight, has been observed in this laboratory from light scattering experiments (Underdown et al. 1981). If this takes place, it will be more difficult for pseudo-chelation to occur. This will result in a diminution in complexation as pH increases. Since the results obtained in this work (and most other

Fig. 30. Bound Cu(II) as a Function of Total Cu(II) Added. (100 mg/L FA, pH 3.50, NSE)

Fig. 31. $\bar{K}_4$ and K_4 as a Function of Total Cu(II) Added.

Fig. 32. Gibbs Standard Free Energy as a Function of χ_C

Table 17

A Comparison of Chelation Equilibrium Data Obtained
for the Two Cu-FA Titrations at pH 3.50
and 6.00^a

pH	Binding Capacity ($\pm 4\%$)	Weighted Average Equilibrium Function ($\bar{K}_4$) $\pm 10\%$	Differential Equilibrium Function (K_4) $\pm 20\%$	$-(\Delta G^\circ_{RT} \ln T)$ (KJ/mole) $\pm 8\%$
3.50	$2.4 \times 10^{-4} M$ (2.6 mmole/gFA)	57.9-11.4	174.5-1.69	12.8-3.59
6.00	$3.9 \times 10^{-4} M$ (4.7 mmole/gFA)	80.0-0.18	1420-1.78	18.0-1.43

^a Both titrations were with a FA concentration of 100 mg/L and no supporting electrolyte.

work) show the contrary, it can be assumed that the increase in complexation is simply due to the fact that more functional groups are being ionized and available as binding sites. This effect is dominant over the lack of pseudo-chelation as pH increases.

The Effect of Ionic Strength

Using ion selective electrodes, Gamble (1973) has demonstrated that fulvic acid like other polyelectrolytes binds both Na^+ and K^+ . The ability of humic substances to bind alkali metal ions must undoubtedly affect the binding of other metals such as transition metals, particularly when sodium or potassium salts are used as supporting electrolytes. Schnitzer and Hansen (1970), Mantoura et al. (1978) also reported the decrease in stability constants and complexation as ionic strength increases.

The effect of ionic strength on Cu-FA complexation was studied here first on 10.0 mg/L FA samples. The potentiometric titration results of the Cu-FA system with 10.0 mg/L FA, pH 6.00, and ionic strength 0.1 M (KCl) can be discussed here. These will be compared with the results of the system determined at low ionic strength reported earlier in this chapter under the section, "The Effect of FA concentration".

The values of both the equilibrium functions, the weighted average $\bar{K}_4$ and differential, K_4 are given in Table 18. As in previous cases, K_4 values are higher at low Cu(II) loadings. A comparison of chelation equilibrium data from the two systems with ~ 0.0 M and 0.1 M ionic strengths is given in Table 19. The values of equilibrium functions

Table 18

Weighted Average and Differential Equilibrium Functions

for a Cu-FA system

(FA conc. = 10.0 mg/L, pH 6.00, μ 0.1 M)

<u>mmole Cu(II)/gFA</u>	<u>Weighted Average Equilibrium Function (K_4) ($\pm 10\%$)</u>	<u>Differential Equilibrium Function (K_4) ($\pm 20\%$)</u>
0.25	1.39	7.14
0.50	0.84	2.01
0.75	0.67	1.16
1.00	0.59	0.76
1.25	0.56	0.54
1.50	0.56	0.45

Table 19

A Comparison of Chelation Equilibrium Data
 from two Cu-FA systems of Different Ionic Strengths
 (10 mg/L FA, pH 6.00)

Ionic Strength (μ)	Binding Capacity ($\pm 4\%$)	Weighted Average Equilibrium Function ($\bar{K}_4$) $\pm 10\%$	Differential Equilibrium (K_4) $\pm 20\%$	$-(\Delta G^\circ + RT \ln \tau)$ (KJ/mole) $\pm 8\%$
0.0 M	$2.9 \times 10^{-5} \text{ M}$ (3.3 mmole/g FA)	1.81-0.35	20.6-1.36	7.50-0.76
0.1 M	$1.4 \times 10^{-5} \text{ M}$ (1.6 mmole/g FA)	1.39-0.56	7.14-0.45	4.87-0.37

and standard Gibbs free energies of these two systems are noticeably different with the system 0.0 M ionic strength having the higher values. The possibility that K^+ ions are bound in a crown ether type environment was investigated by running two sets of experiments, one in 0.1 M Na^+ and the other in 0.1 M K^+ , but both at pH 6.00 and 100 mg/L FA. Due to the difference in ionic radii of the two alkali ions, the binding capacity of Cu(II) with FA in the 0.1 M K^+ would be expected to differ from that of the 0.1 M Na^+ medium, if the binding was indeed crown ether type. The values obtained were $1.8 \pm 4\%$ mmole/g FA for the 0.1 M K^+ and $1.7 \pm 4\%$ mmole/g FA for 0.1 M Na^+ . These values were close enough to rule out the crown ether postulate. The alkali ions are assumed to be electrostatically and weakly bound in the neighborhood of ionized functional groups (Gamble, 1973). Their ability to compete with Cu(II) for the binding sites could probably be due to territory advantages.

Fig. 33 illustrates a plot of bound copper (II) vs total Cu(II) added for the Cu-FA system with 100 mg/L FA, pH 6.00 and variable ionic strengths. The values of maximum Cu(II) bound or binding capacities are listed in Table 20. It can be seen that the binding of Cu(II) ion decreases as ionic strength increases.

SUMMARY

The copper-FA equilibrium system was studied by ion selective potentiometry in this chapter. Binding capacities of different systems under different conditions were estimated. Equilibrium functions were

Fig. 33. Bound Cu(II) vs Total Cu(II) Added for Cu-FA System with 100 mg/L FA, pH 6.00, and Variable Ionic Strength.

Table 20
Binding Capacities for Cu-FA Systems of Different
Ionic Strength
(100 mg/L FA, pH 6.00)

<u>Ionic Strength μ</u> (M)	<u>Binding Capacity ($\pm 4\%$)</u> (mmole Cu(II)/gFA)
~0.0	4.7
0.03	2.9
0.07	2.1
0.1	1.8
0.2	0.9

calculated using Gamble's treatment (1973, 1980). The difference between the weighted average and the differential equilibrium functions were found to be real, with the latter having higher values at low Cu(II) loadings. The study of the effect of FA concentrations indicated that FA of higher concentrations probably binds metal ions by "pseudo-chelation" process. The study at pH values 3.50 and 6.00 shows that increase in binding at higher pH is due to increase in ionization of functional groups. Ionic strength of the system also exerts an important effect; the binding of Cu(II) decreases with increase in ionic strength.

CHAPTER 5

STUDIES OF MAGNESIUM-FULVIC ACID EQUILIBRIUM

SYSTEM BY ION SELECTIVE ELECTRODE

POTENTIOMETRY

The results of binding capacity determinations and Mg-FA complexation studies are presented in this chapter. All the experiments were performed at three pH levels, namely, pH 6.00, 8.00 and 10.00. The high pH values were feasible because no hydrolytic precipitation occurred under the experimental conditions.

Table 21 lists the Mg-FA potentiometric titration results (pH 6.00, 100 mg/L FA, no background electrolyte) from replicate runs. The Nernstian plot ($\log[Mg(II)]_{Total}$ vs electrode potential in millivolt) is shown in Fig. 34. The calibration curve (without FA) has a slope of 26.5 mV per decade change in magnesium (II) concentration. The Mg(II)-FA titration curve approaches the calibration curve asymptotically. The potentials of the Mg-FA system are converted to concentrations from the calibration curve and these values correspond to free magnesium. The bound magnesium is obtained by difference (Table 22). The binding capacity of FA for Mg(II) is obtained from a plot of bound Mg(II) vs total Mg(II) depicted in Fig. 35. This was a "best fit" curve obtained using the polynomial least square Fortran IV program TE04. The portion of the curve with zero slope corresponds to the binding capacity.

Table 21

Titration of Mg-FA (Averaged data, 100 mg/L

FA, pH 6.00, no background electrolyte)

Initial Volume = 100 mL

<u>mmole Mg(II)/gFA</u>	<u>Total Mg(II)</u> ($\times 10^5$ M)	<u>$-\log[Mg(II)]$</u>	<u>Potential</u> (-mV)
0.10	0.952	5.022	184.4
0.12	1.14	4.943	182.0
0.14	1.33	4.876	179.5
0.16	1.52	4.818	178.0
0.18	1.71	4.767	173.0
0.20	1.90	4.722	171.6
0.25	2.36	4.627	166.5
0.30	2.82	4.549	160.8
0.35	3.29	4.483	157.0
0.40	3.76	4.425	154.8
0.45	4.22	4.374	151.0
0.50	4.69	4.329	148.5
0.55	5.16	4.287	146.0
0.60	5.62	4.250	144.7
0.65	6.09	4.215	143.1
0.70	6.55	4.184	141.4
0.75	7.02	4.154	139.9
0.80	7.48	4.126	138.5
0.85	7.94	4.100	137.5
0.90	8.40	4.076	136.7
0.95	8.87	4.052	135.5

Table continued...

1.00	9.33	4.030	134.6
1.20	11.2	3.952	131.3
1.40	13.0	3.886	128.8
1.60	14.8	3.829	126.4
1.80	16.7	3.779	124.5
2.00	18.5	3.734	123.4
2.50	23.0	3.639	120.0
3.00	27.4	3.562	117.0
3.50	31.8	3.487	115.7
4.00	36.2	3.441	113.4
4.50	40.5	3.392	111.8
5.00	44.8	3.348	110.6
5.50	49.1	3.309	108.5
6.00	53.3	3.273	107.9
6.50	57.5	3.240	106.9
7.00	61.6	3.210	106.0
7.50	65.7	3.182	105.3
8.00	69.8	3.156	104.4
8.50	73.9	3.132	103.0
9.50	81.8	3.087	102.0
10.00	85.8	3.067	101.8

Fig. 34. The Titration of Mg-FA (Averaged Data, 100 mg/L FA , pH 6.00, NSE).
Upper Curve= Calibration, lower=titration.

Table 22

Estimated Free and Bound Mg(II)

(100 mg/L FA, pH 6.00, no background electrolyte)

<u>mmole Mg(II)/g FA</u>	<u>-log Free [Mg(II)]</u>	<u>Free Mg(II)</u> <u>($\times 10^6 M$)</u>	<u>Bound Mg(II)</u> <u>($\times 10^3 M$)</u>
0.10	7.020	0.096	0.919
0.20	6.460	0.347	1.80
0.30	6.030	0.933	2.65
0.40	5.725	1.88	3.44
0.50	5.500	3.16	4.18
0.60	5.260	5.50	4.82
0.70	5.060	8.71	5.36
0.80	4.890	12.9	5.80
0.90	4.710	19.5	5.99
1.00	4.590	25.7	6.23
1.20	4.430	37.2	6.78
1.40	4.310	49.0	7.29
1.60	4.190	64.6	7.40
1.80	4.095	80.4	7.49
2.00	4.035	92.3	7.65
2.50	3.880	13.2	7.75
3.00	3.740	18.2	7.75
3.50	3.660	21.9	7.70

Fig. 35. Bound Mg(II) vs Total Mg(II) Added. (100 mg/L FA, pH 6.00, NSE)

The value obtained in this case was $7.75 \times 10^{-5} \text{ M}$ or 0.84 mmole Mg(II) per gram FA.

The uncertainty in the free Mg(II) ion concentration is about $\pm 2.5\%$ based on a standard deviation calculation for replicate titrations. The uncertainty in the estimation of maximum bound Mg(II) or the binding capacity is about $\pm 5\%$.

The binding capacity of FA for Mg(II) seems to be an increasing function of the pH of the Mg-FA system. Table 23 illustrates these values obtained at three different pH levels.

Using the binding capacity obtained, the mole fractions of the un-ionized, ionized and complexed chelation sites were calculated as in the Cu-FA systems for the Mg-FA system at pH 6.00 and illustrated in Fig. 36. The mole fractions are important for describing the composition of the system. Any two of the three mole fractions, together with temperature and FA concentration, will specify the sample composition. The most useful choice for this purpose is the use of X_C and X_{SH_2} . As in previous cases, X_{SH} and X_{SH_2} decrease differently with increasing X_C . X_{SH} is seen to be used up at a much faster pace than X_{SH_2} .

With magnesium binding from $X_C = 0.119$ to $X_C = 0.785$, the weighted average equilibrium function $\bar{K}_4$ values decrease by an order of magnitude as shown in Table 24 and Fig. 37. The values obtained by Schnitzer and Hansen (1970) using ion exchange and continuous variation methods and recalculated by Gamble and Schnitzer (1973) fall entirely within this range. Although the pH values at which Schnitzer and Hansen (1970) obtained their

Table 23.

The Binding Capacities of FA for Mg(II) at Different
pH Levels

(100 mg/L FA, no background electrolyte)

<u>pH</u>	<u>Binding Capacity ($\pm 5\%$)</u>
6.00	0.84 mmole Mg(II)/g FA ($7.75 \times 10^{-5} \text{ M}$)
8.00	1.7 mmole Mg(II)/g FA ($1.62 \times 10^{-4} \text{ M}$)
10.00	2.0 mmole Mg(II)/g FA ($1.92 \times 10^{-4} \text{ M}$)

Fig. 36. Mole Fractions of Un-ionized, Ionized and Complexed Chelation Sites (100 mg/L FA., pH 6.00, HSE) of Mg-FA System.

Table 24

Weighted Average and Differential Equilibrium Functions
for Mg-FA System (100 mg/L FA, pH 6.00, no background electrolyte)

χ_c	Weighted Average Equilibrium Functions ($\bar{K}_4$) ($\pm 12\%$)	Differential Equilibrium Function (K_4) ($\pm 24\%$)
0.119	1.48	2.38
0.166	1.36	2.24
0.211	1.15	2.11
0.286	0.60	1.89
0.395	0.53	1.56
0.493	0.43	1.27
0.583	0.35	1.00
0.659	0.29	0.78
0.721	0.25	0.59
0.765	0.22	0.46
0.785	0.17	0.40

Fig. 37. The Weighted Averaged Equilibrium Function $\bar{K}_4$ as a Function of Total Mg(II) Added.
(no supporting electrolyte)

values were slightly lower, the FA concentrations were approximately the same. This demonstrates that different methods appear to give consistent results. The values of the differential equilibrium function K_4 , were calculated using Equation 19 from Chapter 2. This does not produce a state function for the direct comparison of different samples, unlike those of Equations 35 and 36 (from Chapter 2) which give K_4 as a state function having a constant χ_{SH_2} . The values of K_4 were obtained as in Chapter 4 by fitting $\chi_{SH} \bar{K}_4$ vs χ_{SH} data to the polynomial least square program (TEO₄). Table 24 lists the values of K_4 and Fig. 38 illustrates a plot of K_4 as a function of total Mg(II) added. The difference between $\bar{K}_4$ and K_4 is again demonstrated. The standard Gibbs free energy ΔG^0 values calculated using the K_4 values obtained are plotted in Fig. 39 as a function of χ_c . As in the Cu-FA equilibrium systems, the ΔG^0 values are seen to decrease systematically with increasing Mg(II) loading. Again, this is to be expected because in a mixture, the strongest chelate or complex will form first. The standard Gibbs free energy ΔG^0 values reported here for Mg^{2+} are in agreement with the literature values for Mn^{2+} complexes with organic ligands which are evidently outer sphere type reactions (Gillen and Martell, 1964; Li et al. 1957). The term outer sphere complex is used when the metal ion and the ligand are separated by a water molecule layer. An inner sphere complex on the other hand, is formed when a direct interaction of the metal ion and ligand occurs.

Fig. 38. K_4 as a Function of Total Mg(II) added.

Fig. 39. Gibbs Standard Free Energy as a Function of x_C .

OUTER SPHERE COMPLEX

INNER SPHERE COMPLEX

From their proton nuclear magnetic resonance studies on Mn^{2+} -FA complex, Gamble et al. (1975, 1977) also argued that Mn^{2+} complexation with FA was outer sphere electrostatic type interaction. From the comparison of ΔG^0 values, it seems likely that Mg^{2+} chelation with FA is also outer sphere type binding. Further evidence is, however, needed and this will be furnished in part by the stopped flow kinetic studies in chapter 7.

In the experiments described earlier (performed at pH 6.00), the following equilibrium expression has been used.

where SH^- is a singly ionized chelation site. For Mg^{2+} , the chelation sites are most likely dicarboxylic sites. To be able to compare equilibrium data of pH 6.00 with those of pH 8.00 and 10.00, where all carboxylic groups are essentially ionized, the following equilibrium was assumed.

Note that it has only recently been clearly shown that Mg(II) ion is 6-coordinate in solution (Langford and Sasti; 1972).

Therefore,

$$\bar{K} = M_C / (M_S M_M)$$

where M_C , M_S and M_M are the molarities of the complex, the deprotonated chelation site and free Mg^{2+} ion respectively. $\bar{K}$ is a weighted average function analogous to $\bar{K}_4$ described earlier. The following equation gives the result for this case (Gamble; 1973).

$$\bar{K} = \frac{1}{X_S} \int_0^{X_S} K(X_S) dX_S$$

$$X_S = M_S / T_{FA}$$

X_S is the mole fraction of carboxylic groups free from both H^+ and metal ions, T_{FA} is the maximum binding sites or binding capacity.

K which is a differential function could be calculated by $K = d(X_S \bar{K}) / dX_S$.

Table 25 lists the range of $\bar{K}$ and K values calculated for the three pH levels from $X_S = 0.90$ to 0.10 . No significant difference among the three pH values have been observed. Fig. 40 depicts the change in standard Gibbs free energies as a function of X_S . The closeness of the ΔG^0 values indicates that Mg^{2+} ions are not too specific in choosing binding sites at the three pH levels. This again lends support to the postulate that Mg^{2+} ions are bound electrostatically as outer sphere complexes to FA molecules.

In natural waters where the concentration of dissolved organic

Table 25

$\bar{K}$ and K Values for Mg-FA Equilibrium System
at three pH Levels

<u>pH</u>	<u>T_{FA}</u> (<u>± 5%</u>)	<u>$\bar{K}$</u> (<u>$\times 10^{-6}$</u>) (<u>± 12%</u>)	<u>K</u> (<u>$\times 10^{-6}$</u>) (<u>± 24%</u>)
6.00	$7.75 \times 10^{-5} \text{ M}$	1.4 - 0.16	5.2 - 0.52
8.00	$1.62 \times 10^{-4} \text{ M}$	1.1 - 0.16	6.2 - 0.16
10.00	$1.98 \times 10^{-4} \text{ M}$	1.1 - 0.14	5.1 - 0.32

Fig. 40. Gibbs Standard Free Energy as a Function of X_S . (100 mg/L FA, NSE).

matter may be low and where competition between trace metals such as Cu(II) and major cations such as Mg^{2+} for available binding sites may be significant, the importance of metal-FA complexes has been questioned (Stumm and Morgan, 1970). The effect of Ca^{2+} competition on the complexation of Cu(II) by FA was examined by Gamble and Schnitzer (1973). The calculation was later extended by Reuter and Perdue (1976) and they predict that substantial quantities of Cu(II) could still be complexed by FA even in the presence of a 40,000-fold excess of Ca^{2+} .

To investigate the effect of Mg^{2+} ion on Cu(II) complexation with FA, an experiment was designed with the use of cupric ion selective electrode monitoring the Cu-FA system in the presence of 10^{-3} M Mg^{2+} . The Nernstian plot and the equilibrium data were shown earlier in Fig. 12 and Table 10 (Chapter 4). The shape of the titration curve is not unlike the rest of the Cu-FA titration curves. The binding capacity as estimated from Fig. 13 is 3.6 mmole Cu(II) per g FA. For the sake of convenience, the binding data of Cu-FA equilibrium systems with and without Mg^{2+} are shown together in Table 26. Under the experimental conditions (100 mg/L FA, pH 6.00), it can be seen that the presence of 10^{-3} M Mg^{2+} decreases the amount of Cu(II) bound by 1.1 mmole per g FA or about 30% binding sites. Admittedly, the level of Mg^{2+} in fresh water is less than 10^{-3} M. But in seawater the concentration of Mg^{2+} is about 10^{-3} M or higher (Chapter 2, Table 2). The competition of Mg^{2+} for the available binding sites, would therefore, be quite significant.

Table 26

The Comparison of Binding Capacities for Cu-FA
Systems with and without Mg^{2+} (100 mg FA, pH 6.00)

Cu(II)-FA SystemBinding Capacities
($\pm 4\%$)

No Background Electrolyte

4.7 mmole/g FA

 $(3.9 \times 10^{-4} \text{ M})$ $10^{-3} \text{ M Mg}^{2+}$

3.6 mmole/g FA

 $(3.4 \times 10^{-4} \text{ M})$

The following calculations were done to find out whether direct competition can account for the lowering of Cu(II) binding capacity in the Cu(II)-Mg²⁺-FA system.

First, the total carboxyl groups available for Mg²⁺ binding was estimated. This was done assuming the total carboxyls in the FA as 7.7 mmole/g as reported in the literature (Gamble, 1970; Gamble and Schnitzer, 1973). From this value, the binding sites occupied by protons (2.7 mmole/g) and Cu(II) (3.9 mmole/g) were subtracted. The remaining carboxyls of 1.1 mmole/g FA or dicarboxylic sites of 0.55 mmole/g FA (0.55×10^{-4} M) were therefore potentially available for Mg²⁺ binding. This value is a theoretical upper limit and might not be reached in the actual situation.

The amount of Mg²⁺ actually bound was next estimated keeping in mind that $M_C + M_S = 0.55 \times 10^{-4}$ M. The $\bar{K}$ value for the Mg²⁺-FA complexation was not obtainable in the Cu(II)-Mg²⁺-FA system because only the cupric ion concentration was monitored in the experiment.

Therefore, its value was estimated from a plot of $\bar{K}$ vs χ_S or $\bar{K}$ vs χ_C of the Mg(II)-FA system. This estimation was the best alternative available. A value of 1.60×10^5 was thus obtained at the plateau region where the loading of binding sites by Mg²⁺ ion was high. The $\bar{K}$ value was then used in the calculation of M_C , the molarity of complexed Mg²⁺. Therefore,

$$\bar{K} = 1.60 \times 10^5 = \frac{M_C}{M_S \times M_M}$$

A value of $5.5 \times 10^{-5} \text{ M}$ was obtained for M_C after substituting in total Mg^{2+} ($M_C + M_M$) = $1 \times 10^{-3} \text{ M}$ and $M_C + M_S = 0.55 \times 10^{-4} \text{ M}$ and solving the quadratic equation. This indicates that M_S , the molarity of deprotonated binding sites, is almost non-existent in the solution. The implication of this is that at a Mg^{2+} concentration of $1 \times 10^{-3} \text{ M}$, the Mg^{2+} ions would compete for the available binding sites in the FA molecules. This result agrees well with the competitive binding study of Mn^{2+} , K^+ and H^+ by Gamble et al. (1976). In a similar study with eleven metal ions at various pH values ranging from 2.4 to 5.8, Gamble, Schnitzer and et al. (1981) found that all the binding sites in the humic acid were almost totally occupied by the metal ions with a total concentration of around $2 \times 10^{-3} \text{ M}$.

The amount of binding sites in FA molecules due to electrostatic could be evaluated from Table 26. Assuming Mg^{2+} ions are bound to FA electrostatically, about $5 \times 10^{-5} \text{ M}$ of binding sites are due to electrostatic.

SUMMARY

In this chapter, the Mg-FA equilibrium system was studied by ion selective electrode potentiometry. Binding capacities at three different pH levels were estimated. Equilibrium functions were also calculated. From the standard Gibbs free energy values obtained, it is postulated that Mg^{2+} ion is bound electrostatically as outer sphere complex to the ionized acidic functional groups of the FA

molecule. The functional groups in question were assumed to be ionized carboxylics. This assumption was substantiated by the standard Gibbs free energies obtained which show relative insignificant change at the three pH levels. The effect of Mg^{2+} on trace metal binding in natural waters could be significant since calculation shows that Mg^{2+} would compete for the available binding sites in the FA molecules.

CHAPTER.6

STUDIES OF MAGNESIUM-FULVIC ACID-EQUILIBRIUM SYSTEM BY LIGHT SCATTERING TECHNIQUE

Study of the Mg-FA complexation equilibrium by light scattering method is reported in this chapter. The change in particle size or degree of aggregation as Mg^{2+} was being titrated into the FA solution was monitored continuously by the light scattered. Also, the intensity of scattered light from a FA solution was studied as a function of pH.

While the light scattering technique has proven to be one of the most versatile and useful methods to study macromolecules, especially in the determination of molecular weights, it has rarely been applied to the studying of humic substances. This is probably a consequence of the experimental difficulty. Humic solutions are strongly absorbing at concentrations where light scattering is usually conducted and light of wavelengths can induce fluorescence of humic materials. These two difficulties are minimized by the use of a He-Ne laser source which emits at 633 nm, a highly monochromatic very well collimated red light. The light scattering technique is especially difficult with smaller molecular species such as fulvic acids (diameter $\sim 200 \text{ \AA}$). However with care and proper instrumentation, the technique can be very useful. Saar and Weber (1980) studied the aggregation of Pb^{2+} and soil FA by nephelometry. They were able to show that Pb-fulvate precipitates at a much lower mole ratio of metal ion to FA than either Cu-fulvate or Cd-fulvate. They did not, however, explore

colloid particle size. Langford et al. (1981) confirmed an aggregation effect in Fe(III): FA complexes using a He-Ne laser source light scattering technique. Underdown et al. (1981) studied the polydispersity of FA and estimated the weight average molecular weights of different FA factions.

In the present work, the light scattering technique was used only as a monitor of relative aggregation in the Mg-FA system. No attempt was made to determine the molecular weights and the actual size of the particles. The Brice-Phoenix Universal Light Scattering Photometer used in this work is a versatile instrument designed for the measurement of micro-scattering, micro-fluorescence, micro-luminescence, and very low transmittance and reflectance by dark materials. It is a completely self-contained instrument for measuring the angular dependence of light scattering. The original light source is replaced by a 5 M.W. He-Ne laser providing radiation at 633 nm. The actual quantity measured was R_θ , the Rayleigh scattering ratio or reduced intensity which is proportional to the intensity of the light scattered at an angle θ given by

$$R_\theta = \frac{i_\theta r^2}{I_0(1+\cos^2 \theta)}$$

where I_0 is the incident intensity, r is the distance from the scattering center to the photodetector and i_θ is the intensity of the scattered light at the angle θ . At $\theta = 90^\circ$, the scattering is completely plane-polarized and this angle is the one usually chosen for observation when the scattering has no dissymmetry. This has the further advantage that the scattering from extraneous dust is at a minimum. R_{90} can also be related to turbidity τ as

$$\tau = \frac{16 \pi}{3} R_{90}$$

R_{θ} can be calculated from the observed scattering ratios G_{θ}/G_0 (the observed ratio of the recorder pen deflection for the light scattered from the solution at θ^0 to that for the transmitted light at the 0^0 position) by means of the following operation (Brice-Phoenix Light Scattering Photometer operation manual):

$$R_{\theta} = \frac{TD \cdot a n^2 (R_w/R_c)}{1.049 \pi h} \left(\frac{r}{r'} \right) \left(\frac{\sin \theta}{1 + \cos^2 \theta} \right) \left[\frac{1}{(1-R)^2 (1-4R^2)} \right] \\ \times \left\{ \left[\left(\frac{G_{\theta} F}{G_w} \right)_{\text{solution}} - \left(\frac{G_{\theta} F}{G_w} \right)_{\text{solvent}} \right] - 2R \left[\left(\frac{G_{180-\theta}}{G_w} \right)_{\text{solution}} - \left(\frac{G_{180-\theta} F}{G_w} \right)_{\text{solvent}} \right] \right\}$$

where TD is the experimentally determined product of the diffuse transmittance of the opal glass reference standard and a correction factor (unity), a is a constant relating the working standard to the opal glass reference standard, n is the refractive index of the solution, which for dilute solutions can be replaced by the refractive index of the solvent, R_w/R_c is an experimentally determined correction for incomplete compensation for refraction effects (given in the manual), h is the width of the laser beam, r/r' is a calibration factor necessary only for small or cylindrical cells, $\sin \theta$ corrects for the volume change on viewing the solution at different angles, F is the product of transmittances of neutral filters and R is $(n-1/n+1)^2$ where n is the refractive index of the glass used in the cell. This equation can be reduced to the following equations by applying published data of R_{θ} for benzene and using ultrapure benzene for calibration:

$$R_{90} = 5.16 \times 10^{-3} \frac{G_{90}}{G_0} F$$

$$R_{45} \text{ or } R_{135} = 2.54 \times 10^{-3} \left(\frac{G_{\theta}}{G_0} F - 4.06 \times 10^{-2} \frac{G_{180-\theta}}{G_0} F \right)$$

By using the above equations, R_0 's of the FA solutions (100 mg/L) without Mg^{2+} and background electrolyte at three different pH levels were calculated and tabulated in Table 27. The data were the average of two separate determinations. The R_0 values appear to decrease as pH increases from 6 to 10, although the changes are small. Not enough data were available, however, to prove that the decrease was in fact real. Nevertheless, Wershaw and Pinckney (1977) reported a reduction in aggregation of HA solutions from pH 5 to 9. Also, the result obtained here is consistent with those of Langford et al. (1982) and Underdown (1982). They found that in an unfiltered FA solution, the light scattered at 90° slightly decreased from pH 6 to 10. Gamble (1972) reported that at pH 6, the H-bonding may be phasing out just as the titration end point is not sharp. Since the decrease of H-bonding is gradual, the aggregation is therefore, slowly phasing out resulting in the gradual decrease of the light scattered.

When Mg^{2+} solution was titrated into FA solutions of different pH's and without background electrolyte the change in R_{90} together with error bar for each data point is illustrated in Figs. 41, 42, 43. All the scattering intensities were corrected for dilution when necessary. The least square fitted results of these data points are shown in Table 28. From the figures and the data calculated as shown in Table 28, it appears that Mg^{2+} is causing a small increase in particle

Table 27

The Rayleigh Ratios R_θ of FA solutions (100 mg/L) at
Three Different pH Levels

pH	R_θ^*		
	45° ($\times 10^5$)	90° ($\times 10^5$)	135° ($\times 10^6$)
6	4.18	2.89	7.54
8	3.51	2.70	6.79
10	3.40	2.65	6.70

* The experimental error of R_{90} is estimated to be $\pm 3\%$

Fig. 41. Rayleigh Scattering Ratio R_{90} as a Function of Added Mg(II) ,pH 6.00.

Fig. 42. Rayleigh Scattering Ratio R_{90} as a Function of Added Mg(II), pH 8.00.

Fig. 43. Rayleigh Scattering Ratio R_{90} as a Function of Added Mg(II), pH 10.0.

Table 28

Calculated (Least Square Fitted) Data for Three pH Levels

pH	Intercept	Slope	Standard Error
6	2.98×10^{-5}	4.18×10^{-7}	4.05×10^{-7}
8	2.92×10^{-5}	2.26×10^{-7}	8.27×10^{-7}
10	2.64×10^{-5}	4.64×10^{-7}	5.25×10^{-7}

size or aggregation of the FA solutions. Although the effect is small as compared to that of Cu^{2+} (Langford et al., 1982, Underdown, 1982), it is apparently outside the experimental errors. No pH effect, however, is observed based on the similarity in the values of the slopes calculated from the plots. This reinforces the argument from Chapter 5 that Mg^{2+} is bound to carboxylic acid groups which by pH 6, are practically all ionized. From Figs. 41, 42, 43 and Fig. 44, where R_{90} values are plotted against x_{Mg} (the mole fraction of binding sites occupied by Mg^{2+} ions), increase in the scattered light at 90° induced by bound Mg^{2+} can be seen as sites are progressively occupied. The values of R_{90} is seen to increase beyond the apparent binding capacity. Apparently the FA scattering is relatively more sensitive to small increases than ISE measurements by which apparent maximum binding capacity is determined. Also, in the ISE experiments, the error of measurement is being magnified when calculation is done near the end point region where the titration curve approaches the standard calibration curve asymptotically. In addition, small particles in the FA solution previously not causing appreciable scattering might well be converted to scatterers by a small change of Mg^{2+} ion concentration (Langford et al. 1982). This will make R_{90} a more sensitive indicator of small increase in bound Mg than the ISE measurement near the asymptotic approach.

In the Cu-FA systems, the R_{90} changes very little in the beginning of the titration. The R_{90} values appear to rise very rapidly only when apparent occupation of salicylic acid type sites is reached. In the Mg-FA systems, however, the R_{90} values increase gradually from the beginning of the titration although the increase is relatively

Fig. 44. Effect of Bound Mg(II) on FA Aggregation (100 mg/L). X_{Mg} is the Mole Fraction of Total Chelation Sites Occupied by Mg(II).

small. This seems to indicate that Mg^{2+} is apparently causing aggregation through some unknown combinations of salting-out effect and pseudo-chelation (see Chapter 4 for definition).

SUMMARY

A light scattering technique was utilized to monitor the change in particle size or degree of aggregation of Mg-FA titrations in this chapter. The scattered light or the Rayleigh scattering ratios were also determined for FA solutions of three different pH values. Although not enough data were available, it does appear that the scattered light intensity or degree of aggregation decreases slightly as pH varies from 6 to 10. Mg^{2+} was also found to induce aggregation in FA solutions similar to Cu^{2+} ion. But the scattered light due to Mg^{2+} induced aggregation is much less in intensity than that of Cu^{2+} . This is in good agreement with the results of Pagenkopf and Whitworth (1981).

CHAPTER 7

MAGNESIUM COMPLEXATION STUDIES BY STOPPED-FLOW SPECTROFLUORIMETRIC METHOD

In this chapter, stopped-flow kinetic method was utilized to study Magnesium complexation and to verify the conclusion drawn from the last two chapters that Mg (II) is bound to fulvic acid in an outer sphere complex. To be able to use the fast kinetic method, a suitable fluorescent reagent, (ligand) for Mg (II) has to be found and characterized.

- (a) Characterization of a fluorescent reagent for the determination of Mg (II) by kinetic analysis.

When a reagent is adopted for the specific purpose of kinetic analysis, parameters of the kinetic behaviour of the system under investigation have to be considered. In general, the following requirements have to be met before an analytical reagent is deemed suitable for kinetic analysis.

- (i) High sensitivity and specificity towards the analyte in question. This implies not only a low detection limit, but also the ability of the reagent to remain resilient towards potential interferences present in the matrix. These criteria are basic requirements for all complexometric analyses. In model systems such as ours, of course, the interferences are not severe.

- (ii) The chemistry associated with the complex formation should be strongly thermodynamically favourable and well defined. For example, both the data interpretation and experimental design aspects of kinetic analysis can be drastically improved if the complexometric system selected produces a stoichiometrically well defined, preferably 1:1 complex which is highly favoured thermodynamically so that 100% of the metal is converted to the detected species. The reagent of choice should also be compatible in a medium in which the analyte of interest is found.
- (iii) The product of a complexometric analysis is expected to have radically different properties from the original analyte and the reagent, with respect to the detection mode. When the latter is spectroscopic, an ideal reagent should effect a well defined and substantial spectral shift on complexation. This feature is particularly important in kinetic analysis since the kinetics can then be followed in the presence of excess reagent which allows simplification of the rate law.
- (iv) The kinetic data should be manageable. Most simple complexation reactions are second order, a situation which is generally reduced to pseudo first-order by using an excess of the reagent. Thus, potentially very complex multi component second-order kinetics are reduced to relatively simple first-order kinetics. In a multicomponent system, the composite rate can become extremely complex. It is therefore essential, when selecting an analytical reagent in kinetic analysis not to complicate the kinetic any further further.

Undoubtedly, it is difficult to find a reagent that can fulfil all of the above criteria. Therefore, when deciding for or against a given reagent, priorities for these criteria have to be set judiciously. When the detection mode is absorption spectrophotometry, appropriate reagents are often more readily available. Generally speaking, the absorption characteristics of a complex are less susceptible to the influences of external parameters such as pH and medium composition so that choices dictated by other factors may be freely made. For fluorescence a variation of one of these parameters will often produce adverse consequences. For instance, the intensity of fluorescence is a function of both the concentrations of quenchers and absorbers present; consequently, any change in the concentrations of the medium components can represent a potential experimental failure. In the present work a fluorimetric reagent was preferred for sensitivity. The following priorities govern the final choice.

- The reagent should be highly sensitive and specific towards $Mg^{(II)}$ species; the resulting complex should have a relatively high fluorescence quantum yield.
- There should also be a sufficiently large Stokes shift between the excitation and emission spectrum of the complex.
- When the reagent luminesces independently it is necessary that the emission spectrum of the free reagent and that of the complex do not overlap extensively.
- The reagent should be able to form a reasonably strong complex in an aqueous medium, preferably at an acidic or basic pH where the analyte complexes tend to be unstable.

- The complex formation kinetics, when not simple and straightforward must be at least manageable and most of all, well characterized.

Like many other metal ions, Mg (II) forms chelates of appropriate stability with O-O'-dihydroxyazo compounds that give strong fluorescence. Of all these, O-O'-dihydroxyazobenzene was found to combine with Mg (II) to give the strongest fluorescence (Shchenbor, 1968; Olsen and Diehl, 1963; Diehl et al, 1963). The drawback was the use of 95% ethanol as solvent. This eliminated it as a serious contender because of refractive index transients in stopped flow. Another reagent, lumomagneson, IREA was also rejected for more or less the same reason. In an attempt to prepare a fluorescent ligand O-O'-dihydroxy compound from coupling of 8-hydroxyquinoline (oxine) and a diazo compound, 1-amino-2-hydroxynaphthalene-4-sulfonic acid, Badrinas (1963) obtained two isomers. One of these coupled in the 5-position of the 8-hydroxyquinoline, and the other in the 7-position. But neither of these was found to be suitable because the former was reported to have poor affinity for alkaline earth metals and the latter would be interfered by heavy metals such as Fe(III) which was originally planned to be involved in an adsorption study. Mordant Blue 31, 2-(2-hydroxy-5-sulfophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid was tested by Hiraki (1972) as a fluorescent reagent for Al, Ga, Sc and Mg. Although the sensitivity for the Al-complex was high, it was not so for the Mg-complex. Moreover, the high optimum pH at 11 makes the reagent Mordant Blue 31 unsuitable for Mg. Two more reagents were

rejected because of the non-aqueous solvents they require for dissolution (Conde et al., 1974, Dagnall et al, 1967). One of these N N'-Bis-salicylidene-2,3-diaminobenzofuran was reported to be very sensitive. But because they require organic solvent such as methanol, they could not be used in this work.

Two reagents were finally chosen for detailed testing and evaluation. They were tetracycline and 8-hydroxyquinoline-5-sulfonic acid.

Tetracycline (Pfizer Ltd) was first used as a fluorescent indicator for the complexometric titrations of Ca, St and Mg at pH 10 by Ashton (1966). It has the formula shown,

Fig. 45 illustrates the dependence of fluorescent intensity of Mg-tetracycline complex on pH (λ_{ex} and λ_{em} were 400 and 465 nm respectively). The fluorescent intensity was seen to peak at about pH 11 which was considered to be less than ideal. The reagent was rejected, however, on its low sensitivity. It was found that the lowest detection limit of Mg^{2+} by tetracycline was about 1×10^{-4} M which was considered to be slightly too high. Moreover, ions such as Cu, Bi, Pb, Fe(III) etc were reported to interfere with the determinations.

The next reagent studied was 8-hydroxy-quinoline-5-sulfonic acid (oxine). It has a structural formula as shown,

Fig. 45. Dependence of Fluorescent Intensity of Mg-Tetracycline complex on pH

The reagent is a yellow crystalline powder and is a derivative of 8-hydroxyquinoline, which has been widely used as an analytical reagent. Polar substituents, such as hydroxy, sulfonic groups etc in the five position render the complexes soluble in water and other polar solvents (Liu and Bailar, Jr, 1951). These workers also reported that these polar substituents did not affect the stability of the complex significantly. Stevens (1959) reported that the existence of only one stable cationic valency-state, in which the remaining electron shells are empty, half-full or complete are necessary conditions for the fluorescence of a metal-8-hydroxyquinoline complex.

The stereochemistry of Zn-8-hydroxyquinoline-5-sulfonic acid was studied by Liu and Bailar, Jr (1951). They reported that the complex has a stoichiometry of 1-2 (metal to ligand ratio) and that it has a tetrahedral configuration. Bis complexes of 8-hydroxyquinoline with other metals such as Mg, Mn, Co, Ni, Cd were also reported (Perrin, 1964). An attempt to verify the stoichiometry by the continuous variation method using fluorescence was not successful probably due to energy transfer processes. Similar result was obtained by Mak (1980) in his Al-Calcein Blue complexation studies.

In his studies of fluorescence with metal-8-hydroxyquinoline complexes, Mg^{2+} complex was found to give strong fluorescence comparing to a quinine sulfate standard (Stevens 1959). The

strong fluorescence of Mg-8-hydroxyquinoline-5-sulfonic acid complex (Mg-Oxine) was verified when the detection limit of Mg^{2+} was found to be about $10^{-6}M$. The strongest fluorescence was obtained at about pH 9 as shown in Fig. 46. In Fig. 47 the excitation and emission spectra of both the reagent and the complex are depicted. The maxima of the reagent are $\lambda_{ex} = 400\text{ nm}$, $\lambda_{em} = 510\text{ nm}$ and the Mg-complex - $\lambda_{ex} = 380\text{ nm}$, $\lambda_{em} = 500\text{ nm}$. Although the difference between the Stokes shift of the complex and the reagent is only 10 nm, the shortcoming of the system could be alleviated by using the zero suppressor in the instruments. This would allow the fluorescent intensity due to the reagent to be made almost non-detectable.

The dependence of the emission intensity of Mg-complex upon the Mg concentration was investigated in two separate calibration studies. Various concentrations of the complex were prepared in reactions which were pseudo zero-order in the reagent. The first calibration study was carried out on a Perkin-Elmer (Model 204S) Spectrometer with a 10 mm pathlength cuvet, and the result is shown in Fig. 48. The other study was observed in the 2 mm pathlength stopped flow cell and the result is depicted in Fig. 49. A good linear relationship was observed in both of the curves within the range of working concentrations of magnesium. While the sensitivity may not be called exceptionally high, it was termed satisfactory and acceptable. Subsequently the reagent 8-hydroxy-quinoline-5-sulfonic acid (oxine) was chosen to undergo further kinetic experiments.

Fig. 46. Dependence of Fluorescent Intensity of Mg-8-hydroxyquinoline-5-sulfonic Acid Complex on pH. Mg^{+2} conc. = 6×10^{-6} M.

Fig. 47 . Excitation and Emission Spectra of the Mg-Oxine Complex and the Reagent. Upper curve = Mg-Oxine Complex, lower curve= Oxine reagent.

Fig. 48. Calibration Curve of the Mg-Complex (10 mm cuvet)

(b) Rate of formation of Mg(II)-8-hydroxyquinoline-5-sulfonic acid complex in aqueous Mg(II) solution

The formation of Mg(II)-8-hydroxyquinoline-5-sulfonic acid involves the replacement or substitution of coordinated water molecules by the ligand, i.e. 8-hydroxyquinoline-5-sulfonic acid molecule.

In the scheme of Eigen and Wilkins chelate formation mechanism (e.g. a bidentate complex):

The overall forward and reverse rate constants, K_f and K_r , obtained by imposing a steady state condition on the intermediate, i.e.

$d[\text{MS}_5 \cdot \text{L-L}]/dt = 0$, are;

$$K_f = K_0 K_{11} K_{22} / (K_{22} + K_{-11})$$

$$K_r = K_{-11} K_{-22} / (K_{22} + K_{-11})$$

It is assumed that chelate ring enclosure is much faster than the breaking of the first metal-ligand bond, i.e. $K_{22} \gg K_{-11}$,

K_f can then be expressed as,

$$K_f = (1/S) K_0 K_{ex}$$

where $(1/S)$ is a statistical factor first proposed by Langford and Stengle (1967) to account for the discrepancy between first-order rate constants of solvent substitution and exchange; K_o is the encounter equilibrium constant or the outer-sphere association constant of the ion-pair, and K_{ex} is the NMR determined solvent or water exchange rate constant of the metal ion. Using the Fuoss's equation (Fuoss, 1958)*, the calculated value of K_o is 5.5 M^{-1} when the closest approach between the two ions is taken to be $5 \text{ \AA}$ which is a reasonable value in aqueous solution (Pearson and Ellgen, 1967). From the relationship

$$K_f^* = \left(\frac{1}{S}\right) K_o K_{ex}$$

K_f could be estimated to be around $4.6 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ when K_{ex} is taken to be $5.3 \times 10^5 \text{ s}^{-1}$ at 25°C (Neely and Connick, 1970; Langford and Sastri, 1972). As can be seen below, the experimentally determined value of K_f is in reasonably good agreement with theory considering the uncertainty in the value of

K_o .

In the kinetic experiments, a Mg^{2+} solution with a concentration of $2.30 \times 10^{-5} \text{ M}$ is mixed with the fluorescent reagent (30 mg of 8-hydroxyquinoline-5-sulfonic acid dissolved in 250 ml of pH 10

* The Fuoss's ion-pairing equation gives the encounter equilibrium constant as $K_o = 4\pi N a^3 / [3000 \exp(Z)]$, where $Z = Z_a Z_b e^2 / \epsilon a K T$, N is Avogadro number, a is the distance of closest approach of the two ions, ϵ is the dielectric constant of the medium and the other symbols have their usual meaning.

3 3

OF / DE

NaHCO₃/NaOH buffer solution) making the final effective concentrations, i.e. [Mg²⁺]_{eff} = 1.15 x 10⁻⁵ M and [Reagent]_{eff} = 2.34 x 10⁻⁴ M and an ionic strength of 0.1 M. pH 9 buffer could be used instead of 10 to give the same kinetic results. The latter was used because the control of pH was more critical at pH 9 (Fig. 46).

An oscilloscope trace of the fluorescent rise signal of one of the experimental runs is shown in Fig. 50. A plot of $\ln(P_{\infty} - P_t)$ vs time t was produced from this trace (by the computer program LFIT) and illustrated in Fig. 51. The linearity of the plot indicates a single component system. From the equation

$$\ln(P_{\infty} - P_t) = -K_A t + \ln[A_0]$$

the pseudo first-order rate constant K_A and the original concentration $[A_0]$ could be obtained from the slope and intercept respectively. The kinetic results of five replicate runs are shown in Table 29.

It can be seen that the average second-order rate constant K ($K_A = K[\text{Reagent}]$) of the Mg-oxine complex formation is $4.3 \pm 0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (the pseudo first-order rate constant $K_A = 1.00 \times 10^3 \text{ s}^{-1}$) and the average percent recovery of Mg²⁺ is 99.6 ± 1.3 . The former is equal to K_f and is remarkably close to the value from simple theory of $4.6 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. The experimental value was confirmed using a Durrum-Gibson stopped-flow instrument at the University of Ottawa (courtesy of Dr. K.J. Laidler). As argued above, the rate-determining step is the conversion of the outer-sphere complex to the inner-sphere complex which in turn is governed by the inner water exchange rate of the $\text{Mg}(\text{H}_2\text{O})_6^{2+}$ ion. The reaction appeared

Horizontal Scale 10 ms/cm

Vertical Scale 1 V/cm

Fig. 50. An Oscilloscope Trace of the Fluorescent Rise Signal. [Mg + Oxine reagent]

Fig. 51. A Plot of $\ln(P_{\infty} - P_t)$ vs Time (Mg + Reagent)

Table 29

Kinetic Analysis of Mg-Oxine Complex Formation

Run No.	Second-order ^a Rate Constant $K(\times 10^{-5} \text{ M}^{-1} \text{ s}^{-1})$	Equilibrium Infinity	Kinetic Infinity*	%Mg Recovery
1	4.1	28.71	28.99	101
2	4.3	27.91	27.80	99.6
3	4.1	28.60	27.90	97.6
4	4.4	28.00	27.80	99.3
5	4.4	28.90	29.01	100

$$\bar{A}_v = 4.3 \pm 0.2$$

$$\bar{A}_v = 99.6 \pm 1.3$$

* Both infinities are given by arbitrary unit. Equilibrium infinity is the emission intensity of the reaction mixture 10 min after mixing.

% Mg Recovery = $100 \times (\text{Kinetic Infinity} / \text{Equilibrium Infinity})$

^a Pseudo first-order rate constant = $1.00 \times 10^3 \text{ s}^{-1}$

to proceed via a straightforward dissociative mechanism.

In the above experiments, the $\text{NaHCO}_3/\text{NaOH}$ pH 10 buffer was found to be adequate in holding the pH at about 10 when the Mg^{2+} solution was at an original pH of not less than 6 before mixing. The lack of dependence of the second order rate constants on the original pH of the Mg^{2+} is shown in Table 30. The insensitivity of the rate constant toward the different Mg:oxine ratio (1:10 and 1:20) is also shown in Table 30. The kinetics of the present analytical system can therefore be considered to be simple and uniform in behaviour. It should be readily implemented without much difficulty. Thus, the system meets the criteria for a good reagent system for kinetic analysis.

(c) Kinetic study of interactions between Mg^{2+} and fulvic acid

Since FA was known to fluoresce (Kononova 1966; Ghosh and Schnitzer, 1980), its excitation and emission spectra were taken to determine if the fluorescence interferes in the Mg-oxine complex fluorescence studies. Two sets of excitation and emission maxima were obtained. They were $\lambda_{\text{ex}} = 470$, $\lambda_{\text{em}} = 520$ and $\lambda_{\text{ex}} = 340$ and $\lambda_{\text{em}} = 460$. From these values, it appears unlikely that the fluorescence of FA will interfere. Moreover, the fluorescence intensity of Mg-oxine complex is much stronger than that of FA. It was also found that when FA solution was mixed with oxine reagent at pH 10, the fluorescence of FA dropped sharply to a negligible level. It can be thus, assumed that the fluorescence of FA will not interfere in the fluorimetric kinetic study of the Mg-FA system.

Table 30

Kinetic Results of Mg-Oxine Complex Formation at
Different Mg^{2+} pH and Mg/Oxine Ratios

Mg^{2+} pH of solution ^a	Mg^{2+} Concentration (M)	Reagent Concentration (M)	Second Order Rate Constant ($\times 10^{-5} M^{-1} s^{-1}$)
6	2.30×10^{-5}	4.68×10^{-4}	4.3
6	4.68×10^{-5}	"	4.4
7	2.30×10^{-5}	"	4.3
7	4.68×10^{-5}	"	4.3
8	2.80×10^{-5}	"	4.4
8	4.68×10^{-5}	"	4.1

^a pH adjustment of Mg^{2+} solution was achieved by titrating with Tris (Tham) solution.

The preliminary experiments (Mg-FA-oxine systems) were run manually (injecting the reactants into the cuvet through a syringe) using a Perkin-Elmer spectrofluorimeter (model 204S). They were done in order to establish the time duration required for the reaction systems of interest to reach experimental equilibria. Thus, the emission intensities were monitored at various time intervals after mixing and are tabulated in Table 31. The concentration of FA in the Mg-FA mixtures were 100 mg/L. The amount of Mg added was taken from the ion selective electrode potentiometric study of Mg-FA equilibrium in Chapter 5. At pH 6.00, the concentration was 3.00×10^{-4} M, which was a total Mg concentration well passed the titration end point or the binding capacity. Accordingly, the concentration of oxine reagent used was 3.00×10^{-3} M, in order that pseudo first-order kinetics could be assumed. The Mg recoveries at various time intervals were calculated using a theoretical 100% Mg recovery derived by substituting the reaction mixture in question with a reference solution of Mg at pH 6.00. The concentration of the reference solution was identical to the total Mg concentration of the mixture. The final emission intensity obtained from equilibrating this reference with the oxine reagent for 10 min (see Table 9 in Chapter 3) was used as the value of total possible recovery. It is interesting to note that no significant change in Mg recovery (about 100%) was observed even after 24 hours; a situation which indicates that either no Mg is bound to the binding sites of FA or they are very weakly bound. The Mg-FA ISE experiments showed

Table 31

Time Dependent Equilibrium Recoveries of Magnesium
from Mg-FA Mixtures at pH 6.00^a

<u>Time</u>	<u>% Magnesium Recoveries</u>
1. min	99.8
2. min	100.1
4. min	100.0
6. min	99.9
8. min	100.2
10. min	100.0*

* No significant change in % recoveries was observed after 24 hrs.

^a See text for concentrations used.

that a certain amount of Mg was bound in the present experimental conditions. Stopped flow fast kinetic experiments were run next to investigate the rate constants. Based on the above observation, all the experimental reaction systems were prepared by equilibrating the mixtures for about an hour.

Fig. 52 is an oscilloscopic trace of the fluorescent rise signal of one of the experimental runs at pH 6.00. The concentrations of the reactants were the same as in previous experiments done at pH 6.00 manually, i.e. $[Mg] = 3.0 \times 10^{-4} M$, $[oxine] = 3.00 \times 10^{-3} M$ and $[FA] = 100 \text{ mg/L}$. This was to ensure that part of the Mg^{2+} was bound to FA leaving the rest as free Mg^{2+} ion since the concentrations were taken past the end-point or the apparent binding capacity in the titration curve. In the fast kinetic study, then it might be possible to see two components corresponding to the two species of Mg (compare to Al(III) system where several components were found, Mak (1980)) assuming the binding of Mg to FA is reasonably strong and yet labile enough to be displaced by the fluorescent reagent in a reasonable time. The kinetic results however, shows that only one component can be detected in the system (Table 32) as indicated by the % Mg recovered. The pseudo first-order or second order rate constants were surprisingly similar to that of a Mg-oxine complex formation system as seen in Table 29. The kinetic results of the Mg + FA systems at pH 8.00 and 10.0 also gave similar results. The implication of this is obvious. It is that the Mg^{2+} ions bound

Horizontal Scale 10 ms/cm

Vertical Scale 1 V / cm

Fig: 52. An Oscilloscope Trace of the Fluorescent Rise Signal
[Mg + FA reacting with Oxine at pH 6.00]

Table 32
Kinetic Results of Mg + FA System Reacting
with Oxine Reagent at pH 6.00

Run No.	Second Order ^a Rate Constant $K(\times 10^{-5} \text{ M}^{-1} \text{ s}^{-1})$	Equilibrium Infinity*	Kinetic Infinity*	% Mg Recovery
1	4.4	28.91	29.00	99.7
2	4.3	27.99	28.09	99.6
3	4.4	29.00	29.10	99.7
4	4.3	28.99	28.99	100

Av = 4.4

Av = 99.8

* Both infinities are given by arbitrary unit. Equilibrium infinity is the emission intensity of this reaction mixture 10 min after mixing.

% Mg Recovery = (Kinetic Infinity/Equilibrium Infinity) $\times$ 100

^aPseudo first-order rate constant = $1.00 \times 10^3 \text{ s}^{-1}$.

to FA (as determined from the ISE experiments) were not kinetically distinguishable from the free Mg^{2+} ions. This means the Mg^{2+} -FA complex must be labile on the time scale of formation of Mg-oxine complex. This is striking since Shuman (1982) was able to detect between 7-35% (depending on conditions) of Cu^{2+} bound to FA extracted from streams. The detected portions of Cu^{2+} reacted slow enough to be distinguished by stopped flow kinetic from reaction of free Cu^{2+} with a color-forming reagent. The dissociation of a simple $Cu-L_2$ chelate in acid is in the range of 10^7 to $10^9 s^{-1}$ and the formation of some Cu^{2+} complexes such as Cu^{2+} -tetramethylethylenediamine and Cu^{2+} -pyridine-2-azodimethyl-aniline are 10^9 and $10^8 M^{-1} s^{-1}$ respectively (Eigen and Wilkins, 1965). This phenomena is supposedly due to a combination of Jahn-Teller labilization of the apical position as well as rapid changes within the coordination structure (Eigen and Wilkins, 1965). It thus seems that FA binds some Cu^{2+} strongly enough to reduce its lability by between 10^5 and 10^8 . Since the intrinsic lability of Mg(II) chelates is nearer to $10^5 s^{-1}$, it is very interesting to note that no FA site reduces the lability enough to produce a system distinguishable kinetically from reactions of free Mg^{2+} with the fluorescent reagent. It is known, of course, that Cu(II) binding is inner sphere. The evidence obtained here for Mg^{2+} is clear. It is indicating that Mg^{2+} binding to FA is not inner sphere but rather, outer sphere.

(d) Kinetic Studies of Mg(II) in the presence of Fe(III) polymers

The adsorption of Mg(II) on Fe(III) polymers is briefly described in this section. The stopped flow kinetic method was used. Adsorption on hydrous oxide colloids in general, is important in natural water systems. It has been exploited for preconcentration in analytical chemistry and in engineering processes. It also finds application in electro-analytical chemistry, ion-exchange, froth flotation, coagulation, and concentration and collection of trace contaminants. However, very little is known of the kinetics of adsorption of foreign metal ions in hydrous oxides (Fe(III) colloids).

In natural water systems, one of the more common hydrous oxides is some Fe(III) hydrous oxide species. The amorphous species initially formed during Fe(III) precipitation may undergo crystallization to eg. α - FeOOH (goethite).

Dousma and deBruyn (1976) proposed a hydrolysis/precipitation scheme for formation of amorphous $\text{Fe}(\text{OH})_3$ involving rapid and reversible formation of iron-hydroxo monomers and dimers followed by slower, irreversible polymerization and finally oxolation of the polymers with simultaneous release of protons.

According to the study of Hsu (1973), it was observed that in dilute solutions of Fe(III), ($< 0.001 \text{ M}$), the initial polymerization product was amorphous Fe(III) hydroxide which yielded x-ray diffraction peaks for FeOOH only after prolonged aging or not at all. In higher concentrations, such as 0.01 M solutions, crystalline FeOOH was the major product from the very beginning of polymerization. In the present

adsorption study, it appears that the Fe(III) species obtained was amorphous Fe(III) hydroxide because of the low concentration (10^{-4} - 10^{-5} M) and short aging time (24 hr). The experiments were performed by titrating the Fe(III) solutions of the desired concentration to pH 6.00 with Tris (2-amino-2-(hydroxy-methyl)-1,3-propanediol) in the presence of 10^{-6} M Mg(II). After an aging period of 24 hr, the solutions were analyzed kinetically for Mg(II) by using the stopped flow spectrofluorimeter and the oxine reagent in the presence of pH 10 buffer and at 25°C.

The data from kinetic analysis indicate that only one species of Mg(II) was present in the system, the pseudo first-order rate constants were directly comparable to those of free Mg(II). It thus appears that Mg(II) adsorbed very weakly (or not at all), on the Fe(III) polymers or that the adsorbed species were labile on the time scale of the formation of Mg-oxine complex. There is also a slight possibility that Fe(III) polymers redissolved rapidly to produce species such as $\text{Fe}(\text{OH})_4^-$ on the change of pH from 6.00 to 10.0 so that sorbed or occluded Mg(II) was released at a fast rate and then picked up by the oxine reagent. These preliminary experiments tend to suggest that the problem of kinetics of release of hydrous oxide (or colloids) adsorbed metal will not be most effectively approached by studies of Mg(II). The initiative was abandoned.

SUMMARY

The Mg(II)-FA binding equilibrium was studied by kinetic methods.

A fluorescent reagent - 8-hydroxyquinoline-5-sulfonic acid was found

to be suitable and to meet the criteria for a good reagent system for kinetic analysis. The second-order and pseudo first-order rate constants for Mg-reagent complex formation are $4.3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $1.00 \times 10^3 \text{ s}^{-1}$ respectively at 25°C and under the conditions adopted.

Almost identical rate constants were obtained for Mg-FA-reagent system and only one component was identified. This indicates that Mg-FA complex is labile on the time scale of formation of Mg-Oxine complex.

The lability of Mg(II)-FA species indicates that Mg(II) binds to FA as outer sphere complexes. An attempt was made to study Mg(II) in the presence of Fe(III) polymers in aqueous systems by kinetic method.

This was not rewarding.

CONCLUSIONS

This study has examined the complexations or interactions between a well-characterized naturally occurring organic material derived from soil called fulvic acid and metal ions such as Cu(II) and Mg(II) in aqueous solutions. Although the experimental systems are simple in comparison to natural aqueous systems, it is now possible and with more confidence to categorize the roles of fulvic acid and similar natural complexing ligands and their significance in controlling the distribution of metal ions. Throughout this work, the philosophy of approach to the fulvic acid is that it is characterized in terms of its functional groups- rather than structure.

Three different analytical techniques were successfully used in this work. Potentiometry with ion selective electrodes (ISE) was found satisfactory in the speciation studies of Cu-FA and Mg-FA equilibrium systems. The ISE's are able to distinguish between free metal ions and complexed metal ions. The major disadvantage of ISE's was found to be in their relatively low sensitivity. Although Cu²⁺ ISE can be used for concentrations as low as 10^{-7} M, which is comparable to the upper limit in natural waters, Mg²⁺ ISE (divalent cation electrode) was found to be less stable and can not be used for concentrations less than 10^{-6} M.

The light scattering technique was found useful for studying aggregation although its potential is not limited to this. Extreme care

however, is required in handling sample solutions and glassware to ensure cleanliness and reproducible results.

Stopped-flow spectrofluorimetric technique was very useful in studying fast kinetics involving metal ion-FA complexation. It has the capability to determine both qualitatively and quantitatively, the species involved. The detection mode in this study was fluorescence. A fluorescent reagent, namely, 8-hydroxyquinoline-5-sulfonic acid was found to be satisfactory for the kinetic analysis of Mg^{2+} ion with a detection limit for Mg^{2+} of about 10^{-6} M. The kinetics of the analytical system was simple and uniform in behavior and met the criteria for a good reagent system for kinetic analysis.

The studies of Cu-FA and Mg-FA equilibrium systems were approached at two levels. The first was stoichiometric and the second was thermodynamic. At the stoichiometric level, the concentration of bound (equals total minus free) metal was plotted versus the total metal added. As this function approaches a limit, it defines the apparent number of binding sites or the "binding capacity". The term "apparent" is used here because values are fairly sensitive function of pH, ionic strength, and concentration of fulvic acid. The apparent binding capacity of Cu^{2+} is seen to approach a maximum value approximating the theoretical limit of 5.4 mmole/g FA chelating sites only for high FA concentration (100 mg/L), at high pH (little protonation of sites) and low ionic strength. The results for Cu-FA system indicate that a decrease in pH, or an increase in ionic strength, or a decrease in total FA concentration would decrease the apparent

binding capacity. In fact, at low FA concentration (10 mg/L), only the salicylic acid type bidentate binding sites of about 3 mmole/g FA is available for binding. At high FA concentration (100 mg/L), aggregation makes farther carboxylic groups available in pairs for additional Cu^{2+} complexing. It is also true that 10^{-3} M Mg^{2+} has a quite significant effect on the binding capacity for Cu^{2+} , by occupying about 30% of binding sites normally available to Cu^{2+} .

To be able to understand this changeable apparent stoichiometry, a proper understanding of the thermodynamics of binding of a metal ion by a mixture ligand is required (since FA is known to be a mixture).

A suitable formalism for analysis of binding of an ion by a mixture was given by Gamble and Schnitzer and was used in this work. The values of K_4 , the differential equilibrium function was found to vary (decrease) considerably as the sites became progressively occupied by Cu^{2+} (and Mg^{2+}). In the course of a titration, the values of K_4 which were produced by the ion selective electrode, covered a range of more than 10^2 as the weighted average measured $\bar{K}_4$, varied by an order of magnitude. This seems to satisfy the stoichiometric results as determined. This is reinforced by results from standard Gibbs free energies of binding which found to decrease as the binding sites were progressively occupied by Cu^{2+} or Mg^{2+} ions.

Mg^{2+} was found to be less strongly bound by FA than Cu^{2+} in general. But the first sites of Mg^{2+} binding in the mixture ligand corresponded to larger values of K_4 than those associated with weaker sites for Cu^{2+} . This means that there will be no simple calculation

of the competition between Cu^{2+} and Mg^{2+} using single values of constants, K .

From the standard Gibbs free energies obtained, it is postulated that Mg^{2+} is bound electrostatically as outer sphere complex to the ionized carboxylic groups. This assumption was substantiated by the standard Gibbs free energies obtained (for different pH's) which show relative insignificant change at three pH levels and also by light scattering results.

The question of variation of stoichiometry with changing conditions finds its explanation in the variability of K_4 . This has become more apparent when calculations of the fraction of total carboxylate sites occupied by various cations (ie. Cu^{2+} , H^+ , Mg^{2+}) were done. It emerged that whenever enough cations were available, the total was constant and all binding sites bore cations. The essential point of this is that strongly bound ions such as Cu^{2+} , compete well for initial selective sites. But at high coverage, K_4 is smaller and selectivity is greatly reduced. There are no ions sufficiently strongly bound by these later sites to exclude the less strongly bound ions such as Mg^{2+} . Therefore, the apparent stoichiometry is variable.

FA is observed to have a slight decrease in aggregation from pH 6 to pH 10 in the light scattering measurements. pH 6 is at the neighborhood of the first acid-base titration end point, and the end point is not a sharp one. The consequence is at pH 6 there's a small residue of bound protons which finally vanished at high pH. The H-bonding contribution to the aggregation may still

exist (to a small extent) at pH 6. More experiments however, are necessary.

Mg^{2+} ion is found to induce aggregation in the FA solutions at pH's 6, 8, and 10. The overall induced aggregation is relatively small in comparison to Cu^{2+} induced aggregation. The fact that no pH effect was apparent indicates that Mg^{2+} is indeed bound to ionized carboxylic groups. No bidentate chelating site (on the same FA molecules) seems to be strong enough to prevent Mg^{2+} from binding with different FA molecules. This appears to be in contrast to Cu-FA system where the first Cu(II) added is seen to be taken up by the strong bidentate chelating sites (salicylic acid type) on the same FA molecule. In any case, light scattering measurement is found to be more sensitive to bound Mg^{2+} than ISE measurement in the region of the asymptotic approach in a titration curve.

The Mg-FA kinetic dissociation rate was investigated based on the appearance of a fluorescent complex of Mg with 8-hydroxyquinoline-5-sulfonic acid (HQSA). The overall reaction can be written

The rate constants k_1 and k_{-1} were estimated by stopped-flow technique under pseudo first-order conditions (HQSA in excess). The values

obtained were $4.3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (pseudo first-order rate constant = $1.0 \times 10^3 \text{ s}^{-1}$) and $1.1 \times 10^{-2} \text{ s}^{-1}$ respectively at 25°C . Since these values were similar to those obtained for free hydrated Mg^{2+} ion, and since only one component was observed in the kinetic system, it was concluded that Mg-FA complex must be labile on the time scale of formation of Mg-Oxine (HQSA) complex. Interestingly, certain amounts of both Cu^{2+} and Al^{3+} were reported to be complexed strong enough with FA to be detected by stopped-flow kinetic analysis.

Another conclusion can also be drawn from the kinetic experiments. This is based on the lability of the Mg-FA complex and indicates that Mg^{2+} is bound to FA molecules electrostatically as an outer sphere complex. Although no definite and unequivocal proof such as NMR method is available, the postulate is reinforced by the standard Gibbs free energies. In this respect, Mg^{2+} is similar to Mn^{2+} ion, but is different from the strongly binding Cu^{2+} and Fe^{3+} which are known to form inner sphere complexes with FA molecules.

APPENDIX

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1.000 1 INSTRUMENTAL ANALYSIS WITH A STANDARD CURVE
2.000 2 STANDARDS FITTED TO POLYNOMIALS BY METHOD OF LEAST
3.000 3 SQUARES PROGRAM TLO4
4.000 4 FORMAT(3I3)
5.000 5 FORMAT(E12.6)
6.000 6 FORMAT(E11.6,E14.7,F6.15)
7.000 7 FORMAT(4X,I3,10X,D12.5,2X,D12.5,3X,D12.5)
8.000 8 FORMAT(//I4,D12.6,4X,D12.6,8X,14//)
9.000 9 FORMAT(14,F20.6)
10.000 10 FORMAT(F12.6,3X,D12.6,3X,D12.6,3X,D12.6,3X,D12.6,3X)
11.000 11 FORMAT(20A4)
12.000 12 FORMAT(1H1,20A4)
13.000 13 DOUBLE PRECISION X(300),Y(200),A(16,16),SUMX(31)
14.000 14 SUMY(15),XE(300)
15.000 15 DOUBLE PRECISION S1,S2,S3,S4,TOL,C,YE,DYE
16.000 16 DIMENSION W(200),ITITLE(20)
17.000 17 IN=105
18.000 18 IOUT=108
19.000 19 READ(IN,10,END=200)ITITLE
20.000 20 WRITE(IOUT,11)ITITLE
21.000 21 READ(IN,2)N,NE,LAST
22.000 22 OUTPUT N,NE
23.000 23 TOL=1.0D-20
24.000 24 DO 40 I=1,N
25.000 25 READ(IN,4)X(I),Y(I),W(I)
26.000 26 OUTPUT Y(I)
27.000 27 IF(W(I).LT.1.0D-15)W(I)=1.
28.000 28 CONTINUE
29.000 29 DO 20 I=1,NE
30.000 30 READ(IN,3)XE(I)
31.000 31 OUTPUT XE(I)
32.000 32 SUMX(1)=0.
33.000 33 SUMX(2)=0.
34.000 34 SUMX(3)=0.
35.000 35 SUMY(1)=0.
36.000 36 SUMY(2)=0.
37.000 37 DO 90 I=1,N
38.000 38 SUMX(1)=SUMX(1)+W(I)
39.000 39 SUMX(2)=SUMX(2)+W(I)*X(I)
40.000 40 SUMX(3)=SUMX(3)+W(I)*X(I)*X(I)
41.000 41 SUMY(1)=SUMY(1)+W(I)*Y(I)
42.000 42 SUMY(2)=SUMY(2)+W(I)*X(I)*Y(I)
43.000 43 NORD=1
44.000 44 L=NORD+1
45.000 45 KK=L+1
46.000 46 DO 101 J=1,L
47.000 47 DO 100 J=1,L
48.000 48 IK=J-1+I
49.000 49 A(I,J)=SUMX(IK)
50.000 50 A(I,KK)=SUMY(I)
51.000 51 DO 140 I=1,L
52.000 52 A(KK,I)=-1.
53.000 53 KKK=I+1
54.000 54 DO 110 J=KKK,KN
55.000 55 A(KK,J)=0.
56.000 56 C=1./A(1,1)
57.000 57 DO 120 II=2,KN
58.000 58 DO 120 J=KKK,KN
59.000 59 A(II,J)=A(II,J)-A(1,J)*A(1,1)*C
60.000 60 DO 140 II=1,L

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61.000      DO 140 J=1,N
62.000      S1=0.
63.000      S2=0.
64.000      DO 160 J=1,N
65.000      S1=0.
66.000      S1=S1+A(I,KK)
67.000      DO 150 I=1,NORD
68.000      150 S1=S1+A(I+1,KK)*X(J)**I
69.000      160 S2=S2+(S1-Y(J))*(S1-Y(J))
70.000      B=N-L
71.000      S2=DSQRT(S2/B)
72.000      163 WRITE(IOUT,7)NORD,TOL,S2,N
73.000      DO 164 I=1,L
74.000      J=I-1
75.000      164 WRITE(IOUT,8)N,A(I,KK)
76.000      162 DO 169 I=1,N
77.000      S1=0.
78.000      S4=0.
79.000      S1=A(I,KK)
80.000      DO 168 J=1,NORD
81.000      S4=S4+J*A(J+1,KK)*X(I)**(J-1)
82.000      168 S1=S1+A(J+1,KK)*X(I)**J
83.000      S3=Y(I)-S1
84.000      169 WRITE(IOUT,9)X(I),Y(I),S1,S3,S4
85.000      WRITE(IOUT,71)
86.000      71 FORMAT(//T10,'ANALYSIS RESULTS'//)
87.000      WRITE(IOUT,72)
88.000      72 FORMAT(//T2,'EXPERIMENT NO.',T22,'READING',T34,
89.000      1'CONCENTRATION',T51,'DERIVATIVE'//)
90.000      DO 188 I=1,NE
91.000      YE=0.
92.000      DYE=0.
93.000      YE=A(I,KK)
94.000      DO 187 J=1,NORD
95.000      YE=YE+A(J+1,KK)*XE(I)**J
96.000      187 DYE=DYE+J*A(J+1,KK)*XE(I)**(J-1)
97.000      188 WRITE(IOUT,5)I,XE(I),YE,DYE
98.000      IF(NORD-LAST)170,1,1
99.000      170 IF(S2-TOL)1,1,171
100.000      171 NORD=NORD+1
101.000      J=2*NORD
102.000      SUMX(J)=0.
103.000      SUMX(J+1)=0.
104.000      SUMY(NORD+1)=0.
105.000      DO 172 I=1,N
106.000      SUMX(J)=SUMX(J)+X(I)**(J-1)*W(I)
107.000      SUMX(J+1)=SUMX(J+1)+X(I)**J*W(I)
108.000      172 SUMY(NORD+1)=SUMY(NORD+1)+Y(I)*X(I)**NORD*W(I)
109.000      GO TO 93
110.000      200 CALL EXIT
111.000      ENP

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REFERENCES

- Alexander, J.E. and E.F. Corcoran, *Limn. Ocean.* 12, 236, (1967).
- Ammerman, C.B., *J. Dairy Sci.*, 53, 1097, (1970).
- Andelman, J.B., "Incidence, Variability and Controlling Factors for Trace Elements in Natural, Fresh Waters" in "Trace Metal and Metal-Organic Interactions in Natural Waters", P.C. Singer (Ed.), Ann Arbor Science Pub., Chap. 3 (1973) and references therein.
- Ardakani, M.S. and F.J. Stevenson, *Soil Sci. Soc. Amer. Proc.*, 36, 884, 1972.
- Badrinas, A., *Talanta*, 10, 704 (1963).
- Barsdate, R.J., Progress Report SAN 310 P4-3 U.S. Atomic Energy Commission.
- Benes, P., E.T. Gjessing, E. Steinnes, *Water Research*, 10, 711 (1976).
- Benes, P., and E. Steinnes, *Water Research*, 9, 741 (1975).
- Benes, P., and E. Steinnes, *Water Research*, 9, 741 (1975).
- Benjamin, M.M., Ph.D. thesis, Stanford University, Stanford CA (1978).
- Berner, R.A., *Estuaries: Amer. Assoc. Adv. Soc. Publ.* 83, 268 (1967).
- Bilinski, H., R. Hurton, and W. Stumm, *Anal. Chim. Acta.*, 84, 157 (1976).
- Borggaard, O.K., *Acad. Chem. Scand. Acta*, 28, 121 (1974).
- Brady, B. and G.K. Pagernkopf, *Can. J. Chem.* 56, 2331 (1974).
- Bresnahan, W., L.L. Grant, and J.H. Weber, *Anal. Chem.*, 50, 1675 (1978).
- Bresnahan, W., M.S. Thesis, University of New Hampshire, Durham, N.H. (1977).
- Brooks, R.R., "Pollution through Trace Elements", in "Environmental Chemistry, J.O.M. Bockris (ed.), Plenum Press, New York, Chapt. 14.
- Bryan, G.W., *Proc. Roy Soc. London* 13, 177, 389 (1971).
- Buffle, J., P. Deladoey, F.L. Greter and W. Haerdi, *Anal. Chem. Scand. Acta* (1980).
- Buffle, J., and F.L. Greter, *J. Electroanal. Chem.*, 101, 231 (1979).
- Buffle, J., and F.L. Gaeter, and W. Haerdi, *Anal. Chem.*, 49 (2), 216 (1977).
- Burch, R.D., C.H. Langford and D.S. Gamble, *Can. J. Chem.*, 56, 1196 (1978).

Caldin, E.F., J.E. Crooks, and A. Queen, J. Of Physics E , Scientific Instruments, 6, 930 (1973).

Carrp11, D., Geochim. Cosmochim. Acta., 14, 192 (1958).

Chaberek, S., and A.E. Bartell, "Organic Sequestering Agents", John Wiley and Sons, New York (1959).

Chau, Y.K., R. Gachter and K. Lum-Shue-Chan, J. Fish. Res. Board Can., 31, 1515 (1974).

Cheam, V. and D.S. Gamble, Can. J. Soil. Sci., 54, 413 (1974).

Conde, C.P., J.A. Perez. Bustamante, F.B. Marti, Anal. Chim. Acta, 73, 191 (1974).

Dagnall, R.M., R. Smith, T.S. West, Analyst, 92, 20, (1967).

Davey, E.W., M.J. Morgan and S.J. Erickson, J. Limnol. Oceanogr. 18, 993 (1973).

Davis, J.A. III, Ph.D. thesis, Stanford University, Stanford, CA., (1977).

Davis, J.A., and J. Jacknow, J. Water Poll. Control Fed., 47, 2292, (1975).

Diehl, H. et al., Anal. Chem. 35, 1144 (1963).

Dousma, J., and P.L. de Bruyn, (J. Coll. Int. Sci 56, 527 (1976).

Dubach, P. and N.C. Mehta, Soils Fertil., 26, 293 (1963).

Eigen, M., R.G. Wilkins, Adv. Chem. Sci. 49, 55 (1965).

Ernst, R., H.E. Allen and K.H. Mancy, Water Research, 9, 969 (1975).

Florence, T.M. and G.E. Batley, Talanta, 24, 151 (1977).

Fuoss, R.M., J. Am. Chem. Soc., 80, 5059, (1958).

Gamble, D.S., Can. J. Chem., 48, 2662 (1970).

Gamble, D.S., ibid, 50, 2680 (1972).

Gamble, D.S., ibid, 51, 3217 (1973).

Gamble, D.S., C.H. Langford and J.P.K. Tong, Can. J. Chem. 54, 1239 (1975).

Gamble, D.S., J.A. Marinsky and C.H. Langford, in "Humic-Trace Metal in Equilibria in Natural Waters", submitted to Commission V.6. on Equilibrium Data, Anal. Chem. Div. of the I.U.P.A.C. (1981).

Gamble, D.S. and M. Schnitzer, "The Chemistry of Fulvic Acid and the Reactions with Metal Ions" in "Trace Metals and Metal-Organic Interactions in Natural Waters", P.C. Singer (ed.), Ann. Arbor Science Pub., Michigan, 1973.

Gamble, D.S., M. Schnitzer and I. Hoffman, Can. J. Chem., 48, 3197 (1970).

Gamble, D.S., M. Schnitzer, and D.S. Skinner, Can. J. Soil Sci. 57, 47 (1977).

Gamble, D.S., A.W. Underdown and C.H. Langford, Anal. Chem. 52, 1901 (1980).

Gardiner, J., Water Research, 8, 23, (1974).

Gardiner, L.R., Geochim. et. Cosmo Acta, 38, 1297 (1974).

Garrels, R.M. and M.E. Thompson, Am. J. Sci., 260, 57 (1960).

Ghosh, K. and M. Schnitzer, Soil Science, 129, 266, (1980).

Greter, F.L., J. Buffle, W. Haerdi, J. Electroanal. Chem., 101, 211 (1979).

Guy, R.D., Ph.D. thesis, Carleton University, Ottawa, (1976).

Guy, R.D. and C.L. Chakrabarti, Can. J. Chem., 54, 2600 (1976).

Hiraki, K.; Bull. Chem. Soc. Jpn, 45, 789 (1972).

Horne, R.A., in "Marine Chemistry", John Wiley and Sons, Inc., 1969, Chap. 6.

Hsu, P.H., Clays and Clay Minerals, 21, 267 (1973).

Irving, H., and R.J.P. Williams, Nature, 162, 746 (1948).

Jenne, E., Advances in Chemistry Series, 73, 337, (1968).

Kerndorfz, H., and M. Schnitzer, Water, Air and Soil Pollution, 12, 319, (1979).

Khan, T.R. and C.H. Langford, Can. J. Chem., 54, 3192 (1976).

Khanna, S.S. and F.J. Stevenson, Soil Sci., 93, 298 (1962).

Kharkar, D.P., K.K. Tarekian and K.K. Bertine, Geochim., Cosmochim. Acta, 32, 285 (1968).

Kononova, M.M., "Soil Organic Matter", 2nd ed., Pergamon Press, Oxford, 1966.

Krauskopf, K., Geochim. et Cosmo. Acta., 9, 1, (1956).

Langford, Inorg. Nucl. Chem. Letters, 9, 679 (1973).

Langford, C.H., Water Quality Parameters, ASTM S1P 573. American Society for Testing and Materials, 414, (1975).

Langford, C.H., D.S. Gamble, A. Underdown, S. Lee, "The Interaction of Metal Ions with a Well Characterized Fulvic Acid", in "Terrestrial and Aquatic Humic Substances", R.F. Christman and E. Gjessing, editors, Ann Arbor Scientific, Ann Arbor, M.I. (1982) in press.

Langford, C.H., S.M. Wong, A.W. Underdown, Can. J. Chem., 59, 181, (1981).

Langford, C.H., and T.R. Khan, Can. J. Chem., 53, 2979 (1970).

Langford, C.H. and T.R. Khan, Can. J. Chem., 54, 100, (1978).

Langford, C.H. and V.S. Sastri in "Reaction Mechanisms in Inorganic Chemistry", Vol. 9, Chapter 6, Edited by M.L. Tobe, Butterworths, London (1972).

Langford, C.H. and T.R. Stengle, Coord. Chem. Rev., 2, 349 (1967).

Leckie, J.O., and R.O. James (1975), "Control Mechanisms for Trace Metals in Natural Lakes in Aqueous-Environmental Chemistry of Metals", A.J. Rubin (Ed.) Ann Arbor Science Pub., Ann Arbor, Mich., Chap. 1.

Lee, S., M.Sc. thesis, Brock University, St. Catharines Ontario, (1977).

Li, N.C., W.M. Westfall, A. Lindenbaum, J.M. White and J. Schubert, J. Am. Chem. Soc. 79, 5864 (1957).

Liu, J., C.I. and J.C. Barlar, Jr., J. Am. Chem. Soc., 73, 5432 (1951).

Manahan, S.E., "Environmental Chemistry", 2nd Ed., Willard Grant Press, Boston, Mass., Chap. 4.

Mantoura, R.F.C., A. Dickson and J.P. Riley, Estuarine Coastal Mar. Sci., 6, 387 (1978).

Mantoura, R.F.C., and J.P. Riley, Anal. Chim. Acta, 76, 97 (1975).

Mak, M.K.S., Ph.D. thesis, Carleton University (1980).

Mak, M.K.S., C.H. Langford and T.R. Khan, J. Indian Chem. Soc., LIV, Jan., Feb., Mar., 51, (1977).

Margerum, D.W., J.B. Pausch, G.A. Nyssen and G.F. Smith, Anal. Chem., 41 (2), 233 (1969).

Margerum, D.W. and R.K. Steinhaus, Anal. Chem., 37, 222, (1965).

Martin, D.F., "Coordination Chemistry of the Oceans" in "Equilibrium Concepts in Natural Waters Systems", Advances in Chem. Ser. 67, (1967).

Matson, W.R. Ph.D. Thesis, Mass. Inst. Tech., (1968).

Mortenson, J.L., Soil Sci. Soc. Am. Proc. 27, 179, (1963).

Moss, M., Presented at the 49th Annual Meeting of the Royal College of Physicians and Surgeons of Canada, 1980.

National Academy of Sciences, Copper, Series on Medical and Biological Effects of Environmental Pollutants, Washington, D.C. (1977).

Neely, J. and R. Connick, J. Am. Chem. Soc., 92, 2476 (1970).

Neyroad, J.A. and M. Schnitzer, Soil Sci. Soc. Am. Proc., 38, 907, (1974).

Nielson, E.S. and W. Wium-Andersen, Marine Biology, 6, 93 (1970).

Nissenbaum, A., and D.J. Swine, Geochimica et Cosmochim Acta, 40, 809, (1976).

Ochiai, El-Ichiro, in "Bioinorganic Chemistry, An introduction", Allyn and Bacon Inc. Boston, Mass. (1977). Chap. 1.

Odier, M. and Y. Plichon, Anal. Chim. Acta, 55, 209, (1971).

Olsen, R.L. and H. Diehl, Anal. Chem. 35, 1142 (1963).

Pagenkopf, G.K. and C. Whitworth, J. Inorg. Nucl. Chem. 43, 1219, (1981).

Pal, B.K., D.E. Ryan, Anal. Chim. Acta, 47, 35, (1969).

Pausch, J.B. and D.W. Margerum, Anal. Chem., 41(2) 226, (1969).

Pearson, R.G. and P. Ellgen, Inorg. Chem., 6, 1379 (1967).

Perrin, D.D. in "Organic Complexing Reagents", Chap. 9, Interscience Publishers, John Wiley & Sons, Inc. (1964).

Ramamoorthy, S., and D.J. Kuohner, Nature, 25b, 399 (1975a).

Rashid, M.A., Soil Sci., 111(5), 298, (1971).

Reuter, D.H. and E.M. Perdue, Geochimica et. Cosmochim Acta, 41, 325 (1977) and references therein.

Ryan, D.E., and B.K. Pal, Anal. Chim. Acta, 44, 377 (1969).

Saar, R.A., and J.H. Weber, Can. J. Chem., 57, 1263, (1979) and references therein.

Saar, R.A., and J.H. Weber, Geochim. Cosmochim. Acta, 44, 1381 (1980).

Scherbina, V.V., Geochimiya, 5, 486 (1956).

Schnitzer, M., International Atomic Energy Agency, Vienna, Soil Organic Matter Studies II, pp 117-132 (1977).

Schnitzer, M., in "Soil Organic Matter", Schnitzer, M., and S.U. Khan, Editors, Elsevier Scientific Publishing Co., The Netherlands (1978), Chap. 1.

Schnitzer, M., "Effect of Low pH on the Chemical Structure and Reactions of Humic Substances" in "Effects of Acid Precipitation of Terrestrial Ecosystems", T.C. Hutchinson and M. Havas Eds., Plenum Pub. Corp. (1980).

Schnitzer, M., Dejardin, J.S., Soil Sci. Soc. Am. Proc., 26, 362 (1962).

Schnitzer, M., and E.H. Hansen, Soil Science, 109, 333, (1970).

Schnitzer, M. and H. Kerndorff, J. Environ. Sci. Health, B15 (4), 431 (1980).

Schnitzer, M. and S.U. Khan, in "Humic Substances in the Environment", Marcel Dekker, Inc., New York (1972).

Schnitzer, M., Skinner, S.I.M., Soil Sci., 96, 86 (1963).

Schnitzer, M., and S.I.M. Skinner, Soil Sci., 99(4), 278, (1965).

Schnitzer, M., and S.I.M. Skinner, Soil Sciences, 102, 361 (1966).

Shaw, D.J., "Introduction to Colloid and Surface Chemistry", Butterworth and Co. (pub.), London-Boston, Second Edition, Chapter 3, (1970).

Shcherbov, D.P., "Fluorescence Analysis of Inorganic Materials (Review), translated from Zavodskaya Laboratoriya, 34, 641 (1968).

Shuman, M., in "Terrestrial and Aquatic Humic Substances in, R.F. Christman and E. Gjessing editors, Ann Arbor Scientific, Ann Arbor, M.I., (1982) in press.

Sillan, L.G. and A. Mantell, "Stability Constants of Metal ion Complexes". Section II. Organic Ligands. Special publication No. 17, The Chemical Society, Burlington House, W. 2, London (1964).

Sipos, S., E. Sipos, I. Dekany, F. Szanto, and B. Lakatos, International Peat. Cong., 4th Otaniemi, Finland, Proc., V. 4, 255 (1972).

Stowey, J.F. and D.W. Wood, Geochim. Cosmochim. Acta, 35, 121 (1971).

Stowey, J.F., L.M. Jeffrey, and D.W. Hood ; Nature 214, 377, (1967).

Stacey, K.A., "Light-Scattering in Physical Chemistry", Butterworth Scientific Publications, London Chap. 1 (1956).

Stevens, H.M. Anal. Chim. Acta, 20, 389 (1959).

Stevenson, F.J., Soil Sci. Soc. Amer. Proc., 40, 665 (1976).

Stevenson, F.J., Soil Sci., 123, 10, (1977).

- Thompson, M.E., Science, 153, 866 (1966).
- Truitt, R.E., J.H. Weber, Anal. Chem., 53, 337 (1981).
- Turekian, K.K., and M.R. Scott, Env. Sci. Tech., 1, 940 (1967).
- Underdown, A.W., Ph.D. thesis, Carleton University, Ottawa, (1982).
- Underdown, A.W., C.H. Langford, D.S. Gamble, Anal. Chem., 53, 2139 (1981).
- Underwood, E.J., Trace Elements in Human and Animal Nutrition, 3rd ed. Academic Press, New York page 543.
- Van Dijk, H., Geoderma, 5, 53, (1971).
- Vesely, J., D. Weiss and K. Stubik, in "Analysis with Ion-Selective Electrodes", Ellis Horwood Ltd. Pubs., Sussex, England (1978) and references therein.
- Weast, R.C., "Handbook of Chemistry and Physics", 56th Edition, Weast, R.L. editor, CRC Press, Ohio 44128.
- Weber, J.H. and S.A. Wilson, Water Research, 2, 1079 (1975).
- Wershaw, R.L. and D.J. Pinckney, Journ. Research U.S. Geol. Survey 1, 361, May-June (1973).
- Wershaw, R.L. and D.J. Pinckney, Journ. Research U.S. Geol. Survey 5, 571, Sept.-Oct. (1979).
- Wershaw, R.L., D.J. Pinckney and S.E. Booker, Jour. Research U.S. Geol. Survey, 5, 565, Sept.-Oct. (1977).
- Whitworth, C. and G.K. Pagenkopf, J. Inorg. Nucl. Chem., 41, 317 (1979).
- Willis, G., W.H. Woodruff, J.R. Iryinger, D.W. Margerum and H. Pardue, Anal. Chem., 42, 1350 (1970).
- Wilson, S.A., T.C. Huth, R.E. Arndt, and R.K. Skogerboe, 52, 1515 (1980) and references therein.
- Wilson, D.E. and P. Kinney, Limnol. Oceanogr., 22, 283, (1977).
- Zirino, R., and S. Yamamoto, Limn. and Oceanogr., 17, 661, (1972).

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