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**TRACE METAL ION BINDING TO FULVIC ACIDS IN MODEL
SYSTEMS AND FRESHWATERS**

by

AMINA L.R. SEKALY

M.Sc. Carleton University

Dottorato di Ricerca in Science Chimiche, University of Rome "La Sapienza"

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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acceptance of the thesis,

**TRACE METAL ION BINDING TO FULVIC ACIDS IN MODEL SYSTEMS
AND FRESHWATERS**

submitted by

AMINA L.R. SEKALY, M.Sc.

Dottorato di Ricerca in Science Chimiche, University of Rome, "La Sapienza"

in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

Chair, Department of Chemistry
Thesis Co-Supervisor
Thesis Co-Supervisor
External Examiner

2000, Carleton University

ABSTRACT

Bioavailability of trace metals is known to be often correlated with free metal ion activity in freshwaters. The free metal ion activity of trace metals is dependent on the dissociation of metal complexes with humic substances which are ubiquitous in freshwaters. Trace metal complexation by humic substances determines at least to some extent solubility, mobility and transport of trace metals in freshwaters. An experimental study of dissociation of fulvic acid complexes of selected trace metals in model systems and in freshwaters is described.

Metal binding characteristics were investigated as a function of the [metal]/[FA] mole ratio by Competing Ligand Exchange Method using Chelex-100 as a competing ligand and inductively-coupled plasma mass spectrometry to measure the first-order dissociation of metal complexes. The kinetic data of metal-complex dissociation were analyzed by non-linear regression analysis. The model systems consisted of Ni(II), Cu(II), Co(II), Pb(II), and Zn(II) and two well-characterized fulvic acids. The freshwater samples were unpolluted river surface water from the Rideau River, and metal-polluted streams from Sudbury (Canada) mines area.

The dissociation rate coefficient and the percentage of the faster kinetically distinguishable component increased with increasing [metal]/[FA] mole ratio. The percentage of the faster kinetically distinguishable component of Ni(II) complexes in freshwaters from the Sudbury mines area increased with increasing [Ni(II)]/[DOC] ratios. The order of increase in the lability of the complexes in the model systems and the freshwater samples was $\text{Co(II)} > \text{Zn(II)} > \text{Ni(II)} > \text{Cu(II)}$. Even in the presence of excess of the FA, Cu(II) and Co(II) competed with Ni(II) for strong binding sites of the FA.

Since fulvic acids are heterogeneous complexants, the dissociation rate coefficients of their metal complexes are modelled more satisfactorily as a distribution function. Decay curves of the dissociation of metal fulvic acid complexes were analyzed using a Distribution Analysis Method. Simulated data were first subjected to the Distribution Analysis Method to ferret out its capabilities and limitations. Analysis of the experimental data by the Distribution Analysis Method shows that metal-FA complexes have a broad range of dissociation rate coefficients rather than a single value.

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SECTION 1

THEORETICAL BACKGROUND

CHAPTER 1

INTRODUCTION

The main objective of this work was to study the interactions between selected metal ions and two well-characterized, naturally-occurring organic materials, called fulvic acids, in model systems and in freshwaters. The Competing Ligand Exchange Method using inductively-coupled plasma mass spectrometry to measure the rates of dissociation of metal complexes was employed for this study in the estimation of the dissociation rate coefficients together with their analysis by the Non-Linear Regression Analysis Method and the Distribution Analysis Method.

Complexation of metal ions in freshwaters is largely controlled by the colloidal ligands: humic substances and hydrous oxides. Naturally-occurring dissolved organic matter in freshwaters include humic and non-humic substances. The latter include polymers such as polypeptides, polysaccharides, nucleic acids, and small molecules such as sugars and amino acids [1-3]. Their concentrations remain low because they are easily degradable by the most common hydrolitic enzyme systems. The hydrolytic breakdown products are always found at low concentrations because they are rapidly assimilated by biological systems. Humic substances are therefore the main contributors to dissolved organic carbon in freshwaters.

Fulvic acid, which is the acid-soluble fractions of humic substances which are ubiquitous in the natural environment. Humic substances are derived from the chemical

and biological degradation of plant materials and/or synthetic activities of microbes. In this study, fulvic acid was used as model for the laboratory examination of the nature of interactions between dissolved organic substances and metal ions in model systems and freshwaters.

In view of the importance of humic substances as complexants in freshwaters, the experiments in this work were designed to study the metal-organic complexation in model systems and freshwaters using two stoichiometrically-defined, well-characterized, soil-derived (pedogenic) fulvic acids (FA) as representative of humic substances. The use of soil fulvic acid is justified since the major fraction of organic matter in freshwaters resembles soil fulvic acids. The metals studied were Ni(II), Cu(II), Co(II), Pb(II) and Zn(II).

This thesis is divided into three sections. Section 1 describes theoretical background and contains three chapters. Chapter 1 gives a brief introduction, explaining the rationale behind this thesis research and the general lay out of the thesis. Chapter 2 presents the metal complexation properties of humic substances and the effects of trace metal competition. Chapter 3 presents various models for studying metal-fulvic acid interactions and introduces the topics of chemical speciation and bioavailability of metals, and metal speciation dynamics. Section 2 describes the experimental determination of dissociation rate coefficients and contains three chapters. Chapter 4 describes methodology and materials. Experimental results on kinetics of dissociation of metal-fulvic acid complexes are presented and discussed in Chapter 5; the important contributions of the electron configuration of the first series of the 3d transition metal ions on the lability of the metal-fulvic acid complexes are also presented and discussed.

Chapter 6 presents the results of trace metal competition for binding sites of fulvic acid. Section 3 describes the evaluation of a method of kinetic data analysis and contains two chapters. Chapter 7 presents the Distribution Analysis Method using simulated data. Chapter 8 presents the application of the Distribution Analysis Method to model systems. The environmental significance of the results (the effects of other trace metal competition on the lability of Ni(II)-FA complexes) is presented in CONCLUSIONS.

CHAPTER 2

HUMIC SUBSTANCES IN THE NATURAL ENVIRONMENT

Natural organic matter

Natural organic matter (NOM) is ubiquitous in the aquatic and the terrestrial environment. NOM plays an important role in regulating trace metal speciation, transport and bioavailability in the natural environment [4-6]. The NOM found in the aquatic environment possesses a large variety of properties and is composed of an extremely complex mixture of compounds. The nature of NOM found in aquatic environment depends primarily on whether the source is aquagenic, i.e. derived from water, or pedogenic, i.e. derived from soils [7]. Aquagenic organic matter is produced in the water mass itself by excretion by, and decomposition of, plankton and aquatic bacteria. Pedogenic organic matter is that due to rainwater leaching of the soluble fraction of soil organic matter, which is itself a result of the decomposition of higher plants by bacteria and fungi.

Humic substances constitute a major fraction of soil organic matter (60-70%) and (30-50%) of aquatic organic matter [8]. Humic substances are traditionally divided into three fractions based on their solubility in acids and bases; (i) fulvic acid (FA) is the low molecular weight fraction of humic substances and is soluble in alkaline and acidic solutions and in water; (ii) humic acid (HA) is the fraction that is soluble in dilute

alkaline solution, but is precipitated in acid solution and in water; and (iii) humin is the fraction that is not soluble in alkaline or acid solutions [9-10].

Humic substances are large, structurally-complex macromolecules. They consist of carbon, oxygen, hydrogen and sometimes small amounts of nitrogen and occasionally phosphorous and sulphur. Despite many decades of research, a universally accepted structure for humic substances does not exist. Numerous tentative models have been proposed [11-13] that can be modified when new data become available. The HA structure shown in Figure 1 [14] is a tentative model on the basis of the result of pyrolysis-GC/MS [15] and pyrolysis –FIMS [16], ^{13}C NMR [17] chemical [18], oxidative and reductive degradation [19], colloid-chemical [20] and electron microscope [21].

In general terms, the structures can be described as assemblies of covalently linked aromatic and aliphatic residues carrying carboxyl, phenolic and alkoxy groups, although sulphate esters, alanine moieties, semiquinone, phosphate ester, and hydroquinone [22] have been proposed to exist in some humic isolates.

A comprehensive review of the different techniques that can be used to characterize humic material can be found in the literature [23]. Elemental analysis is most commonly used to characterize humic substances, but it does not provide an absolute molecular formula; however, it does provide general compositional information and sets limits as to possible molecular composition.

The nature of aquatic humic substances is quite different from soil humic substances [24]. Aquatic humic substances consist of carbohydrate–protein complexes, whereas terrestrial or soil humic substances are mainly ligno-protein complexes [25]. When the percent composition data are expressed as the atom ratio: C/H, O/C, and C/N,

some general characteristics of humic substances (HS) become visible. The carbon content of aquatic humic substances is greater, whereas the oxygen content is less than in soil humic substances [9]. Carbon/nitrogen ratios for aquatic FA (45-55:1) and HA (18-30:1) are considerably greater than carbon/nitrogen ratios in soil FA (average 20:1) and HA (average 10:1) and in HS from aquatic sediments [9]. Aquatic humic substances appear, therefore, to be depleted of nitrogen as compared to adjacent soils and sediments. Between the two types of humic substances there are also differences in the functional groups. The phenolic OH content of aquatic HS ($1-2 \text{ meq g}^{-1}$) is considerably lower than that in soil HS. The total acidity, which is attributed to the sum of the carboxyl and phenolic-OH group content, shows some variations, which are due to the origin. High total acidity value indicates high cation exchange capacity (CEC) and complexing power. Comparison of HA and FA, both aquatic and terrestrial, reveals differences in elemental composition, molecular weight and functional group content [9]. The FA fraction exhibits lower molecular weight and lower levels of total and aromatic carbons. In general, humic acid is higher in C and N, but lower in O and S content than fulvic acid. Oxygen and oxygen-containing functional group levels are higher in FA than HA [9]. HA contains fatty-acid products with longer chains than FA, suggesting a higher hydrophobicity than FA. ^{13}C NMR spectra indicate that approximately 65% of the carbon in aquatic FA is aliphatic and many of the COOH and OH groups are attached to aliphatic carbons. Aquatic FA, similar to soil FA, exhibits low molecular weight (500-2000 Da), whereas aquatic HA ranges in molecular weight from 2000 Da to much higher molecular weights, and is more polydisperse and colloidal [9].

Figure 1 A tentative structure proposed for humic acid [14].

Metal ion binding by humic substances

Knowledge of the interaction between metal ions and humic substances is important in understanding chemical speciation, bioavailability and transport of metal ions in the natural environment [4, 26-29]. Because of the binding of metal ions by humic and fulvic acids, the availability of the metals for uptake by various organisms may decrease; on the other hand, the mobility of the metals may increase because these naturally-occurring complexing agents may act as a solubilizer in the aquatic environment.

Humic substances are indispensable for ensuring the chemical regulation of different constituents, in particular, for buffering local excess or deficiencies of metals. In other words, it is the basis for the *chemostasis* [30] of an aquatic system, an indispensable property for the maintenance of life.

Humic substances as complexants

The vast majority of chemical reactions that occur between metal cations and ligands can be classified as *complexation* reactions. The complexants in the aquatic environment can be roughly divided into two groups: “**simple**” and “**heterogeneous**” complexants, based on their structural complexity.

Simple complexants are those for which:

- 1) The actual molecular structure (composition and geometry) is well defined;
- 2) The amount of complexant present and its complexes present can be expressed in molar concentration units;
- 3) The stoichiometry of each complex formed with all the metal ions is known, and

- 4) The standard free energy of formation (ΔG^θ) of each metal-ligand complex has a unique value and is known for the range of system conditions to be studied.

Heterogeneous complexants include those ligand-site-containing structures found in a given aqueous environment that do not satisfy all the criteria for a “simple” complexant described above. Humic substances are heterogeneous complexants. Heterogeneity refers to both *physical and chemical properties* [10, 31-32]. The *physical heterogeneity* is due to different molecular masses and shapes of the constituent parts of the molecules. These properties make metal binding with humic substances highly complex and variable depending on experimental conditions such as metal ion concentration, metal/humic substances mole ratio, ionic strength, and pH.

In general the heterogeneity of humic substances results from the following three characteristic properties:

- 1) **Polyfunctionality** — Each molecule contains a number of different functional groups. Fulvic and humic acids primarily have carboxylic or phenolic groups, but moieties bearing sulphur and nitrogen are also known.
- 2) **Oligoelectrolytic properties** — Each individual molecule of humic substances can acquire a large number of electric charges (negative charge) because of ionization of its functional groups. The oligoelectrolytic properties are dependent on pH, and are important at pH 5-8, typical of freshwaters because of dissociation of the functional groups at this pH range [4, 33-34, 32].
- 3) **Conformational properties** — Factors such as pH and ionic strength affect *conformational changes, degree of aggregation, coiling and uncoiling* of the various molecules of humic substances and consequently metal ion binding. This is probably due

to changes in the accessibility of some ligand sites to metal ions because of conformation changes [35, 36]. It has been reported that at low pH, humic substances have a tightly coiled, cross-linked (via H-bonding) conformation in which metal binding sites are not readily available, but at higher pH, they have a more open conformation [37, 38]. Consequently, the conformation and degree of aggregation of humic substances affect metal ion binding to HS [39, 40]. In particular, the conformation depends on the hydration-dehydration process, the formation of hydrogen bonds, the formation of electrostatic interactions among the various functional groups on a single molecule, and the formation of metallic bridges between two hydrodynamically separate molecules, which themselves depend on the degree of occupation of the complexing sites [41]. In all cases, the relative importance of these properties varies with pH, ionic strength and the degree of occupation of the complexing sites.

Humic substances associate with nearly everything found in water, including trace metals, hydrous metal oxides, calcium carbonate, herbicides, hydrocarbons and protein. They are sufficiently hydrophilic to be transported easily, thereby affecting the distribution of any metals associated with them. They act as oxidizing or reducing agents, depending on environmental conditions. For example, humic substances have been known to reduce Hg(II) to volatile Hg^0 under natural pH conditions, thereby providing a potential pathway for the mobilization of mercury in the environment.

Binding sites in humic substances

The functional groups of humic substances can be conceptually subdivided into *major* and *minor* sites [26, 36, 42-44]. The *major sites* include those sites types, few in

number, forming the majority of the sites of humic substances (e.g., carboxylate and phenolate groups). Since they represent the largest proportion of dissociable groups, they will play an important role in the polyelectrolytic properties of humic substances. The importance of this effect on the overall complexing strength of humic substances will therefore depend largely on the degree of saturation of those major sites. On the other hand, since the chemical diversity of the site types included in the major sites is poor, their polyfunctional properties are restricted. Indeed, although the heterogeneity of major sites is not negligible, it is mainly due to physical factors like conformational changes. It is therefore more limited than that of minor sites, which differ from each other both by their physical and chemical nature.

The *minor sites* include a small fraction of the total sites [42-43], but a large number of site types with a broad range of associated complexation constants spanning several orders of magnitude [36]. This group may often be the most important as far as the control of trace metals in aquatic systems is concerned, because the values of their formation constants are much higher than those of the major sites. The major sites (which are hard Lewis bases) are oxygen-containing groups, for example, carboxylate and phenolate groups which will react preferentially with hard Lewis acids (mostly, metals having 18-electron or 20-electron valence shells). The minor sites (soft Lewis bases) of humic substances, which include in particular N and S bearing groups, preferably bind to soft Lewis acids (metals) [45]. Soft cations form bonds which are more covalent and for which the free energy of complexation is more enthalpic in origin [46].

Humic substances typically bear an overall negative charge in the pH range of natural waters (5-8); hence, the principal complexation reactions they engage in aqueous

systems are those with protons and metal cations, that is, with Brönsted and Lewis acids. In particular, humic substances will bind with all transition metals, including the lanthanides, the actinides, and the alkaline earth metals [27], although the strength of the binding depends very strongly on the electron-configuration of metal ion with humic substances favouring softer Lewis bases such as copper and lead over harder Lewis bases, such as zinc and calcium [47-48].

Metal competition

In aqueous systems, protons (or hydroxyl ions) are by definition present. Since protons largely determine the state of the functional groups and the charge of the humic substances, a proton effect on the metal ion binding is the least that is to be expected. Additionally, in freshwaters, trace metals such as Ni(II), Cd(II), Cu(II) and Co(II) coexist with major cations such as Ca^{2+} , Mg^{2+} , Na^+ and K^+ . The major cations are usually present in freshwaters in concentrations ($\sim 10^{-3}$ M), which are several orders of magnitude greater than those of trace metal ions ($\sim 10^{-8}$ M). Since cations co-exist and interact with all ligands, and each ligand interacts with all cations, competition between the various cations and ligand anions [32] must be considered. The effect of competition between selected trace metals will be discussed in Chapter 6.

CHAPTER 3

METAL COMPLEXATION WITH FULVIC ACIDS

From a modelling perspective, the simple ligands of known structure have reactions of well-defined stoichiometry and can be dealt with using the mass action approach. In contrast, the fulvic and humic acids are oligoelectrolyte macromolecules of unknown structure. They have high molecular weights, which range from 500-1500 Da for fulvic acids to 1500-300,000 Da for humic acids. Humic and fulvic acids contain a range of organic functionalities; for example, they possess several carboxylic functional groups with a range of acidity constants. To complicate matters further, these organic macromolecules develop surface charge giving rise to electrostatic interactions, which influence the acidity of each functional group. Therefore, dissociation rate coefficients and equilibrium constants of metal-humate complexes cannot be interpreted by classical molecular models (which are based on the existence of very limited number of coordinating sites in a given complexant). Even after extensive separations humic substances still consist of mixtures of fulvic and humic acids that contain an extremely large number of similar but non-identical functional groups. These functional groups differ in their affinity for binding cations; this leads to chemical heterogeneity at the molecular level.

To overcome the above problems, a number of modelling approaches have been developed in recent years to describe some of the observed behaviour of humic substances. Some of these models are described in the following sections.

Discrete Multi-Ligand Model

Discrete Multi-Ligand models have been used to model humic substances. The Discrete Multi-Ligand model assumes that different independent, or discrete, complexant sites are present on humic substances [49]. The different site types in the model can be part of a larger molecule or can be present as a mixture of simple ligands in the solution. Mattigod and Sposito [50] were the first to propose the Discrete Multi-Ligand Model. Mixtures of citric, malonic and aspartic acids have been used to simulate copper binding [51]. In order to use the Discrete Multi-Ligand Models, simplifying assumptions about the chemistry of humic substances have to be made because the exact molecular structure of the humic macromolecule is unknown.

An alternative to the above approach that attempts to address the problem of chemical heterogeneity of acidic functional groups and the numerical spread of their associated equilibrium constants is the Continuous Distribution Model.

Continuous Distribution Model

Continuous Distribution Models assume that the property of humic substances, such as rate or equilibrium constant, cannot be described by a unique value, or even a few discrete values, but by a continuous distribution. This statistical concept has been used now for some time and it is physically more realistic since humic substances contain a distribution of site types [52-53]. The Continuous Distribution Model is statistical in concept and does not allow us to distinguish the nature of each individual reaction on the molecular scale. However, it permits a detailed prediction of the behaviour of the entire system studied. Furthermore, the advantage of the Continuous Distribution Model for

continuous heterogeneity is the small number of parameters involved. In many cases only two parameters suffice to describe the metal binding: a parameter that determines the width of the distribution and a median affinity constant. Disadvantages are that the extension to multi-component binding is complicated and in most cases for all sites the same type of binding equation needs to be used [54].

Distribution Analysis Methods can be used to characterize binding under equilibrium conditions [55-57] and non-equilibrium conditions [58-59, 65]. Several models have been developed for analysis of kinetic data: discrete multi-component model [60-63], discrete kinetic spectra [64], continuous kinetic spectra [65], and log-normal distribution [64, 66].

In this thesis, a Continuous Distribution Method that is capable of retrieving a distribution of rate coefficients from experimental data has been adopted. This methodology is applied for the estimation of dissociation rate coefficients of metal-fulvate complexes. The algorithm was developed originally for the determination of lifetime distribution for fluorescence decay, and the application of the method has been described by Gakamasky et al. [67]. Although the rate coefficients for fluorescence decay are many orders of magnitude smaller than the rate coefficients for metal-complex dissociation found in our study, the ratio of the rate coefficients used by Gakamasky et al. [67] in their simulations are similar to ours, and their results compare well with ours. To our knowledge, the Distribution Analysis Method has not yet been applied to obtain the dissociation rate coefficients of metal complexes with humic substances. The Distribution Analysis Method will be described in detail in Chapters 7 and 8.

Chemical speciation and bioavailability of metals

There is no universally accepted definition of *chemical speciation*, and various meanings have been attributed to the term by various workers [68]. In the present context, chemical speciation is defined as the occurrence, distribution and characterization of various chemical species of an element [68]. Chemical speciation involves the analysis of chemical species leading to their identification in a defined sample; the measurement of the concentration of species of an element in a defined sample; the *reactivity* of a given species; and the *transformation* of one species into another. The chemical speciation of trace metals, or their distribution among various metal complexes and particulate forms, varies both spatially and temporally in freshwaters.

Metal speciation has been shown to govern important biotic uptake processes including the acquisition of essential metals, the regulation of growth in phytoplankton, the entry of toxic metals into aquatic food chains, and the toxic effects of metals on higher organisms, such as fish. Most of the chemical speciation studies in aquatic systems have been done with the assumption that all chemical reactions in the system have attained equilibria, and the distribution is controlled by equilibrium constants [42, 69-72]. Modelling studies have also been done using an equilibrium approach [73].

Equilibrium speciation, however, is not adequate to represent chemical species in aquatic systems such as freshwaters because these are multiphase, multicomponent, open systems, in which a myriad of interconnected chemical reactions, including those involving biota, proceed simultaneously between the various phases. It is in this complicated milieu that chemical speciation must be applied. Hence, a quantitative description of chemical speciation of metal ions in freshwaters in terms of the various

chemical species cannot be considered complete without adequate characterization of the *rates* of the chemical reactions [71, 74-75]. Furthermore, many metal complexes in natural waters dissociate slowly, and equilibrium conditions may not be established during the time of the experiment [76]. Coordination equilibrium of trace metal complexes of humic substances in soils and freshwaters are typically slow, making distributions of trace metals among various thermodynamically possible chemical species kinetically controlled.

Bioavailable metal ions can be defined as the chemical form (chemical species) of the metal that can be absorbed by biota. Over the period 1976 to 1983, a convincing body of evidence was developed to support the theory that the biological response elicited by a dissolved metal is usually a function of the free-metal-ion concentration, $M(H_2O)_n^{n+}$. The free-metal-ion concentration is determined not by the total dissolved metal concentration, but by the concentration and nature of the ligands present in solution. To rationalize these experimental observations, Morel [77-78] formulated the Free-Ion Activity Model (FIAM). The FIAM assumes fast transport and adsorption/desorption kinetics such that a (pseudo-) equilibrium is established between metal species in the bulk solution and those at the biological surface. It follows that the identity of the metal form(s) reacting with the cell surface is of no biological significance. No single species in solution can be considered more or less bioavailable than another. This suggests that the equilibrium speciation approach will be optimal for slowly-dissociating metal complexes. Therefore, both bioavailability for growth and toxicity for inhibition, may depend on the rate of release of free metal ion into the solution. Consequently, rate coefficients for the dissociation of metal complexes are important parameters of metal complexes; hence,

there is a need for investigating the factors that determine rate coefficients for dissociation of metal complexes in freshwaters.

Throughout the genesis and evolution of the FIAM, there has been awareness that non-equilibrium conditions could change the apparent rate-controlling species for metal uptake. Jackson and Morgan [79] were the first to explore the implication of *chemical kinetics* for metal uptake by aquatic organisms [80], examining theoretically both the possibility that diffusion-limited uptake of a metal could be enhanced by the dissociation of *labile* metal complexes. In other words, the concentrations of the free metal ion is not completely controlled by equilibria.

Metal speciation dynamics

There is a growing awareness of the importance of a kinetic approach for chemical speciation. However, there is little information on the rates of chemical reactions in natural aquatic systems. This is mainly due to the complexity of naturally-occurring organic ligands such as humic substances. Metal-exchange reactions involving such complexants are difficult to study and very few estimates of dissociation rate coefficients of metal complexes have appeared in the literature. One of the methods that has been developed to study kinetic speciation is described in the next section.

The role of kinetic factors for the reactions of **simple** complexant L has been discussed by Pankow and Morgan [81], who have compiled the rate coefficients for pertinent chemical reactions in aquatic systems. However, data are rare for **heterogeneous** complexants such as humic substances, and the dissociation rate

coefficient, k_d , can usually only be estimated from the corresponding equilibrium constant, K :

$$K = \frac{k_f}{k_d} \quad (3.7)$$

where k_f is the formation constant and k_d is the dissociation coefficient for the complex. With non-chelating, simple ligand, L , the rate of ML formation is independent of L and limited only by the rate of ligand exchange of $M(H_2O)$. In this case, k_f is the second order rate coefficient for the replacement of the ligand water of the inner co-ordination sphere of M . For a given metal M , $k(H_2O)$ is a constant and known, so k_d can be calculated from K :

$$k_d = \frac{k(H_2O)}{K} \quad (3.8)$$

However, for heterogeneous complexants such as humic substances this is only a rough approximation that give the order of magnitude for the maximum value of k_d for the reaction at the complexing sites L_i [82].

Kinetic speciation by ligand exchange kinetics

Langford and Gutzman [63] developed a method for kinetic speciation by ligand exchange kinetics using direct molecular spectrophotometric detection. In this method, the sample containing a metal ion is swamped with an excess of colour-forming reagent to impose pseudo-first-order kinetics and the first-order growth in the complex-formation

was spectrometrically measured. The relevant equation is (the charges on the ions are ignored for simplicity):

where M is the metal ion and R is the excess reagent and MR the complex formed between M and the reagent R. The number of kinetically distinguishable components obtained by analysis of the kinetic spectra provides a means for identifying a distinguishable set of components. The kinetic data are treated by using the expression:

$$d[MR]/dt = \sum_i A_{o,i} [1 - \exp(-k_i t)] + X \quad (3.10)$$

where MR is the complex formed, $A_{o,i}$ represents the initial ($t = 0$) concentration of the i th species expressed in units consistent with the product (MR), k_i is the pseudo-first-order rate coefficient for the i th species, and X represents contributions from reactions that are too fast to be resolved during the time scale of the experiment, plus the contribution from the sample blank. The task is to fit the observed time course to a set of rate coefficient and concentration parameters, k_i and $C(o,i)$ (related stoichiometrically to $A_{o,i}$). Since the equation is non-linear, this is a difficult task. In order to obtain reproducible kinetics, it is necessary to control the kinetically crucial parameters such as temperature, pH and ionic strength.

Several other authors have also used 4-(2-pyridylazo) resorcinol (PAR) for the kinetic speciation of Cu(II) [64,66] and Ni(II) [60,76]. The ferrozine ligand has been used for the measurements of iron [83]. The ferrozine coupled with spectrophotometry is able to distinguish Fe(II) and Fe(III) forms of various labilities from each other. A similar approach for iron speciation was developed by Sojo et al. [61] using 2,4,6-tri-(2-pyridyl)-s-triazine as the competing ligand. Sojo and de Haan have applied their kinetic method to

investigate the speciation of iron in the water of Lake Tjeukemeer in the Netherlands. The results of the Lake Tjeukemeer water samples suggested that all the iron was present in colloidal, and polymeric inorganic and organic complexes, which take between 1 min and 1 h to dissociate [61].

Direct reaction with ferron [84] and pyrocatechol violet (PCV) [85] was employed for the kinetic speciation of aluminium. Seip et al. [86] compared ferron and PCV for the determination of labile monomeric and organic aluminium species, and found good agreement between the two methods. PCV was preferred, owing to greater sensitivity and freedom from interferences. Kramer [87] reported a similar kinetic method based on the reaction with lumogallion and the measurement of the resulting complex by fluorescence spectrometry. Mak et al. [88] determined the kinetically distinguishable species of Al(III) in the presence of a soil fulvic acid using the fluorimetric Al(III) reagent, calcein blue. These methods are, however, limited in their application because of the limited analytical sensitivity provided by molecular spectrometry. Laser thermal lensing technique was proposed by Langford et al. [71] as a means of improving the analytical sensitivity. This technique improved the sensitivity of the determination of rate coefficients in homogeneous solutions, but other problems associated with this technique limited its applications [71].

By continuously measuring the formation of the detectable metal complex with time, it is possible to apply more sophisticated methods of data analysis. For instance, Olson and Shuman [65,90] examined copper complexation by humic substances in estuarine waters by adding an excess of 4-(2-pyridylazo) resorcinol (PAR) which formed a coloured complex with copper. It was assumed that the ensuing reactions could be fitted

to pseudo-first order kinetics. By numerical differentiation of the absorbance time curve, a “kinetic spectrum” was obtained from which dissociation rate coefficients were derived. The formation of $\text{Cu}(\text{PAR})_2$ in ultrapure water took about 20 ms, and this defined the upper limit for the rates of the dissociation reactions that could be observed by this method. For the estuarine water samples, only 87% of copper reacted within 30 min, indicating that a significant fraction of the copper was not labile. Owing to the limited sensitivity of the spectrometric analysis, the method could not be applied directly to unpolluted freshwaters and the water samples had to be amended by additions of copper before the experiment could be done.

Chakrabarti et al. [89] have adapted the Kinetic Model which was originally proposed by Olson and Shuman [90] and have used it to develop a kinetic method for chemical speciation, called Competing Ligand Exchange Method (CLEM). CLEM uses Chelex-100 cation exchange resin as the competing ligand. The dissociation rate coefficients of $\text{Ni}(\text{II})$, $\text{Cu}(\text{II})$, $\text{Co}(\text{II})$, $\text{Zn}(\text{II})$, $\text{Pb}(\text{II})$ and $\text{Al}(\text{III})$ by Chelex-100–100 in model solutions of a well-characterized fulvic acid, as measured by inductively coupled plasma-mass spectrometry (ICP-MS), was about 10^{-2} s^{-1} [91-94], and this defined the upper theoretical limit of rates of the dissociation reactions that could be measured by inductively-coupled plasma mass spectrometry. In this study, species concentrations and species rate coefficients were determined by using the Competing Ligand Exchange Method (CLEM), and this method is described in the next Section.

SECTION 2

EXPERIMENTAL DETERMINATION

CHAPTER 4

MATERIALS, REAGENTS AND PROCEDURES

Materials and reagents

Screw-capped Teflon bottles (500, 1000, and 2000 mL) were used both as Reactors and as storage vessels for all the water samples. These bottles were pre-cleaned following the procedure described below. A 0.45- μm membrane filter (CN-6 MetriceL, 47 mm diameter, 150 μm thickness, Gelman Sciences, Inc.) was used to separate particulates from dissolved species in the water samples in this study. This filter is made of mixed cellulose esters and has the advantage of providing filtrates having low blanks for the metals.

Chelex-100-100 cation exchange resin (100-200 mesh), a styrene-divinylbenzene copolymer with iminodiacetate functional groups, supplied by Bio-Rad Laboratories in the sodium form, was employed as the competing ligand in all the kinetic experiments. Chelex-100-100 cation exchange resin was pre-treated by equilibrating it with the HOAc-NaOAc buffer at pH 5. The HOAc-NaOAc buffer was prepared as follows: 4.0 mL of 2 M sodium acetate trihydrate (BDH, Analar) (which had been purified of all metals by controlled-potential electrolysis for several days immediately prior to its use) was added to about 2 L of ultrapure water and the pH was adjusted to 5 using ultrapure nitric acid (ULTREX II, J.T. Baker Inc., Phillipsburg, NJ, USA).

Stock solutions of (1000 $\mu\text{g/mL}$) Ni(II), Cu(II), Co(II) and Pb(II) were prepared separately for each metal by dissolving appropriate quantities of the metals as follows:

nickel metal powder (SPEX 99.999 %), copper metal (99.9 %), cobalt metal (SPEX 99.999 %) and $\text{Pb}(\text{NO}_3)_2$ (Fisher, A.C.S. reagent) in ultrapure nitric acid (ULTREX II, J.T. Baker Inc., Phillipsburg, NJ, USA) with heating and diluting to the appropriate volume with ultrapure water. The final solution (1000 $\mu\text{g/mL}$) contained 1% (v/v) ultrapure nitric acid. A standard solution (1000 $\mu\text{g/mL}$) of zinc was purchased from BDH Chemicals. A mixed-metal, high-purity standard, ICP-MS-2 (Delta Scientific) containing Ni(II), Cu(II), Co(II), Zn(II), and Pb(II) and other metals, each 10 $\mu\text{g/mL}$, formulated specifically for use in ICP-MS, was also used for this study.

A stock solution of ethylenediamine tetraacetic acid (EDTA) was prepared by dissolving an appropriate quantity of EDTA as a salt (Fisher Scientific Co., Certified ACS) in ultrapure water. Ultrapure water of resistivity 18.2 $\text{M}\Omega \cdot \text{cm}$, obtained from a Milli-Q-Plus water purification system (Millipore Corporation) was used to prepare all standard and test solutions. All standard solutions were prepared to contain 1% (v/v) HNO_3 (ULTREX II, J.T. Baker Inc., Phillipsburg, NJ, USA). Standard buffers (supplied by Fisher Scientific) were used for the calibration of the pH-meter.

The fulvic acids

Two well-characterized fulvic acids, Armadale fulvic acid and Laurentian fulvic acid, both supplied by Dr. D.S. Gamble of Agriculture Canada, Ottawa, Canada, were used in this study. The Armadale fulvic acid was extracted from the Bh horizon of a soil, collected from Armadale, Prince Edward Island, Canada, and Laurentian fulvic acid was extracted from pozolic soil sample collected from the Laurentian forest preserve of Laval University, Quebec, Canada. Both fulvic acids have been “well-characterized” by

Gamble and Schnitzer [95]. Armadale FA was characterized by Gamble using potentiometric titration [95, 96-97]. Gamble [96, 97] has dealt with the errors involved in the potentiometric titration of the FA. The total number of phenolic OH and carboxyl groups determined by potentiometric titration of Armadale fulvic acid are 3 mmole/g phenolic OH groups [95] and 7.71 mmol/g carboxyl groups [95,96-97], respectively (Table 1). The bidendate complexing capacity of Armadale FA was therefore estimated to be approximately 5.4 mmol/g [98], and the molarity of this FA was calculated based on this complexing capacity.

An estimate of the background metal concentration in Armadale fulvic acid was obtained by quantitative determination of its metal contamination using inductively-coupled plasma mass spectrometry. The naturally-occurring concentration of the metals of interest in the Armadale fulvic acid were found to be: Ni(II) 5.2 $\mu\text{g/g}$, Cu(II) 18.4 $\mu\text{g/g}$, Pb(II) 6.5 $\mu\text{g/g}$, and Co(II) was below its limit of detection. The concentration of these metals in the stock solution of fulvic acid (1 g/L, i.e. 10^{-3} M FA) was: Ni (II) 8.6×10^{-8} M, Cu (II) 2.9×10^{-7} M and Pb(II) 3.1×10^{-8} M. These concentrations are negligible compared to even the lowest concentration of the metals in our experiments. Therefore no account was taken of the naturally-occurring metals in the fulvic acid used, either in computing the experimental results or in their interpretation.

The number of phenolic and carboxyl acid groups of Laurentian soil FA was determined by potentiometric titration by Tramonti [99], who reported 3.03 and 3.59 mmol/g phenolic and carboxyl acid groups, respectively (Table 2).

Stock solutions of the fulvic acids were prepared separately by dissolving appropriate amounts of the freeze-dried fulvic acids in ultrapure water. The pH of the model solutions

containing fulvic acid was adjusted to $\text{pH } 5.0 \pm 0.2$ using 1% (g/v) NaOH solution. The sodium hydroxide solution was purified by electrodeposition at -1.2 V (vs E_{sce}) for at least 48 hours immediately prior to use.

Cleaning procedures

The following cleaning procedure was used. All containers were sequentially soaked in 1:1 HCl:H₂O (AR grade HCl) and 1:1 HNO₃:H₂O (AR grade HNO₃). The containers filled with each of the above solutions were left to stand for one week at room temperature, sequentially, in order to leach out trace metals and other impurities. The containers were then rinsed five times with the ultrapure water. The containers were then completely filled with the ultrapure water and were left to stand until they were used; the filling water was renewed periodically to ensure continued contact with the ultrapure water.

Test solutions

To investigate the effect of [metal]/[FA] mole ratios on the lability of the metal-FA complexes, test solutions were prepared to contain a constant concentration in the range of $\sim 10^{-7}$ - 10^{-8} M of one of the following metals, Ni(II), Cu(II), Co(II) and Pb(II) and varying concentrations of the FA. It should be noted that for a naturally occurring complexant, such as FA, the molar mass is not directly accessible. Therefore, a molar concentration was calculated based on the bidentate complexing capacity (mmole g^{-1}) of the fulvic acids.

Table 1 Functional groups in Armadale fulvic acid. Concentrations are reported in meq/g [100].

Functional groups	Fulvic Acid
Phenolic-OH	3.9
Alcoholic-OH	4.0
Ketonic C O	1.4
COOH	8.1
Quinoid C O	0.6
Methoxyl	0.4

Table 2 Elemental analysis and functional group analysis of Laurentian fulvic acid [89].

Element	Composition (%)	Functional groups	Concentration (mmole g ⁻¹)
C	45.1	Carboxylate A	5.11
O + S	49.7	Carboxylate B	3.49
H	4.1	Phenol	3.03
N	1.1	-----	-----

To investigate the effect of the electron configuration of selected metal ions of the 3d transition series on the lability of the metal complexes, the following test solutions were used: 1) model systems containing Laurentian fulvic acid (FA) and a mixture containing equal concentrations of Cu(II), Ni(II) and Zn(II) (their concentrations are specified later); 2) freshwater samples from Rideau River surface water at Carleton University; 3) freshwater samples from Sudbury mines area.

1) To prepare the test solutions, known amounts of the Laurentian fulvic acid solution were added to known amounts of the above mixture of elements and diluted to a known volume. The molar concentration of the FA was calculated based on the mmole g⁻¹ of phenolic and carboxylic groups of this sample of fulvic acid as determined by Tramonti [99]. The combined total concentration of the metals was 2.1×10^{-6} M in all the mixtures. After preparation of the test solutions, the pH was adjusted to 5.0 ± 0.2 using 1% NaOH (purified electrolytically). The solutions were then equilibrated overnight.

2) Samples of Rideau River surface water (RRSW) were collected from a location close to the Steacie Building at Carleton University in Ottawa, Ontario, Canada, using an acid pre-cleaned Teflon container. Immediately after the collection, the samples were filtered through 0.45 μ m polycarbonate membrane (Millipore) using a peristaltic pump. The pH and the conductivity of the original samples were measured using an Accumet 20 pH/conductivity meter. Since it was known from previous experiments that the concentration of the metals in the RRSW was too low for accurate kinetic analysis, the filtered samples were spiked with an appropriate amount of a standard solution containing one of the following metals: Ni(II), Cu(II), or Zn(II). The pH was then adjusted to 5.0 ± 0.2 and the sample was left to stand overnight for equilibration.

3) Freshwater samples were collected from different sites of the Sudbury mines area in October 1998 (see Table 8) in pre-treated 2 L Teflon containers. On the following day when samples were brought to our laboratory, they were immediately filtered through 0.45 μm filters. The filtrates were studied at their initial pH values. The pH and conductivity of these samples are shown in Table 9. These results are presented in Chapter 5.

To investigate the effect of metal competition between a target trace metal Ni(II) and other trace metals such as copper and cobalt in model systems containing a well-characterized fulvic acid (at constant pH and ionic strength) on the lability of the target metal complex, three types of model systems were used: 1) test solutions containing a constant concentration of Ni(II) [10^{-8} M] plus FA [10^{-5} M] and varying concentrations of Cu(II); (2) test solutions containing a constant concentration of Ni(II) [10^{-8} M], plus FA [10^{-5} M] and varying concentrations of Co(II); (3) test solutions containing a constant concentration of Ni(II) [10^{-8} M], plus Cu(II) [10^{-8} M] plus FA [10^{-5} M] and varying concentrations of Co(II). Model systems were prepared by spiking ultrapure water with appropriate volumes of the standard solutions of the metals and of the fulvic acid solution (1.0219 g / L). These results are presented in Chapter 6.

For the study of the distribution of the rate coefficients, two complexing agents, EDTA and FA, were used with the following test solutions: 1) aqueous solutions containing a single metal and EDTA; 2) aqueous solutions containing a mixture of the metals: Al(III), Cd(II), Cu(II), Ni (II), Zn (II) and Pb(II), with and without the Laurentian fulvic acid. Solutions of Zn(II) with EDTA were prepared containing a constant concentration of Zn(II) (6.4×10^{-6} M) and the following mole ratios of Zn:EDTA: 0.5, 1,

1.5 and 2. The ICP-MS high-purity metal standards (Delta Scientific), each containing only one of the following metals: Al(III), Cd(II), Cu(II), Ni(II), Pb(II), and Zn(II) (10mg/L each) were used. These results are presented in Chapter 8.

Determination of Dissolved Organic Carbon (DOC) in freshwater samples

The concentration of dissolved organic carbon (DOC) in the Rideau River surface water samples was measured using a Total Organic Carbon Analyzer (Shimazu Model TOC-5000) after filtration of the water samples through a 0.45 μm filter. The concentrations of DOC in the freshwater samples from Sudbury mines area were measured using Rosemount-Dohrmann DC-190 High-Temperature TOC analyzer. The principle of operation of the two instruments is the same. A water sample containing organic matter is automatically introduced into a combustion tube via an air-actuated injection port. Through catalytic oxidation, the sample is completely oxidized to CO_2 and H_2O . The gas flow sweeps the CO_2 containing steam out of the combustion tube, through a condenser, and into a gas/liquid separator reactor to trap most of the H_2O . Final H_2O removal is accomplished by a dehumidifier operating at 0-10 $^\circ\text{C}$. The combustion product CO_2 is carried by purified air and oxygen at a flow rate of about 200 mL/min and is detected by a specific non-dispersive infrared gas analyzer [101-102].

CHAPTER 5

INVESTIGATION OF THE RATES OF DISSOCIATION OF Ni(II), Cu(II), Co(II), Pb(II) AND Zn(II) COMPLEXES IN MODEL SYSTEMS CONTAINING ARMADALE FULVIC ACID AND IN FRESHWATERS

Introduction

The interactions between metal ions and humic substances are known to determine at least to some extent the solubility, transport, and bioavailability of trace metals. In freshwaters, trace metal ions, such as Ni(II), Cu(II), Co(II) or Pb(II), exist predominantly as complexes with humic substances [103]. Freshwater systems are usually non-equilibrium systems [71], and the importance of non-equilibrium processes in controlling metal ion speciation in the aquatic environment is becoming increasingly apparent [104-105]. Recently, several studies investigating the kinetics of metal-humic interactions have been reported [106-108].

For heterogeneous complexants, since the nature of complexing sites changes with the metal to ligand mole ratio, the latter will influence both the equilibrium and kinetic properties of the complexes [109]. Unfortunately, in kinetic studies the metal:ligand dependence of kinetics is not usually detected. Morel [110] has suggested that this is probably due to the fact that complexation experiments are most often conducted at high metal concentrations, which may reveal only the characteristics of the

more abundant and weaker complexing sites. Unless complexation experiments are run at sufficiently low metal to ligand mole ratios, the strongest binding sites cannot be identified, and the results may be misleading, i.e. may not be environmentally significant because the metal and the ligand concentrations are too high to be applicable to freshwater conditions [107-109, 111]. The possible existence of high affinity humate sites at low concentrations is a major issue in assessing the extent of trace metal complexation by humic compounds in unpolluted freshwaters where the metal concentrations themselves are very low [112]. This emphasizes the need for experimental conditions to be as close as possible to those prevailing in the freshwaters environment.

Sunda and Hanson for the first time reported the existence of strong sites [113]. It is interesting to note that these authors used an initial concentration (10^{-8} M) that was an order of magnitude lower than most other workers. Morel [110] reported that the use of the low concentration was probably the reason why these authors found these strong sites.

Since humic substances are polyfunctional complexants, the geometric location of functional groups plays a significant role in their metal-binding properties. From the consideration of the geometric location, Zunino and Martin [114] divided the functional groups, such as carboxylic acid and phenolic hydroxyl groups, into the following categories: (a) functional groups capable of forming chelate rings with high bonding energy, (b) functional groups participating in open complex formation with intermediate binding energy, and (c) functional groups lying deeply buried within a macromolecule with low binding energy.

The objectives of the study presented in this chapter are: i) to investigate the progressive occupation of strong and weak binding sites of humic substances; ii) to

extend the study to freshwaters and investigate the effect of [metal]/[DOC] ratio on the dissociation rate coefficients of M-DOC complexes in freshwaters; iii) to investigate the effect of the characteristics of the metal ions on the dissociation of their complexes in model systems and in freshwaters.

In the first part of this Chapter, the results of our investigations of the progressive occupation of strong and weak binding sites of humic substances by selected metals will be reported. In order to do this study Armadale fulvic acid (a pedogenic fulvic acid) was used as a representative of humic substances. The metals selected for this study are Pb(II), Ni(II), Cu(II), and Co(II).

The dissociation of metal-FA complexes were studied over a range of [metal]/[FA] mole ratios. Test solutions were prepared with constant concentrations in the range of $\sim 10^{-7}$ - 10^{-8} M of the metals Pb(II), Ni(II), Cu(II), Co(II), and varying concentrations of the Armadale FA to make [metal]/[FA] mole ratios of 0.0018, 0.018 and 0.18. The molar concentration of the fulvic acid was calculated based on the bidentate complexing capacity (mmole g^{-1}) of the Armadale fulvic acid. These complexing capacities were reported by Gamble et al. [115] for the Armadale fulvic acid.

In the second section of this Chapter, the results of the investigation on the effect of the mole ratio of [metal]/[DOC] on the dissociation rate of metal complexes in freshwaters will be reported. Nickel was chosen for this study because nickel is important for the Canadian environment. Canada is one of the world's foremost producers of nickel. Nickel ore mining, processing and refining operations may discharge nickel-containing effluents to the Canadian natural environment.

Nickel is an essential micronutrient for some marine algae, but micromolar Ni^{2+} is toxic to a variety of algae, invertebrates, and fish [116]. However, interactions of Ni (II) with FA and other dissolved organic matter (DOM) have not been much studied [116, 117] perhaps because of the absence of convenient, sensitive methods for measuring free $[\text{Ni}^{2+}]$ ion in the presence of organic complexants [118]. Free Ni^{2+} ion, which is $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$, is reported to be toxic [107].

In the third and last part of this Chapter, the results of the investigations of the kinetics of dissociation of Co(II), Ni(II), Cu(II) and Zn(II) complexes in model systems containing a well-characterized fulvic acid (Laurentian), and in freshwater samples, has been reported. The freshwater samples were collected from the Rideau River surface water near Carleton University, Ottawa, and from the Sudbury mines area. Since the concentrations of the metals in the Rideau River surface water samples were below their limits of detection, the samples were spiked to contain 20 $\mu\text{g/L}$ of each metal. In the freshwater samples collected from the Sudbury mines area the concentration of metal ions were already high and those samples were therefore not spiked. It is noteworthy that the samples collected from the Sudbury mines area contained the metals in different concentrations; for example, the concentration of Ni(II) was one order of magnitude higher than that of Co(II), Cu(II) and Zn(II), whereas in the Rideau River surface water samples and in the model systems, the metal ions were present at about the same concentrations.

The kinetic model [89, 92-94]

Competing ligand exchange method [119-120, 91]

Adapting the kinetic model proposed by Olson and Shuman [90], the following kinetic model has been developed [89-93] to study the dissociation kinetics of ML, where M is a metal ion and L is a macromolecular polyfunctional, multisite, heterogeneous, organic complexant, such as fulvic acid.

Consider an aqueous mixture of n components, in which each component, designated ML_i , exists in equilibrium with its dissociation products

where k_1 and k_{-1} are the rate coefficients for the forward and the reverse reaction, respectively, and ML_i refers to a complex in which the metal ion is bound to the i th ligand site on a polyfunctional complexant. The charges on M and L have been omitted for simplicity.

Competing Ligand Exchange Method (CLEM) [119-120] was employed for the determination of the dissociation kinetics of metal-ligand complexes using Chelex-100, a chelating cation exchange resin, as a competing ligand. Chelex-100 reacts with M as follows:

where k_2 is the rate coefficients for formation of the complex Chelex-M. The model is based on the following assumptions: i) M-Chelex complex is thermodynamically much stronger than ML complex, so that the net reaction of eq. (5.1) and eq. (5.2):

is driven to the right; ii) that reaction (5.2) is much faster than the reverse reaction of equation (5.1), so that the forward reaction of (5.1) becomes the rate-limiting step and the measured kinetics then represents the kinetics of the dissociation of the metal complex, ML_i ; iii) that $[\text{Chelex-100}] \gg [M]$. Since $[\text{Chelex-100}]$ is used in large excess over M , $[\text{Chelex-100}]$ can be considered a constant and reaction (5.2) becomes pseudo-first-order. Since k_2 is large, as has been determined by inductively-coupled plasma mass spectrometry (ICP-MS) [94], and with Chelex-100 in sufficient excess, the condition $k_2 [\text{Chelex-100}] \gg k_{-1} [L_i]$ is valid and the overall reaction (5.3) is irreversible. If the above three assumptions are valid, then the rate of formation of the M-Chelex complex is determined by the rate of dissociation of ML_i in reaction (5.1) and the rate expression is equation (5.4):

$$\frac{d[M - \text{Chelex}]}{dt} = -\frac{d[ML_i]}{dt} = k_1 [ML_i] \quad (5.4)$$

The three assumptions made above were found to be valid [89].

If each complex, ML_i , undergoes independently and simultaneously, a first-order or pseudo-first-order dissociation reaction, then $C(t)$, the concentrations of all components remaining undissociated in the test solution at time, t , can be described as:

$$C(t) = \sum_{i=1}^n C_i^0 \exp(-k_i t) \quad (5.5)$$

where C_i^0 is the initial concentration of ML_i , the i th component, and k_i is the rate coefficient for dissociation of the i th site. $C(t)$ is quantified by ICP-MS by measuring the metal complexes remaining undissociated in solution over time.

The number of components is not a simple issue. Within a polyelectrolyte mixture such as fulvic acid, the actual number of components may be very large. In fact, where a limited set is appropriate, the components may be grouped about a few central tendencies that can adequately model the system. However, the assignments of a set of components must always pass chemical test of significance. This last step is important. A chemically significant partition into a few kinetically defined components will have two characteristics. First, the rate coefficients will be fairly stable and only the concentration of the components will vary significantly as pH and concentration ratios vary. Secondly, the changes in concentrations of components will follow trends required by mass action law consideration. Unless the above two test are satisfied there is little assurance of the chemical significance of the components [63, 71].

Measurement of dissociation kinetics of ML complexes

The decrease in concentrations of lead, nickel, copper, cobalt, and zinc in the model systems and in the freshwater samples due to binding by Chelex-100 as a function of time was determined by inductively-coupled plasma mass spectrometry. The inductively-coupled plasma mass spectrometer used was a Perkin-Elmer SCIEX Elan 5000, fitted with a pneumatic nebulizer as the sample introduction system. The instrument operating conditions and the data acquisition protocol are described in Table 3.

The Chelex-100 cation-exchange resin was pre-treated for this work as follows: Chelex-100 cation exchange resin was equilibrated with the acetic acid-sodium acetate (HOAc-NaOAc) buffer solution ($\text{pH } 5.0 \pm 0.1$) for 24 hours. After this pre-treatment, the

Chelex-100 resin was kept in the buffer solution until it was used. This pre-treatment of the Chelex-100 resin was used to maintain the pH at 5 (a buffer could not be used for this purpose, as will be explained later on). Because the dryness of the resin affected its properties and changed the pH of the sample during the kinetic run, the resin was isolated from the buffer solution immediately before the kinetic measurements and was rinsed at least three times with ultrapure water of resistivity $18.2 \text{ M}\Omega \cdot \text{cm}$.

One per cent (w/v) of Chelex-100 resin was added to the test solution in a Teflon Reactor (cylindrical, 500 mL volume) and the test solution was stirred continuously with a Teflon-coated stirring bar (Figure 2). The data acquisition was initiated 15 seconds before the resin was added to the Teflon Reactor. Therefore, any contamination of the resin from the pre-treatment of the resin would be indicated by a sudden increase in the signal. In the case of any contamination, the experiment was terminated, and the results discarded.

The test solution was continuously delivered to the plasma torch through the solution nebulizer using a peristaltic pump at a flow rate of 1-mL min^{-1} . The interval between the data points and the total time of the data acquisition was set for each experiment based on the rate of decrease of the metal ions. In the case of rapid decrease of the metal ions, both the time interval and the total time for the data acquisition were kept short. Longer times for data acquisition were adopted for slowly-dissociating metal-FA complexes together with correspondingly longer time intervals.

Table 3 Instrumental operating conditions and data acquisition protocol for the inductively-coupled plasma mass spectrometry.

Inductively-coupled plasma	
RF Power	1 kilowatt
Coolant argon flow rate	15 L min ⁻¹
Carrier argon flow rate	0.6 L min ⁻¹
Auxiliary argon flow rate	2.4 L min ⁻¹
Data acquisition parameters	
Dwell time	100-1000 ms
Scan mode	Peak hop
Signal measurement	Counts/s
Points/spectral peak	1
Resolution	Normal

Figure 2 The Teflon Reactor used for the kinetic experiments

Methods for the kinetic data analysis

Analyzing experimental data to obtain physical parameters is a crucial point in the kinetic speciation method. In this thesis, two methods of data analysis have been used. In the first part of thesis the data was fitted to the kinetic model by non-linear regression analysis using the Marquadt-Levenberg algorithm. The minimum number of parameters required to accurately fit the data was determined by finding the number of components in which the sum of square of the weighted residuals achieved a minimum value.

$$\text{Sum of squares of the weighted residuals} = \sum \left[\frac{C(t) - C_T(t)}{C(t)^{1/2}} \right]^2 \quad (5-6)$$

In the above equation, $C(t)$ is the experimental value and $C_T(t)$ is the calculated value using the parameters obtained from the regression analysis. The non-linear regression analysis gave up to four components in the mixtures of metal and fulvic acid with specific values of the rate coefficients for dissociation of each component.

For a polyfunctional, complexant such as fulvic acid the number of components is not a simple issue. The small number of components with their specific rate coefficients may not accurately describe the chemistry of the binding sites of the fulvic acid [63, 71]. A binding site may have a range of binding energy due to the heterogeneous nature of fulvic acid. This in turn will lead to a distribution of values for the dissociation rate coefficients for the complexes. Nevertheless the specific rate coefficients may represent an average value for a group of complexes on a particular site.

It may therefore be more realistic to assume a continuum of values for the rate coefficients rather than single values. For this purpose, the second part of this thesis examines the dissociation of metal complexes using a Distribution Analysis Method. The Distribution Analysis Method will be presented in Chapter 7.

RESULTS AND DISCUSSION

Effect of the [metal]/[fulvic acid] mole ratio on the rate of dissociation of the metal-fulvic acid complexes.

Figures 3-6 show the effect of varying [metal]/[FA] mole ratios on the percent composition of Ni(II), Cu(II), Co(II) and Pb(II) bound to fulvic acid as a function of time in model systems. In Figures 3-6, the [metal]/[FA] mole ratio was varied from 0.0018, 0.018 to 0.18 (from top curve to bottom curve). The points in Figures 3-6 are the experimental data, and the solid lines are the results from the non-linear regression analysis. At the mole ratio of 0.0018 of the [metal]/[FA], the concentration of the metals is so low relative to the available number of strong binding sites of the FA, that all the metal binds to strong sites, forming strong complexes, which are inert ($k \leq 10^{-5}$). The rate of dissociation of the metal-FA complexes at this [metal]/[FA] mole ratio is very slow and hardly detectable over the entire period of the measurement. The decay was too slow to be analyzed statistically but a rate coefficient could be estimated in the following way. If, for example, 10% of the metal had decayed after 6000 seconds the rate coefficient would be given as follows:

$$\ln \frac{100}{100-10} = k \times 6000 \quad (5.7)$$

$$k \sim 1.8 \times 10^{-5} \text{ s}^{-1} \quad (5.8)$$

This represents an approximate lower limit to the rate coefficient measured by the present method. Thus, at low metal concentration and low [metal]/[FA] mole ratio, the strong binding sites are expected to control metal speciation.

When the mole ratio of [metal]/[FA] was increased by one order of magnitude to 0.018 (middle curve of Figures 3-6), the rate of dissociation increased, suggesting that some of the metals Pb(II), Ni(II), Cu(II) and Co(II) must be bound to weaker sites of the FA forming labile complexes which dissociated more rapidly. When the concentration of FA was further decreased by one order of magnitude to [metal]/[FA] mole ratio 0.18 (bottom curve of Figures 3-6), a rapid initial decrease was observed which levelled off to a slower rate of dissociation at longer times. The curves were analyzed using non-linear regression analysis and their dissociation rate coefficients are presented in Tables 4-7.

The analysis showed that a minimum of two components was required to describe the dissociation kinetics of the metal-FA complexes at the [metal]/[FA] mole ratios of 0.018 and 0.18 for the four metals studied. This is consistent with the presence of two distinct classes of binding sites in the Armadale FA.

Sunda and Hanson [113], using an initial metal concentration of 10^{-8} M, reported for the first time the existence of the strong sites of humic substances. Earlier workers had failed to detect the existence of the strong sites probably because the earlier workers had used higher concentrations of the metals in their titration experiments [113,121] and a lower concentration of the ligand. The results are in agreement with the hypothesis that as the ratio of [metal]/[FA] mole ratio is increased, the strong binding sites become

saturated and the excess metal then binds to the weaker sites, forming weak complexes which are labile. This observation was common for all the metals studied.

Tables 4 -7 present the average values of the dissociation rate coefficients. At the $[\text{metal}]/[\text{FA}]$ mole ratio of 0.0018, only one rate coefficient was estimated of about $k_d \sim 10^{-5} \text{ s}^{-1}$ for all the metal ions studied. With further increase in the mole ratio of $[\text{metal}]/[\text{FA}]$ by one order of magnitude from 0.0018 to 0.018, a second faster kinetically distinguishable component appeared for all the metals. With further increase in the ratio $[\text{M}]/[\text{FA}]$ by one order of magnitude from 0.018 to 0.18, the percentage of the faster component increased, whereas the percentage of the slower component decreased. There was also a change in the dissociation rate coefficients of the faster and slower kinetically distinguishable components. These results show that the dissociation rate coefficients of the metal-FA complexes decrease with decreasing $[\text{metal}]/[\text{FA}]$ mole ratio.

The change in the dissociation rate coefficient measured for each component of (metal-FA complex) provides experimental evidence of the polyfunctionality of the fulvic acid. Although carboxyl groups are reported to play a predominant role in the binding of metal ions, mixed complexes [122-124], and complexes with ligands belonging to two fulvic acid molecules [125] probably formed also. In other words, the nature of the complexing site may change with the metal/ligand ratio. When metal ions are added to the fulvic acid solution, metal-fulvic acid complexes having different dissociation rate coefficients are formed. Slow dissociation rates may result from a chemical mechanism operating at the heterogeneous binding sites of fulvic acid and from secondary effects, such as slow diffusion of the metal ion into or out of the fulvic acid gel.

Figure 3 Effect of the mole ratio on the dissociation of Pb-FA complexes. Lead remaining bound to the FA as a function of time in model systems containing varying concentration of the FA, measured by inductively-coupled plasma mass spectrometry. The same concentration of lead was used for all curves. Pb(II) 4.8×10^{-8} M; ○, $[\text{Pb(II)}]/[\text{FA}] = 0.0018$; □, $[\text{Pb(II)}]/[\text{FA}] = 0.018$; Δ, $[\text{Pb(II)}]/[\text{FA}] = 0.18$. pH = 5.0 ± 0.1 ; ionic strength ~ zero.

Figure 4 Effect of the mole ratio on the dissociation of Ni-FA complexes. Ni(II) remaining bound to the FA as a function of time in model systems containing varying concentration of the FA, measured by inductively-coupled plasma mass spectrometry. The same concentration of nickel was used for all curves. Ni(II) 4.3×10^{-8} M; ○, $[\text{Ni(II)}]/[\text{FA}] = 0.0018$; □, $[\text{Ni(II)}]/[\text{FA}] = 0.018$; Δ, $[\text{Ni(II)}]/[\text{FA}] = 0.18$. pH = 5.0 ± 0.1 ; ionic strength ~ zero.

Figure 5 Effect of the mole ratio on the dissociation of Cu-FA complexes. Cu(II) remaining bound to the FA as a function of time in model systems containing varying concentration of the FA, measured by inductively-coupled plasma mass spectrometry. The same concentration of copper was used for all curves. Cu(II) 6.3×10^{-8} M; ○, $[\text{Cu(II)}]/[\text{FA}] = 0.0018$; □, $[\text{Cu(II)}]/[\text{FA}] = 0.018$; Δ, $[\text{Cu(II)}]/[\text{FA}] = 0.18$. pH = 5.0 ± 0.1 ; ionic strength ~ zero.

Figure 6 Effect of the mole ratio on the dissociation of Co-FA complexes. Co(II) remaining bound to the FA as a function of time in model systems containing varying concentration of the FA, measured by inductively-coupled plasma mass spectrometry. The same concentration of copper was used for all curves. Co(II) 6.8×10^{-8} M; ○, $[\text{Co(II)}]/[\text{FA}] = 0.0018$; □, $[\text{Co(II)}]/[\text{FA}] = 0.018$; Δ, $[\text{Co(II)}]/[\text{FA}] = 0.18$. pH = 5.0 ± 0.1 ; ionic strength ~ zero.

The dissociation rate coefficient measured here and presented in Tables 4-7 are an average value of a group of closely related rate coefficients. Caution should be exercised when considering such dissociation rate coefficients as they are derived from a minimum set of parameters to fit the experimental curves within the experimental error. There may well be a more complicated distribution of dissociation rate coefficients than the minimum set [126]. The binding sites in fulvic acid are known to involve a range of binding energies [127]. Even among the strong and weak binding sites, as revealed by the present experiments, a distribution of sites stronger or weaker than the experimentally measured averages more probably represents the actual binding characteristics. As more metal becomes available for binding, a broader range of binding sites becomes occupied and the rate coefficients for dissociation show a corresponding distribution. Since the strong sites have a large variation in the equilibrium constant, a large distribution of dissociation rate coefficients would be expected at low metal to fulvic acid mole ratios. The distribution of the rate coefficients for dissociation will be discussed in Chapters 7 and 8. The results show how kinetic data can detect the progressive occupation of strong and weak binding sites of a well-characterized fulvic and the existence of strong and weak binding sites in fulvic acids.

Table 4 Effect of $[\text{Pb(II)}]/[\text{FA}]$ mole ratio on percentage distributions and dissociation rate coefficients of lead-FA complexes in model systems, at a constant concentration of Pb(II) , and varying concentrations of FA (Armada). pH 5.0 ± 0.1 ; ionic strength $\sim$ zero. $[\text{Pb(II)}] 4.8 \times 10^{-8} \text{ M} + [\text{FA}]$.

$[\text{FA}], \text{M}$	$[\text{Pb (II)}]/[\text{FA}]$ mole ratio	$\%C_1$	$k_1 \times 10^2, \text{s}^{-1}$	$\%C_2$	$k_2 \times 10^3, \text{s}^{-1}$	$\%C_3$	$k_3 \times 10^5, \text{s}^{-1}$
2.6×10^{-5}	0.0018	----	----	----	----	100 ± 10	2.1 ± 0.2
2.6×10^{-6}	0.018	----	----	----	----	100 ± 1	5.8 ± 0.2
2.6×10^{-7}	0.18	7.4 ± 0.2	2.1 ± 0.1	56 ± 2	1.3 ± 0.2	36 ± 1	5.8 ± 0.2

----- Not recovered.

k_1 , and k_2 are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. $\%C_1$, and $\%C_2$, are percentage relative distributions of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Table 5 Effect of [Ni(II)]/[FA] mole ratio on percentage distribution and dissociation rate coefficients of nickel-FA complexes in model systems, at a constant concentration of Ni(II), and varying concentrations of FA (Armada). pH 5.0 $\pm$ 0.1; ionic strength $\sim$ zero. [Ni(II) 4.3×10^{-8} M] + [FA].

[FA], M	[Ni (II)]/[FA] mole ratio	%C ₁	k ₁ x 10 ² , s ⁻¹	% C ₂	k ₂ x 10 ⁵ , s ⁻¹
2.3×10^{-5}	0.0018	-----	-----	100 $\pm$ 3	8.4 $\pm$ 0.8
2.3×10^{-6}	0.018	18 $\pm$ 2	1.8 $\pm$ 0.2	83 $\pm$ 1	30 $\pm$ 3
2.3×10^{-7}	0.18	49 $\pm$ 1	0.8 $\pm$ 0.3	52 $\pm$ 2	100 $\pm$ 10

----- Not recovered.

k₁, and k₂ are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C₁, and % C₂, are percentage relative distributions of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Table 6 Effect of [Cu(II)]/[FA] mole ratio on percentage distributions and dissociation rate coefficients of copper-FA complexes in model systems, at a constant concentration of Cu(II), and varying concentrations of FA (Armadale). pH 5.0 $\pm$ 0.1; ionic strength $\sim$ zero. [Cu (II) 6.3×10^{-8} M] + [FA].

[FA], M	[Cu (II)]/[FA] mole ratio	%C ₁	k ₁ x 10 ² , s ⁻¹	% C ₂	k ₂ x 10 ⁵ , s ⁻¹
3.5×10^{-5}	0.0018	-----	-----	98 $\pm$ 2	^s Inert
3.5×10^{-6}	0.018	4 $\pm$ 1	0.49 $\pm$ 0.05	96 $\pm$ 1	5.6 $\pm$ 0.2
3.5×10^{-7}	0.18	47 $\pm$ 3	0.58 $\pm$ 0.04	56 $\pm$ 1	23 $\pm$ 3

----- Not recovered.

^sInert = k < 10⁻⁵ s⁻¹.

k₁, and k₂ are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C₁, and % C₂, are percentage relative concentrations of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Table 7 Effect of $[\text{Co(II)}]/[\text{FA}]$ mole ratio on percentage distributions and dissociation rate coefficients of cobalt-FA complexes in model systems, at a constant concentration of Co(II), and varying concentrations of FA (Armada). pH 5.0 $\pm$ 0.1; ionic strength $\sim$ zero. $[\text{Co(II)}] 6.8 \times 10^{-8} \text{ M} + [\text{FA}]$.

$[\text{FA}], \text{M}$	$[\text{Co(II)}]/[\text{FA}]$ mole ratio	%C ₁	$k_1 \times 10^2, \text{s}^{-1}$	% C ₂	$k_2 \times 10^5, \text{s}^{-1}$
3.8×10^{-5}	0.0018	----	----	98 ± 2	1.5 ± 0.4
3.8×10^{-6}	0.018	9 ± 3	1.1 ± 0.5	91 ± 1	7.4 ± 0.4
3.8×10^{-7}	0.18	45 ± 1	1.6 ± 0.1	56 ± 2	79 ± 6

----- Not recovered.

k_1 , and k_2 are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C₁, and % C₂, are percentage relative distributions of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Effect of the [Ni(II)]/[DOC] mole ratio on the dissociation rate coefficients of Ni(II) complexes in freshwater samples collected from the Sudbury mines area

The metal complexation properties of Dissolved Organic Carbon (DOC) in freshwaters are well known but are poorly understood. The previous section of this Chapter has dealt with the effect of the [metal]/[FA] mole ratio on the dissociation rate of metal-FA complexes in model systems. The above study is now extended to dissociation of metal complexes in freshwaters with Ni(II) as the target metal. In particular, the results of the effect of [Ni(II)]/[DOC] ratio on the dissociation rate coefficient of nickel complexes in metal-polluted freshwaters collected from the Sudbury mines area (Canada) are reported. Interactions of Ni(II) with dissolved organic carbon (DOC) have not been extensively studied [116-117].

The concentration of Dissolved Organic Carbon in the aquatic environment is typically in the range 10^{-1} to 10 mM [128]. In bogs, the concentration may reach 50 mM carbon, while in the ocean it varies from 40 μ M of carbon in deepwaters to six times this value in highly productive coastal surface waters [128]. The chemical nature of the dissolved organic carbon is poorly characterized. Humic compounds represent typically some 40% to 90% of the DOC [128].

Tables 8 and 9 present a list of the sampling sites from Sudbury mines area, and some properties of these freshwater samples such as pH and conductivity. In these samples, the concentration of nickel varied over a range spanning two orders of magnitude, whereas the concentration of the Dissolved Organic Carbon (DOC) was

almost the same (~ 5 mg / L) for all the samples. Consequently, the $[\text{Ni(II)}]/[\text{DOC}]$ mole ratio varied from sample to sample.

Figure 7 presents the dissociation kinetics of nickel complexes in the freshwater samples collected from the Sudbury mines area. The points in Figures 7 are the experimental data, and the solid lines the results from the non-linear regression analysis. The curves of Figure 7 (from top to bottom) show that the lability of the Ni(II) complexes increased with increasing total concentration of Ni(II) , and hence, increasing $[\text{Ni(II)}]/[\text{DOC}]$ mole ratio. This is because with increasing concentrations of nickel at the fixed concentration of the DOC, the strong binding sites of the DOC were completely saturated. The remaining nickel was then bound to the weak binding sites of the DOC, forming weak nickel complexes which were labile. The results of the data analysis of Figure 7 are presented in Table 10. Component 1 is the metal aqua ion plus any other labile complexes. Component 2 is a mixture of moderately labile complexes and the slowly labile complexes. Sample No. 6 shows enhanced lability at extremely low Ni:DOC ratio (the second lowest among the samples in Table 10), which goes against the above trend. Sample 6 has unusually high conductivity (Table 9). Since the other trace elements were found to be present in Sample No. 6 at low concentrations, this high conductivity was probably due to the presence of large amounts of Ca^{2+} and Mg^{2+} , which outcompete the target metal nickel for the binding sites of the DOC, and also decrease the electrostatic bonding component of the nickel binding by the DOC by screening the non-reacting, neighbouring negative charges on the DOC. A previous study done in our laboratory on model systems [129] showed that the presence of overwhelming amounts

of major ions such as Ca^{2+} and Mg^{2+} resulted in release of large fractions of organically-bound nickel from the Ni-FA complex as $\text{Ni}(\text{H}_2\text{O})_6^{2+}$ to the water.

Study of the kinetics of dissociation of Co(II)-, Ni(II)-, Cu(II)- and Zn(II) complexes in model systems containing a well-characterized fulvic acid and in freshwaters.

Figure 8 presents the exponential decay curves for the dissociation of Cu-FA, Ni-FA, and Zn-FA complexes in model systems at the same [metal]/[FA] mole ratio 0.8. The metal ions were present in the model systems at the same concentration of 20 $\mu\text{g/L}$ and the complexant used was the Laurentian fulvic acid. The test solutions were adjusted to $\text{pH } 5.0 \pm 0.2$. Figure 8 shows that at 600 s, about 98% of the Zn-FA and Ni-FA complexes, and only about 20% of the Cu-FA complexes dissociated in 600 s. The points in Figure 8 are the experimental data and the solid lines are the results from the fitting using non-linear regression analysis. Figure 8 shows that the order in the lability of these complexes is: $\text{Zn(II)} > \text{Ni(II)} > \text{Cu(II)}$. The results from the non-linear regression analysis are presented in Table 11 which shows that the dissociation rate coefficient of the faster distinguishable component increased in the order: $\text{Zn(II)} > \text{Ni(II)} > \text{Cu(II)}$.

Figure 9 shows the decay curves for the dissociation of Ni(II)-, Cu(II)-, and Zn(II)-DOC, complexes in the Rideau River surface water (RRSW) sample collected on June 13, 1996 from a site at Carleton University. The concentration of Dissolved Organic Carbon in some samples of the Rideau River surface water is shown in Table 12. The points in Figure 9 are the experimental data and the solid lines are the results from the fitting using non-linear regression analysis.

Table 8 List of sampling sites from Sudbury mines area, collected in October 1998.

Site #	Description of sites
1	Moose Creek upstream of the Onaping River. Treated effluent from Inco and Falconbridge
2	Kelly Lake. Mixed water from Copper Cliff effluent and Junction Creek after municipal sewage treatment plant.
3	Coniston Creek downstream.
4	Falconbridge conservation area. Treated effluent from the smelter.
5	Onaping River downstream of Moose Creek. Mixed water from Inco/Falconbridge and municipal pollution
6	Moose Lake treated discharge. Treated effluent from Moose Lake tailings.
7	Onaping River downstream of sewage treatment plant and upstream of Moose Creek. Municipal impact only.

Table 9 pH and conductivity of water samples from the Sudbury Mines area, collected in October 1998.

Sample No.	pH	Conductivity ($\mu\text{S}/\text{cm}$)
1	7.5	1060
2	7.9	1250
3	7.6	528
4	7.5	898
5	7.3	83.5
6	7.2	1520
7	6.9	54.5

Figure 7 Effect of the Ni:DOC ratio on the dissociation of Ni(II) complexes in freshwater samples from the Sudbury mines area. ▽, Sample No. 7; Δ, Sample No. 5; □, Sample No. 2; ○, Sample No. 1.

Table 10 Effect of Ni:DOC ratio on percentage distribution and dissociation rate coefficients of nickel complexes in the freshwater samples collected from Sudbury mines area.

Sample	[Ni(II)] M	[Ni(II)]/[DOC] mole / mg	% C ₁	k ₁ x 10 ³ s ⁻¹	% C ₂	k ₂ x 10 ⁴ s ⁻¹
1	4.7 x 10 ⁻⁵	9.4x10 ⁻⁵	90±2	1.6± 0.2	10±5	4.7±0.2
2	1.4 x 10 ⁻⁵	1.4 x 10 ⁻⁵	80±2	1.3± 0.4	22±3	4.5±0.2
3	3.7 x 10 ⁻⁶	7.4x10 ⁻⁷	50±4	1.4±0.2	55±6	4.0±0.2
4	3.5 x 10 ⁻⁶	7.0x10 ⁻⁷	43±3	1.3±0.2	47±1	4.1±0.2
5	1.6 x 10 ⁻⁶	3.2x10 ⁻⁷	32±2	1.1±0.2	72±3	3.0±0.2
6	9.5 x 10 ⁻⁷	1.9x10 ⁻⁷	90±5	1.8±0.2	11±2	4.8±0.2
7	6.5 x 10 ⁻⁷	1.3x10 ⁻⁷	25±2	1.0±0.1	78±3	2.8±0.2

k₁, and k₂ are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C₁, and % C₂, are percentage relative distributions of the first and second components, respectively. Values after ± signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

The order of lability observed in the Rideau River surface water (Figure 9) is the same as observed in the model systems containing Laurentian fulvic acid as the complexing agent. The decay curves were analyzed by the non-linear regression analysis and the results are presented in Table 13. As expected from the order of lability in Figure 9, Zn(II) has proportionally more of the fast component than Ni(II) or Cu(II). The Zn(II) species also have somewhat larger dissociation rate coefficients than the Ni(II) species. Figure 10 shows the dissociation kinetics of Co(II), Ni(II), Cu(II), and Zn(II) complexes in a freshwater sample (No. 4) collected from Sudbury mines area. In Figure 10, the points are the experimental data and the solid lines are the results from the curve fitting using non-linear regression analysis. The results from the non-linear regression are presented in Table 14. The concentration of Dissolved Organic Carbon (DOC), about 5 mg mL⁻¹, was almost the same for all the samples from the Sudbury mines area. The order of lability observed in these samples was: Co(II) > Zn(II) > Ni(II) > Cu(II). This trend was also observed in model systems containing these metal ions and fulvic acid, and in the Rideau River surface water sample, which have been presented earlier. In the Sample No. 4 from the Sudbury mines area, the concentration of Ni(II) was one order of magnitude higher than the concentrations of the other trace metals. In the Rideau River water samples, the samples were spiked so that the samples contained all the metals at the same concentration of 20 µg/L of each metal.

Figure 8 Comparison of the decay curves of dissociation of Ni-FA, Cu-FA, and Zn-FA complexes in a model system (Laurentian FA), measured by inductively-coupled plasma mass spectrometry. O, $[\text{Cu(II)}]/[\text{FA}] = 0.8$; Δ , $[\text{Ni(II)}]/[\text{FA}] = 0.8$; $\square$, $[\text{Zn(II)}]/[\text{FA}] = 0.8$; pH 5.0 ± 0.2 ; ionic strength 4.2×10^{-5} M

Table 11 Percentage distribution and dissociation rate coefficients of metal-fulvic acid (Laurentian fulvic acid) complexes in model systems at the mole ratio $[M]/[FA] = 0.8$, at pH 5.0 ± 0.1 ; ionic strength = 4.2×10^{-5} M.

Metal	%C ₁	k ₁ x 10 ² , s ⁻¹	%C ₂	k ₂ x 10 ³ , s ⁻¹
Cu(II)	32 ± 1	0.24 ± 0.3	68 ± 1	1.3 ± 0.1
Ni(II)	65 ± 3	1.3 ± 0.3	41 ± 3	3.0 ± 0.1
Zn(II)	76 ± 1	3.0 ± 0.2	24 ± 1	4.5 ± 0.3

k₁, and k₂ are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C₁, and % C₂, are percentage relative distributions of the first and second components, respectively. Values after ± signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Figure 9 Comparison of the decay curves for the dissociation of Ni(II)-, Cu(II)-, and Zn(II)- DOC complexes in a Rideau River surface water sample, collected on June 13, 1996, measured by inductively-coupled plasma mass spectrometry., ∇ , Cu(II)-DOC complexes; $\square$, Ni(II)-DOC complexes; Δ , Zn(II)-DOC complexes; pH = 5.0 ± 0.1 ; Conductivity = 308 ($\mu\text{S}/\text{cm}$)

Table 12 Concentration of total organic carbon and dissolved organic carbon in Rideau River surface water samples.

Season	Date of sample collection	Total Organic Carbon, mg of C/L	Dissolved Organic Carbon, mg of C/L
	Blank (ultrapure water)	0.3 ± 0.1	
	DOC Standard (10 mg/L)	7.6 ± 0.6	
Late Spring	June 12, 1996	11 ± 0.7	7.5 ± 0.1
Late Spring	June 13, 1996	10 ± 0.1	7.7 ± 0.2
Fall	September 24, 1997	----	9.1 ± 0.3
Early Spring	March 12, 1998	7.6 ± 0.7	5.6 ± 0.2
Late Spring	June 8, 1998	10 ± 0.1	6.5 ± 0.3

Table 13 Percentage distribution and dissociation rate coefficients of Cu(II)-, Ni(II)- and Zn(II)-DOC complexes in a Rideau River surface water sample, collected on June 13, 1996.

Metal	%C ₁	k ₁ x 10 ² , s ⁻¹	%C ₂	k ₂ x 10 ³ , s ⁻¹	%C ₃	k ₃ x 10 ⁵ , s ⁻¹
Cu(II)	----	----	47 ± 4	0.9 ± 0.2	51 ± 4	9.9 ± 2
Ni(II)	41 ± 1	1.7 ± 0.4	36 ± 1	2.0 ± 0.3	23 ± 0.7	15 ± 1
Zn(II)	55 ± 1	2.9 ± 0.4	37 ± 3	3.7 ± 0.1	8 ± 0.1	27 ± 1

---- Not recovered.

k₁, k₂, and k₃ are the dissociation rate coefficients of the faster, the slower and the slowest, kinetically distinguishable components, respectively. % C₁, and % C₂, are percentage relative distributions of the first and second components, respectively. Values after ± signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Figure 10 Comparison of the decay curves for the dissociation of Co(II)-, Ni(II)-, Cu(II)- and Zn(II)-DOC complexes in the freshwater samples collected from the Sudbury mines area (Sample No.7), measured by inductively-coupled plasma mass spectrometry. ○, Cu(II)-DOC complexes; Δ, Ni(II)-DOC complexes; □, Zn(II)-DOC complexes; ▽, Co(II)-DOC complexes.

Table 14 Total concentration, percentage distribution and dissociation rate coefficients of Cu(II)-, Ni(II)- and Zn(II)-DOC, complexes in the Sample No. 7, collected from the Sudbury mines area. pH = 7.5 ± 0.1

Metal	$\mu\text{g/L}$	% C ₁	$k_1 \times 10^2, \text{s}^{-1}$	% C ₂	$k_2 \times 10^3, \text{s}^{-1}$	% C ₃	$k_3 \times 10^5, \text{s}^{-1}$
Cu(II)	8.2	----	----	10 ± 2	1.5 ± 0.2	89 ± 3	7.5 ± 0.3
Ni(II)	88	10 ± 1	1.3 ± 0.1	51 ± 2	1.9 ± 0.1	39 ± 3	11 ± 1
Zn(II)	4.7	24 ± 1	3.8 ± 0.2	69 ± 1	3.9 ± 0.2	7 ± 2	10 ± 1
Co(II)	2.1	31 ± 1	5.6 ± 0.4	67 ± 1	4.0 ± 0.2	-----	-----

---- Not recovered.

k_1 , and k_2 are dissociation rate coefficients of the faster and slower kinetically distinguishable components. % C₁, and % C₂, are percentage relative distributions of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Lability and d-Electron Configuration

Properties of the metal ions such as ionic charge and size affect rates of ligand substitution reactions [130], and these factors may also affect relative rates of dissociation of metal-ligand complexes [130]. In comparing the water exchange rates of metals in general, one has to take into consideration that metal ions with high charge density (Z^2/r), such as Cr(III), have very low rates of water exchange compared to those with low charge density, such as Pb(II), suggesting that a principal contributor to the activation energy is the breaking of the bond of the leaving water molecule [144]. Although the decreasing ionic radius with increasing atomic number in the 3d transition metals primarily determines the “natural order of stability” (the Irving-William series [131]) of metal complexes ($Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$), the above order is also partially due to ligand field stabilization energy [132] (and the Jahn Teller effect for Cu(II) [132]). In humic substances the most important donor atoms are oxygen, nitrogen and sulphur. In order to fully understand the inertness/lability of complexes of transition metal ions with humic substances two factors should be considered: a) Hard-Soft Acid Base (HSAB) theory, and b) Ligand-Field Stabilization Energy (LFSE).

For a given soft metal, stability constant (K) increases with the overall softness of the donor atom, e.g. for bidentate sites in the order: (O,O) < (O,N) < (N,N) < (N,S); the reverse order is observed for hard metals [133]. If the formation rate constants for a reaction of a single metal with a series of ligands show little variation being determined by the nature of the metal ion only, then the dissociation rate constants should show an inverse correlation with the stability constant (K) [134].

To estimate the degree of relative hardness of Ni(II), Cu(II) and Zn(II), the hardness, η , was calculated according to the Mullikan definition which is as follows:

$$\eta = \frac{1}{2} (\text{I.P.} - \text{E.A.}) \quad (5.9)$$

where Ionization Potential (I.P.) is the energy required to remove an electron from the valence shell, and Electron Affinity (E.A.) is the energy required to introduce another electron into the valence shell. According to the definition of hardness given above, a hard atoms will have high I.P. and low E.A. Table 15 shows that Cu(II), which has d^9 electronic configuration, is the softest of the above metals and therefore binds with the softest of the ligands, forming strong complexes which are inert [135].

One way of viewing the relationship of electron configuration to lability is to note that the metals that form inert complexes are those with very high Ligand-Field Stabilization energies: d^3 and low-spin d^4 , d^5 , and d^6 . The d^8 configuration is borderline. A large LFSE in the initial octahedral complex results in a large sacrifice on going to a transition state of lower symmetry. Thus, the ease of ligand substitution may be expected to parallel the magnitude of the LFSE. This expectation is upheld by the data for one of the simplest substitution processes, the substitution of a water molecule in a hexaaqua ion by another ligand molecule. The Ligand-Field Stabilization energies and rate coefficients of water exchange for some of the 3d transition metals are presented in Table 16. The rate coefficients of water exchange are inversely proportional to the Ligand-Field Stabilization Energies, and indicate that inert complexes are formed by those metals that have a high LFSE. The exception is Cu(II), for which its low LFSE is compensated by the Jahn-Teller effect which adds greater stability to Cu(II) complexes by lengthening the

axial bonds and shortening the equatorial bonds, making the equatorial bonds stronger. In the tetragonally distorted structure of the Cu(II) complexes, the ground-state geometry of Cu(II) thus is close to that of its transition state geometry for water exchange, and the axial water ligands can exchange much more rapidly than the undistorted octahedral complex. Thus, Cu(II) forms stronger complexes than predicted by its Ligand-Field Stabilization Energy alone, but has a higher rate coefficient for water exchange.

Figures 8, 9, 10 and Tables 11, 13, and 14 show that the relative lability of complexes of the 3d transition metals is: Ni(II) $d^8 >$ Cu(II) $d^9 <$ Zn(II) d^{10} . This trend parallels that of the HSAB theory and of the LFSE with the exception of the enhanced stability of Cu(II) complexes (relative to Ni(II) complexes) which is due to the Jahn-Teller effect; Zn(II) with its d^{10} electron configuration cannot have any LFSE contribution to its bond energy. The above trend was first reported for the stability constants (which are inversely related to the dissociation rate coefficients) of complexes of 3d transition metal ions with simple ligands [136], and later was observed with humic substances by Gamble et al. [137], who reported that such a trend in the stability constants indicated that the metals were bound to structurally similar binding sites of humic acid. A similar conclusion is logical for the rate coefficients for dissociation of the metal complexes found in the model solutions of the FA, in the freshwater samples of Rideau River, in the freshwater samples from the Sudbury mines area. Gamble et al. [137] also suggested that variation in the metal loading of sites tended to obscure the above trend *via* higher-order interactions.

Table 15 Hardness of selected 3d transition metal ions.

Element	Hardness kJmol^{-1}
Ni	391.65
Cu	292.1
Zn	411.85

Table 16 Exchange rates for solvent water in hexaaqua complexes of the 3d transition metal ions [138].

Metal Ion	Mn²⁺	Fe²⁺	Co²⁺	Ni²⁺	Cu²⁺	Zn²⁺*
dⁿ	d⁵	d⁶	d⁷	d⁸	d⁹	d¹⁰
k_w, s⁻¹	3 x 10 ⁷	3 x 10 ⁶	1 x 10 ⁶	3 x 10 ⁴	8 x 10 ⁹	7 x 10 ⁷
LFSE (Dq)	0	6	8	12	6	0

* The data for Zn²⁺ are from various other sources.

Disjunctive and Adjunctive pathways

The Competing Ligand Exchange Method can follow two possible mechanistic pathways, disjunctive and adjunctive [139]: i) slow dissociation of ML to give M and L, followed by a fast reaction with the competing ligand, Chelex-100, (equations 5.10 and 5.11), which is known as the disjunctive pathway (analogous to an S_N1 pathway),

and ii) direct attack by the competing ligand followed by loss of the original ligand, L, which is known as the adjunctive pathway (analogous to an S_N2 pathway), (equations 5.12 and 5.13):

The first-order rate coefficient for the disjunctive pathway is simply the rate coefficient for the slow step of dissociation of the metal complex, ML [71], which is a fundamental process of the natural sample. Furthermore, it is the type of reaction for which the theory of ligand substitution processes allows for a significant degree of reliable extrapolation over concentrations and pH. The disjunctive pathway is determined by the rate coefficient for reaction of the incoming metal with the steady-state concentration of the free or protonated ligand intermediate and is directly proportional to the formation rate coefficient for the final metal complex [140]. It is thus inversely

proportional to the conditional equilibrium constant for the formation of the initial metal complex, ML. Hence, dissociation rate coefficient for a strong metal complex, ML, will be small and vice versa. In the adjunctive pathway, the observed rate coefficients are influenced by the nature of the arbitrarily chosen probe ligand (i.e. steric and electrostatic factors and by protonation of the incoming ligand). Consequently, very little relevant information can be inferred from the adjunctive pathway about the rates of processes in the natural system [71]. In reality, both the disjunctive and adjunctive pathways may contribute to the observed, overall rate coefficient [141]; in a given situation, one pathway may predominate over the other depending on the experimental conditions:

$$k_{\text{overall}} = k_{\text{disjunctive}} \frac{1}{[L]} + k_{\text{adjunctive}} \quad (5.14)$$

where k with its subscript stands for the rate coefficient. The adjunctive and disjunctive pathways are stoichiometric mechanisms inferred from rate laws and do not imply any characterization of the transition state of the substitution reaction.

The Kinetic Model and the Competing Ligand Exchange Method employed in the Kinetic Model have yielded results that show that the lability of the investigated 3d transition metal complexes agree with the trend shown by the Ligand-field stabilization energy $\text{Co(II)} d^7 > \text{Ni(II)} d^8 > \text{Cu(II)} d^9 < \text{Zn(II)} d^{10}$). Since the Kinetic Model assumes the dissociation of the metal complex, ML, as the rate-determining step, the disjunctive path probably predominates in the dissociation of the metal complexes investigated in the fulvic acid model systems and in the freshwater samples. The significance of the above result is that the Competing Ligand Exchange Method and the Kinetic Model presented earlier can correctly diagnose the relative labilities of the metal complexes in the freshwater environment.

CHAPTER 6

COMPETITION OF Ni(II), Cu(II), AND Co(II) FOR BINDING SITES OF ARMADALE FULVIC ACID

Introduction

Metal ions vary greatly in their affinity for the binding sites of humic substances (e.g. humic and fulvic acids). Release of bound metals in freshwaters as free metal ions (i.e. metal-aquo complexes) and hence, bioavailability of free metal ions, depends on the competition of various metal ions for the binding sites of the above polyfunctional, organic complexants, especially at low metal to ligand mole ratios typical of unpolluted freshwaters, and/or in an organic-complexant-limited aquatic environment typical of heavily-polluted freshwaters (the term 'polluted' is used in this context to mean 'contaminated with metals only').

Published literature dealing with the competition between trace metals for binding sites of naturally-occurring, organic complexants in freshwaters are scarce. Xue et al. [142] have investigated the competition of copper and zinc for strong ligands in a eutrophic lake using cathodic and anodic stripping voltammetry and have concluded that the release of zinc from electrochemically inert complexes upon addition of copper indicates that copper and zinc compete for strong organic complexants. If, and as long as, the organic complexant is present in excess, metals do not compete with one other, as would be expected [143].

Nickel ore mining, processing, refining, and metallurgical operations may discharge nickel-containing effluents to the Canadian natural environment. Since nickel in the mineral phase is associated with copper and cobalt, the aqueous effluents from nickel ore mining, processing, refining and metallurgical operations have nickel present with copper and cobalt in aqueous effluents. Therefore, copper and cobalt have been included in this research.

RESULTS AND DISCUSSION

Effect of varying concentrations of Cu(II) on the lability of the Ni-FA complexes

Figure 11 shows that in the case of the Armadale fulvic acid (FA), Ni(II) and Cu(II) in model systems, occupy the small fraction of the strong binding sites of the FA (~1-10% [26, 36, 42, 43]), thereby forming strong Ni-FA and Cu-FA complexes, which are inert (top curves). The sharply-increasing concentrations of Cu(II) (by one order of magnitude for each curve from top to bottom) has a dramatic effect on the lability of the Ni-FA and the Cu-FA complexes. The small fraction of the strong binding sites of the FA become completely filled by Cu(II) and Ni(II), both of which are in large excess over the small fraction of strong binding sites of the FA; the remaining Cu(II) and Ni(II) are then bound by the large fraction of weak binding sites of the FA, forming weak complexes, which are labile. The result is that most of the Cu(II) and the Ni(II) (in the [metal]/[FA] mole ratio used in this work to simulate the freshwater environment) form weak Ni-FA and Cu-FA complexes, which are labile. It is, however, significant that about 20% of the

Ni-FA complexes [the bottom curve in (a)] are still inert (within the time scale of measurements (8000 s)), probably a reflection of the strong Ni(II) bonding (both covalent and electrostatic) with the strong sites of the FA even when Cu(II) is present in large excess over Ni(II) (by three orders of magnitude). Comparison of curve (a) with curve (b) of Figure 11 shows that, under the same experimental conditions, the Ni-FA complexes are more labile than the Cu-FA complexes. The kinetic parameters of the Ni-FA and Cu-FA complexes in model systems, in the presence of varying concentrations of Cu(II) and a constant concentration of the FA, are presented in Tables 17 and 18. For the Ni-FA complexes, two kinetically distinguishable components of the Ni-FA complexes were resolved in all the samples except the first one (in the presence of 10^{-8} M Cu(II)). For the Cu-FA complexes in the presence of 10^{-8} M and 10^{-7} M Cu(II), one kinetically distinguishable component of Cu-FA complexes, and in the presence of 10^{-6} M and 10^{-5} M Cu(II), two kinetically distinguishable components of the Cu-FA complexes have been resolved. Tables 17 and 18 show that the concentrations and the dissociation rate coefficients of the slower components for both the Ni-FA and the Cu-FA complexes are almost the same (within the experimental error), but with the increasing concentrations of Cu(II), both the concentrations and the dissociation rate coefficients of the faster and the slower component of the Ni-FA and Cu-FA complexes vary greatly.

Effect of varying concentrations of Co(II) on the lability of Ni-FA complexes

Figure 12 shows that with a large excess of the FA (three orders of magnitude greater than those of Ni(II) and Co(II)), Ni(II) (and also Co(II)) occupy the small fraction

of the strong binding sites of the FA, thereby forming strong Ni-FA and Co-FA complexes, which are inert (top curves).

The sharply-increasing concentrations of Co(II) (by one order of magnitude for each curve from top to bottom) has a dramatic effect on the lability of the Ni-FA and the Co-FA complexes - the small fraction of strong binding sites of the FA become completely filled by Co(II) and Ni(II), both of which are in large excess over the small number of strong binding sites of the FA; the remaining Co(II) and Ni(II) are then bound by the large fraction of weak binding sites of the FA, forming weak complexes, which are labile. The result is that most of the Co(II) and the Ni(II), in the [metal] / [FA] mole ratio used in this work to simulate the freshwater environment, form weak Ni-FA and Co-FA complexes, which are labile. It is, however, significant that < 20% of the Ni-FA complexes [the bottom curve in (a)] are still inert, probably a reflection of the strong Ni(II) bonding (both covalent and electrostatic) with the strong sites of the FA even when the Co(II) is present in large excess over Ni(II) (by three orders of magnitude).

Comparison of curve (a) with curve (b) of Figure 12 shows that, under the same experimental conditions, the Co-FA complexes are more labile than the Ni-FA complexes. The kinetic parameters of the Ni-FA and Co-FA complexes in model systems, in the presence of varying concentrations of Co(II) and a constant concentration of the FA are presented in Tables 19 and 20. For the Ni-FA and Co-FA complexes, two kinetically distinguishable components were resolved in all the samples except the first one (in the presence of 10^{-8} M Co(II)).

Tables 19 and 20 show that the concentrations and the dissociation rate coefficients of the slower component for both the Ni-FA and the Co-FA complexes are

almost the same, but with the increasing concentrations of Co(II), both the concentrations and the dissociation rate coefficients of the faster and the slower component of the Ni-FA and Co-FA complexes vary greatly.

Effect of varying concentrations of Co(II) in the presence of Cu(II) on the lability of Ni-FA complexes

Figure 13 shows that when the FA is present in great excess (with its concentration three orders of magnitude greater than those of the Cu(II), Ni(II), and Co(II)), these three metals are in such small molar concentrations compared to the FA that sufficient number of the strong sites of the FA are available for binding all the Cu(II), Ni(II) and Co(II), and all of these three metals therefore occupy the strong binding sites of the FA, thereby forming strong metal-FA complexes, which are inert (top curves). Figures 13(b) and 13(c) show that with the sharply-increasing concentration of Co(II) by one order of magnitude (the second curve from top), the Ni(II) and Cu(II) still occupy the strong binding sites of the FA, thereby forming strong Ni-FA and Cu-FA complexes, which are inert. However, Figure 13(a) shows that under the same experimental conditions, i.e. with Co(II) 10^{-7} M, ~80% of Co(II) are bound to the strong binding sites of the FA; the remaining Co(II) are bound to the large fraction of weak binding sites present in the FA (the second curve from top), resulting in the formation of weak Co-FA complexes, which are labile. With further increase in the Co(II) concentration, all the strong binding sites of the FA are filled by Cu(II), Co(II) and Ni(II), all of which are present in excess of that required for binding the strong sites of the FA.

Figure 11 Effect of varying concentrations of Cu(II) on the lability of (a) Ni-FA and (b) Cu-FA complexes in model systems

containing constant concentrations of Ni(II) and the Armadale FA. Metal remaining in solution as a function of time, measured by inductively-coupled plasma mass spectrometry. Chelex-100 was the competing ligand. pH 5.1; ionic strength $\sim$ zero. a) Ni(II); b) Cu(II). O , $[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Cu(II)}] 10^{-8} \text{ M} + [\text{FA}] 10^{-5} \text{ M}$; $\diamond$, $[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Cu(II)}] 10^{-7} \text{ M} + [\text{FA}] 10^{-5} \text{ M}$; $\square$, $[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Cu(II)}] 10^{-6} \text{ M} + [\text{FA}] 10^{-5} \text{ M}$; Δ , $[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Cu(II)}] 10^{-5} \text{ M} + [\text{FA}] 10^{-5} \text{ M}$.

Table 17 Effect of varying concentrations of Cu(II) on percentage distributions and dissociation rate coefficients of nickel-FA complexes in model systems, in the presence of constant concentrations of Ni(II) and the fulvic acid.

pH 5.0 ± 0.2 . [Ni (II)] 10^{-8} M + [Cu(II)] + [FA] 10^{-5} M.

[Cu(II)], M	% C ₁	k ₁ ×10 ³ , s ⁻¹	% C ₂	k ₂ ×10 ⁵ , s ⁻¹
10 ⁻⁸	----	----	100 ± 2	[§] Inert
10 ⁻⁷	12 ± 4	2.5 ± 0.4	87 ± 3	2.9 ± 0.3
10 ⁻⁶	21 ± 9	5.7 ± 0.2	78 ± 2	9.3 ± 0.2
10 ⁻⁵	34 ± 2	8.9 ± 0.3	66 ± 1	24 ± 2

---- Not recovered.

[§]Inert = k < 10⁻⁵ s⁻¹.

k₁, and k₂ are the dissociation rate coefficients of the faster and the slower kinetically distinguishable component, respectively. % C₁, and % C₂, are percentage relative distributions of the first and second components, respectively. Values after ± signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Table 18 Effect of varying concentration of Cu(II) on percentage distributions and dissociation rate coefficients of copper-FA complexes in model systems, in the presence of constant concentrations of Ni(II) and the fulvic acid. pH 5.0 ± 0.2 . $[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Cu(II)}] + [\text{FA}] 10^{-5} \text{ M}$.

$[\text{Cu(II)}], \text{ M}$	% C_1	$k_1 \times 10^3, \text{ s}^{-1}$	% C_2	$k_2 \times 10^5, \text{ s}^{-1}$
10^{-8}	----	----	100 ± 5	$^{\S}\text{Inert}$
10^{-7}	----	----	98 ± 6	$^{\S}\text{Inert}$
10^{-6}	32 ± 3	0.5 ± 0.2	70 ± 2	3.5 ± 0.2
10^{-5}	76 ± 5	5.1 ± 0.2	35 ± 1	5.8 ± 0.5

----- Not recovered.

$^{\S}\text{Inert} = k < 10^{-5} \text{ s}^{-1}$.

k_1 , and k_2 are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C_1 , and % C_2 , are percentage relative distributions of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Figure 12 Effect of the varying concentrations of Co(II) on the lability of (a) Ni-FA and (b) Co-FA complexes in model systems containing constant concentrations of Ni(II) and the Armadale FA. Metal remaining in solution as a function of time, measured by inductively-coupled plasma mass spectrometry. Chellex-100 was the competing ligand. pH 5.1; ionic strength $\sim$ zero. a) Ni(II); b) Co(II); O, [Ni(II) 10^{-8} M] + [Co(II) 10^{-8} M] + [FA 10^{-5} M]; ◇, [Ni(II) 10^{-8} M] + [Co(II) 10^{-7} M] + [FA 10^{-5} M]; Δ, [Ni(II) 10^{-8} M] + [Co(II) 10^{-5} M] + [FA 10^{-5} M].

Table 19 Effect of varying concentrations of Co(II) on percentage distributions and dissociation rate coefficients of nickel-FA complexes in model systems, in the presence of constant concentrations of Ni(II) and the fulvic acid. pH 5.0 ± 0.2 .
 $[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Co(II)}] + [\text{FA}] 10^{-5} \text{ M}$.

$[\text{Co(II)}], \text{ M}$	% C_1	$k_1 \times 10^3, \text{ s}^{-1}$	% C_2	$k_2 \times 10^5, \text{ s}^{-1}$
10^{-8}	-----	-----	100 ± 5	$^{\S}\text{Inert}$
10^{-6}	10 ± 3	1.4 ± 0.2	90 ± 2	2.9 ± 0.6
10^{-5}	84 ± 5	9.8 ± 0.7	17 ± 5	12 ± 3

----- Not recovered.

$^{\S}\text{Inert} = k < 10^{-5} \text{ s}^{-1}$.

k_1 , and k_2 are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C_1 , and % C_2 , are percentage relative distributions of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Table 20 Effect of varying concentrations of Co(II) on percentage distributions and dissociation rate coefficients of cobalt-FA complexes in model systems, in the presence of varying concentrations of Co(II) and constant concentrations of Ni(II) and the fulvic acid.

pH 5.0 ± 0.2 . $[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Co(II)}] + [\text{FA}] 10^{-5} \text{ M}$.

$[\text{Co(II)}], \text{ M}$	% C_1	$k_1 \times 10^3, \text{ s}^{-1}$	% C_2	$k_2 \times 10^5, \text{ s}^{-1}$
10^{-8}	----	----	100 ± 5	$^{\S}\text{Inert}$
10^{-6}	22 ± 3	7.2 ± 0.1	83 ± 2	4.4 ± 0.4
10^{-5}	72 ± 4	12 ± 1	25 ± 5	54 ± 3

----- Not recovered.

$^{\S}\text{Inert} = k < 10^{-5} \text{ s}^{-1}$.

k_1 , and k_2 are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C_1 , and % C_2 , are percentage relative concentrations of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Figure 13 Effect of varying concentrations of Co(II) in the presence of Cu(II) on the lability of (a) Ni-FA, (b) Co-FA and (c) Cu-FA complexes in model systems containing constant concentrations of Ni(II), Cu(II) and the Armadale FA.

Metal remaining in solution as a function of time, measured by inductively-coupled plasma mass spectrometry.

Chelex-100 was the competing ligand. pH 5.2; ionic strength $\sim$ zero. a) Co(II) b) Ni(II); c) Cu(II).

O, $[\text{Ni(II)} 10^{-8} \text{ M}] + [\text{Cu(II)} 10^{-8} \text{ M}] + [\text{FA} 10^{-5} \text{ M}]$; $\diamond$, $[\text{Ni(II)} 10^{-8} \text{ M}] + [\text{Cu(II)} 10^{-8} \text{ M}] + [\text{Co(II)} 10^{-8} \text{ M}] + [\text{FA} 10^{-5} \text{ M}]$; $\square$, $[\text{Ni(II)} 10^{-8} \text{ M}] + [\text{Cu(II)} 10^{-8} \text{ M}] + [\text{Co(II)} 10^{-6} \text{ M}] + [\text{FA} 10^{-5} \text{ M}]$; Δ , $[\text{Ni(II)} 10^{-8} \text{ M}] + [\text{Cu(II)} 10^{-8} \text{ M}] + [\text{Co(II)} 10^{-5} \text{ M}] + [\text{FA} 10^{-5} \text{ M}]$.

Table 21 Effect of varying concentrations of Co(II) on percentage distributions and dissociation rate coefficients of nickel-FA complexes in model systems, in the presence of constant concentrations of Ni(II), Cu(II) and the fulvic acid, pH 5.0 ± 0.2 . $[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Cu(II)}] 10^{-8} \text{ M} + [\text{Co(II)}] + [\text{FA}] 10^{-5} \text{ M}$.

$[\text{Co(II)}], \text{ M}$	% C_1	$k_1 \times 10^3, \text{ s}^{-1}$	% C_2	$k_2 \times 10^5, \text{ s}^{-1}$
10^{-8}	----	----	100 ± 5	$^{\text{§}}\text{Inert}$
10^{-7}	----	----	95 ± 5	1.2 ± 0.2
10^{-6}	11 ± 2	2.5 ± 0.2	89 ± 1	2.5 ± 0.1
10^{-5}	83 ± 2	5.9 ± 0.3	19 ± 4	11 ± 0.6

----- Not recovered.

$^{\text{§}}\text{Inert} = k < 10^{-5} \text{ s}^{-1}$.

k_1 , and k_2 are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C_1 , and % C_2 , are percentage relative concentrations of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Table 22 Effect of varying concentrations of Co(II) on percentage distributions and dissociation rate coefficients of cobalt-FA complexes in model systems, in the presence of constant concentrations of Ni(II), Cu(II) and the fulvic acid, pH 5.0 ± 0.2 . [Ni(II)] 10^{-8} M + [Cu(II)] 10^{-8} M + [Co(II)] + [FA] 10^{-5} M.

[Co(II)], M	% C ₁	k ₁ × 10 ³ , s ⁻¹	% C ₂	k ₂ × 10 ⁵ , s ⁻¹
10 ⁻⁸	----	----	100 ± 5	^s Inert
10 ⁻⁷	23 ± 1	1.0 ± 0.2	77 ± 2	1.7 ± 0.3
10 ⁻⁶	45 ± 2	1.0 ± 0.1	50 ± 3	14 ± 0.4
10 ⁻⁵	65 ± 1	6.6 ± 0.3	35 ± 5	30 ± 1.5

----- Not recovered.

^sInert = k < 10⁻⁵ s⁻¹.

k₁, and k₂ are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C₁, and % C₂, are percentage relative concentrations of the first and second components, respectively. Values after ± signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

Table 23 Effect of varying concentrations of Co(II) on percentage distributions and dissociation rate coefficients of copper-FA complexes in model systems, in the presence of constant concentrations of Ni(II), Cu(II) and the fulvic acid, pH 5.0 ± 0.2 . $[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Cu(II)}] 10^{-8} \text{ M} + [\text{Co(II)}] + [\text{FA}] 10^{-5} \text{ M}$.

$[\text{Co(II)}], \text{ M}$	% C_1	$k_1 \times 10^3, \text{ s}^{-1}$	% C_2	$k_2 \times 10^5, \text{ s}^{-1}$
10^{-8}	----	----	100 ± 5	$^{\S}\text{Inert}$
10^{-7}	----	----	98 ± 2	1.1 ± 0.2
10^{-6}	10 ± 3	1.8 ± 0.2	91 ± 2	2.0 ± 0.2
10^{-5}	59 ± 2	3.0 ± 0.2	40 ± 2	6.7 ± 0.3

----- Not recovered.

$^{\S}\text{Inert} = k < 10^{-5} \text{ s}^{-1}$.

k_1 , and k_2 are the dissociation rate coefficients of the faster and the slower kinetically distinguishable components, respectively. % C_1 , and % C_2 , are percentage relative concentrations of the first and second components, respectively. Values after $\pm$ signs are standard deviations of non-linear regression analysis. Since the total uncertainties of the analytical method, including that of the regression analysis, are greater than the values of the standard deviations shown above, only two significant figures in the values of the rate coefficients are justified.

The remaining metals are then bound by the weak binding sites (which are present in much larger numbers) of the FA, forming weak Co-FA, Ni-FA, and Cu-FA complexes, which are labile.

Figure 13 makes it clear that Ni(II) and Co(II) cannot compete successfully with Cu(II) for the relatively small fraction of strong binding sites of the FA, as would be expected from their relative rate coefficients for water exchange [144]. Kinetic parameters of the Co-FA, Ni-FA and Cu-FA complexes are presented in Tables 21, 22 and 23, respectively. The increasing lability of the Ni-FA complexes in the presence of Cu(II) and Co(II) indicates that copper and cobalt successfully compete with Ni(II) for the strong binding sites of the FA, as predicted by their relative rate coefficients for water exchange.

The results described above are the combined effect of the following factors [145]: (1) The binding properties of the fulvic acid, both covalent bonding due to Lewis-basicity and coulombic interactions (long-range) due to its oligoelectrolytical properties, are important determinants of the free energy of interactions between a metal ion and the functional groups of the fulvic acid. The polyanionic character of fulvic acid is augmented by low ionic strength, and is decreased by increased metal loading because of partial neutralization of the charge on the neighbouring, non-reacting, negative sites of functional groups of the fulvic acid, all of which contribute greatly to the long-range coulombic interactions [145]. 2) In the formation of a metal-ligand complex, since the rate coefficients for water exchange for ligands are very large 10^9 - 10^{10} s⁻¹, their rate coefficients have been neglected in this treatment [146]. The water exchange rate coefficient of the metal-aquo complex, i.e., the first-order rate coefficient for water spans

16 orders of magnitude from Pb^{2+} and Cu^{2+} with 10^9 s^{-1} to Cr^{3+} 10^{-7} s^{-1} . For a given ligand, the metals such as Pb^{2+} and Cu^{2+} having very high water exchange rate coefficient, form strong complexes, which are likely to be inert, whereas the metals such as Al^{3+} , Cr^{3+} , having very low water exchange rate coefficients form weak complexes, which are likely to be labile [144]. When several metals, such as Cu(II) , Ni(II) , and Co(II) are present with the FA in water samples, metals such as Cu(II) and Pb(II) which have much higher water exchange rate coefficient than the metals such as Ni(II) and Co(II) will form stronger complexes which are likely to be inert.

Strong binding sites of ligands tend to form strong complexes with a given metal. Most complexation reactions are elementary reactions. For elementary reactions, the rate coefficient for complex dissociation can be related to formation rate coefficient through detailed balancing:

where K_{eq} is the equilibrium constant (stability constant), and k_a and k_d are the formation and dissociation rate coefficient, respectively. Because of the inverse relationship (assuming elementary reactions) of k_d and K_{eq} , when K_{eq} is very high, k_d is very low, if k_a is a constant, resulting in an inert complex. This also holds for the reaction of metals with multidentate ligands (such as the salicylic and the phthalic acid functional groups in humic substances, which form chelate rings with metals) if the rate-limiting step for complexation is the formation of the first metal-ligand bond. The metals such as Pb^{2+} and Cu^{2+} which have very high water exchange rate coefficient, form strong complexes, which are inert.

SECTION 3

EVALUATION OF METHODS FOR KINETIC DATA ANALYSIS

CHAPTER 7

EVALUATION OF THE DISTRIBUTION ANALYSIS METHOD FOR KINETIC DATA USING SIMULATED DATA

Introduction

Under the assumption of a series of first-order reactions, the dissociation rate of M-FA is essentially a multi-exponential decay. The question then arises whether the overall decay is a sum of a limited number of exponentials or a continuous distribution. In the previous chapters, the decay curves of dissociation of metal-FA and metal-DOC complexes have been analyzed assuming the overall decay curves of dissociation as a sum of a limited number exponentials using Non-Linear Regression analysis which analyzes the data assuming first that the decay is a sum of two exponential terms. If the quality of the fit is unsatisfactory, a third exponential term is added. In most cases, the addition of a fourth component does not improve the quality of the fit. A satisfactory fit with a sum of two, three, or four exponential terms means that the actual number of fitting parameters is enough considering the limited accuracy of experimental data, but it does not prove that the actual number of components is two (or three or four) unless it is already known that the system can be described by a physical model involving distinct exponentials [147]. To answer the question whether the overall decay consists of a few distinct exponentials or should be interpreted in terms of a continuous distribution, it is advantageous to use an approach without a *priori* assumption of the shape of the

distribution. Such an approach is provided by the Distribution Analysis Method which is presented later in this Chapter.

Application of statistical concepts to metal complexes of heterogeneous complexants

As presented in Chapter 4, the sum of the concentrations of all dissociating components at time t is given by equation (7.1):

$$C(t) = \sum_i^n C_i^0 \exp(-k_i t) \quad (7.1)$$

where $C(t)$ represents the decrease over time of the overall concentration of the metal complexes (e.g. M-FA complexes), C_i^0 is the initial concentration of the i th component, and k_i is the rate coefficient for dissociation of the i th component. For polyfunctional complexants, the summation in equation 7.1 may be replaced by an integral either as an approximation to a finite sum or as the representation of the essentially continuous distribution of binding sites that may arise in the case of polyfunctional heterogeneous complexants such as fulvic and humic acids:

$$C(t) = C(0) \int_0^\infty e^{-kt} F(k) dk \quad (7.2)$$

where $F(k)$ is the normalized function that describes the probable distribution of binding sites, and $C(0)$ is the normalization constant that express that at $t = 0$ all ligands have to be occupied equally with M. Practically speaking, $C(0)$ is the total amount of ML at $t = 0$. The function $F(k)$ gives the probability of finding a site which forms a complex having a particular value of rate coefficient (k). When applying a probability function on to complexation properties, k is used as the independent variable of the distribution (i.e. k is

considered a continuous variable parameter and is plotted along the abscissa). The mathematical form of the function $F(k)$ is such that any surface area under the curve corresponding to a given interval of k (e.g. k^A to k^B) represents the probability of finding sites whose values of the rate coefficient are in this interval ($k^A > k > k^B$). This probability can be seen, more simply, as being equivalent to the fraction ^{AB}S of the total sites present that has k values in the A-B defined interval [127]. In general for heterogeneous complexants, the $F(k)$ function is unknown and certainly not Gaussian [127]; those that are known have been found to be highly asymmetrical [100]. In fact, when studying a heterogeneous complexant, the $F(k)$ function is the information sought since it represents the entirety of its complexation properties [127].

Since the value of the rate coefficient may vary over several orders of magnitude, it is appropriate to switch to a normalized distribution function “ f ” that is a function of $\log k$ instead of k and equation (7.2) becomes:

$$C(t) = C(0) \int_0^\infty e^{-kt} f(\log k) d(\log k) \quad (7.3)$$

Equations (7.2) and (7.3) are members of the general class of Fredholm integral equations of the first kind.

Advantage and disadvantage of applying statistical concepts to metal complexes of heterogeneous complexants

The application of statistical concepts to complexation properties of heterogeneous complexants is potentially useful for understanding the qualitative effects of certain physicochemical factors on the overall complexation properties of L , since any change in

chemical conditions which influence its properties will be reflected by a change in the shape of the $F(k)$ function (i.e. change in chemical conditions will influence the shape of the distribution) [127]. Despite the above-mentioned advantage there is a practical limitation on the use of $F(k)$ functions for interpretation of the complexation properties of heterogeneous complexant, which is due to the great complexity of the mathematical transformations of the function $F(k)$ [127].

The Distribution Analysis Method

The Distribution Analysis Method is a part of a commercial software program FL900, supplied by Edinburgh Analytical Instruments [67], which is able to give a distribution of rate coefficients. This method was developed originally for the determination of lifetime distribution for fluorescence decay. The Distribution Analysis Method utilizes equations 7.2 and 7.3. The mathematical description of the method used by the authors of the algorithm to solve equation 7.2 and 7.3, and some applications of the method have been described by Gakamasky et al. [67].

The Distribution Analysis Method requires no *a priori* assumptions about the number of components, initial estimates of the values of the rate coefficient, or shape of the distribution (e.g. Gaussian). The software computes the function $F(k)$ as a histogram of n columns within the interval $[k_{\min}, k_{\max}]$. The histogram is an estimation of the actual normalized function $F(k)$ that describe the distribution. This distribution is obtained when $\Delta \log k$ becomes infinitely small ($\Delta \log k \rightarrow d \log k$). The surface area within an interval $\Delta \log k$ directly yields the corresponding value of ΔC_{ML} where C_{ML} is the concentration of

the metal complexes ML that have a dissociation rate coefficient within the interval $\Delta \log k$.

The program has a value of $k_{\min}$ that for all k less than $k_{\min}$, the exponential $\exp(-kt)$ decays slowly to be reliably distinguished from a coefficient. On the other hand, there also exists a value of $k_{\max}$ such that for k larger than $k_{\max}$ the exponential $\exp(-kt)$ decays so fast that k cannot be measured.

After a successful fit, the program displays the original decay curve and the fitted decay curve as well as the distribution of rate coefficients (in lifetimes) and the residuals. The mean values of distribution of lifetimes are displayed with the standard deviations. The standard deviation corresponds to the width of the distribution. This mean lifetime for a peak can be thought of as a single exponential in the Non-linear regression analysis. When this program is used to study rate of dissociation of metal-humate complexes, the mean lifetime is converted to mean dissociation rate coefficient and the width of the calculated distribution of the rate coefficient is represented by a standard deviation. Therefore, the dissociation rate coefficients presented in the tables of this Chapter and in Chapter 8 are the mean rate coefficients of the distribution of rate coefficients, and the standard deviations represent the width of the calculated distribution of the rate coefficients.

The results of the data fitting procedures were judged by the randomness of the weighted residuals and the statistical parameter Chi-square (χ^2) that is defined as follows:

$$\chi^2 = \sum [Y(t) - F(t) / \delta t]^2 \quad (7.4)$$

where $Y(t)$ is the original data, $F(t)$ is the result of the fitting and $\delta(t)$ is the statistical uncertainty of $Y(t)$. The model adjusts the fitting parameters to yield the lowest possible value of χ^2 .

Figure 14 shows an example of the output file of the program. The left-hand half of Figure 14 shows the experimental decay curve and the fitted data plus the plot of the residuals. The right hand-side of Figure 14 shows a graph of the lifetime distributions and a table of the mean lifetime, the width of the calculated distribution given by a standard deviation, and the percentage relative weight for each peak of the lifetime distribution.

The software program FL900 has also a feature referred to us as Level I that fits the data assuming discrete values of the rate coefficient. Level I is similar to the Non-linear regression analysis since it uses the Marquardt algorithm [148], but the former is limited to a sum of four exponentials plus a coefficient. Level I does not require initial guesses of the concentrations of the components, but does require initial guesses of the number of components and the values of the rate coefficients. The Distribution Analysis Method was used to generate the initial guesses of the number of components and the values of the rate coefficients when Level I was used. The results obtained using both Level I and the Distribution Analysis Method have been compared in this Chapter and in Chapter 8.

Generation of simulated data

In this chapter we will attempt to answer the following questions using simulated data:

- (1) How does experimental noise affect the distribution of the rate coefficients?

Figure 14 Example of an output file from the Distribution Analysis Method Software

- (2) What values of the ratio of the dissociation rate coefficients for the kinetic components in the system are necessary to obtain an adequate resolution (i.e. an adequate separation of the distribution of the rate coefficients (peaks))?
- (3) What is the effect of the concentration of each component on the resolution of the distribution of the rate coefficients?

To answer the first question, and to illustrate the method in the case of random error in the experimental data, simulated decay curves with and without noise in $C(t)$ were used. The noise added to the simulated data were two examples of experimental noise taken from our previous determinations of copper and aluminum in Rideau River surface water by inductively-coupled plasma mass spectrometry. Noise from the determinations of copper (0.1%) was smaller in magnitude and less scattered than that from the aluminum determinations (10%). The simulated decay curves used were mono-exponential or the sum of two or three exponentials according to equation (7.1). However, only the results of a one-component system will be reported in this thesis as an example since the effect of noise was the same for all the systems.

To answer the second question, simulated decay curves with rate coefficients differing by a factor of two and five were used. The values of the rate coefficients were chosen to match the rate coefficients that are usually observed for metal-fulvic acid complexes in model systems using the Competing Ligand Exchange Method with Chelex-100 as the competing ligand and inductively-coupled plasma mass spectrometry as the analytical method. Thus, the results may be directly compared to the experimental results shown in Chapter 8. The effect of time increments between the data points and the total time for accumulation of the experimental data was also studied.

To answer the third question, three-component systems with different initial concentrations of the kinetic components were used. The rate coefficients of the three components differed by a factor of five. Both levels of noise mentioned earlier were added to the simulated data.

RESULTS AND DISCUSSION

Effect of experimental noise on the distribution of dissociation rate coefficients.

To study the effect of experimental noise on the distribution of rate coefficients, a system with one kinetic component and a rate coefficient of 0.001 s^{-1} was simulated and analyzed by the Distribution Analysis Method with and without the addition of noise. The noises added to the data were two examples of experimental noise taken from our previous determinations of copper and aluminum in freshwaters by inductively-coupled plasma mass spectrometry. Noise from the determinations of copper (0.1%) was smaller in magnitude and less scattered than that from aluminum (10%).

Figure 15 shows the distribution of the rate coefficients obtained for one component system with and without noise. The mean values of the rate coefficients plus the width of the distributions represented by the standard deviations are summarized in Table 24. For the first set of data, which represents data without noise, a narrow distribution of the rate coefficients (narrow peak) was obtained (Fig. 15a). The recovered mean value of the rate coefficient was within 1% of the input rate coefficient and the width of the distribution was 3% (Table 24). By the addition of 0.1% noise to the

simulated data, the distribution of the rate coefficients obtained (Fig. 15b) was broader than the distribution of the rate coefficients obtained in the first case (Fig. 15a), but the mean value of the rate coefficients recovered was still within 1% of the input value. The width of the distribution of the rate coefficients, however, was increased from 3% to 13%. When the level of noise was increased from 0.1% to 10 %, the distribution of the rate coefficients became broader (Fig. 15c). By increasing the level of noise from 0.1% to 10%, the width of the distribution of the rate coefficients increased from 13% to 30% (Table 24). From this set of experiments it is clear that the level of noise has a strong effect on the width of the distribution of the rate coefficients; however, the recovered mean values of the rate coefficients are less affected.

Effect of the ratio of dissociation rate coefficients on the resolution of the dissociation rate coefficients

The ability of the Distribution Analysis Method to resolve a three-component system with different ratios of the rate coefficients was examined next. Decay curves for a three-component system with rate coefficients that differ by factors of two and five were analyzed. In the first case, the input values of the rate coefficients were k_1 0.004 s^{-1} , k_2 0.002 s^{-1} , and k_3 0.001 s^{-1} , whereas in the second case, the values of the input rate coefficients were k_1 0.025 s^{-1} , k_2 0.005 s^{-1} , and k_3 0.001 s^{-1} . The concentration of each component was equal and expressed as signal intensities. The values of the signals were chosen to be similar to the values found in the determinations of trace metals using inductively-coupled plasma mass spectrometry.

Figure 15 Effect of magnitude of noise on the distribution of the rate coefficients recovered from simulated data. One-component system.
 $C(t) = 15000 \cdot \exp(-0.001 \cdot t)$. Time increment = 8 s. Total time = 4000 s.
 (a) zero noise; (b) 0.1% noise; (c) 10% noise.

Table 24 Effect of magnitude of noise on rate coefficients recovered from simulated data.

Level of noise	%C ₁	k x 10 ³ , s ⁻¹	% Relative standard deviation
no-noise	100	1.00 ± 0.03	3
0.1%-noise	99.9	1.00 ± 0.13	13
10%-noise	99.9	1.00 ± 0.3	30

Total time = 4000 s; Time increment= 8 s; Total number of points was 500. One-component system; k = 0.001 s⁻¹.

The effects of noise, time increments between the data points, and the total time were also investigated.

Figure 16 presents an example of the analysis of the three-component system with different ratios of rate coefficients. The two systems analyzed have time increments of 4 s, a total time range of 2000 s, and a percent added noise of 0.1%. When the rate coefficients of the three components differed by a factor of two (Fig. 16a), only two components were recovered. The middle component was not separated from the other two. For the system with rate coefficients that differ by a factor of five, three well-separated peaks were recovered (Fig. 16b). From Figure 16, it is clear that the recovery of the rate coefficients depends critically on the separation of the rate coefficients.

Table 25 shows the results obtained for a three-component system with rate coefficients that differ by a factor of 2. In an ideal case (i.e., using data without noise), the input values of the three rate coefficients can be obtained with an uncertainty that varies between 2.5% - 5%. The width of the distribution of the rate coefficients varies from 5% to 10%. When 0.1% noise was added to the simulated data, the slowest component with dissociation rate coefficient of $k_1 0.001 \text{ s}^{-1}$ could not be retrieved, and the width of the distribution of the rate coefficients of the second component increased from 9% to 40%. Furthermore, the value of the rate coefficient recovered for the second component ($k_2 0.0014 \text{ s}^{-1}$) appeared to be an average of the input values of the rate coefficients of the second and third components ($k_2 0.002 \text{ s}^{-1}$ and $k_3 0.001 \text{ s}^{-1}$). When the level of noise was increased from 0.1% to 10%, only one component was observed with a mean rate coefficient equal to 50% of the input value. Table 25 shows that if the time increment was decreased from 8 s to 4 s while keeping the total time constant, the

number of points increased from 500 to 1000, resulting in a better fit. However, with a further decrease of the time increment, a still better fit was not obtained. This was probably due to the large weighting factor of the end of the decay curve, where signal to noise ratio approaches zero.

Table 26 shows the kinetic parameters obtained for a three-component system with rate coefficients that differ by a factor of five. For an ideal case (i.e. using data without noise), the width of the distribution of the rate coefficients recovered were 4% of the input first component (fastest, k_1), 4% for the second component (k_2) and 10% for the third component (slowest, k_3). When 0.1% noise was added, the rate coefficient recovered for the first component (k_1) was within 4% of the input value, that for the second component (k_2) was within 8% and for the third component (k_3) was within 4%. When 10% noise was added, three components were still recovered, but the agreement between the recovered values and the input values was worse. Moreover, the number of points taken for the set of data had a significant effect on the values of the rate coefficients. With the smallest number of data points, the fastest component was not recovered. In the case of the largest number of data points, the width of the distribution of the rate coefficients for the first component (fastest, k_1) was 29%, for the second component was 43% (k_2) and for the third component (slowest, k_3) 45%.

The examples of the analysis of the three-component system with different ratios of rate coefficients are presented in Figure 16, which illustrates these results for the systems with 0.1% noise (which was typical of the inductively-coupled plasma mass spectrometry measurement of the Cu decay). Both systems had a time increment of 4s, and a total time range of 2000 s.

Figure 16 Effect of the ratio of the dissociation rate coefficients on the resolution of three-component systems, with equal initial concentrations.

$C_1^0 = C_2^0 = C_3^0 = 5000$; 0.1% noise was added to both systems.

(a) $C(t) = C_1^0 \exp(-0.004t) + C_2^0 \exp(-0.002t) + C_3^0 \exp(-0.001t)$;

(b) $C(t) = C_1^0 \exp(-0.025t) + C_2^0 \exp(-0.005t) + C_3^0 \exp(-0.001t)$.

Table 25 Rate coefficients recovered from simulated data for three-component systems. Effect of time increment and magnitude of noise. $k_1/k_2 = k_2/k_3 = 2$. Range 4000 s. $k_1 = 0.004 \text{ s}^{-1}$; $k_2 = 0.002 \text{ s}^{-1}$; $k_3 = 0.001 \text{ s}^{-1}$.

Level of Noise	Time		# of Points	% C_1	$k_1 \times 10^3, \text{s}^{-1}$	% C_2	$k_2 \times 10^3, \text{s}^{-1}$	% C_3	$k_3 \times 10^3, \text{s}^{-1}$
	Increments, s								
No noise	8	500		35	4.0±0.2	33	2.1±0.2	32	1.0±0.1
	4	1000		34	3.9±0.2	33	2.0±0.2	33	1.0±0.1
	2	2000		34	4.0±0.3	33	2.1±0.2	33	1.0±0.1
0.1% noise	8	500		11	4.3±0.9	88	1.4±0.5	---	---
	4	1000		20	4.3±0.9	80	1.4±0.6	---	---
	2	2000		100	1.7±0.9	---	---	---	---
10% noise	8	500		100	2.3 ± 0.7	---	---	---	---
	4	1000		100	3.0 ± 0.6	---	---	---	---
	2	2000		100	2±1	---	---	---	---

-----Not Recovered. C_i relative concentration recovered

Table 26 Rate coefficients recovered from simulated data for three-component systems. Effect of time increment and magnitude of noise. $k_1/k_2 = k_2/k_3 = 5$. Range 2000 s. k_1 0.0250 s⁻¹; k_2 0.0050 s⁻¹; k_3 0.0010 s⁻¹.

Level of noise	Time Increment, s	# of Points	%C ₁	$k_1 \times 10^2, \text{s}^{-1}$	% C ₂	$k_2 \times 10^3, \text{s}^{-1}$	% C ₃	$k_3 \times 10^3, \text{s}^{-1}$
No noise	8	250	35	2.5 ± 0.1	33	5.0 ± 0.2	32	1.0 ± 0.1
	4	500	34	2.5 ± 0.1	33	5.0 ± 0.2	33	1.0 ± 0.1
	2	1000	34	2.5 ± 0.1	33	5.0 ± 0.2	33	1.0 ± 0.1
0.1% noise	8	250	34	2.6 ± 0.4	32	5.4 ± 0.9	34	1.0 ± 0.2
	4	500	33	2.6 ± 0.6	33	5.4 ± 0.9	34	1.0 ± 0.2
	2	1000	32	2.5 ± 0.3	33	5.4 ± 0.9	35	1.0 ± 0.2
10% noise	8	250	-----	-----	50	15 ± 9	50	1.3 ± 0.9
	4	500	25	3.1 ± 0.9	35	7.0 ± 3	39	1.1 ± 0.5
	2	1000	22	3.2 ± 0.8	35	7.0 ± 3	39	1.1 ± 0.5

-----Not Recovered

When the rates of dissociation of the three components differed by a factor of two (Fig. 16a), only two components were recovered. The middle component was not separated from the other two. For the system with rate coefficients that differ by a factor of five, three well-separated peaks were recovered (Fig. 16b). From Figure 16, it is clear that the recovery of the rate coefficients depends critically on the separation of the rate coefficients.

Effect of the initial concentrations of the kinetically distinguishable components on the resolution of the dissociation rate coefficients.

The ability to recover the rate coefficients for a three-component system with different initial concentrations of the kinetic components was tested. For these systems, the rate coefficients differed by a factor of 5 and the values of the rate coefficients chosen are shown in Table 27. Table 27 summarizes the results with the two levels of noise, considered earlier. Table 27 illustrates the ability of the program to resolve three-component systems with different initial concentrations of the components, and with different levels of noise. At both levels of noise, 0.1% and 10%, all the three components were recovered when the initial concentration of the three kinetic components were equal. When the noise level was 0.1%, the three components were resolved for all the cases when one component was present at a concentration ten times less than the other two. When the initial concentration of the first component was ten times lower than that of the other components, the recovered rate coefficient was 68 % of the input value (Table 27); when the initial concentration of the second component was ten times lower than that of the other components, the recovered rate coefficient was 74% of the input

value (Table 27); and when the initial concentration of the third component was ten times lower than that of the other components the error in the recovered rate coefficient was 90 % (Table 27). When the noise level was 10%, the component present at the lower concentration was not recovered, although reasonable values for the rate coefficients of the other components were obtained. The exception was the case where the slowest component was present at the lower concentration. For this case, the fastest component was also not recovered. Only one broad peak was obtained and the rate coefficient recovered was an average of the three values.

The results of Table 27 for the systems with 10% noise are illustrated in Figure 17. All systems had a time increment of 4 s and a total time range of 2000 s. Figure 17a shows the case where the concentrations of all the components were equal and all three components were recovered. Figure 17b shows a case in which the concentration of the fastest component was 10 times less than that of the other two components. In this case, the fastest component was not recovered and the rate coefficients obtained for the second and third components were within 20% and 10% of the input value, respectively. Figure 17c shows a case in which the concentrations of the fastest and the slowest component are equal, whereas the concentration of the middle component was decreased by a factor of ten. In this case, the middle component was not recovered, but the rate coefficients obtained for the first and third components were in better agreement with the input values. Figure 17d shows a case in which the concentration of the slowest component was decreased by a factor of ten. In this case, only one broad peak was recovered.

Tables 25, 26 and 27 illustrate the ability of the program used here to resolve the kinetic components of equation 1. Clearly, the resolution depends on the degree in which

the rate coefficients differ as well as on the relative concentration of each kinetic component. Each of these factors also depends on the level of noise in the data so that arbitrary stipulation of the ratio of rate coefficients or of their relative concentrations cannot be made. The data for each set of experimental conditions must be examined independently.

For comparison, the data sets in Table 26 were also analyzed using Level 1, which gives single values for the rate coefficients for each kinetic component. In Table 28, the results from the Level 1 and the Distribution Analysis are presented. In general, good agreement was obtained between the rate coefficients recovered by the two methods.

Since this simulated data was generated using single values for the rate coefficients of metal-complex dissociation for each kinetic component, analysis by Level I should be adequate. For experimental systems, however, the characteristics of the metal-complexes undergoing dissociation may not be known. Analysis by both types of methods should therefore be advantageous.

However, it must be remembered, that a satisfactory fit of the experimental data indicates only that the values obtained are compatible with the experimental data and does not prove the actual existence of the components or a particular distribution. For example, the fitting of a typical decay curve by Level 1 may indicate two kinetic components with single values for the rate coefficients, but the fitting by the Distribution Analysis may indicate a single component with a broad distribution. Therefore, interpretation of the results requires a good knowledge of the physico-chemical characteristics of the system and of the reliability of the data.

Table 27 Effect of the initial concentrations of the kinetically distinguishable components on rate coefficients recovered from simulated data. Time interval 4 s; Number of points 500; $k_1 = 0.025 \text{ s}^{-1}$, $k_2 = 0.005 \text{ s}^{-1}$, $k_3 = 0.001 \text{ s}^{-1}$.

Level of Noise	C^0_1	C^0_2	C^0_3	ICP-MS signal, counts/s			$k_2 \times 10^3, \text{ s}^{-1}$	$k_3 \times 10^3, \text{ s}^{-1}$
				$k_1 \times 10^2, \text{ s}^{-1}$				
0.1% noise	10000	10000	10000	2.6±0.0	5.3±0.8	1.0 ±0.2		
	1000	10000	10000	4.2±0.4	5.3±0.8	1.0±0.0		
	10000	1000	10000	2.4±0.4	8.7±0.8	1.0±0.0		
	10000	10000	1000	2.7±0.5	5±1	0.1±0.2		
10% noise	10000	10000	10000	3.1±0.9	7±3	1.1±0.5		
	1000	10000	10000	-----	6±2	1.1±0.5		
	10000	1000	10000	2.4±0.6	-----	1.0±0.4		
	10000	10000	1000	-----	7±8	-----		

-----Not Recovered

Figure 17 Resolution of three-component systems as a function of the initial concentrations of the components. All systems have time increment 4 s and a total range 2000 s. 10% noise was added to all systems.

$$C(t) = C_1^0 \cdot \exp(-0.025 \cdot t) + C_2^0 \cdot \exp(-0.005 \cdot t) + C_3^0 \cdot \exp(-0.001 \cdot t).$$

(a) $C_1^0 = C_2^0 = C_3^0 = 10000$; (b) $C_1^0 = 10000$; (c) $C_1^0 = 1000$; $C_2^0 = 10000$; $C_3^0 = 1000$; (d) $C_1^0 = 1000$; $C_2^0 = 10000$; $C_3^0 = 1000$.

Table 28 Comparison of the results of Level I and Distribution Analysis Method.

$k_1/k_2 = k_2/k_3 = 5$. $C_1^0 = C_2^0 = C_3^0$. Range 2000 s. Time increment = 4 s;

$k_1 = 0.0250 \text{ s}^{-1}$; $k_2 = 0.0050 \text{ s}^{-1}$; $k_3 = 0.0010 \text{ s}^{-1}$;

Level of noise	Method	$k_1 \times 10^2, \text{ s}^{-1}$	$k_2 \times 10^3, \text{ s}^{-1}$	$k_3 \times 10^3, \text{ s}^{-1}$
0.1%- noise	Level I	2.5 ± 0.1	5.1 ± 0.1	1.0 ± 0.1
	Distribution Analysis	2.6 ± 0.3	5.3 ± 0.8	1.0 ± 0.2
10%-noise	Level I	2.8 ± 0.1	6.0 ± 0.1	1.0 ± 0.1
	Distribution Analysis	3.1 ± 0.9	7 ± 0.12	1.0 ± 0.4

CHAPTER 8

ANALYSIS OF DISSOCIATION RATE COEFFICIENTS OF METAL COMPLEXES IN MODEL SYSTEMS USING THE DISTRIBUTION ANALYSIS METHOD

Introduction

In Chapters 5 and 6 it has been shown that the binding of the metal by the well-characterized fulvic acids occurred over a range of binding energies. Rate coefficients for dissociation were measured over the range of 10^{-2} - 10^{-5} s⁻¹. It was also shown that the rates of dissociation varied with various experimental conditions, particularly the [metal]/[fulvic acid] mole ratio. In this Chapter, the distribution of the dissociation rate coefficients of kinetically distinguishable components of selected metal complexes in model systems containing EDTA is compared with that of the Laurentian fulvic acid as complexants. In particular, the effects of the [metal]/[fulvic acid] mole ratio on the distribution of the rate coefficients are examined. The following test solutions were used: 1) aqueous solutions containing a single metal and EDTA; 2) aqueous solutions containing a mixture of Al(III), Cd(II), Cu(II), Ni(II), Zn(II) and Pb(II), with and without the Laurentian fulvic acid.

The metal-EDTA complex has been used previously [149] to test the Distribution Analysis Method because it is expected that a metal complexed with a simple ligand (EDTA) would not give a broad distribution of rate coefficients compared with the distribution of rate coefficients of the metal-FA complexes. EDTA was chosen because it

is an anthropogenic complexant which is much stronger than naturally-occurring complexants, such as humic and fulvic acids, and it forms 1:1 simple, stable and well-characterized complexes with Zn(II), Pb(II), Ni(II), Cu(II), and Cd(II). Since the results were similar for all the methods only the results of Zn-EDTA system are presented in this thesis

RESULTS AND DISCUSSION

Effect of the [Zn(II)]/[EDTA] mole ratio on the distribution of rate coefficients for the dissociation of Zn-EDTA complexes

Figure 18 shows the results of the analysis by the Distribution Analysis Method of the decay curves for the Zn-EDTA complex for the two mole ratios [Zn]/[EDTA] of 0.5 and 1. The mean values of the rate coefficients of the kinetically distinguishable components obtained by the Distribution Analysis Method are presented in Table 29, which shows that on increasing the Zn(II)/EDTA mole ratio, the distribution of the complexes changes. The percentage of the slower dissociating complex increased.

At pH 5, the major EDTA forms having negative charges are: H_3Y^- , H_2Y^{2-} , HY^{3-} , and Y^{4-} . At the [Zn]/[EDTA] mole ratio of 0.5, the EDTA is in large excess and 1:1 Zn-EDTA complexes are formed between Zn and the various major complexing forms of EDTA stated above. Similar Zn-EDTA complexes are formed also at the [Zn]/[EDTA] mole ratio of 1. The three kinetically distinguishable components of the Zn-EDTA complexes observed are probably these Zn-EDTA complexes. At pH 5, at the [Zn]/[EDTA] mole ratio of 0.5 and 1.0, almost all the Zn is complexed by EDTA, leaving

the free aqueous ion, $[\text{Zn}^{2+}]$ concentration $\sim 9.1 \times 10^{-11}$ M, which is negligible, and hence, is not one of the observed (three) kinetically distinguishable components. Comparison of the results obtained with EDTA as complexing agent and fulvic acid (Laurentian) will be discussed later.

Distribution analysis of the rate of uptake of metal ions by Chelex-100 in model systems having no complexing ligands other than aqua ligand.

Figure 19 shows the (exponential) decay curves for the uptake of Pb(II), Ni(II), Zn(II), Cu(II) and Cd(II) by the Chelex-100 resin from aqueous model systems measured by inductively-coupled plasma mass spectrometry. This Figure shows that the rate of uptake of all the metals by the Chelex-100 resin is similar within the experimental error, and more than 98% of all the metals are taken up from the test solution without fulvic acid in 500 s. The rate of removal of metal aqua ion from solution using Chelex-100 and inductively-coupled plasma mass spectrometry is equal within the experimental error for all the metals studied in this thesis. The experimental data of Figure 19 were analyzed by the Distribution Analysis Method and the results are presented in Table 30. The relative standard deviation of the values for the rate coefficients which represent the width of the distribution of the rate coefficients of each metal ion is about 15-25%. This value is not greater than the width observed with the simulated data with 10% added noise. Therefore, as expected, a broad distribution of the rate coefficients was not observed for the removal of the metal ion by Chelex-100.

Figure 18 Distribution of the dissociation rate coefficients of Zn-EDTA complexes; a) $[Zn(II)]/[EDTA]$ mole ratio 0.5; b), $[Zn(II)]/[EDTA]$ mole ratio 1; $pH = 5.5 \pm 0.1$; $I = 1 \times 10^{-8} M$.

Table 29 Distribution analysis of the dissociation rate coefficient of the Zn(II)-EDTA complexes

$[\text{Zn(II)}]/[\text{EDTA}]$ mole ratio	%C ₁	$k_1 \times 10^2, \text{s}^{-1}$	% C ₂	$k_2 \times 10^3, \text{s}^{-1}$	% C ₃	$k_3 \times 10^4, \text{s}^{-1}$
Zn only	97 ± 3	2.9 ± 0.8	----	----	----	----
0.5	24 ± 1	6 ± 1	72 ± 1	7 ± 2	4 ± 0.3	14 ± 2
1	4 ± 0.2	5.3 ± 0.7	91 ± 1	8 ± 3	4 ± 0.3	16 ± 4

----- Not recovered

Figure 19

Uptake of metal ions by Chelex-100 100 measured by inductively-coupled plasma mass spectrometry at pH 5.0 ± 0.2 ; $I = 4.2 \times 10^{-5}$ M; $\circ$, Pb (II); ∇ , Ni(II); $\diamond$, Zn(II); $\square$, Cu(II); Δ , Cd(II).

Effect of the [metal]/[fulvic acid] mole ratio on the distribution of rate coefficients for the dissociation of metal-fulvic acid complexes

Zinc

Figure 20 shows the distribution of the dissociation rate coefficients obtained from the decay curves for Zn(II) obtained by the Distribution Analysis Method in the absence and in the presence of the Laurentian fulvic acid. Figure 20a shows the distribution of rate coefficients for uptake of Zn(II) ions by Chelex-100 in an aqueous solution without the fulvic acid. Only one sharp peak corresponding probably to Zn(II) aqua complex was obtained; the width of the peak, which represents the width of the distribution of the rate coefficients, is not greater than the width observed with the simulated data with added 10% noise. Figure 20b shows the effect of the [Zn(II)]/[FA] mole ratio on the distribution of the rate coefficients of Zn-FA complexes at two mole ratios. In these experiments, the concentration of zinc was kept constant and the concentration of the fulvic acid was varied. Figure 20b, (triangles) shows the distribution rate coefficients of Zn-FA complexes obtained at the [Zn(II)]/[FA] mole ratio 3.5 ($[\text{Zn(II)}] 2.1 \times 10^{-6} / [\text{FA } 0.61 \times 10^{-6} \text{ M}] = 3.5$). At this mole ratio, only one broad peak was obtained with dissociation rate coefficient of $k_1 = 9.5 \times 10^{-3} \text{ s}^{-1}$. The distribution observed in the dissociation rate coefficients of Zn-FA complexes is broader (57%) than the distribution observed in the dissociation rate coefficients of Zn-EDTA complexes, (~35%), and much higher than that obtained with the simulated data (~13%) with the signal to noise ratio of 1000. When the mole ratio of [Zn(II)]/[FA] was decreased from

3.5 to 0.8 at the same ionic strength of 4.2×10^{-5} M (Fig. 20b, open squares), two kinetically distinguishable components were resolved with dissociation rate coefficients: $k_1 = 3.0 \times 10^{-2} \text{ s}^{-1}$, $k_2 = 4.5 \times 10^{-3} \text{ s}^{-1}$. Figure 20b also shows that with decreasing [Zn(II)]/[FA] mole ratios, the broad peak is now resolved and a complex with a slower rate of dissociation has appeared. The results obtained are presented in Table 30.

Lead

Figure 21 shows the distribution of the dissociation rate coefficients obtained from the decay curves for Pb(II) obtained by the Distribution Analysis Method in the absence and in the presence of the fulvic acid. Figure 21a shows the distribution of rate coefficients for uptake of aqueous Pb(II) ions by Chelex-100 in an aqueous solution without the fulvic acid. Although two peaks were observed, the small peak can probably be ignored because it represents < 2% of the total area. The large peak (98%, $k = 2.0 \times 10^{-2} \text{ s}^{-1}$) corresponds to the Pb(II) aqua complex. The width of the distribution of the rate coefficients of is not greater than the width observed with the simulated data with added 10% noise.

Figure 21b shows the effect of the [Pb(II)]/[FA] mole ratio on the distribution of the rate coefficients of Pb-FA complexes at mole ratios 3.5 and 0.8. In these experiments, the concentration of Pb(II) was kept constant ($[\text{Pb(II)}] = 2.1 \times 10^{-6} \text{ M}$), and the concentration of fulvic acid was varied. Figure 21b, (triangles) shows the distribution rate coefficients of the Pb-FA complexes obtained at the [Pb(II)]/[FA] mole ratio 3.5 ($[\text{Pb(II)}] = 2.1 \times 10^{-6} \text{ M}$)/[FA $0.61 \times 10^{-6} \text{ M}$] = 3.5).

Figure 20 (a) Distribution of the rate coefficients for uptake of Zn(II) aqua ions by Chelex-100 from a solution without the Laurentian FA; (b) Effect of the mole ratio on the distribution of the dissociation rate coefficients of Zn-FA complexes in model systems containing the Laurentian FA; Δ , $[Zn(II)]/[FA] = 3.5$; $\square$, $[Zn(II)]/[FA] = 0.8$; pH 5.0 ± 0.2 ; $I = 4.2 \times 10^{-5}$ M.

Table 30 Distribution analysis of the dissociation of metal-FA complexes in model systems at two mole ratios for various metals. pH = 5.0 ± 0.2 ; $I = 4.2 \times 10^{-5}$ M. $[M(II)] = 2.1 \times 10^{-6}$ M. Laurentian fulvic acid.

[FA], M	[M(II)]/[FA]	%C ₁	k ₁ x 10 ² , s ⁻¹	%C ₂	k ₂ x 10 ³ , s ⁻¹	%C ₃	k ₃ x 10 ⁴ , s ⁻¹
Pb(II)-FA complexes							
zero	----	98	2.0 ± 0.1	----	----	----	----
0.6 x 10 ⁻⁶	3.5	27	3.9 ± 1.8	73	1.7 ± 1.6	----	----
2.6 x 10 ⁻⁶	0.8	21	7.4 ± 1.8	27	16 ± 4.6	52	15 ± 5.5
Ni(II)-FA complexes							
zero	----	100	3.9 ± 0.9	----	----	----	----
0.6 x 10 ⁻⁶	3.5	96	1.8 ± 0.5	4	0.25 ± 0.1	----	----
2.6 x 10 ⁻⁶	0.8	97	1.2 ± 0.5	----	----	3	3.3 ± 0.6
Zn(II)-FA complexes							
zero	----	100	2.2 ± 0.2	----	----	----	----
0.6 x 10 ⁻⁶	3.5	100	9.5 ± 1.6	----	----	----	----
2.6 x 10 ⁻⁶	0.8	62	3.0 ± 1.3	38	4.5 ± 2.5	----	----
Cu(II)-FA complexes							
zero	----	100	2.2 ± 0.1	----	----	----	----
0.6 x 10 ⁻⁶	3.5	25	15 ± 4.2	75	14 ± 4.6	----	----
2.6 x 10 ⁻⁶	0.8	----	----	----	----	98	3.9 ± 1.2
Cd(II)-FA complexes							
zero	----	100	3.2 ± 0.4	----	----	----	----
0.6 x 10 ⁻⁶	3.5	100	1.1 ± 1.6	----	----	----	----
2.6 x 10 ⁻⁶	0.8	59	3.6 ± 1.7	41	5.1 ± 2.9	----	----

The rate coefficients are the mean rate coefficient for each peak and the value after ± signs is the standard deviation which represents the width of the distribution obtained.

Two peaks (components) were obtained by the Distribution Analysis Method. The two peaks were partially separated. It has been shown in Chapter 7 using simulated data that the dissociation rate coefficients should differ at least by a factor of 5 and the kinetically distinguishable components should be present in equal amounts to be well separated by this method. In this experiment, the dissociation rate coefficients obtained were different by one order of magnitude. However, the separation was not complete, probably because the concentrations of the two components were not equal (Table 30). Furthermore, the distribution of the rate coefficients was considerably broader than that in the simulated data. The value of the dissociation rate coefficient of the faster, kinetically distinguishable component was of the same order of magnitude as that of the dissociation rate coefficient for uptake of the free metal ions. This apparent broad distribution may be due to Pb-FA complexes that have dissociation rates similar to the rate of uptake by Chelex-100. The second peak is also broad and shows that the Pb-FA complexes have a distribution of dissociation rate coefficients.

When the concentration of the fulvic acid was increased to 2.4×10^{-6} M, with consequent decrease of the mole ratio of $[\text{Pb(II)}]/[\text{FA}]$ from 3.5 to 0.8 at the same ionic strength of 4.2×10^{-5} M (Figure 21b, open squares), three kinetically distinguishable components were resolved with dissociation rate coefficients with values of $k_1 = 7.4 \times 10^{-2} \text{ s}^{-1}$, $k_2 = 1.6 \times 10^{-2} \text{ s}^{-1}$ and $k_3 = 1.5 \times 10^{-3} \text{ s}^{-1}$. Figure 21b shows that when the $[\text{Pb(II)}]/[\text{FA}]$ mole ratio was decreased, the peaks were well-separated into two narrower peaks. The rate coefficients of dissociations of the complexes shifted to lower values. It can be concluded that with the decrease of $[\text{Pb(II)}]/[\text{FA}]$ mole ratio, the shape and distribution of the rate coefficients of Pb-FA complexes changes.

Figure 21 (a) Distribution of the rate coefficients for uptake of Pb(II) aqua ions by Chelex-100 from a test solution without the FA; (b) Effect of the mole ratio on the distribution of the dissociation rate coefficients of Pb-FA complexes in model systems containing the Laurentian FA: Δ , $[Pb(II)]/[FA] = 3.5$; $\square$, $[Pb(II)]/[FA] = 0.8$; pH 5.0 ± 0.2 ; $I = 4.2 \times 10^{-5}$ M.

The results from the distribution analysis of Pb-FA complexes are presented in Table 29. The distribution observed in the dissociation rate coefficient of Pb-FA complexes was broader than the distribution obtained with the simulated data with 10% noise and the distribution obtained for the Pb-aqua complexes.

Nickel

Figure 22 shows the distribution of the dissociation rate coefficients obtained from the decay curves for Ni(II) obtained by the Distribution Analysis Method in the absence and in the presence of the fulvic acid. Figure 22a shows the distribution of rate coefficients for uptake of aqueous Ni(II) ions by Chelex-100 in an aqueous solution without the fulvic acid. Only one sharp peak corresponding probably to Ni(II) aqua complex was obtained; the width of the peak which represents the width of the distribution of the rate coefficients was not greater than the width observed with the simulated data with added 10% noise. Figure 22b shows the effect of the [Ni(II)/[FA] mole ratio on the distribution of the rate coefficients of Ni-FA complexes at two mole ratios.

The [Ni(II)]/[FA] mole ratios were 3.5 and 0.8. In these experiments, the concentration of Ni(II) was kept constant ($[Ni(II) \ 2.1 \times 10^{-6} \text{ M}]$), and the concentration of the fulvic acid was varied. Figure 20b, (triangles) shows the distribution rate coefficients of Ni-FA complexes obtained at the [Ni(II)]/[FA] mole ratio 3.5 ($[Ni(II) \ 2.1 \times 10^{-6} \text{ M}]/[FA \ 0.61 \times 10^{-6} \text{ M}] = 3.5$). A large component obtained from the Distribution Analysis Method had a rate coefficient of $k_1 = 1.1 \times 10^{-2} \text{ s}^{-1}$, indicating that most of the Ni(II) ion formed very weak complexes with the FA, probably as a result of the presence of Pb(II), Cu(II) and Zn(II) metal ions in the test solutions.

Figure 22 (a) Distribution of the rate coefficients for uptake of Ni(II) aqua ions by Chelex-100 from the model system without the FA; (b) Effect of the mole ratio on the distribution of the dissociation rate coefficients of Ni-FA complexes in the model systems containing the Laurentian FA: Δ , $[Ni(II)]/[FA] = 3.5$; $\square$, $[Ni(II)]/[FA] = 0.8$; pH 5.0 ± 0.2 ; $I = 4.2 \times 10^{-5}$ M.

A small component slowly dissociating with a rate coefficient of about $k_2 = 0.52 \times 10^{-3} \text{ s}^{-1}$ was observed. The results of the Distribution Analysis are shown in Table 30. When the concentration of the fulvic acid was increased to $2.4 \times 10^{-6} \text{ M}$ with the consequent decrease of the mole ratio of $[\text{Ni(II)}] / [\text{FA}]$ from 3.5 to 0.8 at the same ionic strength of $4.2 \times 10^{-5} \text{ M}$ (Fig.20b, open squares), the slow component was still very minor, but had shifted to lower values. The rate of dissociation of the strongly bound nickel complexes thus follows the trend clearly observed for the other metals.

Copper

Figure 23 shows the distribution of the dissociation rate coefficients obtained from the decay curves for Cu(II) obtained by the Distribution Analysis Method in the absence and in the presence of the fulvic acid. Figure 23a shows the distribution of rate coefficients for uptake of aqueous Cu(II) ions by Chelex-100 in an aqueous solution without the fulvic acid. Figure 23b shows the effect of the $[\text{Cu(II)}]/[\text{FA}]$ mole ratio on the distribution of the rate coefficients of Cu-FA complexes at two mole ratios. In these experiments, the concentration of copper was kept constant and the concentration of the fulvic acid was increased. Figure 23b, (triangles) shows the distribution rate coefficients of Cu-FA complexes obtained at the $[\text{Cu(II)}]/[\text{FA}]$ mole ratio 3.5 ($[\text{Cu(II)}] 2.1 \times 10^{-6} / [\text{FA}] 0.61 \times 10^{-6} \text{ M}] = 3.5$). At this mole ratio, two kinetically distinguishable components were resolved (two peaks; Figure 23b (triangles)). When the mole ratio was decreased from 3.5 to 0.8, the later ratio being obtained by $[\text{Cu(II)}] 2.2 \times 10^{-6} \text{ M} / [\text{FA}] 2.4 \times 10^{-6} \text{ M}]$, at the same ionic strength of $4.5 \times 10^{-5} \text{ M}$ (Figure 23b open squares), only one slow kinetically distinguishable component was resolved with a dissociation rate coefficient of $k_1 = 4.9 \times 10^{-4} \text{ s}^{-1}$.

Figure 23 (a) Distribution of the rate coefficients for uptake of Cu(II) aqua ions by Chelex-100 from the model system without the FA; (b) Effect of the mole ratio on the distribution of the dissociation rate coefficients of Cu-FA complexes in the model systems containing the Laurentian FA: Δ , $[Cu(II)]/[FA] = 3.5$; $\square$, $[Cu(II)]/[FA] = 0.8$; pH 5.0 ± 0.2 ; $I = 4.2 \times 10^{-5}$ M.

Cadmium

Figure 24 presents the distribution of the dissociation rate coefficients obtained from the decay curves for Cd(II) obtained by the Distribution Analysis Method in the absence and in the presence of fulvic acid. Figure 24a shows the distribution of rate coefficients for uptake of aqueous Cd(II) ions by Chelex-100 in an aqueous solution without the fulvic acid. Only one sharp peak corresponding probably to Cd(II) aqua complex was obtained. Figure 24b shows the effect of the [Cd(II)]/[FA] mole ratio on the distribution of the rate coefficients of Cd-FA complexes at two mole ratios. In these experiments, the concentration of Cd(II) was kept constant and the concentration of fulvic acid was increased. Figure 24b, (triangles) shows the distribution rate coefficients of Cd-FA complexes obtained at the [Cd(II)]/[FA] mole ratio of $[Cd(II) 2.1 \times 10^{-6}] / [FA 0.61 \times 10^{-6} M] = 3.5$. At this mole ratio (Figure 24b, triangles) only one broad peak was obtained; however, the mean rate coefficient of the distribution $k_1 = 1.1 \times 10^{-2} s^{-1}$ was close to the rate of removal of Cd(II) aqua ions by the Chelex-100. Figure 24b shows that when the concentration of fulvic acid was increased to $2.4 \times 10^{-6} M$ with the consequent decrease of the mole ratio of [Cd(II)]/[FA] from 3.5 to 0.8 at the same ionic strength $4.2 \times 10^{-5} M$, two kinetically distinguishable components were resolved with dissociation rate coefficients with values of $k_1 = 3.6 \times 10^{-2} s^{-1}$, $k_2 = 5.1 \times 10^{-3} s^{-1}$. It can be concluded that decreasing the [Cd(II)]/[FA] mole ratio has had the effect of forming more Cd-FA complexes with slower dissociation rate coefficients. The broad peak obtained with the mole ratio of 3.5 was separated into two peaks. The results are presented in Table 30.

Figure 24 (a) Distribution of the rate coefficients for uptake of Cd(II) aqua ions by Chelex-100 from a solution without the FA; (b) Effect of the mole ratio on the distribution of the dissociation rate coefficients of Cd-FA complexes in the model systems containing the Laurentian FA: Δ , $[Cd(II)]/[FA] = 3.5$; $\square$, $[Cd(II)]/[FA] = 0.8$; pH 5.0 ± 0.2 ; $I = 4.2 \times 10^{-5}$ M.

It appears that the dissociation rate of the slow component for the mole of ratio $[\text{Cu(II)}]/[\text{FA}] = 3.5$ was reduced when the mole ratio was reduced to 0.8. The results are presented in Table 30.

Analysis of the experimental data by the Distribution analysis Method shows that metal-FA complexes have a broad range of dissociation rate coefficients rather than a single value. The distribution of the rate constants for the dissociation for the FA complexes was for all the metals broader than the distribution obtained with the aqua ligand complexes or metal-EDTA complexes. Furthermore, the distribution of the rate coefficients the metal-FA complexes were changed by changing the $[\text{metal}]/[\text{FA}]$ mole ratio. These results illustrate the heterogeneity binding properties of FA. Based on the results of Chapter 7 it should be noted that data collected at a low level of precision may give a broad distribution of rate coefficients. The width of the peaks might be due either to the noise in the experimental data or to the heterogeneity of the complexing ligand. Therefore, to study the distribution of the rate coefficients, the experimental data should be collected at high level of precision. In order to ascertain that the recovered distribution shapes have chemical significance, a simulated decay with the same mean dissociation rate coefficient and level of noise should be generated. The distribution of the rate coefficients was not the same for all the metals. This may due to the different characteristics of the metals.

CONCLUSIONS

This thesis has examined the complexation between Ni(II), Cu(II), Co(II), Pb(II) and Zn(II) and two well-characterized fulvic acids in model systems and in freshwaters. Although the model systems are simple in comparison to the freshwater samples, it is now possible to categorize the role of fulvic acid and similar natural complexing ligands with more confidence and to understand their significance in controlling the distribution of metal ions in freshwaters. Throughout this thesis, the philosophy of approach to the fulvic acid is that it is characterized in terms of binding sites or functional groups rather than structure.

For Armadale fulvic acid (FA), the effect of the variation in the mole ratio of the $[\text{metal}]/[\text{FA}]$ on the dissociation rate coefficients of Ni(II)-FA, Cu(II)-FA, Co(II)-FA, Pb(II)-FA complexes is that the dissociation rate coefficients are distributed into two or three groups ranging from labile to inert. This trend is common for all the metals studied. The reported partitioning into two to four kinetically distinguishable components does appear to reflect definite features of the chemistry of fulvic acid systems. This does not mean that the two to four rate coefficients given refer to specific metal complexes. There may still be some diversity of the individual binding sites, but they group into recognizable sets that may be adequately approximated by a small sets of stable rate coefficients. This is consistent with the presence of two distinct classes of binding sites in the fulvic acid. The dissociation rate coefficients of Ni(II)-FA, Cu(II)-FA, Co(II)-FA, Pb(II)-FA increase considerably as the $[\text{metal}]/[\text{FA}]$ mole ratio is increased. This suggests that at low $[\text{metal}]/[\text{FA}]$ mole ratio, the strong binding sites become saturated first, and

after all the strong binding sites are completely filled, the excess metal binds to the weaker sites, forming weak complexes which are labile. The results have demonstrated how kinetic data can detect the existence of strong and weak binding sites in well-characterized fulvic acids and the progressive occupation of strong and weak binding sites of fulvic acids.

The Kinetic Model and the Competing Ligand Exchange Method employed in the Kinetic Model have yielded results that show that the lability of the investigated metal complexes agree with the trend shown by the Ligand-field stabilization energy ($\text{Co(II)} d^7 > \text{Ni(II)} d^8 > \text{Cu(II)} d^9 < \text{Zn(II)} d^{10}$). Since the Kinetic Model is based on the dissociation of the metal complex ML as the rate-determining step, the disjunctive pathway probably predominates in the dissociation of the metal complexes investigated in the fulvic acid model systems and in the freshwater samples presented earlier. The significance of this work is that the Kinetic Model and the Competing Ligand Exchange Method presented in this thesis can correctly diagnose the relative labilities of metal complexes in the freshwater environment.

The increasing order of the lability for the metal-complexes in three different type of water samples: 1) model solutions containing a well-characterized fulvic acid, 2) Rideau River freshwater samples and 3) freshwater samples collected from Sudbury mines area, are as follows: $\text{Co(II)} > \text{Zn(II)} > \text{Ni(II)} > \text{Cu(II)}$. This order arises in part from a decrease in size across the series and in part from ligand field stabilization energies. The relevance of these results is that an explicit quantitative structure activity relationship (QSAR) between the electronic configuration of the 3d transition metal ions and the kinetic lability of the metal-FA and/or the metal-DOC complexes of the 3d

transition metals has been found. The practical importance of this finding is that it is possible to extrapolate results from laboratory experiments (model systems) to field conditions (freshwaters).

The results using simulated data show the importance of high precision in the collection of data. Analysis of the experimental data by the Distribution Analysis Method shows that metal-FA complexes have a broad range of dissociation rate coefficients rather than a single value. The rate coefficients of metal-FA complexes have a broader distribution than that of the metal-EDTA complexes and zinc-aqua ion. The distribution of the rate coefficient changes with $[\text{metal}]/[\text{FA}]$ mole ratio.

The environmental significance of this work for the freshwater environment is that even at relatively low Ni(II) loadings, the metals usually associated with nickel in nickel ore, such as Cu(II) and Co(II), can successfully compete with the Ni(II) for the relatively few strong binding sites of humic substances. The result is that Ni(II) binds to more numerous, weak binding sites, forming labile nickel complexes, with the consequence that Ni(II) exists as free nickel ion (Ni(II)-aqua complex) which is reported to be toxic. This situation is further exacerbated by: 1) the co-presence of other metals that have a high water-exchange rate coefficient such as Pb(II) which form strong complexes with humic substances in freshwaters; 2) when Ni(II) is present in a freshwater environment deficient in DOC.

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