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COMPETITIVE BINDING OF METAL IONS BY HUMIC SUBSTANCES IN MODEL SYSTEMS AND IN FRESHWATERS

by

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

**Department of Chemistry
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October, 2001**

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The undersigned hereby recommended to the Faculty of Graduate Studies and Research
acceptance of the thesis,

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in Model Systems and in Freshwaters”**

submitted by

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ABSTRACT

Metal complexation reactions involving dissolved organic carbon (DOC) in freshwaters play a key role in regulating trace metal speciation, mobility, bioavailability and toxicity. In freshwaters having multimetals and multiligands milieu, many different metal ions are in competition with the same complexant, and many different complexants are in competition with the same metal ion. The above competitions determine the speciation of trace metals and their bioavailability.

Competitive binding of Cu(II), Co(II) and Ni(II) by humic substances in model systems and in freshwaters from the Rideau River surface area was investigated employing the Competing Ligand Exchange Method (CLEM). CLEM is a kinetic speciation method based on ligand-exchange kinetics. The CLEM was applied using a cation-exchange resin, Chelex-100, as the competing ligand, and Graphite Furnace Atomic Absorption Spectrometry for measurement of the rate of free Ni^{2+} ion release from the dissociation of Ni(II) complexes. It was found that even at relatively low Ni(II) loadings, Cu(II) and Co(II), which were usually associated with nickel in nickel ores, successfully competed with Ni(II) for binding sites of humic substances, and released bound Ni(II) as free Ni^{2+} ions (toxic).

The competition of major cations, Ca^{2+} and Mg^{2+} , with the target trace metal, Ni(II), in model systems and in nickel-polluted natural waters from the Sudbury area (Canada) was also investigated using the same analytical technique as above. It was found that the release of bound Ni(II) as free Ni^{2+} ions is critically dependent on the competition by the

major cations. Bioassay toxicity tests of the water samples from the Sudbury area showed that the released free Ni^{2+} ion was bioavailable and toxic.

The effect of metal competition was also studied using the equilibrium approach involving measurement of stability constants of metal complexes, which gave a measure of the availability of free metal ions at equilibrium. Square Wave Anodic Stripping Voltammetry (SWASV) at a static mercury drop electrode was employed to determine stability constants of metal complexes.

The environmental significance for freshwaters is that it has demonstrated the importance of competition of trace metals and major cations in release of DOC-bound target trace metals as free metal ions (toxic).

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SECTION 1

THEORETICAL BACKGROUND

CHAPTER 1

INTRODUCTION

“Because the hydrologic cycle powers all others, and because life on earth is so intimately tied to water, aquatic chemistry is a central link between the cycles of elements at the surface of the earth and the workings of biological systems. Life adapts to its environment and modifies it. As noted by Alfred Redfield some 50 years ago, the composition of natural waters reflects both the geochemistry of the planet earth and the biochemical requirements of its inhabitants. These are delicately in tune, constantly modifying each other, linked by a few billion years of “co-evolution,” and both ultimately responding to the same fundamental laws of chemistry” [1].

The above quotation is from PRINCIPLES OF AQUATIC CHEMISTRY by F.M.M. Morel.

This thesis deals with the fundamental laws of chemistry as applied to the freshwater environment that Morel referred to above. Although freshwaters represents a small part of the aquatic environment, it merits a much larger share of importance because it is indispensable for the life of humans and most other life forms. As Morel has noted above [1], the composition of natural waters is controlled by both geochemical and geobiological processes. Freshwaters is a complex mixture of natural water and other substances, both of natural (geogenic) and anthropogenic origin, in addition to being the habitats of myriads of aquatic biota.

In the freshwater environment, trace metals are usually present as mixtures of various chemical species: free metal ion (properly called metal-aqua complex) and various metal complexes of naturally-occurring organic and inorganic complexants. The overall goal of environmental metal speciation studies is to provide the data required for environmental impact assessment of trace metals by determining their chemical speciation, distribution, properties, and fluxes. The impact assessment of trace metals in the natural environment is ultimately concerned with how these metals directly or indirectly affect biota. Some of these trace metal ions, within narrow concentration limits, are essential for life, but when they are present outside these narrow limits, toxicity may result. Human life may be continuously functioning at the thin edge between limitations by essential elements and inhibition by toxic ones. The knowledge of the type and concentration of individual species present in the system is, therefore, essential for assessing the impact of metals on the freshwater environment. The distribution of metal ions in the freshwater environment is extremely complex and is governed by a variety of reactions that include complexation with organic and inorganic ligands, ion exchange, adsorption and desorption processes, precipitation and dissolution of solids, and acid-base equilibria [2].

In order to study the interactions of dissolved organic carbon (DOC) with trace metals in freshwaters to predict their impact on water quality, one needs to determine the chemical speciation of metal-DOC complexes using a multiplicity of speciation techniques to cover the entire range of reactivity of metal complexes and the various characteristics of these metal complexes *vis-a-vis* the analytical techniques used to measure them. Results from these techniques should be compared with metal biouptake by organisms to assess the relevance of speciation measurements to bioavailability, and to evaluate the best

predictive parameters. It is generally assumed that the free ion activity is the best predictor of bioavailability. This work will examine the role of kinetically labile metal complexes.

NATURAL ORGANIC MATTER – DISSOLVED ORGANIC CARBON – HUMIC SUBSTANCES

This thesis is about interactions of metals with the natural organic matter (NOM). The NOM predominantly consists of the dissolved organic carbon (DOC). Humic substances represent typically 40 % to 99 % of the DOC [3]. Humic substances are refractory (i.e. non-biodegradable, resistant to oxidation) polymeric substances of variable composition, and are derived from chemical and biological degradation of plant materials and/or from synthetic activities of microbes.

Humic substances are both chemically and physically heterogeneous and this affects their metal complexation properties. The metal-humate complexes, by their adsorption on suspended sediments (solids) or on colloidal substances, are transported over long distances in the freshwater environment, thereby dispersing metals and lowering their concentrations. Humic substances may also mobilize metals from sediments by solubilizing them and thereby increasing the flux of available metals in freshwaters. Also, humic substances, because of their chemical heterogeneity, exert a buffering action on metal ions, resulting in chemostasis, preventing abrupt, major changes in free-ion activity from occurring when freshwaters receive large inputs of metal-containing effluents [4]. This enables aquatic biota to evolve and adjust to slow changes – the very basis of

homeostasis. Through adsorption on solid, organic complexants (e.g. humic acid), and complex-formation in solutions, multimetals and multiligands in freshwaters constitute a sort of interlocking chemical network, which is a part of the chemostasis [4]. However, presence of other organic complexants in freshwaters makes them less chemostatic [5].

The importance of humic substances arises from their ubiquitous presence in freshwaters and from their strongly chelating properties – the chelates are formed by two COOH groups and/or COOH and OH groups in close proximity in the same humic molecule, or, at high concentrations of the humic substance (on its aggregation) on pairs of humic molecules (forming bridges), binding metal ions after these functional groups have been deprotonated, fully or partly, at appropriate pHs. The metal complexation properties of humic substances will be discussed in detail later. In this study, two well-characterized fulvic acids were used for examination of the metal-fulvic acid interactions in laboratory model systems, and the results of these studies were then extrapolated to freshwaters. The studies of these interactions originated from environmental concern over metal-toxicities in freshwater resources.

ORGANIZATION OF THE THESIS

This thesis is divided into three sections. Section 1 contains 3 Chapters and describes theoretical background. Chapter 1 gives a brief introduction, explaining the driving force behind this work, and gives an outline of the thesis. Chapter 2 presents the topics of chemical speciation and various models for studying the interactions between metal ions and humic substances. Chapter 3 presents the metal complexation properties of humic

substances. Section 2 contains 5 chapters and describes the experimental results of competition of trace metals and major cations with the target trace metal for binding by humic substances in laboratory model systems and in freshwaters using the kinetic approach. Experimental results of dissociation kinetics of metal-fulvic acid (FA) complexes in laboratory model systems are presented and discussed in Chapter 4, which also includes discussion on the importance of [metal]/[FA] mole ratio on the kinetic lability of the metal-FA complexes. Chapter 5 describes the results of the competitive binding of Cu(II), Co(II) and Ni(II) by a well-characterized FA, Armadale FA, in laboratory model systems. Chapter 6 presents the results of the above competition in unpolluted freshwater systems (Rideau River surface waters, Ottawa, Canada). Chapter 7 presents the results of the competition of major cations, Ca(II) and Mg(II), with the target trace metal, Ni(II), for binding by the Armadale FA in laboratory model systems. The effect of metal / ligand (mol / g) ratios, trace metal and major metal competition on the kinetic lability of the Ni(II)-DOC complexes in nickel-polluted natural waters from the Sudbury area (Canada) are presented and discussed in Chapter 8, which attempts to establish a linkage between nickel speciation, bioavailability and toxicity. Section 3 contains 2 Chapters and describes the results of trace metal and major metal competition for binding by humic substances using the equilibrium approach. Chapter 9 describes the effects of competition of trace metals, Cu(II), Ni(II), Zn(II), and major cations, Ca(II) and Mg(II), on the thermodynamic stability of the Pb(II)-FA (Laurentian) complexes. Chapter 10 presents the results of the complexation of Pb(II), Cu(II), Cd(II) and Zn(II) by humic substances in freshwaters. The environmental significance of the results is presented in Chapter 11, Conclusions.

CHAPTER 2

CHEMICAL SPECIATION AND BIOAVAILABILITY

Bioavailability of metals to organisms, and hence, their toxicity to biota strongly depends on the chemical speciation of the metals. There is no generally accepted definition of chemical speciation, and various meanings have been attributed to the term by various workers [6]. In the present context, chemical speciation is defined as the occurrence, distribution and characterization of various chemical species of an element.

The interaction of metals with intracellular compartments is highly dependent on chemical speciation of the metals. When the capacity of a cell to detoxify accumulated metal is exceeded, damage to cytoplasmic constituents will occur. This is a toxic effect. Symptoms of intracellular toxicity include ultrastructural deformities, reductions in cell division rate, respiration, photosynthesis, motility, electron transport activity and ATP production. Since only some metal species can penetrate the biomembrane to cause a toxic effect, metal toxicity to aquatic organisms cannot be predicted by determining the total metal concentration. Studies of toxicity suggest that ionic or uncomplexed metal is the toxic form [7], and complexation with organic materials reduces metal toxicity in the oceans [8], rivers and lakes [9]. Two mechanisms of assimilation of dissolved metal species by microorganisms have been proposed and are illustrated [10] in Figure 1:

Figure 1. Schematic diagram of the assimilation of metals (M) by aquatic organisms. L_1 is the natural ligand, L_2 is the transporter ligand, P is a membrane protein channel, and M_a is a metal ion absorbed on a site that will enable transport through the protein channel (Adapted from Wood [10]).

- (1) direct transfer of the species (usually hydrophobic) through the cellular membrane; and
- (2) dissociation of complexes (often hydrophilic or large species) prior to their uptake by the cellular membrane.

The latter mechanism depends on the availability of the free metal ion (i.e. metal-aqua complex), which in turn depends on chemical potential gradients between the external medium, the membrane surface, the intracellular medium, and the nature of the natural organic complexants present in the environment. For these complexes, free metal ion activity has been hypothesized to be a good predictor of metal bioavailability [11]. These mechanisms indicate that the uptake tendency of the dissolved metal species varies with the type of the species present in the external medium.

SPECIATION PARAMETERS

In the freshwaters and soils, it is often impossible to determine the identity and concentration of metal species, many of which are transient. It is therefore necessary to characterize these metal species by the speciation parameters of their metal complexes. Speciation parameters are quantitative measures of chemical characteristics of chemical species, which are independent of the analytical methods that are used to measure them. It often requires a number of speciation parameters to characterize a system fully. Some important speciation parameters are given below:

(1) Diffusion Coefficient: The mobility of a particular chemical species plays a very important role in the biological impact the element may have in the natural environment. Before an element may elicit a toxic response, it must come into contact with a biological membrane and eventually penetrate it [12]. The diffusion coefficient is a direct measure of how quickly the chemical species can reach the biological membrane.

(2) Stability Constant: The thermodynamic stability of a metal complex (which is a measure of the availability of free metal ions at equilibrium) is a very important property for classifying the metal species in the aquatic and the terrestrial environment [13-15]. The determination of stability constants relies on equilibrium or pseudo-equilibrium condition to be established before the measurement can be made. Although measurement of this parameter is straightforward for homogenous systems (such as inorganic complexes), it may be difficult to measure this parameter for heterogeneous systems such as those containing naturally-occurring organic complexants. Owing to the different types of functional groups in humic substances, which are ubiquitous in the natural environment, humic substances are heterogeneous complexants. This chemical heterogeneity is a strong characteristic which determines the metal ion binding, and hence, the stability constants of metal complexes of organic complexants. In such cases, instead of discrete values for stability constants, a large spectrum of stability constants is often observed and the interpretation becomes uncertain. Often an average stability constant is estimated for a group of metal complexes having closely spaced values of stability constants.

(3) Dissociation Rate Coefficients: The dissociation rate coefficients represents how rapidly a metal complex dissociates to form free metal ion (i.e. the metal-aqua complex),

and hence, is a very important speciation parameter, especially in the case where free metal ion is the bioavailable species. Under the Free-Ion Activity Model [FIAM, 11], free ion activity is often a good predictor of the metal's bioavailability and hence, its nutrient or toxic effect.

In the present study, dissociation rate coefficients and stability constants were experimentally determined to characterize metal species in model systems and in freshwaters.

APPROACHES FOR STUDYING CHEMICAL SPECIATION

There are two approaches for studying chemical speciation: equilibrium and kinetic. 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 [16-19]. Modelling studies have also been done using the equilibrium approach [20]. Freshwater systems, however, are often far removed from equilibrium and require kinetic approaches to determine their chemical speciation [18]. A quantitative description of chemical speciation of metal ions in freshwaters in terms of various chemical species cannot be considered complete without adequate characterization of the rates of chemical reactions [18,21,22]. Furthermore, many metal complexes in natural waters dissociate slowly, and equilibrium conditions may not be established during the time of the experiment [23]. 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. Slow coordination equilibrium of trace metals in freshwaters may not be attained in analytical time scales, especially the very-short instrumental response time.

The kinetic approach assumes that each species has its own chemical reactivity, which means its own formation and dissociation rate coefficients. Kinetic studies of metal speciation not only can differentiate chemical species according to their kinetic parameters, but also can give information on the distribution of the chemical species in the system at any time during the kinetic process. The results obtained can, therefore, be used to estimate the bioavailability of metal if a kinetic model can be constructed to represent the process of biological uptake. For non-equilibrium systems, kinetic speciation would give a more realistic description of bioavailability of trace metals than metal speciation based on local equilibrium approximations. Freshwaters are generally non-equilibrium systems, in which slow coordination equilibrium results in non-equilibrium distributions of metal species even though some other reactions may reach equilibrium rapidly, providing partial or local equilibrium.

The importance of kinetic studies is being increasingly realized and in the last few years many studies have been reported. However, there is little information on the kinetics of reactions of metal complexes in freshwaters. This is mainly due to the complexity of naturally-occurring organic complexants in freshwaters. 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. Among the kinetic studies reported is the dissociation of Fe(II)-natural organic matter (NOM) complexes in

lake waters without previous NOM fractionation [24]. Different data interpretation models have been reported in the literature: simple dissociation constants [25], discrete multicomponent models [24,26-28], discrete kinetic spectra [29], continuous kinetic spectra [30], and log normal distribution [29,31]. Hering and Morel [32] have studied the rates of ligand exchange reactions of copper-nitrilotriacetic acid complexes and copper-humate complexes. In their work, they have found that the rate coefficients of ligand exchange were pH-dependent, and depend not only on the properties of the humic substances but also on the experimental conditions.

KINETICS OF COMPLEX FORMATION AND DISSOCIATION [33]

In the Eigen mechanism for complex formation, formation of an outer-sphere complex between a metal and a ligand is followed by rate-limiting loss of water from the inner coordination sphere of the metal [34, 35]. Thus,

First-order rate of loss of coordinated water from $M(H_2O)_6$,

$$\text{Rate} = -\frac{d[M(H_2O)_6, L]^{(2-n)+}}{dt} = k_{-w} [M(H_2O)_6, L]^{(2-n)+} \quad (3)$$

$$K_{OS} = \frac{[M(H_2O)_6, L]^{(2-n)+}}{[M(H_2O)_6]^{2+} [L^{n-}]} \quad (4)$$

$$[M(H_2O)_6L]^{(2-n)+} = K_{OS} [M(H_2O)_6^{2+}] [L^{n-}] \quad (5)$$

Substituting eq. (5) in eq. (3),

$$\text{Rate} = - \frac{d[M(H_2O)_6L^{(2-n)+}]}{dt} = k_{-w} K_{OS} [M(H_2O)_6^{2+}] [L^{n-}] = k_f [M(H_2O)_6^{2+}] [L^{n-}]$$

where the formation rate coefficient $k_f = K_{OS} k_{-w}$, as shown below.

By the Principle of Microscopic Reversibility [36],

$$K_{OS} = \frac{k_f}{k_d}$$

$$k_f = k_d K_{OS}$$

but $k_d = k_{-w}$, therefore, $k_f = K_{OS} k_{-w}$.

The stability constant for the outer-sphere complex, K_{OS} , is primarily dependent on the charges of the reacting species and the ionic strength of the medium. Hence, K_{OS} is the equilibrium constant for an ion-pair complex-formation in which only electrostatic bonding operates. The complex-formation resulting from ligand exchange between the leaving ligand, coordinated water molecule, and the in-coming ligand (say, fulvate or humate ion), is, however, mainly covalent bonding.

The rate coefficient for water-loss, k_{-w} , is characteristic of the reacting metal. For many metals, the magnitude of k_{-w} can be explained by the electrostatic interaction between the metal cation and the coordinated water molecules, which is related to the charge and, inversely, to the size of the metal cation. Water loss from the ligand is considered to be fast relative to metal water loss and to precede formation of the outer-sphere complex [37].

The above mechanism for complex formation also holds for the reaction of metals with multidentate ligands (chelate-formation) if the rate-limiting step for complexation is the formation of first metal-ligand bond. The effects of ligand structure, other than charge, are difficult to generalize. Ligand protonation decreases the rate of complex formation often more than can be accounted for based on the change in ligand charge.

KINETIC SPECIATION BY LIGAND EXCHANGE KINETICS

The Competing Ligand Exchange Method (CLEM) is a kinetic speciation method based on ligand exchange kinetics and can employ Chelex-100 or other cation-exchange resins to measure the kinetics of metal-complex dissociation in aqueous samples including freshwaters [38]. CLEM is used to study dissociation kinetics of metal complexes with heterogeneous, organic complexants such as humic substances. Typically, a spectrophotometrically determinable ligand is used (e.g. 4-(2-pyridylazo)-resorcinol (PAR) for Ni(II) [23,26] and Cu(II) [29,31]; 2,4,6-tri(2-pyridyl)-s-triazine for Fe(III) [24]). Fluorescence spectrometric methods have also been applied by several workers [27,39,40]. Olson et al. [30,41] studied the dissociation of copper complexes of estuarine humic substances. They used PAR as the competing ligand and monitored the dissociation reaction by measuring the concentration of the metal-PAR complex spectrophotometrically. Lavigne et al. [26] also used PAR in their study of the dissociation of nickel complexes of a well-characterized soil fulvic acid. Chakrabarti et al. adapted the kinetic model proposed by Olson and Shuman [41], and developed it further [42,43] to study the dissociation kinetics of metal complexes. In the present study, metal species concentrations and species dissociation rate coefficients were determined

by using the Competing Ligand Exchange Method [42,43], and this method is described in Chapter 4.

Kinetic heterogeneity is an important factor in determining bioavailability [18]. However, interpretation of metal complexation by naturally-occurring, heterogeneous, organic complexants, and assessment of their relationship to biogeochemical processes require combined equilibrium and kinetic approaches [18,44,45]. Since the nature of the complexing site changes with the metal to ligand mole ratio, in heterogeneous, organic complexants, site heterogeneity influences both the equilibrium and the kinetic properties of the metal complexes [28,46]. Thus, in the present study, metal complexation by humic substances was studied using both the kinetic and the equilibrium approaches.

CHAPTER 3

METAL COMPLEXATION BY HUMIC SUBSTANCES

Complexation reactions of metals with natural organic matter (NOM) play a key role in regulating trace metal speciation, bioavailability and toxicity to microorganisms. It also regulates metal ion concentrations in freshwaters and soils, and their transport from one environment to another [47,48]. The nature of the NOM found in aquatic systems depends primarily on whether the sources are aquagenic, i.e. derived from water, or pedogenic, i.e. derived from soil [49]. Organic compounds in aquatic and terrestrial environments that form complexes with metal ions may be grouped into three main classes: (1) organic substances of known molecular structure and chemical properties including biochemicals such as simple aliphatic acids, polysaccharides, amino acids, and polyphenols; (2) xenobiotic organic chemicals deriving from human, agricultural, industrial, and urban activities; and (3) humic substances.

Humic substances (HS) represent a significant proportion of total organic carbon in the global carbon cycle, constituting the major organic fraction in soils (between 70 and 80%), and the largest fraction of natural organic matter in stream, river, wetland, lake, sea, and ground waters (40 to 99% of dissolved organic carbon) [3].

Humic substances can be fractioned on the basis of their solubility characteristics: (i) fulvic acid (FA) is the low molecular weight fraction of humic substances and is soluble

in alkaline, acidic solutions and in water; (ii) humic acid (HA) is the fraction that is soluble in dilute alkaline solution, but is precipitated in acidic solution and in water; and (iii) humin is the fraction that is not soluble in alkaline or acid solutions [50,51].

MOLECULAR STRUCTURES AND PROPERTIES OF HUMIC SUBSTANCES

A comprehensive review of the different techniques that can be used to characterize the humic material can be found in literature [52]. Elemental analysis is the most commonly used procedure to characterize humic substances, but it does not provide an absolute molecular formula; however, it does provide general compositional information. Humic substances are complex mixtures of macromolecules, with a composition that changes in time and space. They consist of carbon, oxygen, hydrogen and sometimes small amounts of nitrogen and occasionally phosphorus and sulphur. On a weight basis C and O are the most important elements. For HA, the contribution of C, S and N elements is larger, the content of H is about equal and the content of O is smaller than that of FA.

Since HS consist of a heterogeneous mixture of compounds, no single structural formula is valid, and it is virtually impossible to describe uniquely the molecular configuration of HA and FA. Numerous tentative models have been proposed [53-55]. A tentative structure of HA is shown in Figure 2 [56]. For most FA the molecular weight ranges from 500-5000 g / mol. The molecular weight of HA is larger than that of FA and ranges from 1500 up to $> 10^5$ g / mol [47,51,52,57].

Figure 2. A tentative structure of humic acid [56].

NATURE OF COMPLEXING SITES AND COMPLEXING CAPACITY

One of the important characteristics of humic substances is the type and the content of the functional groups. The functional groups are of great importance since metal ion binding occurs at the functional groups of the humic substances. The functional groups of humic substances can be divided into major and minor sites [4,58-60]. Major site type are those present in large proportions in a given complexant system (~99-90 % of the total sites present). Major sites can be considered to be chemically homogeneous, including only one type of functional group, and are weak Lewis bases. Examples of major sites include the carboxylate and phenolate functional groups of humic and fulvic compounds, the carboxylate groups of polysaccharides and proteins, and the $\equiv \text{Fe-OH}$ surface groups of iron hydroxides. Minor sites are those present in trace amounts (~ 1-10 % of the total sites present), but whose chemical diversity may be very large. They can be considered to be chemically heterogeneous, and are strong Lewis bases. Examples of minor sites are the N and S complexing functional groups of fulvic/humic compounds, the -SH groups of proteins, and the $\equiv \text{Mn-OH}$ groups of iron hydroxides containing Mn impurities.

Carboxyl groups play a prominent role in the binding of metal ions by humic substances. Mixed complexes are probably formed, the most prominent one involving both phenolic and COOH groups i.e. a salicylate-type site [61,62] (eq. 6)

Other possible combinations include two COOH e.g. a phthalic-type site (eq. 7), two phenolic OH, quinone groups, NH₂ groups, sulfhydryl groups, and conjugated ketonic structures [61-64] (eq. 8).

Two main types of complexes may be formed between metal ions and HS: (1) inner-sphere complexes, resulting in the formation of covalent bonds between the ligand atom and the metal ion, both completely or partially dehydrated; and (2) outer-sphere complexes (ion pairs) that result in the electrostatic interaction between the ligand and the metal ion that remains hydrated. For simplicity, all reaction schemes described in eqs. (6-8) show formation of inner-sphere complexes, but they also represent outer-sphere HS complexes with a solvated cation (eq. 9).

Humic substances are heterogeneous complexants. Heterogeneity refers to both physical and chemical properties [47,51,65]. These properties make metal binding by humic substances highly complex and variable depending on experimental conditions such as metal ion concentration, metal / HS ratio, ionic strength and pH.

In general, the chemical and physical heterogeneity of humic substances results from the following four 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, nitrogen and phosphorus are also known.
- 2) **Polydispersity.** Humic substances represent a variety of different molecules with a wide range of molecular sizes.
- 3) **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 [65-68].
- 4) **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 [4,69]. 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 [70]. In all cases, the relative importance of these properties varies with pH, ionic strength and the degree of occupation of the complexing sites.

One of the most striking characteristics of HS is their ability to interact with metal ions to form water-soluble, colloidal, and water-insoluble complexes of varying properties and widely differing chemical and biological stabilities [71,51]. The evidence suggests that almost every aspect of the chemistry of trace metals in soils, waters, and sediments is related in some way to the formation of complexes with HS. The topic is of considerable practical interest because of the continuous and increasing release of various trace metals to environmental systems by numerous human activities.

Four key areas of importance in metal ion binding to humic substances in freshwaters are:

1. **Variable Stoichiometry.** Trace metal ions can be bound to humic substances in various stoichiometries; for instance, by forming monodentate complexes to a single site, or multi-dentate complexes to structure in which several functional groups are coordinated, or by forming chelate complexes with two aromatic rings of the same molecule, or by forming chelate complexes which bridge two hydrodynamically distinct humic ligands.
2. **Chemical and Physical Heterogeneity.** Humic substances are chemically heterogeneous polymers since they contain a variety of functional groups having

different binding affinities. They are physically heterogeneous, polydisperse molecules, and are of various sizes. The reactivity of a functional group depends on its type and its environment. For the bulk of the metal binding the carboxylic and the phenolic OH groups are of importance. At very low concentrations binding mainly occurs at sites which have a high and specific affinity. Among these high affinity sites there are nitrogen, sulphur or phosphorus containing functional groups in humic substances [47,51,72].

3. **Variable Charge and Potential: Electrostatics.** Because of ion binding to humic molecules exhibit a variable electric charge that creates a variable electric field adjacent to the particle, and in turn this electric field influences further cation binding. These electrostatic interactions can be strong and long-ranging. Humic substances develop a pH-dependent negative charge because of the dissociation of functional groups. The negative charge promotes the adsorption of cations in two ways. First of all, the concentration of the cations in the double layer around the humic colloids is larger than in the bulk, due to coulombic interactions. The binding by coulombic interaction is non-specific and depends only on the valency of the ion. The second effect is that a higher concentration of metal ions near the functional groups will result in a larger specific binding than expected on the basis of the concentration in the bulk solution. The electrostatics or variable charge effects do also influence the secondary properties of the humic molecules like their conformation and the aggregation of molecules in larger complexes [47,52,70]. Both conformational changes and aggregation will in turn affect the metal ion binding and vice versa.

4. **Competition.** In aqueous systems, protons (or hydroxyl ions) are by definition present. Because protons largely determine the state of the functional groups and the charge of the humic substances, at least a proton effect on the metal ion binding is to be expected. Additionally in freshwaters having multimetals and multiligands milieu, many different metal ions such as trace metals, Ni(II), Cu(II), Co(II), Cd(II) and Pb(II), and major cations, Ca(II) and Mg(II), are in competition with the same complexants, and many different complexants are in competition with the same metal ion. The above competitions determine the speciation of trace metals and their bioavailability. In considering competition of metal binding in freshwaters, one should consider all metals, H^+ and all ligands present, which form a complicated network of interactions. Because each cation interacts and equilibrates with all ligands, and each ligand interacts and equilibrates with all cations, the concentration of free metal ions and the distribution of both cations and ligands depend on the total concentrations of all the other constituents of the system. The addition of Ni(II), Cu(II), Co(II) (from nickel mining, refining, and metallurgical sources, for example), or of any other metals to freshwater systems produces significant reverberations in the interdependent 'web' of metals and ligands, and may lead to a redistribution of all trace metals [73].

HYPOTHESIS

Within the framework outlined above, the hypothesis to be tested is that in the freshwater environment, release of target trace metal ions bound by polyfunctional, polyelectrolytical, macromolecular, heterogeneous, organic complexants such as humic

substances (which are ubiquitous in the aquatic environment), is determined by competition of other trace metal ions and major cations with the target trace metal ions for the binding sites of the organic complexants. The key hypothesis to be tested is that the polyfunctionality (the existence of a few strong binding sites (~ 1-10 %) and much larger number of weak binding sites (~99-90 %)) of these organic complexants dominate the metal release process.

OBJECTIVES AND HYPOTHESIS TESTING

The overall objective was to test the above hypothesis using three water samples of different sources: (1) laboratory model systems containing two well-characterized fulvic acids, (2) unpolluted freshwater samples from the Rideau River surface area, and (3) metal-polluted freshwaters from the Sudbury area. The specific short-term objectives were (i) to demonstrate that the above competition is responsible for release of bound metals as free metal ions which are reported to be toxic; and (ii) to determine whether major cations, Ca^{2+} and Mg^{2+} , do really outcompete trace metals for the binding sites of humic substances where electrostatic interactions dominate. The long-term objective was to gain a comprehensive understanding of binding of trace metals when trace metals and major cations compete with the target trace metals. The metals studied were: trace metals, Ni(II), Cu(II), Co(II), Cd(II) and Pb(II), and major cations, Ca(II) and Mg(II).

The above hypothesis was tested by making rigorous studies of metal competition for binding sites of humic substances in laboratory model systems and in freshwaters. The results of these investigations are presented in Chapters 4-10. Two approaches were used

in this study. The first one was the kinetic approach that involves the measurement of the dissociation kinetics of metal complexes. The Competing Ligand Exchange Method (CLEM) with Chelex-100 as the competing ligand and Graphite Furnace Atomic Absorption Spectrometry (GFAAS) to measure the rate of dissociation of metal complexes were employed in the estimation of the dissociation rate coefficients. The CLEM is described in Chapter 4. The second one was the equilibrium approach that involves the measurement of average stability constants of metal complexes, which gives a measure of the availability of free metal ions at equilibrium. Square Wave Anodic Stripping Voltammetry (SWASV) at a static mercury drop electrode was employed to determine the average stability constants of the metal complexes.

SECTION 2

EXPERIMENTAL RESULTS OF METAL COMPETITION FOR BINDING BY HUMIC SUBSTANCES USING THE KINETIC APPROACH

CHAPTER 4

EFFECT OF [METAL] / [FULVIC ACID] MOLE RATIOS ON THE KINETIC LABILITY OF METAL COMPLEXES IN MODEL SYSTEMS

INTRODUCTION

The study of metal binding by naturally-occurring organic complexants is of primary importance in understanding their behaviour and fate in aquatic systems [32,44]. However, information on the rates of chemical reactions of heterogeneous organic complexants in freshwater systems is scarce [44].

For heterogeneous complexants such as humic substances, since the nature of the complexing site changes with the metal to ligand ratio, site heterogeneity will influence both the equilibrium and the kinetic properties of the complexes. However, the metal/ligand ratio dependence of kinetics is not usually detected. This is probably due to the fact that either only one metal/ligand mole ratio is studied [29] or the results are not environmentally significant because the ligand and metal concentrations and the mole ratios are far from freshwater conditions [23,27,31]. Complexation experiments are most often conducted at high metal concentrations, which reveal only the characteristics of the more abundant and weaker complexing sites. Unless the complexation experiments are

done at very low metal/ligand mole ratios, the strongest binding sites cannot be identified, and the results may not be environmentally significant. 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 natural waters where the metal concentrations themselves are very low. This emphasizes the need for experimental conditions to be as close as possible to those prevailing in the environment.

Sunda and Hanson for the first time reported the existence of strong sites [74]. It is noteworthy that they used an initial metal concentration of 10^{-8} M that was one order of magnitude lower than most of the other workers. Morel [75] reported that the use of the low metal concentration was probably the reason why these authors found these strong binding sites. Nevertheless, most of the results confirm the existence of a distribution of dissociation rates of fulvic and humic complexes, with dissociation increasing with increasing metal to ligand ratio [23,26,31,32,76].

The specific objective of the study presented in this Chapter is to investigate the progressive occupation of strong and weak binding sites of a well-characterized fulvic acid by selected metals. This study shows how kinetic data lead to differentiation between strong and weak binding sites in FA. The metals selected for the study were Pb(II), Cu(II), Cd(II) Co(II), and Ni(II).

THE KINETIC MODEL

Competing Ligand Exchange Method (CLEM)

Adapting the kinetic model proposed by Olson *et al.* [41], the following kinetic model have been developed further [42,43] to study the dissociation kinetics of complex ML, where M is a metal ion and L is a macromolecular, polyfunctional, multisite, heterogeneous, organic complexant, such as a humic compound.

Consider an aqueous mixture of n components in which each component, designated ML_i , undergoes a first-order or pseudo-first-order reaction simultaneously with all other components

where k_1 and k_{-1} are the rate coefficients for the forward and the backward reaction, respectively. The charges on M and L have been neglected in this general statement of the reaction.

In CLEM with solid Chelex-100 cation-exchange resin, the latter reacts with M as follows:

The model assumes that

- (a) reaction (11) is much faster than reaction (10),
- (b) M-Chelex complex is thermodynamically much stronger than ML complex,

(c) $C_{\text{Chelex}} \gg C_M$, and

(d) Chelex does not bind with the ML.

Because $[\text{Chelex}] \gg [M]$, reaction (11) is pseudo-first-order. Since k_2 is large, as has been determined by inductively-coupled plasma mass spectrometry [77,78], and Chelex-100 is in sufficiently large excess, the condition $k_2 [\text{Chelex}] \gg k_{-1}[L]$ is valid. Hence, the overall reaction (12) is irreversible.

The rate of formation of the M-Chelex complex is hence determined by the rate of dissociation of ML_i in reaction (10), and the rate expression is simply as follows:

$$\frac{d[\text{M} - \text{Chelex}]}{dt} = - \frac{d[\text{ML}_i]}{dt} = k_1 [\text{ML}_i] \quad (13)$$

If each complex, ML_i , undergoes, independently and simultaneously, a first-order or pseudo-first-order dissociation reaction, the sum of the concentrations of all components remaining in the solution at time t , $C_{\text{ML}}(t)$, decreases exponentially as:

$$C_{\text{ML}}(t) = \sum_{i=1}^n C_i^0 \exp(-k_i t) \quad (14)$$

where C_i^0 is the initial concentration of ML_i , the i th component. The concentration of the metal complexes remaining in the test solution, $C_{\text{ML}}(t)$, is measured as a function of time. The time taken for completion of reaction (11), i.e. its rate will establish the theoretical upper limit of the dissociation reaction that can be observed with this technique.

EXPERIMENTAL

Reagents and materials

Stock solutions (1000 $\mu\text{g/mL}$) of Ni(II), Co(II) and Pb(II) were prepared separately for each metal by dissolving an appropriate quantity of nickel metal powder (SPEX 99.999%), cobalt metal (SPEX 99.999%), and $\text{Pb}(\text{NO}_3)_2$ (Fisher, ACS reagent) in ultrapure nitric acid (ULTREX II, J.T. Baker, Phillipsburg, NJ, USA) with heating, and diluting to the appropriate volume with ultrapure water; the final solutions contained 1% (v/v) ultrapure nitric acid. Standard solutions (1000 $\mu\text{g/mL}$) of Cu(II) and Cd(II) were purchased from BDH. Ultrapure water of resistivity 18.2 $\text{M}\Omega\cdot\text{cm}$ was obtained direct from a Milli-Q-Plus water purification system (Millipore Corporation).

Chelex-100 cation exchange resin (Bio-Rad, 100-200 mesh, Sodium form) was soaked in ultrapure water, stored and then filtered just before use. The pH of the solution was adjusted to 5.0 ± 0.5 using ultrapure nitric acid (ULTREX II, J. T. Baker Inc., Phillipsburg, NJ, USA).

A well-characterized FA [79] extracted from a Bh horizon soil and collected from Armadale, Prince Edward Island, was supplied by Dr. D. S. Gamble, Agriculture Canada, Ottawa, Ontario, Canada. However, the “well-characterized FA” term requires some elaboration. The FA was characterized by potentiometric titration [57,79-81]. The total number of phenolic OH and carboxyl groups determined by potentiometric titration are 3.0 mmol / g fulvic acid [79,57] and 7.71 mmol / g fulvic acid [79-81], respectively

(Table 1). The bidentate complexing capacity is therefore approximately 5.4 mmol / g fulvic acid [82].

An estimate of the background metal concentration in the Armadale fulvic acid was obtained by quantitative determination of its metal contamination using inductively-coupled plasma mass spectrometry [83]. 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^{-8} 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.

Apparatus

Screw-capped Teflon cylindrical bottles (500 mL capacity) were used to fabricate the Reactors for the kinetic studies of the samples using Graphite Furnace Atomic Absorption Spectrometry (GFAAS) [84], for which a Perkin-Elmer Zeeman atomic absorption spectrophotometer, model 5000, equipped with an AS-70 autosampler, an HGA-500 graphite furnace, pyrolytically-coated graphite tubes (Perkin-Elmer) and

Table 1. Functional groups in the Armadale fulvic acid [85].

Functional groups	Concentrations of functional groups in the FA, (mmol/g)
Phenolic-OH	3.0
Alcoholic-OH	4.0
Ketonic C O	1.4
COOH	7.7
Quinoid C O	0.6
Methoxyl	0.4

laboratory-made pyrolytic graphite platforms were used. The instrumental parameters used in the GFAAS determination of the metals Ni(II), Cu(II), Pb(II), Cd(II) and Co(II) are shown in Table 2. An Accumet 20 pH/mV/conductivity meter (Fisher Scientific) was used for the pH and conductivity determinations.

Cleaning procedure

All containers were made of Teflon, and were pre-cleaned by soaking in 10% HNO₃ (AR grade) for one week at room temperature, in order to leach out trace metals and other impurities. The containers were then rinsed five-times with ultrapure water. Finally, they were kept soaked in ultrapure water until used.

Test solutions

The model solutions were prepared to contain a constant concentration (10^{-8} M) of one of the following metals, Ni(II), Cu(II), Co(II), Pb(II) and Cd(II), and varying concentrations of the Armadale fulvic acid to give the [metal] / [FA] mole ratios of 0.0018, 0.018 and 0.18. It should be noted that for a naturally-occurring, heterogeneous complexant, such as the FA the molar mass is not directly accessible. However, a molar concentration was calculated, based on the bidentate complexing capacity, 5.4 mmole g⁻¹, of the Armadale fulvic acid, as determined by Gamble et al. [82]. A stock solution (1.0219 g / L) of the Armadale FA was prepared by dissolving appropriate amount of the freeze-dried FA in ultrapure water. The stock solution was stored in the dark at 4°C until use. The stock solution of the Armadale fulvic acid 1.0219 g/L corresponded

Table 2. Analysis lines and instrumental conditions for Zeeman 5000 GFAAS.

Elements	Wavelength (nm)	Ashing temperature (°C)	Atomization parameters	
			Temperature (°C)	Duration (s)
Ni	232.0	1000	2300	6
Pb	217.0	700	2300	6
Cu	324.7	900	2500	6
Cd	228.8	250	2100	6
Co	240.7	900	2500	6

to 5.5×10^{-3} mol / L solution of the FA, calculated on the basis of the bidentate complexing capacity of the FA. The pH of the model solutions containing FA was adjusted to 5.0 ± 0.2 using 1 % (g/v) NaOH solution. The NaOH solution was purified by electrodeposition at -1.2 V (vs. E_{SCE}) for at least 48 hours immediately prior to use.

Method for kinetic measurement

The kinetics of uptake by Chelex-100 of free plus other small labile metal species arising from the dissociation of metal-FA complexes as a function of time was measured by GFAAS as follows. One percent (w/v) of pre-treated Chelex-100 resin was added to a 300 mL of the above samples placed in the Reactor, and the mixture was stirred with a Teflon-coated magnetic stirring bar (Figure 3). Test solutions for the kinetic run were withdrawn from the Reactor through a nylon membrane filter placed at the end of the Reactor tube, which filtered out the Chelex-100 resin and injected a clear filtrate into the graphite furnace. The zero time for the kinetic measurement was taken as the time at which the Chelex-100 resin first came in contact with the test solution in the Reactor. The concentrations of the metal complexes remaining in the test solution, $C_{ML}(t)$, (eq. 14) was measured by GFAAS as a function of time. For the determination of metal concentration, 10 μ L of the test solution was injected into the graphite furnace, where it was dried, ashed and atomized. The signal was measured in the peak area mode. Each completed determination was followed by a 4 s clean-up cycle of the graphite furnace at 2600°C . During the drying, ashing and clean-up cycles, the internal argon gas was passed through the graphite furnace at 250 mL / min. The internal argon gas flow was interrupted during the atomization cycle, but was restored for the clean-up cycle. The changes in the pH of

Figure 3. The Teflon Reactor used for the kinetic experiment.

the test solutions between the beginning and the end of the experiment were found to be $< \pm 0.5$ pH unit.

QA /QC

Quality control for metal analyses included repeated injections and periodic (two in every two-hour operation of the equipment) analysis of Certified Reference Standard, NIST 1643d. A prior set of samples was re-run if the analysed value differed from the Reference Standard's certified value by $> 20\%$. The relative standard deviation among replicate determinations was typically $\leq 5\%$.

Kinetic data analysis

The experimental data were analyzed for discrete values of the dissociation rate coefficients by a non-linear regression method based on the Marquardt-Levenberg algorithm [86]. All the experimental data were used in the fitting. For kinetic model, the first condition which must be satisfied is that the regressions converge rapidly, and there should be a reasonable fit (as measured by correlation coefficient, r^2) with the kinetic data. A typical example of one-component fitting of kinetic data is shown in Figure 4.

RESULTS AND DISCUSSION

Effect of [metal] / [FA] mole ratios on the kinetic lability of the metal-FA complexes

Figures 4-8 show the effect of [metal] / [FA] mole ratios on the rates of dissociation of the FA complexes of Pb(II), Cu(II), Cd(II), Co(II) and Ni(II). The [metal] / [FA] mole

ratios were varied by keeping the metal concentration constant (10^{-8} M), and varying the FA concentration. In Figures 4-8, the [metal] / [FA] mole ratios were varied from 0.0018 to 0.18. For the sake of simplicity of calculating [metal] / [FA] mole ratio, the FA will be considered to have ~ 1% strong binding sites, and ~ 99 % weak binding sites. If FA has ~ 1% strong binding sites, then the mole ratio of metal to FA for occupation only of the strong binding sites of the FA was ~ 0.01. To be on the safe side, the experiments were started with a [metal] / [FA] mole ratio of 0.001.

In Figures 4-8, the top curves represent the [metal] / [FA] mole ratio of 0.0018. At this mole ratio, the rates of dissociation of the metal-FA complexes were very slow and hardly detectable over the entire period of the measurement. When the [metal] / [FA] mole ratios were increased by one order of magnitude to 0.018 (the middle curves in Figures 4-8), the rate of dissociation was increased initially, but becomes slow again at longer time. When the [metal] / [FA] mole ratios were further increased by one order magnitude to 0.18 (the bottom curves in Figures 4-8), a rapid initial decrease was observed which levelled off to a slower rate of dissociation at longer times.

These observations can be interpreted as follows. At the [metal] / [FA] mole ratio of 0.0018, the metal concentrations were much lower (10^{-8} M) than that of the FA (10^{-5} M), and the FA had enough strong binding sites to occupy all the metal ions, resulting in the formation of strong metal-FA complexes, which are inert. With further increase in the [metal] / [FA] mole ratio to 0.018, and still further to 0.18, all the strong sites (~1%) are now completely filled, leaving the remaining metals to bind to the weak binding sites (~

99 %) of the FA, resulting in the formation of weak metal-FA complexes, which are labile.

The experimental data presented in Figures 4-8 were fitted to the kinetic model (eq. 14), and analyzed using a non-linear regression method to obtain the numerical values of the kinetically distinguishable components and the associated dissociation rate coefficients. The values for kinetic parameters of the metal-FA complexes are presented in Tables 3-7. The points in Figures 4-8 are the experimental data, and the solid lines are the results from the non-linear regression analysis. At the [metal] / [FA] mole ratio of 0.0018, only one dissociation rate coefficient was estimated, whereas two kinetically distinguishable components and two rates of dissociation were appeared to be involved for the mole ratios of 0.018 and 0.18. This is consistent with the presence of two distinct classes of binding sites in the Armadale FA.

Figures 4-8 and Tables 3-7 show that the fraction of the free plus other small labile species and their corresponding dissociation rate coefficients increase with increasing [metal] / [FA] mole ratios. This observation was common for all the metal studied. The results are in agreement with the hypothesis that as the [metal] / [FA] mole ratio is increased by keeping the metal concentration constant and varying the FA concentration, the strong binding sites become saturated and the excess metal then binds to the weaker sites of the FA. These weak metal complexes are labile, releasing free metal ions, which are probably bioavailable.

The kinetic lability of the metal species varies in a reasonable way with the [metal] / [FA] mole ratio, suggesting that the kinetic speciation model gives chemically significant

metal species. The change in the dissociation rate coefficient measured for each component of the metal-FA complexes provides experimental evidence of the polyfunctionality of the fulvic acid. The results show how kinetic data can detect the progressive occupation of strong and weak binding sites of a well-characterized FA and the existence of strong and weak binding sites in fulvic acids.

Figure 4. Effect of $[\text{Pb(II)}] / [\text{FA}]$ mole ratio on the kinetic lability of the Pb(II) -FA complexes in model systems containing varying concentrations of the Armadale FA, measured by Zeeman 5000 GFAAS. Concentrations of lead were same for all curves. $\text{pH } 5.1 \pm 0.1$; ionic strength $\sim 5 \times 10^{-8} \text{ M}$. The error bar represents 95.5 % confidence intervals.

(----), one-component fitting ($r^2 < 0.65$); (—), two-component fitting ($r^2 > 0.98$).

(○), $[\text{Pb(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-5} \text{ M}] = 0.0018$; (□), $[\text{Pb(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-6} \text{ M}] = 0.018$; (Δ), $[\text{Pb(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-7} \text{ M}] = 0.18$.

Figure 5. Effect of $[\text{Cu(II)}] / [\text{FA}]$ mole ratio on the kinetic lability of the Cu(II) -FA complexes in model systems containing varying concentrations of the Armadale FA, measured by Zeeman 5000 GFAAS. Concentrations of copper were same for all curves. $\text{pH } 5.1 \pm 0.1$; ionic strength $\sim 5 \times 10^{-8} \text{ M}$. The error bar represents 95.5 % confidence intervals.

(○), $[\text{Cu(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-5} \text{ M}] = 0.0018$; (□), $[\text{Cu(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-6} \text{ M}] = 0.018$; (△), $[\text{Cu(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-7} \text{ M}] = 0.18$.

Figure 6. Effect of $[\text{Cd(II)}] / [\text{FA}]$ mole ratio on the kinetic lability of the Cd(II) -FA complexes in model systems containing varying concentrations of the Armadale FA, measured by Zeeman 5000 GFAAS. Concentrations of cadmium were same for all curves. $\text{pH } 5.1 \pm 0.1$; ionic strength $\sim 5 \times 10^{-8}$ M. The error bar represents 95.5 % confidence intervals.

(○), $[\text{Cd(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-5} \text{ M}] = 0.0018$; (□), $[\text{Cd(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-6} \text{ M}] = 0.018$; (△), $[\text{Cd(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-7} \text{ M}] = 0.18$.

Figure 7. Effect of $[\text{Co(II)}] / [\text{FA}]$ mole ratio on the kinetic lability of the Co(II) -FA complexes in model systems containing varying concentrations of the Armadale FA, measured by Zeeman 5000 GFAAS. Concentrations of cobalt were same for all curves. $\text{pH } 5.1 \pm 0.1$; ionic strength $\sim 5 \times 10^{-8} \text{ M}$. The error bar represents 95.5 % confidence intervals.

(○), $[\text{Co(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-5} \text{ M}] = 0.0018$; (□), $[\text{Co(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-6} \text{ M}] = 0.018$; (Δ), $[\text{Co(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-7} \text{ M}] = 0.18$.

Figure 8. Effect of $[\text{Ni(II)}] / [\text{FA}]$ mole ratio on the kinetic lability of the Ni(II) -FA complexes in model systems containing varying concentrations of the Armadale FA, measured by Zeeman 5000 GFAAS. Concentrations of nickel were same for all curves. $\text{pH } 5.1 \pm 0.1$; ionic strength $\sim 5 \times 10^{-8} \text{ M}$. The error bar represents 95.5 % confidence intervals.

(○), $[\text{Ni(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-5} \text{ M}] = 0.0018$; (□), $[\text{Ni(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-6} \text{ M}] = 0.018$; (Δ), $[\text{Ni(II)} 10^{-8} \text{ M}] / [\text{FA } 10^{-7} \text{ M}] = 0.18$.

Table 3. Kinetic parameters of the Pb(II)-FA complexes in model systems, as a function of [Pb]/[FA] mole ratios, at a constant concentration of Pb(II) and varying concentrations of the FA. pH = 5.1±0.1, ionic strength $\sim 5 \times 10^{-8}$ M. [Pb(II)] = 10^{-8} M.

[FA], M	[Pb(II)] / [FA]	Kinetically distinguishable components		Dissociation rate coefficients	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁵	0.0018		100 ± 0.9		0.3 ± 0.1
10 ⁻⁶	0.018	8.7 ± 0.2	91.2 ± 0.8	2.5 ± 0.1	2.1 ± 0.1
10 ⁻⁷	0.18	20.1 ± 0.5	79.8 ± 0.7	5.0 ± 0.1	8.2 ± 0.2

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 4. Kinetic parameters of the Cu(II)-FA complexes in model systems, as a function of [Cu]/[FA] mole ratios, at a constant concentration of Cu(II) and varying concentrations of the FA. pH = 5.1±0.1, ionic strength ~ 5 × 10⁻⁸ M. [Cu(II)]=10⁻⁸ M.

[FA], M	[Cu(II)] / [FA]	Kinetically distinguishable components		Dissociation rate coefficients	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁵	0.0018		100 ± 0.7		0.2 ± 0.1
10 ⁻⁶	0.018	7.8 ± 0.2	91.0 ± 0.6	1.2 ± 0.1	1.1 ± 0.1
10 ⁻⁷	0.18	18.0 ± 0.1	81.5 ± 0.7	3.6 ± 0.1	6.1 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 5. Kinetic parameters of the Cd(II)-FA complexes in model systems, as a function of [Cd]/[FA] mole ratios, at a constant concentration of Cd(II) and varying concentrations of the FA. pH = 5.1±0.1, ionic strength ~ 5 × 10⁻⁸ M. [Cd(II)] = 10⁻⁸ M.

[FA], M	[Cd(II)] / [FA]	Kinetically distinguishable components		Dissociation rate coefficients	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁵	0.0018		100±0.8		0.2±0.0
10 ⁻⁶	0.018	14.7±0.2	85.4±1.0	6.8±0.0	4.7±0.1
10 ⁻⁷	0.18	40.0±0.1	60.1±0.7	10.2±0.0	23.1±0.3

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 6. Kinetic parameters of the Co(II)-FA complexes in model systems, as a function of [Co]/[FA] mole ratios, at a constant concentration of Co(II) and varying concentrations of the FA. pH = 5.1±0.1, ionic strength ~ 5 × 10⁻⁸ M. [Co(II)] = 10⁻⁸ M.

[FA], M	[Co(II)] / [FA]	Kinetically distinguishable components		Dissociation rate coefficients	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁵	0.0018		100 ± 0.9		0.3 ± 0.1
10 ⁻⁶	0.018	11.5 ± 0.1	88.5 ± 0.9	6.0 ± 0.1	4.0 ± 0.1
10 ⁻⁷	0.18	32.7 ± 0.2	67.9 ± 0.5	9.5 ± 0.3	19.4 ± 0.3

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 7. Kinetic parameters of the Ni(II)-FA complexes in model systems, as a function of [Ni]/[FA] mole ratios, at a constant concentration of Ni(II) and varying concentrations of the FA. pH = 5.1±0.1, ionic strength $\sim 5 \times 10^{-8}$ M. [Ni(II)] = 10^{-8} M.

[FA], M	[Ni(II)] / [FA]	Kinetically distinguishable components		Dissociation rate coefficients	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁵	0.0018		98.8 ± 1.0		0.3 ± 0.1
10 ⁻⁶	0.018	9.5 ± 0.1	90.5 ± 0.8	5.1 ± 0.1	3.5 ± 0.1
10 ⁻⁷	0.18	29.7 ± 0.2	70.2 ± 0.6	8.5 ± 0.3	15.4 ± 0.3

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

CHAPTER 5

COMPETITION OF Cu(II) AND Co(II) WITH Ni(II) FOR BINDING BY A WELL-CHARACTERIZED FULVIC ACID (ARMADALE) IN MODEL SYSTEMS

INTRODUCTION

Metal ions vary greatly in their binding affinity for ligands. Humic substances (e.g. humic and fulvic acids) are essentially polydisperse systems, i.e. they contain a mixture of compounds of different molecular weights; they are also ubiquitous in freshwaters. Humic substances are chemically and physically heterogeneous, polyfunctional, multifunctional, organic complexants having numerous polar functional groups. 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 [87] 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 general consensus is that for the purpose of binding of metals humic substances can be classified as having both minor (~1-10%), strong binding sites, and major (~99-90%), weak binding sites [4,58-60]. Strong

complexes of metals with humic substances are generally inert (i.e. do not dissociate, or dissociate extremely slowly). Release of bound metals in freshwaters as free metal ions (i.e. metal-aqua 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').

Papers dealing with the competition between metals for naturally-occurring, organic complexants in freshwaters are scarce. Xue et al. [88] 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 from the Law of Mass Action as applied to chemical equilibrium [89].

This Chapter presents the results of the investigations of competitive binding of Cu(II), Co(II) and Ni(II) by the Armadale FA in model systems, and the results of the above competition in freshwaters is presented in the next Chapter. Nickel was chosen as the target trace metal for this study because nickel is an essential micronutrient for some marine algae, invertebrates, and fish [90]. Nickel is especially important for the Canadian environment since Canada is one of the world's foremost producers of nickel, and nickel

mining, refining, and metallurgical operations may discharge nickel-containing aqueous effluents to the Canadian aquatic environment. Since nickel in the mineral phase is associated with copper and cobalt, the effluents from nickel beneficiation, refining and metallurgical operations have nickel present with copper and cobalt. In the freshwater environment, where organic complexants are ubiquitous, copper is 100% complexed, which means that in the presence of adequate concentration of copper in an organic-complexant-limited aquatic environment in which the associated copper loading is relatively high, nickel is probably present as the uncomplexed species, i.e. as free nickel ion, $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$, which is reported to be toxic [90]. Similar possibilities exist when cobalt is present with nickel in freshwaters. Therefore copper and cobalt have been included in this research.

The objective of this Chapter is to study the effect of competition of Cu(II) and Co(II) on the kinetic lability of the Ni(II) complexes in model systems containing low nickel ion concentration (10^{-8} M), typical of unpolluted freshwaters.

EXPERIMENTAL

The competitive binding of Cu(II), Co(II) and Ni(II) by the Armadale FA in model systems was investigated using the CLEM with Chelex-100 as the competing ligand. Perkin-Elmer Zeeman 5000 GFAAS was employed for monitoring the rate of uptake of the metals by Chelex-100. The kinetic model (CLEM), method of the kinetic measurement and the properties of the Armadale FA have been described in the previous Chapter.

Test solutions

Three types of model aqueous solutions were used: (1) solutions containing constant concentration of Ni(II) [10^{-8} M] and varying concentrations of the FA; (2) solutions containing constant concentration of Ni(II) [10^{-8} M] and the FA [10^{-5} M], and varying concentrations of Cu(II); (3) solutions containing constant concentration of Ni(II) [10^{-8} M], Cu(II) [10^{-8} M] and the FA [10^{-5} M], and varying concentrations of Co(II). Model aqueous solutions were prepared by spiking ultrapure water with appropriate volumes of standard solutions of the metals and of the fulvic acid solution (1.0219 g / L). The pH of all the model solutions were measured using a Fisher Scientific Accumet 925 pH / ion meter. The pH meter was calibrated with two standard pH buffer solutions (pH 4 and pH 7). The pH of all the test solutions were adjusted to $\text{pH } 5.0 \pm 0.5$ with dilute HNO_3 (ULTREX II) or dilute NaOH (electrolytically pure) solutions. The changes in the pH between the beginning and the end of the experiment were found to be $< \pm 0.3$ pH unit.

QA /QC

Quality control for metal analyses included repeated injections and periodic (two in every two-hour operation of the equipment) analysis of Certified Reference Standard, NIST 1643d. A prior set of samples was re-run if the analysed value differed from the Reference Standard's certified value by $> 20\%$. The relative standard deviation among replicate determinations was typically $\leq 5\%$.

RESULTS AND DISCUSSION

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

Figures 9 and 10 show that with a large excess of the FA (three orders of magnitude greater than those of Ni(II) and Cu(II)), Ni(II) (and also Cu(II)) occupy the small number (~1%) of the strong binding sites of the FA, thereby forming strong Ni(II)-FA and Cu(II)-FA complexes, which are inert (top curves). The sharply-increasing concentration 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(II)-FA and the Cu(II)-FA complexes – the small number (~1%) of the strong binding sites of the FA become completely filled by Ni(II) and Cu(II), both of which are in large excess over the small number of the strong binding sites of the FA; the remaining Ni(II) and Cu(II) are then bound by the large number (~99%) of weak binding sites of the FA, forming weak complexes, which are labile. The result is that most of the Ni(II) and the Cu(II) (in the [metal] / [FA] mole ratio used in this work to simulate the freshwater environment) form weak Ni(II)-FA and Cu(II)-FA complexes, which are labile.

Comparison of Figure 9 with Figure 10 shows that, under the same experimental conditions, the Ni(II)-FA complexes are more labile than the Cu(II)-FA complexes. The kinetic parameters of the Ni(II)-FA and Cu(II)-FA complexes in model systems containing varying concentrations of Cu(II) and constant concentrations of Ni(II) and the FA are presented in Tables 8 and 9. For the Ni(II)-FA complexes, two kinetically distinguishable components of the nickel complexes were resolved in all the samples

tested except the first one (in the presence of 10^{-8} M Cu(II)). For the Cu(II)-FA complexes, in the presence of 10^{-8} M and 10^{-7} M Cu(II), one kinetically distinguishable component, and in the presence of 10^{-6} M and 10^{-5} M Cu(II), two kinetically distinguishable components of the Cu(II)-FA complexes have been resolved. Tables 8 and 9 show that the concentrations and the dissociation rate coefficients of the slower component for both the Ni(II)-FA and the Cu(II)-FA complexes are almost same ($C_2 = 100\%$, $k_2 = 2.0 \times 10^{-6} \text{ s}^{-1}$), but with the increasing concentration of Cu(II), both the concentrations and the dissociation rate coefficients of the faster and the slower component of the Ni(II)-FA and Cu(II)-FA complexes vary greatly.

Effect of a FA-deficient environment on the competitive binding of Cu(II) and Ni(II) (taken in equimolar concentrations) to the FA

Figure 11 shows when the FA is present in great excess (about three orders of magnitude greater than that of the Cu(II) and the Ni(II) (top curve)), the Ni(II) (along with the Cu(II)) are in such small molar concentrations relative to that of the FA that enough strong binding sites of the FA are available for binding both the Cu(II) and the Ni(II) to strong binding sites of the FA, with the results both the Cu(II) and the Ni(II) form strong metal-FA complexes, which are inert (top curve). However, with decrease in the concentration of the FA (one order of magnitude for each curve from top to bottom) a new situation develops, where there are not enough strong binding sites of the FA available for binding all the Cu(II) and the Ni(II) present in the sample. The result is that more and more Ni(II) (which cannot compete with Cu(II) for the relatively small number of the strong binding sites of the FA because the water exchange rate coefficient for

Figure 9. Effect of varying concentrations of Cu(II) on the kinetic lability of the Ni(II)-FA complexes in model systems containing constant concentrations of Ni(II) and the Armadale FA, measured by Zeeman 5000 GFAAS. pH 5.1 ± 0.2 , ionic strength $\sim 10^{-5}$ M. The error bar represents 95.5 % confidence intervals.

(Δ), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [FA 10^{-5} M]; ($\square$), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-7} M] + [FA 10^{-5} M]; (O), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-6} M] + [FA 10^{-5} M]; ($\diamond$), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-5} M] + [FA 10^{-5} M].

Figure 10. Effect of varying concentrations of Cu(II) on the kinetic lability of the Cu(II)-FA complexes in model systems containing constant concentrations of Ni(II) and the Armadale FA, measured by Zeeman 5000 GFAAS. pH 5.1 ± 0.1 , ionic strength $\sim 10^{-5}$ M. The error bar represents 95.5 % confidence intervals.

(Δ), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [FA 10^{-5} M]; (□), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-7} M] + [FA 10^{-5} M]; (○), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-6} M] + [FA 10^{-5} M]; (◊), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-5} M] + [FA 10^{-5} M].

Table 8. Kinetic parameters of the Ni(II)-FA complexes in the presence of varying concentrations of Cu(II) in model systems containing constant concentrations of Ni(II) and the Armadale FA, measured by Zeeman 5000 GFAAS. pH 5.1 ± 0.2 , ionic strength $\sim 10^{-5}$ M, temperature 298 K.

$$[\text{Ni (II)}] 10^{-8} \text{ M} + [\text{Cu(II)}] + [\text{FA}] 10^{-5} \text{ M.}$$

[Cu(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
			C ₁	C ₂
	C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁸		100 ± 0.3		0.2 ± 0.1
10 ⁻⁷	42.3 ± 0.3	57.8 ± 0.6	6.4 ± 0.1	3.7 ± 0.2
10 ⁻⁶	74.2 ± 0.6	25.8 ± 0.5	7.5 ± 0.2	8.2 ± 0.2
10 ⁻⁵	92.0 ± 0.4	8.0 ± 0.6	10.1 ± 0.1	9.9 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 9. Kinetic parameters of the Cu(II)-FA complexes in the presence of varying concentrations of Cu(II) in model systems containing constant concentrations of Ni(II) and the Armadale FA, measured by Zeeman 5000 GFAAS. pH 5.1 ± 0.1 , ionic strength $\sim 10^{-5}$ M, temperature 298 K.

$$[\text{Ni(II)}] 10^{-8} \text{ M} + [\text{Cu(II)}] + [\text{FA}] 10^{-5} \text{ M}.$$

[Cu(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	C ₁ $k_1 \times 10^4 (\text{s}^{-1})$	C ₂ $k_2 \times 10^6 (\text{s}^{-1})$
10^{-8}		100 ± 0.3		1.8 ± 0.1
10^{-7}		99.8 ± 0.3		2.2 ± 0.1
10^{-6}	34.1 ± 0.3	63.9 ± 0.5	3.2 ± 0.1	7.8 ± 0.2
10^{-5}	75.8 ± 0.5	24.0 ± 0.2	9.8 ± 0.1	12.1 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Ni(II) is $3 \times 10^4 \text{ s}^{-1}$ which is $\ll 1 \times 10^9 \text{ s}^{-1}$ for Cu(II) [91] is now bound to the more abundant (~99%) of the weak binding sites of the FA, forming weak Ni(II)-FA complexes, which are labile. Table 10 presents the kinetic parameters of the Ni(II)-FA complexes, in the presence of a constant concentration of Cu(II) and varying concentrations of the FA.

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

Figures 12-14 show that when the FA is present in great excess (three orders of magnitude greater than that of the Cu(II), Ni(II), and Co(II)), these three metals are in such small molar concentrations than that of 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). Figure 14 shows that with the sharply-increasing concentration of Co(II) by one order of magnitude (the second curve from top), the Cu(II) still occupies the strong binding sites of the FA, thereby forming strong Cu(II)-FA complexes, which are inert. However, Figures 12 and 13 show that under the same experimental conditions, i.e. with Co(II) 10^{-7} M , ~ 60% of Ni(II) and ~ 70% of Co(II) are bound to the strong binding sites of the FA; the remaining Ni(II) and Co(II) are bound to the large number of weak binding sites present in the FA, resulting in the formation of weak Ni(II)-FA and Co(II)-FA complexes, which are labile. With further increase in the Co(II) concentration, all the strong binding sites of the FA are filled by

Figure 11. Effect of varying concentrations of the FA on the kinetic lability of the Ni(II)-FA complexes in model systems containing constant concentrations of Ni(II) and Cu(II), measured by Zeeman 5000 GFAAS. pH 5.0 ± 0.2 , ionic strength $\sim 10^{-8}$ M. The error bar represents 95.5 % confidence intervals.

(O), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [FA 10^{-5} M]; (□), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [FA 10^{-6} M]; (Δ), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [FA 10^{-7} M]; (◇), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [FA 10^{-8} M]; (●), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [FA 10^{-9} M]; (▽), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [FA 10^{-10} M].

Table 10. Kinetic parameters of the Ni(II)-FA complexes in the presence of varying concentrations of the Armadale FA in model systems containing constant concentrations of Ni(II) and Cu(II), measured by Zeeman 5000 GFAAS. pH 5.0 ± 0.2 , ionic strength $\sim 10^{-8}$ M, temperature 296 K.

[FA], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C_1 (%)	C_2 (%)	C_1 $k_1 \times 10^3 (\text{s}^{-1})$	C_2 $k_2 \times 10^5 (\text{s}^{-1})$
10^{-5}		100 ± 0.3		0.2 ± 0.1
10^{-6}	7.0 ± 0.1	92.9 ± 0.3	0.9 ± 0.1	0.3 ± 0.1
10^{-7}	14.5 ± 0.1	85.5 ± 0.1	1.2 ± 0.1	0.5 ± 0.1
10^{-8}	30.2 ± 0.2	69.8 ± 0.1	1.9 ± 0.2	0.8 ± 0.1
10^{-9}	69.9 ± 0.2	30.1 ± 0.2	3.7 ± 0.2	4.6 ± 0.3
10^{-10}	91.5 ± 0.1	8.5 ± 0.3	4.1 ± 0.2	6.4 ± 0.2

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Cu(II), Co(II) and Ni(II), all of which are present in excess of that required for binding by the strong sites of the FA. The remaining metals are then bound by the weak binding sites of the FA (which are present in much larger numbers), forming weak Cu(II)-FA, Co(II)-FA, and Ni(II)-FA complexes, which are labile. From the above Figures it is clear that Ni(II) and Co(II) cannot compete successfully with Cu(II) for the relatively small number of strong binding sites of the FA, as would be expected from the relative values of their rate coefficients for water exchange [91]. Kinetic parameters of the Ni(II)-FA, Co(II)-FA and Cu(II)-FA complexes are presented in Tables 11, 12 and 13, respectively. The increasing lability of the Ni(II)-FA complexes in the presence of Cu(II) and Co(II) indicates that Cu(II) and Co(II) 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 [92]:

- (1) The binding properties of the fulvic acid, both covalent bonding due to Lewis-basicity (bonding by strong versus weak binding sites) 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 high pH and low ionic strength, and is decreased by increased metal loading because of partial neutralization of the charge on the neighbouring, nonreacting, negative sites of functional groups of fulvic acid, all of which contribute greatly to the long-range coulombic interactions [92].

Figure 12. Effect of varying concentrations of Co(II) in the presence of Cu(II) on the kinetic lability of the Ni(II)-FA complexes in model systems containing constant concentrations of Ni(II), Cu(II) and the Armadale FA, measured by Zeeman 5000 GFAAS. pH 5.2 ± 0.2 , ionic strength $\sim 10^{-5}$ M. The error bar represents 95.5 % confidence intervals.

(Δ), $[\text{Ni(II)} 10^{-8} \text{ M}] + [\text{Cu(II)} 10^{-8} \text{ M}] + [\text{Co(II)} 10^{-8} \text{ M}] + [\text{FA } 10^{-5} \text{ M}]$; (□), $[\text{Ni(II)} 10^{-8} \text{ M}] + [\text{Cu(II)} 10^{-8} \text{ M}] + [\text{Co(II)} 10^{-7} \text{ M}] + [\text{FA } 10^{-5} \text{ M}]$; (○), $[\text{Ni(II)} 10^{-8} \text{ M}] + [\text{Cu(II)} 10^{-8} \text{ M}] + [\text{Co(II)} 10^{-6} \text{ M}] + [\text{FA } 10^{-5} \text{ M}]$; (◇), $[\text{Ni(II)} 10^{-8} \text{ M}] + [\text{Cu(II)} 10^{-8} \text{ M}] + [\text{Co(II)} 10^{-5} \text{ M}] + [\text{FA } 10^{-5} \text{ M}]$.

Figure 13. Effect of varying concentrations of Co(II) in the presence of Cu(II) and Ni(II) on the kinetic lability of the Co(II)-FA complexes in model systems containing constant concentrations of Ni(II), Cu(II) and the Armadale FA, measured by Zeeman 5000 GFAAS. pH 5.2 ± 0.2 , ionic strength $\sim 10^{-5}$ M. The error bar represents 95.5 % confidence intervals.

(Δ), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [Co(II) 10^{-8} M] + [FA 10^{-5} M]; (□), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [Co(II) 10^{-7} M] + [FA 10^{-5} M]; (○), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [Co(II) 10^{-6} M] + [FA 10^{-5} M]; (◇), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [Co(II) 10^{-5} M] + [FA 10^{-5} M].

Figure 14. Effect of varying concentrations of Co(II) in the presence of Ni(II) on the kinetic lability of the Cu(II)-FA complexes in model systems containing constant concentrations of Ni(II), Cu(II) and the Armadale FA, measured by Zeeman 5000 GFAAS. pH 5.2 ± 0.2 , ionic strength $\sim 10^{-5}$ M. The error bar represents 95.5 % confidence intervals.

(Δ), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [Co(II) 10^{-8} M] + [FA 10^{-5} M]; (□), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [Co(II) 10^{-7} M] + [FA 10^{-5} M]; (○), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [Co(II) 10^{-6} M] + [FA 10^{-5} M]; (◇), [Ni(II) 10^{-8} M] + [Cu(II) 10^{-8} M] + [Co(II) 10^{-5} M] + [FA 10^{-5} M].

Table 11. Kinetic parameters of the Ni(II)-FA complexes as a function of varying concentrations of Co(II) in the presence of Cu(II) in model systems containing constant concentrations of Ni(II), Cu(II) and the Armadale FA, measured by Zeeman 5000 GFAAS. pH 5.2 ± 0.2 , ionic strength $\sim 10^{-5}$ M, temperature 297 K.

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

[Co(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	C ₁ k ₁ × 10 ³ (s ⁻¹)	C ₂ k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁸		100 ± 0.3		0.2 ± 0.1
10 ⁻⁷	40.3 ± 0.6	59.7 ± 0.3	5.3 ± 0.1	5.4 ± 0.1
10 ⁻⁶	68.2 ± 0.5	31.8 ± 0.6	6.8 ± 0.1	9.9 ± 0.1
10 ⁻⁵	87.9 ± 0.6	12.1 ± 0.4	7.2 ± 0.1	12.2 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 12. Kinetic parameters of the Co(II)-FA complexes as a function of varying concentrations Co(II) in the presence of Cu(II) in model systems containing constant concentrations of Ni(II), Cu(II) and the Armadale FA, measured by Zeeman 5000 GFAAS. pH 5.2 ± 0.1 , ionic strength $\sim 10^{-5}$ M, temperature 297 K.

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

[Co(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	C ₁ $k_1 \times 10^3 (\text{s}^{-1})$	C ₂ $k_2 \times 10^5 (\text{s}^{-1})$
10^{-8}		100 ± 0.3		0.2 ± 0.1
10^{-7}	31.3 ± 0.5	68.7 ± 0.4	3.1 ± 0.1	3.5 ± 0.1
10^{-6}	57.5 ± 0.3	42.5 ± 0.5	5.2 ± 0.1	7.4 ± 0.1
10^{-5}	84.9 ± 0.2	15.1 ± 0.1	6.5 ± 0.2	10.1 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 13. Kinetic parameters of the Cu(II)-FA complexes as a function of varying concentrations of Co(II) in model systems containing constant concentrations of Ni(II), Cu(II) and the Armadale FA, measured by Zeeman GFAAS. pH 5.2 ± 0.2 , ionic strength $\sim 10^{-5}$ M, temperature 297 K.

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

[Co(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	C ₁ $k_1 \times 10^3 (\text{s}^{-1})$	C ₂ $k_2 \times 10^5 (\text{s}^{-1})$
10^{-8}		100 ± 0.3		0.2 ± 0.1
10^{-7}		99.7 ± 0.5		0.3 ± 0.1
10^{-6}	46.5 ± 0.4	53.5 ± 0.5	2.3 ± 0.1	4.5 ± 0.1
10^{-5}	74.5 ± 0.3	25.5 ± 0.2	4.8 ± 0.1	8.1 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

(2) In the formation of a metal-ligand complex, since the rate coefficient for water exchange for ligands are very large $10^9 - 10^{10} \text{ s}^{-1}$, their rate coefficients have been neglected in this treatment [73]. The water exchange rate coefficient of the metal-aqua complex, i.e., the first-order rate coefficients for water exchange spans 16 orders of magnitude from Pb^{2+} and Cu^{2+} with 10^9 s^{-1} to Cr^{3+} 10^{-7} s^{-1} . When several metals, such as Cu(II) , Ni(II) , and Co(II) are present with the FA in water samples, depending on the mole ratios of the metals and the FA, the Law of Mass Action determines the equilibrium concentrations of the metal-FA complexes; the equilibrium will favour the metal such as Cu(II) which has a much higher water exchange rate coefficient than the metals such as Ni(II) and Co(II) which have much lower water exchange rate coefficients.

Strong binding sites of ligands tend to form strong complexes with a given metal; the metal that has lower water exchange rate coefficient cannot compete with the metal that has higher water exchange rate coefficient, for the strong binding sites of the humic substances. 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, and k_a and k_d are the formation and dissociation rate coefficients, 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, 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.

CHAPTER 6

COMPETITION OF Cu(II) AND Co(II) WITH Ni(II) FOR BINDING BY NATURALLY-OCCURRING ORGANIC COMPLEXANTS IN FRESHWATERS (RIDEAU RIVER SURFACE WATERS)

INTRODUCTION

Freshwaters contain metals and organic complexants, such as humic substances, and other complexants both inorganic and organic. Metals in freshwaters exist in various chemical forms (called chemical species), as dissolved and particulate metals, as colloids, attached to colloids, and sorbed on suspended solids. Human activities introduce most of the metals found in the freshwater environment. The binding of metals by heterogeneous, polyfunctional, organic complexants in freshwaters has important consequences because it affects the mobility and bioavailability of the metals in the freshwater environment [44]. However, rigorous theoretical development of the interactions of metals with organic complexants in the freshwater environment has not been possible. Until now most of the interpretation models for metal-complexation reactions have been developed on the basis of simple reactions in 'pure' solutions – model aqueous solutions in the

laboratory, often containing only one ligand, one metal, and relatively few metal complexes. The lack of progress in this area reflects a lack of concept development.

As has been shown in the previous Chapter, organic, macromolecular, heterogeneous, organic complexants such as humic substances which are ubiquitous in freshwaters, are polyfunctional, i.e., they possess, in the same molecule, functional groups that have widely differing affinity for binding various metal ions. The above properties allow competition between metal ions for strong binding sites on the above organic complexants (humic substances) in freshwaters, resulting in some metal ions binding to the weak binding sites, forming weak complexes which are labile, and hence, release free metal ions which are reported to be toxic. Only if, and as long as, organic complexant is in excess, trace metals do not compete with each other [89]; however, in freshwaters which have appreciable metal loadings, the metals are above the usual concentrations of trace metals, and hence, they do compete with one another, especially, if the aquatic environment is limited in organic complexants, as in the case of heavily-polluted freshwaters (the term “polluted” here refers to “contamination with metals”).

The objective of this Chapter is to study the effect of competition of Cu(II) and Co(II) on the kinetic lability of the Ni(II)-DOC complexes in the Rideau River surface water (RRSW) samples.

EXPERIMENTAL

The competitive binding of Cu(II), Co(II) and Ni(II) by organic complexants in the RRSW sample was investigated using the same analytical technique as described in Chapter 4.

Samples

A 6 L sample of the RRSW was collected from a site at Carleton University using a precleaned Teflon water sampler. The pH and conductivity of the RRSW sample was 8.1 and 450 $\mu\text{S}/\text{cm}$, respectively. The sample was filtered through 0.45 μm membrane filters (Gelman, Ann Arbor, MI, USA) in the laboratory within 24 h of sample collection to filter out the particulate matter. These filters, made of mixed cellulose esters, had the advantage of providing filters having low blanks for the metals. Each 0.45 μm filter was able to filter ~ 1 L water sample without clogging. A sub-sample (250 mL) of the filtered sample was acidified with HNO_3 (Ultrex II) to pH 1.5. The acidified sample was used to determine the total concentrations of trace metals, Cu(II), Co(II) and Ni(II), using GFAAS. Since the concentrations of the trace metals, Cu(II), Co(II) and Ni(II), in the RRSW sample, as determined by GFAAS, were below their limits of detection (i.e. $< 10^{-8}$ M), and the concentrations of Ni(II), Cu(II) and Co(II) added as spikes to the RRSW samples ranged from 10^{-7} M to 10^{-5} M, the total concentrations of nickel, copper and cobalt in the test solutions were taken to be equal to the concentrations of the Ni(II), Cu(II) and Co(II) spikes, i.e. the concentration of the native Ni(II), Cu(II) and Co(II) in the RRSW sample in calculating the total concentrations of Ni(II), Cu(II) and Co(II) in the test solutions have been ignored. The remaining filtered sample was spiked with the

reference standard solutions of Ni(II), Cu(II) and Co(II) (either individually or all together) to make it contain a few micrograms per litre of each metal. Five types of spiked samples were used: (1) samples spiked with varying concentrations of Ni(II); (2) samples spiked with a constant concentration of Ni(II) (10^{-7} M) and varying concentrations of Cu(II); (3) samples spiked with a constant concentration of Ni(II) (10^{-7} M) and varying concentrations of Co(II); (4) samples spiked with a constant concentration of Ni(II) (10^{-7} M) and Co(II) (10^{-7} M), and varying concentrations of Cu(II); (5) samples spiked with a constant concentration of Ni(II) (10^{-7} M) and Cu(II) (10^{-7} M), and varying concentrations of Co(II). If the kinetic measurements were unavoidably delayed because of non-availability of the GFAAS, the filtered spiked samples were stored in the dark at 4 °C until kinetic measurements (within 4-5 days) were made. The pHs of all the samples were measured using a Fisher Scientific Accumet 925 pH / ion meter and the pH was adjusted to $\text{pH } 5.0 \pm 0.4$ with dilute HNO_3 (ULTREX II) solution just before the kinetic measurements were made. The changes in the pH between the beginning and the end of the experiments were found to be $< \pm 0.5$ pH unit.

Dissolved Organic Carbon

The dissolved organic carbon (DOC) in the Rideau River surface water sample was measured using Rosemount–Dohrmann DC-190 high temperature TOC Analyzer, and the DOC in the RRSW sample was 6.5 mg of C/L. Assuming 40 - 99% of the DOC is present as a humic substance [3], the concentration of humic substance in the RRSW sample was estimated to be roughly 1.5×10^{-5} M to 3.5×10^{-5} M, assuming its binding capacity (strong binding sites) for Ni(II) is of the order of 5.4×10^{-3} mol/g [83].

QA /QC

Quality control for metal analyses included repeated injections and periodic (two in every two-hour operation of the equipment) analysis of Certified Reference Standard, NIST 1643d. A prior set of samples was re-run if the analysed value differed from the Reference Standard's certified value by $> 20\%$. The relative standard deviation among replicate determinations was typically $\leq 5\%$.

RESULTS AND DISCUSSION

Effect of varying concentrations of Ni(II) on the kinetic lability of the Ni(II)-DOC complexes in the RRSW sample

Figure 15 shows the effect of the increasing concentrations of nickel on the lability of the Ni(II)-DOC complexes in the RRSW sample. In Figure 15, the top curve can be interpreted as follows: at low concentrations of Ni(II) ($\sim 10^{-7}$ M), there are enough strong binding sites of the DOC (mainly humic substances) in the RRSW sample to bind all the Ni(II) to the strong sites, forming strong Ni(II)-DOC complexes, which are inert. With increase in the concentration of Ni(II) to 10^{-6} M, and still further to 10^{-5} M (the second curve from top and the bottom curve), all the strong sites of the DOC are now filled, leaving the excess Ni(II) to bind to the weak binding sites of the organic complexants, forming weak Ni(II)-DOC complexes, which are labile. The experimental data presented in Figure 15 were analyzed using a non-linear regression method. The kinetic parameters of the Ni(II)-DOC complexes are presented in Table 14, which shows that at the relatively high concentration of Ni(II) (10^{-5} M), the Ni(II) complex has a faster

dissociation rate coefficient ($k_1 = 5.8 \times 10^{-3} \text{ s}^{-1}$) than the Ni(II) complex formed by the lower concentration of Ni(II) (10^{-6} M), ($k_1 = 4.0 \times 10^{-3} \text{ s}^{-1}$), and the Ni(II) complex with still lower concentration of Ni(II) (10^{-7} M), ($k_2 = 2.0 \times 10^{-6} \text{ s}^{-1}$). These results agree with the interpretation of the lability / inertness of the Ni(II)-DOC complex given above.

Effect of varying concentrations of Cu(II) in the presence of constant concentration of Ni(II) on the kinetic lability of the Ni(II)-DOC complexes in the RRSW sample

Figure 16 shows the effects of the increasing concentrations of Cu(II) on the lability of the Ni(II)-DOC complexes in the RRSW sample. The results can be interpreted as follows. In the absence of Cu(II), Ni(II) occupies the small number of the strong binding sites of the DOC, thereby forming strong Ni(II) complexes, which are inert [top curve]. However, in the presence of a low concentration ($\sim 10^{-7} \text{ M}$) of Cu(II) (the second curve from the top), a new situation develops, where there are not enough strong binding sites of the DOC available for binding all the Ni(II) and the Cu(II) present in the sample. In the competition for the strong binding sites, Cu(II) wins over Ni(II) because the rate coefficient for water exchange for Cu^{2+} $1 \times 10^9 \text{ s}^{-1}$ is $\gg 3 \times 10^4 \text{ s}^{-1}$ for Ni^{2+} [91], which means that Cu(II) wins the competition over the Ni(II) for binding to the strong sites, resulting in the Cu(II) now occupying a part of the small number of strong sites that were previously occupied by the Ni(II). The Ni(II) so outcompeted is now bound by the large number of weak binding sites of the DOC, forming weak Ni(II) complexes, which are labile. With the sharply-increasing concentrations of Cu(II) (by one

Figure 15. Effect of varying concentrations of Ni(II) on the kinetic lability of the Ni(II)-DOC complexes in the RRSW samples collected in June 11, 1998, measured by Zeeman 5000 GFAAS. pH 5.1 ± 0.1 , conductivity $450 \mu\text{S}/\text{cm}$. The error bar represents 95.5 % confidence intervals.

(O), $[\text{Ni(II)}] = 10^{-7} \text{ M}$; (□), $[\text{Ni(II)}] = 10^{-6} \text{ M}$; (Δ), $[\text{Ni(II)}] = 10^{-5} \text{ M}$.

Table 14. Kinetic parameters of the Ni(II)-DOC complexes as a function of varying concentrations of Ni(II) in the RRSW sample collected in June 11, 1998, measured by Zeeman 5000 GFAAS. pH 5.1 ± 0.1 , conductivity $450 \mu\text{S/cm}$, temperature 297 K.

[Ni(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	C ₁ $k_1 \times 10^3 \text{ (s}^{-1}\text{)}$	C ₂ $k_2 \times 10^5 \text{ (s}^{-1}\text{)}$
10^{-7}		99.8 ± 0.3		0.2 ± 0.1
10^{-6}	55.0 ± 0.1	45.0 ± 0.2	4.0 ± 0.1	0.5 ± 0.1
10^{-5}	69.9 ± 0.2	25.7 ± 0.3	5.8 ± 0.1	2.0 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

order of magnitude for each curve from the second from top to bottom curve), the strong binding sites of the DOC become completely filled by the Cu(II) and the Ni(II), and increasingly greater portions of the Ni(II) (which cannot compete with the Cu(II) for the strong binding sites of the DOC primarily because of Ni(II)'s much smaller water-exchange rate coefficient relative to that of Cu(II)), become now bound to the more abundant, weak binding sites of the DOC, forming weak Ni(II)-DOC complexes, which are labile.

Figure 17 shows the effect of the increasing concentrations of Cu(II) on the lability of Cu(II) complexes in the RRSW sample. Comparison of Figure 16 with Figure 17 shows that, under the same experimental conditions as above, the Ni(II) complexes are more labile than the Cu(II) complexes.

Effect of varying concentrations of Co(II) in the presence of constant concentration of Ni(II) on the kinetic lability of the Ni(II)-DOC complexes in the RRSW sample

Figure 18 presents the effect of increasing concentrations of Co(II) on the lability of Ni(II) complexes in the RRSW sample, and shows almost the similar behaviour as shown in Figure 16. In the absence of Co(II), Ni(II) occupies the small number of the strong binding sites of the DOC, thereby forming strong Ni(II) complexes, which are inert (top curve). However, the presence of low concentration of Co(II) ($\sim 10^{-7}$ M) (the second curve from top) and the sharply-increasing concentration of Co(II) (by one order of

Figure 16. Effect of varying concentrations of Cu(II) in the presence of constant concentration of Ni(II) on the kinetic lability of the Ni(II)-DOC complexes in the RRSW samples collected in June 29, 1998, measured by Zeeman 5000 GFAAS. pH 5.0 ± 0.2 , conductivity $450 \mu\text{S}/\text{cm}$. The error bar represents 95.5 % confidence intervals.

(Δ), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] \text{ Nil}$; ($\square$), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-7} \text{ M}$; ($\circ$), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-6} \text{ M}$; ($\diamond$), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-5} \text{ M}$.

Table 15. Kinetic parameters of the Ni(II)-DOC complexes in the presence of varying concentrations of Cu(II) and constant concentration of Ni(II) in the RRSW samples collected in June 29, 1998, measured by Zeeman 5000 GFAAS. pH 5.0 ± 0.2 , conductivity $450 \mu\text{S/cm}$, temperature 297 K .

[Ni (II)] 10^{-7} M + [Cu(II)]

[Cu(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	C ₁ $k_1 \times 10^3 (\text{s}^{-1})$	C ₂ $k_2 \times 10^5 (\text{s}^{-1})$
10^{-7}	42.9 ± 0.4	57.1 ± 0.5	2.6 ± 0.1	1.0 ± 0.1
10^{-6}	51.4 ± 0.6	48.6 ± 0.5	3.7 ± 0.1	3.2 ± 0.1
10^{-5}	81.2 ± 0.2	18.8 ± 0.1	12.8 ± 0.1	4.3 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Figure 17. Effect of varying concentrations of Cu(II) in the presence of constant concentration of Ni(II) on the kinetic lability of the Cu(II)-DOC complexes in the RRSW sample collected in June 29, 1998, measured by Zeeman 5000 GFAAS. pH 5.0 ± 0.2 , conductivity $450 \mu\text{S}/\text{cm}$. The error bar represents 95.5 % confidence intervals.

(Δ), [Ni (II)] Nil + [Cu(II)] 10^{-7} M; (□), [Ni(II)] 10^{-7} M + [Cu(II)] 10^{-7} M; (○), [Ni(II)] 10^{-7} M + [Cu(II)] 10^{-6} M; (◇), [Ni(II)] 10^{-7} M + [Cu(II)] 10^{-5} M.

magnitude for each curve from the second from top to the bottom curve) has a dramatic effect on the lability of the Ni(II)-DOC complexes in the RRSW sample. With the sharply-increasing concentrations of Co(II), the small number of the strong binding sites of the DOC become completely filled by Ni(II) and Co(II). Because the water-exchange rate coefficient for Co^{2+} , $2 \times 10^6 \text{ s}^{-1}$ is $\gg 3 \times 10^4 \text{ s}^{-1}$ for Ni^{2+} [91], Co^{2+} wins over Ni^{2+} in the competition for the strong binding sites of the. The Ni(II) so outcompeted is now bound by the large number of the weak binding sites of the DOC. The result is that most of the Ni(II) form weak Ni(II) complexes, which are labile.

Figure 19 shows the effect of the increasing concentrations of Co(II) on the lability of Co(II) complexes in the RRSW sample. Comparison of Figure 18 with Figure 19 shows that, under the same experimental conditions as above, Co(II) complexes are more labile than Ni(II) complexes.

From the above Figures it is clear that in the presence of increasing concentrations of Cu(II) and Co(II), increasingly greater fractions of the Ni(II) in the RRSW sample become labile. This result agreed well with the results of the previous Chapter with model systems containing the metals and the Armadale FA. Comparison of Figure 16 with Figure 18 reveals that Ni(II) complexes are more labile in the presence of Cu(II) than in the presence of Co(II). The values for kinetic parameters of the Ni(II)-DOC complexes in the RRSW sample, in the presence of varying concentrations of Cu(II) and Co(II), and constant concentration of Ni(II) are presented in Tables 15 and 16, respectively. Tables 15 and 16 show that both the concentrations and the dissociation rate coefficients of the faster and the slower component of the Ni(II)-DOC complexes in the

presence of Cu(II) and in the presence of Co(II) vary greatly. The dissociation rate coefficients (k_1 and k_2) of the Ni(II)-DOC complexes in the presence of Cu(II) are always much greater than in the presence of Co(II).

Competition of Cu(II), Co(II), and Ni(II) for the strong binding sites of the DOC in the RRSW sample

Figures 20 and 21 present the result of the competition of the three metals: Ni(II), Cu(II) and Co(II), for the strong binding sites of the DOC in the RRSW sample. Figure 20 shows that in the absence of Cu(II) and in the presence of low concentration of Co(II) (10^{-7} M) (top curve) about 65% of Ni(II) is bound to the small number of strong binding sites of the DOC, resulting in the formation of strong Ni(II)-DOC complexes, which are inert, and the remaining Ni(II) is bound to the large number of the weak binding sites, resulting in the formation of weak Ni(II)-DOC complexes, which are labile. However, in the presence of low concentration of Cu(II) (10^{-7} M) (the second curve from top) and with the increase in the concentration of copper (by one order of magnitude for each curve from the second curve from top to the bottom curve), a new situation develops, where almost all the strong binding sites of the DOC in the RRSW sample are filled by Ni(II), Cu(II) and Co(II) present in the sample. The result is that increasingly larger portions of the Ni(II) are now bound to the much larger number of the weak binding sites of the DOC, forming weak Ni(II)-DOC complexes, which are labile. Figure 21 shows the same characteristics of the Ni(II)-DOC complexes as shown in Figure 20. The values for kinetic parameters of the Ni(II)-DOC complexes in the presence of constant concentrations of Ni(II) and Co(II) and varying concentrations

Figure 18. Effect of varying concentrations of Co(II) in the presence of constant concentration of Ni(II) on the kinetic lability of the Ni(II)-DOC complexes in the RRSW sample collected in July 13, 1998, measured by Zeeman 5000 GFAAS. pH 5.0 ± 0.2 , conductivity $450 \mu\text{S/cm}$. The error bar represents 95.5 % confidence intervals.

(Δ), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Co(II)}] \text{ Nil}$; (□), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Co(II)}] 10^{-7} \text{ M}$; (○), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Co(II)}] 10^{-6} \text{ M}$; (◇), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Co(II)}] 10^{-5} \text{ M}$.

Figure 19. Effect of varying concentrations of Co(II) in the presence of constant concentration of Ni(II) on the kinetic lability of the Co(II)-DOC complexes in the RRSW sample collected in July 13, 1998, measured by Zeeman 5000 GFAAS. pH 5.0 ± 0.2 , conductivity $450 \mu\text{S}/\text{cm}$. The error bar represents 95.5 % confidence intervals.

(Δ), [Ni (II)] Nil + [Co(II)] 10^{-7} M; (□), [Ni(II)] 10^{-7} M + [Co(II)] 10^{-7} M; (○), [Ni(II)] 10^{-7} M + [Co(II)] 10^{-6} M; (◊), [Ni(II)] 10^{-7} M + [Co(II)] 10^{-5} M.

Table 16. Kinetic parameters of the Ni(II)-DOC complexes in the presence of varying concentrations of Co(II) and constant concentration of Ni(II) in the RRSW samples collected in July 13, 1998, measured by Zeeman 5000 GFAAS. pH 5.0 ± 0.2 , conductivity $450 \mu\text{S/cm}$, temperature 298 K.

[Ni (II)] 10^{-7} M + [Co(II)]

[Co(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	C ₁ $k_1 \times 10^3 (\text{s}^{-1})$	C ₂ $k_2 \times 10^5 (\text{s}^{-1})$
10^{-7}	39.0 ± 0.7	61.0 ± 0.6	0.8 ± 0.1	0.8 ± 0.1
10^{-6}	46.9 ± 0.8	53.1 ± 0.2	2.0 ± 0.1	1.8 ± 0.1
10^{-5}	70.3 ± 0.3	29.7 ± 0.2	5.9 ± 0.1	2.0 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

of Cu(II), and in the presence of the constant concentrations of Ni(II) and Cu(II) and varying concentrations of Co(II) are presented in Tables 17 and 18, respectively.

From all these Figures and Tables, it is clear that the enhanced lability of the Ni(II)-DOC complexes in the presence of Cu(II) and Co(II) is the result of the competition of Cu(II) and Co(II) with Ni(II) for the strong binding sites of the DOC in the RRSW samples. The lability of the Ni(II)-DOC complexes is enhanced more by the presence of Cu(II) than by Co(II).

As stated in the previous Chapter, the results described above are the composite effect of the following factors:

- (1) The binding properties of the organic complexants, both covalent bonding due to Lewis-basicity (strong versus weak binding sites) and coulombic interactions (long-range) due to its oligoelectrolytical properties and its polyanionic character which is augmented by high pH and low ionic strength, and is decreased by greater metal loading, which, by neutralizing a part of the anionic charge from the polyanionic, organic complexants considerably decreases the strength of the coulombic interactions [92].
- (2) Metals that have lower water exchange rate coefficients cannot compete with the metals that have higher water exchange rate coefficients, for the strong binding sites of the DOC.

Figure 20. Effect of varying concentrations of Cu(II) in the presence of constant concentrations of Ni(II) and Co(II) on the kinetic lability of the Ni(II)-DOC complexes in the RRSW sample collected in July 20, 1998, measured by Zeeman 5000 GFAAS. pH 5.2 ± 0.2 , conductivity $450 \mu\text{S}/\text{cm}$. The error bar represents 95.5 % confidence intervals.

(Δ), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] \text{Nil} + [\text{Co(II)}] 10^{-7} \text{ M}$; ($\square$), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-7} \text{ M} + [\text{Co(II)}] 10^{-7} \text{ M}$; ($\circ$), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-6} \text{ M} + [\text{Co(II)}] 10^{-7} \text{ M}$; ($\diamond$), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-5} \text{ M} + [\text{Co(II)}] 10^{-7} \text{ M}$.

Figure 21. Effect of varying concentrations of Co(II) in the presence of constant concentrations of Ni(II) and Cu(II) on the kinetic lability of the Ni(II)-DOC complexes in the RRSW sample collected in July 31, 1998, measured by Zeeman 5000 GFAAS. pH 5.2 ± 0.2 , conductivity $450 \mu\text{S}/\text{cm}$. The error bar represents 95.5 % confidence intervals.

(Δ), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-7} \text{ M} + [\text{Co(II)}] \text{ Nil}$; ($\square$), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-7} \text{ M} + [\text{Co(II)}] 10^{-7} \text{ M}$; (o), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-7} \text{ M} + [\text{Co(II)}] 10^{-6} \text{ M}$; ($\diamond$), $[\text{Ni(II)}] 10^{-7} \text{ M} + [\text{Cu(II)}] 10^{-7} \text{ M} + [\text{Co(II)}] 10^{-5} \text{ M}$.

Table 17. Kinetic parameters of the Ni(II)-DOC complexes in the presence of varying concentrations of Cu(II) and constant concentrations of Ni(II) and Co(II) in the RRSW samples collected in July 20, 1998, measured by Zeeman 5000 GFAAS. pH 5.2 ± 0.2 , conductivity $450 \mu\text{S/cm}$, temperature 298 K.

$$[\text{Ni (II)}] 10^{-7} \text{ M} + [\text{Co (II)}] 10^{-7} \text{ M} + [\text{Cu(II)}].$$

[Cu(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	C ₁ k ₁ × 10 ³ (s ⁻¹)	C ₂ k ₂ × 10 ⁴ (s ⁻¹)
10 ⁻⁷	75.0 ± 0.5	25.0 ± 0.2	3.5 ± 0.1	0.3 ± 0.1
10 ⁻⁶	91.6 ± 0.3	8.4 ± 0.3	7.8 ± 0.1	3.5 ± 0.1
10 ⁻⁵	97.4 ± 0.5	2.6 ± 0.1	14.5 ± 0.1	18.3 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 18. Kinetic parameters of the Ni(II)-DOC complexes in the presence of varying concentrations of Co(II) and constant concentrations of Ni(II) and Cu(II) in the RRSW sample collected in July 31, 1998, measured by Zeeman 5000 GFAAS. pH 5.2 ± 0.2 , conductivity $450 \mu\text{S/cm}$, temperature 298 K.

$$[\text{Ni (II)}] 10^{-7} \text{ M} + [\text{Cu (II)}] 10^{-7} \text{ M} + [\text{Co(II)}]$$

[Co(II)], M	Kinetically distinguishable components		Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	C ₁ k ₁ ×10 ³ (s ⁻¹)	C ₂ k ₂ ×10 ⁴ (s ⁻¹)
10 ⁻⁷	74.5 ± 0.4	25.5 ± 0.3	2.5 ± 0.1	0.2 ± 0.1
10 ⁻⁶	83.6 ± 0.1	16.4 ± 0.1	3.3 ± 0.1	1.0 ± 0.1
10 ⁻⁵	95.9 ± 0.2	4.1 ± 0.1	11.4 ± 0.3	16.4 ± 0.4

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

In the above discussion, the contribution of the coulombic interactions to the total bonding have been generalized between the metals and the organic complexants because at the fixed pH and ionic strength, used in this work, the contribution of the coulombic interactions to the total bonding remains constant and is, therefore, not responsible for any of the observed changes. However, the presence of high concentrations ($\sim 10^{-3}$ M) of major cations such as Ca^{2+} and Mg^{2+} in the RRSW sample may have considerable effect on the binding of the metal-DOC complexes, and the results of competition of Ca(II) and Mg(II) with Ni(II) for binding by a well-characterized fulvic acid in model systems are presented in the next Chapter.

CHAPTER 7

COMPETITION OF Ca(II) AND Mg(II) WITH Ni(II) FOR BINDING BY THE ARMADALE FULVIC ACID IN MODEL SYSTEMS

INTRODUCTION

In the previous Chapters, the contribution of the coulombic interactions to the total bonding between the metals and the organic complexants was not explicitly taken into consideration on the grounds that at the fixed pH and ionic strength used in those works, the contribution of the coulombic interactions to the total bonding remained constant, and was therefore not responsible for any of the observed changes. In this Chapter, the contribution of the coulombic interactions between the negatively-charged surfaces of humic substances and the major cations, Ca^{2+} and Mg^{2+} , in the total bonding of the target trace metal ion, Ni^{2+} , by humic substances have been explicitly taken into consideration.

In unpolluted freshwaters, alkali and alkaline earth metal ions may influence the complexation of trace metals by humic substances since Ca^{2+} and Mg^{2+} ions are generally present at many orders of magnitude greater concentrations ($\sim 10^{-3}$ M) than those of trace metal ions ($\sim <10^{-8}$ M), which outweigh their weaker complexing power. The literature

contains several reports of experimental studies on the competition between Ca^{2+} or Mg^{2+} and copper species for binding by humic substances. Hering and Morel [93] have reported that Ca^{2+} does not have any competitive effect on Cu(II) -humate binding in the experimental conditions used by them and have concluded that different ligand sites must be involved in calcium and copper binding. Recently, Cao et al. [94] have reported that Ca^{2+} does have a measurable effect on the binding of trace metals (Cd(II) , Pb(II) and Cu(II)) by humic acid. The effect, however, was pH dependent and at $\text{pH} > 6$, the effect on the Cu(II) -humate binding was negligible. Since Ca^{2+} mainly forms outer-sphere complexes with ligands, the effect of adding Ca^{2+} might have been due to an overall change in electrostatic attraction. Cabaniss and Shuman [95], and Sunda and Hanson [74] also have reported small but measurable competition between calcium and copper in fulvic acid titrations. Buffle et al. [96] have measured copper binding by organic matter as a function of added Ca^{2+} ions in freeze-concentrated marsh waters using an ion-selective electrode over the pH range 5-6.5. They have reported a decrease in bound copper with increasing calcium concentration (from 1×10^{-4} up to 4×10^{-3} M) and that for $\text{pH} > 6$ and $[\text{Ca}^{2+}] < 5 \times 10^{-3}$ M (typical of freshwaters) the influence of $[\text{Ca}^{2+}]$ on the complexation of Cu^{2+} by fulvic acid is negligible. van den Hoop et al. [97] have observed a decreased association of Cd^{2+} and Zn^{2+} with the humate polyanion at pH 5 and at constant ionic strength, suggesting that Ca^{2+} ions compete with the heavy metal ions for binding by humic material. Mathuthu et al. [98] have suggested that Ca^{2+} will have minimal interference in the interactions of strongly complexing metals like Cu^{2+} with FA, but could reduce the binding of weak complexing metals like Cd^{2+} and Zn^{2+} . The different experimental conditions used by the above workers, as summarized in Table 19,

may account for the difference in their observations. Most of the above workers have reported that Ca(II) and Mg(II) have a measurable effect on the binding of trace metals by humic substances, with the exception of Hering and Morel [93] who have found no effect of the competition of Ca(II) on the Cu(II)-humate binding. This may be due to high pH 8.2 and ionic strength 0.5 M used by them, resulting in very strong Cu(II)-humate binding – strong intrinsic (chemical) binding further enhanced by strong coulombic attraction [99]. Apparently, the effect of Ca(II), competing for specific functional groups and decreasing (shielding) the coulombic attraction of the neighbouring ionized groups, is too small to be measurable.

Tipping [100] has used his Humic Ion-Binding Model V to predict competition effects in the binding of trace metal species and alkaline earth cations (Ca^{2+} , Mg^{2+}) by fulvic-type humic substances. Recently, Tipping [101] has proposed Humic Ion-Binding Model VI, which gives an improved description of the interactions of protons and metal ions with humic substances and accounts for competition and ionic strength effects, and for proton-metal exchange.

The specific objective of this Chapter is to study the competitive binding of Ca(II), Mg(II) and Ni(II) by the Armadale FA in model systems containing low Ni^{2+} ion concentration (10^{-7} M) and high concentrations of Ca^{2+} and Mg^{2+} ions ($\sim 10^{-3}$ M), typical of unpolluted freshwaters.

Table 19. Summary of the experimental conditions used by different research groups.

Research groups	pH	Ionic strength, M	[Cu(II)], M	[Organic Matter], mg/L	[Ca ²⁺] or [Mg ²⁺], M
Hering & Morel [93]	8.2	0.50	10 ⁻⁵	0.3	10 ⁻²
Cao et al. [94]	6, 7, 8	0.05	10 ⁻⁵	5.0	10 ⁻⁶ - 10 ⁻⁴
Cabaniss et al. [95]	4-9	0.10	10 ⁻⁶	5.0	10 ⁻⁴ - 10 ⁻²
Buffle et al. [96]	5 - 6.5	0.10	10 ⁻⁵	35	10 ⁻⁴ - 10 ⁻³

EXPERIMENTAL

The competitive binding of Ca(II), Mg(II) and Ni(II) by the Armadale FA in model systems was investigated by employing the CLEM with Chelex-100 as the competing ligand. GFAAS has been employed for monitoring the rate of uptake of the metals by Chelex-100. For the GFAAS, Perkin-Elmer Zeeman atomic absorption spectrophotometer, model 4100ZL, equipped with an AS-70 autosampler and transversely heated graphite tubes (Perkin-Elmer), were used. The kinetic model (CLEM), method of the kinetic measurement and the properties of the Armadale FA have been described in Chapter 4.

Reagents and materials

Standard solutions (1000 $\mu\text{g} / \text{mL}$) of Ca^{2+} (from carbonate) and Mg^{2+} (from nitrate) were purchased from Fisher Scientific Company, NJ, USA. Ultrapure water of resistivity 18.2 $\text{M}\Omega \cdot \text{cm}$ was obtained direct from a Milli-Q-Plus water purification system (Millipore Corporation).

Test solutions

Three types of model aqueous solutions were used: (1) solutions containing constant concentrations of Ni(II) [$3.4 \times 10^{-7} \text{ M}$] and the FA [$2.5 \times 10^{-5} \text{ M}$], and varying concentrations of Ca(II); (2) solutions containing constant concentrations of Ni(II) [$3.4 \times 10^{-7} \text{ M}$] and the FA [$2.5 \times 10^{-5} \text{ M}$], and varying concentrations of Mg(II); (3) solutions

containing fixed concentrations of Ni(II) [3.4×10^{-7} M] and the FA [2.5×10^{-5} M] and varying concentrations of Ca(II) and Mg(II). For this work the [Ni] / [FA] mole ratio of 0.01 was used. If the FA has ~1% strong binding sites, then the mole ratio of metal to the FA for the occupation only of the strong binding sites of the FA was ~0.01. In the previous Chapter, it has been shown that the DOC in the Rideau River surface water samples was 6.5 mg of C/L. Assuming 40%-99% of the DOC was present as a humic substance [3], the concentration of humic substances in the Rideau River surface waters was estimated to be roughly 1.5×10^{-5} M to 3.5×10^{-5} M, assuming its bidentate complexing capacity for Ni(II) is of the order of 5.4×10^{-3} mol/g [82]. Therefore, for this work, 2.5×10^{-5} M FA was used to simulate freshwaters in the laboratory. Model solutions were prepared by spiking ultrapure water with appropriate volumes of standard solutions of the metals and of the FA solution (1.0219 g / L). The spiked samples were equilibrated for 24 hours, and then the pH of the model solutions was measured using a Fisher Scientific Accumet 925 pH / ion meter. The pH meter was calibrated with two standard pH buffer solutions (pH 7 and pH 10). The pH of all the test solutions was adjusted to $\text{pH } 8.0 \pm 0.5$ (to simulate freshwaters) using dilute HNO_3 (ULTREX II) or dilute NaOH (electrolytically purified) solutions. The changes in the pH between the beginning and the end of the experiment were found to be $< \pm 0.5$ pH unit. The variations of the pH units were not systematic. Sodium perchlorate (NaClO_4) was used to fix the ionic strength of all the test solutions at 6×10^{-3} M.

QA /QC

Quality control for metal analyses included repeated injections and periodic (two in every two-hour operation of the equipment) analysis of Certified Reference Standard, NIST

1643d. A prior set of samples was re-run if the analysed value differed from the Reference Standard's certified value by > 20%. The relative standard deviation among replicate determinations was typically $\leq 5\%$.

RESULTS AND DISCUSSION

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

For the sake of completeness of experimental data, the effect of varying concentrations of Ca(II) on the release of Ni(II) and also of Ca(II) was studied using the same model solutions and the same experimental conditions. Figure 22(a) shows the results of the former and Figure 22(b) the results of the latter. Figure 22(a) is interpreted as follows. In the absence of Ca(II), the strong binding sites of the FA are occupied by Ni(II), forming strong complexes which are inert (top curve). However, the presence of high and sharply-increasing concentrations of Ca(II) ($1 \times 10^{-4} - 2 \times 10^{-3}$ M) (each curve from top to bottom), has a dramatic effect on the lability of the Ni(II)-FA complexes, where high concentrations of Ca(II) compensates for its relatively weak fulvic-binding affinities and results in formation of weak Ni(II)-FA complexes which are labile. The experimental data presented in Figure 23(a) were analyzed using a non-linear regression method. The values for kinetic parameters of the Ni(II)-FA complexes in the presence of varying concentrations of Ca(II) and constant concentrations of Ni(II) and the FA are presented in Table 20, which shows that in the presence of the relatively high concentration of Ca(II) (2×10^{-3} M), the Ni(II)-FA complex has a faster dissociation rate coefficient ($k_1 = 7.0 \times$

10^{-2} s^{-1}) than in the presence of the relatively lower concentration of Ca(II) ($1 \times 10^{-3} \text{ M}$), ($k_1 = 6.5 \times 10^{-3} \text{ s}^{-1}$), and in the presence of still lower concentration of Ca(II) ($1 \times 10^{-4} \text{ M}$), ($k_1 = 2.6 \times 10^{-3} \text{ s}^{-1}$), and in the absence of Ca(II) , ($k_1 = 2.0 \times 10^{-4} \text{ s}^{-1}$). These results are consistent with the interpretation of the lability / inertness of the Ni(II)-FA complexes given above. Figure 22(b) shows that the Ca(II)-FA complexes are ~100% labile (almost all the Ca(II) are released within ~120s) even at the relatively low concentration of $1 \times 10^{-4} \text{ M Ca(II)}$, compared to ~90% of Ni(II)-FA complexes remaining inert.

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

Similar studies were done on the effect of varying concentrations of Mg(II) on the release of Ni(II) and also of Mg(II) , using the same model solutions and the same experimental conditions. Figure 23(a) shows the results of the former and Figure 23(b) the results of the latter. Figures 23(a) and 23(b) show a behaviour similar to that in Figures 22 (a) and 22 (b). In the absence of Mg(II) , Ni(II) occupies the small number of strong binding sites of the FA, forming strong Ni(II)-FA complexes which are inert (top curve). However, the presence of high and sharply-increasing concentrations of Mg(II) ($1 \times 10^{-4} - 2 \times 10^{-3} \text{ M}$) (each curve from top to bottom) has a dramatic effect on the lability of the Ni(II)-FA complexes, where high concentrations of Mg(II) compensates for its relatively weak fulvate-binding affinities, resulting in formation of weak Ni(II)-FA complexes which are labile. Figure 23(b) shows that the Mg(II)-FA complexes are ~100% labile (almost all the Mg(II) are released within ~120s) even at the relatively low concentration of $1 \times 10^{-4} \text{ M}$

Figure 22. Effect of varying concentrations of Ca(II) on the kinetic lability of the (a) Ni(II)-FA and (b) Ca(II)-FA complexes in model systems containing constant concentrations of Ni(II) and the Armadale FA. pH 8.0 ± 0.5 , ionic strength 6×10^{-3} M, temperature 296 K. The error bar represents 95.5 % confidence intervals. (O), $[\text{Ni(II)}] 3.4 \times 10^{-7}$ M + $[\text{FA } 2.5 \times 10^{-5}$ M]; (Δ), $[\text{Ni(II)}] 3.4 \times 10^{-7}$ M + $[\text{Ca(II)}] 1 \times 10^{-4}$ M + $[\text{FA } 2.5 \times 10^{-5}$ M]; ($\square$), $[\text{Ni(II)}] 3.4 \times 10^{-7}$ M + $[\text{Ca(II)}] 1 \times 10^{-3}$ M + $[\text{FA } 2.5 \times 10^{-5}$ M]; ($\diamond$), $[\text{Ni(II)}] 3.4 \times 10^{-7}$ M + $[\text{Ca(II)}] 2 \times 10^{-3}$ M + $[\text{FA } 2.5 \times 10^{-5}$ M].

Table 20. Kinetic parameters of the Ni(II)-FA complexes in the presence of varying concentrations of Ca(II) in model systems containing constant concentrations of Ni(II) and the Armadale FA, measured by 4100ZL GFAAS. pH 8.0 ± 0.5 , ionic strength 6×10^{-3} M, temperature 296 K.

[Ca(II)], M	Kinetically distinguishable components			Dissociation rate coefficients	
	C ₁ (%)	C ₂ (%)	inert*	C ₁ k ₁ × 10 ³ (s ⁻¹)	C ₂ k ₂ × 10 ⁵ (s ⁻¹)
nil	6.1 ± 0.1	94.0 ± 0.2	—	0.2 ± 0.1	0.1 ± 0.0
1 × 10 ⁻⁴	12.2 ± 0.3	87.7 ± 0.5	—	2.6 ± 0.1	0.3 ± 0.1
1 × 10 ⁻³	20.0 ± 0.6	79.8 ± 0.4	—	6.5 ± 0.1	3.2 ± 0.1
2 × 10 ⁻³	39.0 ± 2.0	48.9 ± 1.0	12.0 ± 0.4	70.0 ± 0.1	85.0 ± 0.4

*inert component is defined as having $k < 10^{-6} \text{ s}^{-1}$. The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Mg(II), compared to ~90% of Ni(II)-FA complexes remaining inert. The values for the kinetic parameters of the Ni(II)-FA complexes in the presence of varying concentrations of Mg(II) are presented in Table 21.

Effect of varying concentrations of Ca(II) and Mg(II) together on the kinetic lability of the Ni(II)-FA complexes

Figure 24 presents the effect of the varying concentrations of Ca(II) and Mg(II) on the release of free Ni^{2+} ion plus other small nickel species from the Ni(II)-FA complexes in the model systems, and shows a behaviour similar to that in Figures 22 and 23. In the absence of Ca(II) and Mg(II), Ni(II) occupies the small number of the strong binding sites of the FA, forming strong Ni(II)-FA complexes, which are inert (top curve). However, the presence of high and sharply-increasing concentrations of Ca(II) and Mg(II) ($2 \times 10^{-4} - 2 \times 10^{-3}$ M) (each curve from top to bottom) has a dramatic effect on the lability of the Ni(II)-FA complexes, where high concentrations of Ca(II) and Mg(II) compensate for their relatively weak fulvate-binding affinities, resulting in formation of weak Ni(II)-FA complexes which are labile. The values for the kinetic parameters of Ni(II)-FA complexes in the presence of varying concentrations of Ca(II) and Mg(II) and constant concentrations of Ni(II) and the FA are presented in Table 22. Comparison of Figures 22 and 23 with Figure 24 reveals that Ni(II)-FA complexes are more labile in the presence of Ca(II) and Mg(II) when they are together, compared with the lability of the Ni(II)-FA complexes when either Ca(II) or Mg(II) is present alone. We have investigated whether this is simply a mass effect or a synergistic effect.

Figure 23. Effect of varying concentrations of Mg(II) on the kinetic lability of the (a) Ni(II)-FA and (b) Mg(II)-FA complexes in model systems containing constant concentrations of Ni(II) and the Armadale FA. pH 8.0 ± 0.5 , ionic strength 6×10^{-3} M, temperature 296 K. The error bar represents 95.5 % confidence intervals. (O), [Ni(II) 3.4×10^{-7} M] + [FA 2.5×10^{-5} M]; (Δ), [Ni(II) 3.4×10^{-7} M] + [Mg(II) 1×10^{-4} M] + [FA 2.5×10^{-5} M]; ($\square$), [Ni(II) 3.4×10^{-7} M] + [Mg(II) 3.4×10^{-7} M] + [FA 2.5×10^{-5} M]; ($\diamond$), [Ni(II) 3.4×10^{-7} M] + [Mg(II) 2×10^{-3} M] + [FA 2.5×10^{-5} M].

Table 21. Kinetic parameters of the Ni(II)-FA complexes in the presence of varying concentrations of Mg(II) in model systems containing constant concentrations of Ni(II) and the Armadale FA, measured by 4100ZL GFAAS. pH 8.0 ± 0.5 , ionic strength 6×10^{-3} M, temperature 296 K.

[Mg(II)], M	Kinetically distinguishable components			Dissociation Rate Coefficients	
				C ₁	C ₂
	C ₁ (%)	C ₂ (%)	inert*	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
nil	6.1 ± 0.1	94.0 ± 0.2	—	0.2 ± 0.1	0.1 ± 0.0
1 × 10 ⁻⁴	10.1 ± 0.2	90.0 ± 1.0	—	2.6 ± 0.2	0.4 ± 0.1
1 × 10 ⁻³	24.5 ± 1.0	75.0 ± 1.0	—	6.9 ± 0.3	3.6 ± 0.1
2 × 10 ⁻³	41.5 ± 1.0	44.5 ± 1.0	14.0 ± 0.3	75.0 ± 0.3	80.0 ± 0.2

*inert component is defined as having $k < 10^{-6} \text{ s}^{-1}$. The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Figures 22, 24 and Tables 20, 22 show that in the presence of 1×10^{-3} M Ca^{2+} alone (Figure 22, the second curve from bottom), the percentage of the labile fraction of the Ni(II)-fulvic acid complexes (C_1 % = 20.0) is lower than that in the presence of 5×10^{-4} M Ca^{2+} and 5×10^{-4} M Mg^{2+} when they are present together (Figure 24, the second curve from bottom, C_1 % = 32.9). Hence, the enhanced lability of the Ni(II)-FA complexes when Ca(II) and Mg(II) are present together compared with the lability of the Ni(II)-FA complexes when either Ca(II) or Mg(II) is present alone, is due to synergy, and is not a mass effect.

The WHAM predictions and the experimental results

The Windermere Humic Aqueous Model (WHAM), a chemical equilibrium model and a computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances [102], was used to predict the competition effects of Ca(II) and Mg(II) on the binding of Ni(II) by the FA, and to compare its predictions with the above experimental results. The WHAM takes into account the interactions of metals with: (i) inorganic ligands (OH^- , HCO_3^- , CO_3^{2-} , SO_4^{2-} , Cl^-), using conventional equilibrium formulations and equilibrium constants from the literature; and (ii) humic substances using Humic Ion Binding Model V [103]. The WHAM predictions presented in Table 23 agree reasonably well with the experimental results, considering that the WHAM calculations have not taken into account the particular characteristics of the Armadale FA and that the WHAM is based on equilibrium calculations and does not take into account non-equilibrium speciation. Also, the Model V

Figure 24. Effect of varying concentrations of Ca(II) and Mg(II) on the kinetic lability of the Ni(II)-FA complexes in model systems containing constant concentrations of Ni(II) and the Armadale FA. pH 8.0 ± 0.5 , ionic strength 6×10^{-3} M, temperature 296 K. The error bar represents 95.5 % confidence intervals.

(O), [Ni(II) 3.4×10^{-7} M] + [FA 2.5×10^{-5} M]; (Δ), [Ni(II) 3.4×10^{-7} M] + [Ca(II) 1×10^{-4} M] + [Mg(II) 1×10^{-4} M] + [FA 2.5×10^{-5} M]; (□), [Ni(II) 3.4×10^{-7} M] + [Ca(II) 5×10^{-4} M] + [Mg(II) 5×10^{-4} M] + [FA 2.5×10^{-5} M]; (◇), [Ni(II) 3.4×10^{-7} M] + [Ca(II) 1×10^{-3} M] + [Mg(II) 1×10^{-3} M] + [FA 2.5×10^{-5} M].

Table 22. Kinetic parameters of the Ni(II)-FA complexes in the presence of varying concentrations of Ca(II) and Mg(II) in model systems containing constant concentrations of Ni(II) and the Armadale FA, measured by 4100ZL GFAAS. pH 8.0 $\pm$ 0.5, ionic strength 6×10^{-3} M, temperature 296 K.

[Ca(II)], M	[Mg(II)], M	Kinetically distinguishable components			Dissociation Rate Coefficients	
		C ₁ (%)	C ₂ (%)	inert*	C ₁ $k_1 \times 10^3 \text{ (s}^{-1}\text{)}$	C ₂ $k_2 \times 10^5 \text{ (s}^{-1}\text{)}$
nil	nil	6.1 $\pm$ 0.1	94.0 $\pm$ 0.2	—	0.2 $\pm$ 0.1	0.1 $\pm$ 0.0
1×10^{-4}	1×10^{-4}	13.3 $\pm$ 0.3	87.2 $\pm$ 0.5	—	3.5 $\pm$ 0.1	0.4 $\pm$ 0.0
5×10^{-4}	5×10^{-4}	32.9 $\pm$ 0.6	67.1 $\pm$ 0.4	—	8.9 $\pm$ 0.1	4.8 $\pm$ 1.0
1×10^{-3}	1×10^{-3}	48.9 $\pm$ 2.0	39.1 $\pm$ 1.0	10.8 $\pm$ 0.4	85.0 $\pm$ 0.2	83.0 $\pm$ 4.0

*inert component is defined as having $k < 10^{-6} \text{ s}^{-1}$. The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 23. WHAM computer calculations for the speciation of nickel in model systems containing varying concentrations of Ca(II), Mg(II) and constant concentrations of Ni(II) and the Armadale FA.

		WHAM PREDICTIONS			EXPERIMENTAL	
	[Ca(II), M	[Mg(II)], M	Free Ni ²⁺ ions plus other small Ni- species (%)	Non-specific binding of nickel (%)	Intrinsic binding of nickel (%)	Free Ni ²⁺ ions plus other small Ni-species (%)
1 x 10 ⁻⁴	nil		13.4	1.0	85.6	12.2
1 x 10 ⁻³	nil		36.2	0.2	63.6	20.0
2 x 10 ⁻³	nil		45.6	0.1	54.2	39.0
1 x 10 ⁻⁴	1 x 10 ⁻⁴		18.9	0.6	80.4	13.3
5 x 10 ⁻⁴	5 x 10 ⁻⁴		36.2	0.2	63.6	32.9
1 x 10 ⁻³	1 x 10 ⁻³		45.6	0.1	54.2	48.9

(unlike the Model VI [101]) is not optimized for the competition of major ions, Ca(II) and/or Mg(II), and the ionic strength effects.

Figures 22-24 and Tables 20-22 show that when high levels of Ca(II) and Mg(II) ($\sim 10^{-3}$ M) are present with Ni(II) in model solutions, increasing the concentrations of Ca(II) and/or Mg(II) results in releasing greater fractions of the bound nickel from Ni(II)-FA complexes as free Ni^{2+} ion. The results described above are probably the composite effect of the following factors.

When Ca(II) and Mg(II) are present in high molar concentrations (several orders of magnitude greater than that of Ni(II)), the high concentrations of Ca(II) and Mg(II) compensate for their relatively weak fulvic acid-binding affinities. The high concentrations of Ca^{2+} and Mg^{2+} have important effects on the total binding energy involved in the binding of Ni(II) by the FA, i.e. on the sum total of the energy involved in the covalent bonding and the coulombic attraction. It appears that the type of the FA discrete sites which dominate the binding under the above experimental conditions are the sites where electrostatic interactions dominate; for these sites, there is also direct competition among Ca(II), Mg(II) and Ni(II). Also, there is competition for counterion accumulation in the diffuse part of the electrical double layer of the negatively-charged fulvate anions, and reduction in the net electric charge on the fulvic acid molecule by Ca^{2+} and Mg^{2+} ions binding to certain sites, which diminishes the electrostatic contribution to the Ni^{2+} binding at other sites, resulting in formation of weak Ni(II)-FA complexes which are labile.

The predictions of the WHAM are that for the target trace metal Ni^{2+} ions, binding occurs almost exclusively at bidentate sites, at both low and high ionic strengths. However, the concentrations of Ca^{2+} and Mg^{2+} , and its pK_{MHA} and pK_{MHB} values (where A and B are two types of proton-binding groups, one (type A) being relatively acidic (mainly carboxyl groups) and the other (type B) less acidic (phenolic groups)) are such that Ca^{2+} and Mg^{2+} ions bind mostly at the monodentate and diffuse double layer sites [104]. This is significant for the competition, because it means that the target trace metal Ni^{2+} ions and major cations, Ca^{2+} and Mg^{2+} , are substantially separated in their binding, so that to a considerable degree they are not competing for the same sites [100].

CHAPTER 8

CHEMICAL SPECIATION AND TOXICITY OF NICKEL SPECIES IN NICKEL- POLLUTED NATURAL WATERS FROM THE SUDBURY AREA (CANADA)

INTRODUCTION

Trace metal complexation by dissolved organic carbon (DOC) plays an important role in solubilizing metals and in regulating transport, bioavailability, and toxicity of metals in freshwaters. Since most of these metals in freshwaters are DOC-bound, their study is of primary importance in understanding the behaviour and fate of these metals in freshwaters [19,105].

Chapter 4 has dealt with the effect of the [metal] / [FA] mole ratios on the dissociation rate of the metal-FA complexes in model systems. The above study is now extended to the dissociation of metal complexes in freshwaters with Ni(II) as the target metal. Here, the release of free nickel from the Ni(II)-DOC complex using nickel-polluted freshwaters from the Sudbury (Canada) area was investigated. CLEM with Chelex-100 as the competing ligand was used to measure the rate of release of free Ni^{2+} ion plus small nickel species arising from the dissociation of the Ni(II)-DOC complexes. The water

samples were subjected to bioassay toxicity tests in the laboratory of Dr. David R.S. Lean, NSERC-Industrial Research Chair in Ecotoxicology, University of Ottawa, Ottawa, Canada, to relate the nickel speciation to toxicity.

Nickel is an essential micronutrient for some marine algae, but micromolar Ni^{2+} , free ion i.e. $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$, has been reported to be toxic to a variety of algae, invertebrates, and fish [90]. However, interactions of Ni(II) with FA and other dissolved organic matter have not been much studied [90,106], perhaps because of absence of convenient, sensitive methods for measuring free Ni^{2+} ion in the presence of organic complexants [107]. A recent study [108] of nickel speciation and complexation kinetics in freshwaters by ligand exchange and differential pulse cathodic stripping voltammetry has found that more than 99.9 % of dissolved nickel is bound by organic ligands with strong affinity ($\log K$ 12.1 – 14.9). Langford et al. [28] have used Competing Ligand Exchange Method for kinetic speciation of nickel with the chromophore 4-(2-pyridylazo) resorcinol (PAR) as a competing ligand and spectrophotometric detection of the NiPAR complex. Nimmo and Chester [109] have reported the dissolved organic complexes of nickel and cobalt in a series of north western Mediterranean rain water samples by applying cathodic stripping voltammetry to the samples before and after UV radiation to determine the concentrations of dissolved Ni and Co. Their results indicate the existence of very stable, dissolved Ni and Co complexes of organic complexants in the rainwater samples [109]. However, most of the reported investigations of nickel were done on unpolluted freshwaters, and very little is known about the nickel in metal-polluted freshwaters.

Polluted freshwaters from Ni mining and refining areas typically contain Ni(II) along with other 3d transition metals and strong ligands (anthropogenic and natural) along with naturally-occurring humic substances and large concentrations of Ca(II) and Mg(II). The above multimetal and multiligand mixtures set the stage for double exchange reactions (metal-metal exchange and ligand-ligand exchange) to occur, involving reestablishment of coordination equilibrium [110,111], which is sometimes slow. In a ground-breaking paper published recently, Xue et al. [108] highlighted the significance of slow kinetics of Ni(II) complexation in freshwaters, and its consequences – the unpredictable concentration-levels of free-nickel ions, which have been reported to be toxic to freshwater biota [90]. Information on the competition between Ni(II) and major cations for naturally-occurring organic complexants in freshwaters is scarce. The competition of Ca(II) and Mg(II) with Ni(II) for binding sites of a well-characterized fulvic acid in model systems at constant pH and ionic strength is presented in the previous Chapter. In Chapter 7, it has been reported that in model systems containing humic substances and trace quantities of nickel and large excess of major cations, Ca^{2+} and Mg^{2+} , the competition of Ca^{2+} and Mg^{2+} with Ni^{2+} for binding sites on humic substances produces weak Ni(II)-humate complexes that are labile, releasing free Ni^{2+} ions [112]. The linkages between metal speciation, bioavailability and toxicity have been reported by some workers who have proposed models such as Free-Ion Activity Model (FIAM) [11], but the above linkages have not been conclusively demonstrated. The linkages between trace metal speciation, bioavailability and toxicity, are still questionable and must be firmly established.

The specific objectives of the study presented in this Chapter are: (i) to determine the release of free plus labile nickel from the Ni(II)-DOC complex in nickel-polluted freshwaters; and (ii) to determine the effect of major cations, Ca^{2+} and Mg^{2+} , on the release of free plus labile nickel from the Ni(II)-DOC complex; and (iii) to establish linkage between nickel speciation, bioavailability and toxicity.

EXPERIMENTAL

Dissolved Organic Carbon

The dissolved organic carbon (DOC) of the filtered water samples was measured using O I Analytical Model 1010 TOC Analyzer.

Sampling sites and sampling protocol

Water samples (6.6 L) were collected in October 1998 and in June 1999 from several sampling sites in the Sudbury (Canada) area using pre-cleaned Teflon bottles. The sampling sites were chosen with a view to studying the applicability of the nickel speciation techniques developed in our laboratories [112-114] using model systems to nickel-polluted natural waters. The sites were chosen upstream and downstream of the nickel-tailing ponds, and outflows from the treatment tanks receiving effluents from nickel ore processing and smelting operations. A list of the sampling sites, pHs, conductivities and DOC values of all the samples is presented in Table 24. The sample temperatures were 297 K in 1998 and 298 K in 1999. The samples were filtered through 0.45 μm membrane filters (CN-6 Metrice1, 47 mm diameter, Gelman, Ann Arbor, MI) in

our laboratory within 24 h of the sample collection to filter out the particulate matter. These filters, made of mixed cellulose esters, had the advantage of providing filtrates having low blanks for the metals. Each 0.45 μm filter was able to filter ~500 mL water sample without clogging. A subsample (250 mL) of the filtered samples was acidified with HNO_3 (Ultrex II) to pH 1.5. The acidified samples were used to determine the total metal concentrations of trace metals (Ni, Cu, Co, Al, Fe) using GFAAS, and major metals using flame atomic absorption spectrometry (Ca, Mg), and flame atomic emission spectrometry (Na, K), and total anions using ion chromatography (NO_3^- , F^- , Cl^- , Br^- , SO_4^{2-}). The remaining filtered samples were stored in the dark at 4°C and were used for the kinetic measurement within 72 h of filtration.

QA /QC

Quality control for Ni analyses included repeated injections and periodic (two in every two-hour operation of the equipment) analysis of Certified Reference Standard, NIST 1643d. A prior set of samples was re-run if the analysed value differed from the Reference Standard's certified value by > 20%. The relative standard deviation among replicate determinations was typically $\leq 5\%$.

Bioassay toxicity tests

The bioassay toxicity tests using the alga *Selenastrum capricornutum* and the zooplankter *Daphnia magna* were conducted for the water samples collected in 1999 in the laboratory of Dr. David R.S. Lean, NSERC-Industrial Research Chair in Ecotoxicology, University of Ottawa. The detailed experimental procedure of bioassay toxicity tests was described elsewhere [115].

Table 24. pH, conductivity and DOC of the Sudbury area water samples collected in 1998 and 1999 .

Sample No.	Sampling sites	pH		Conductivity, $\mu\text{S/cm}$		DOC, mg/L	
		1998	1999	1998	1999	1998	1999
1	Copper Cliff, wastewater treatment outflow	6.4	6.2	1980	2920	7.09	7.09
2	Junction Creek upstream of Copper Cliff	***	7.8	***	800	***	6.91
3	Kelly Lake	6.6	7.6	1250	1345	6.45	6.84
4	Alice Lake	***	7.2	***	200	***	6.03
5	Conservation Area	6.9	7.6	898	831	6.44	6.47
6	Coniston Creek downstream	6.8	8.1	528	420	6.03	6.98
7	Moose Lake treated discharge	6.9	7.9	1520	1671	6.47	6.39
8	Moose Creek upstream	6.6	7.6	1060	1640	6.40	3.51
9	Onaping River downstream	6.8	***	93.2	***	6.45	***
10	Onaping River downstream of the sewage treatment plant	6.9	7.2	64.1	221	6.51	6.39

*** Sample not collected

RESULTS AND DISCUSSION

Effect of Ni/DOC (mol/g) ratio on the kinetic lability of the Ni(II)-DOC complexes in the Sudbury area water samples

Percentage of the Ni(II)-DOC complexes remaining undissociated in the solution is plotted as a function of time for the water samples collected in October 1998 (Figure 25) and June 1999 (Figure 26). Dissociation rate coefficients and concentrations of the kinetically distinguishable components of the Ni(II) complexes are presented in Tables 25 (1998) and 26 (1999), which show that the fastest kinetically distinguishable component (C_1) representing ~ 30-95 % of the total Ni(II)-DOC complexes has a dissociation rate coefficient similar to that reported for $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$ in the literature [28]. Figures 27 and 28 show that for most of the samples, the concentration of free plus labile nickel (a), and the corresponding dissociation rate coefficients (b), increases with increasing Ni / DOC (mol/g) ratio. If it is assumed that the DOC is mainly humic substances [3], with increasing concentrations of nickel at the fixed, low, concentration of the DOC (the DOC values are almost the same for all the samples, as shown in Table 24), the Ni / DOC ratios become large enough to saturate all the strong binding sites (~1-10% of the total binding sites) of the DOC; the remaining Ni(II) then binds to the weak binding sites (~99-90% of the total binding sites) of the DOC. These weak Ni(II) complexes are labile, releasing free Ni^{2+} ion, which are probably bioavailable. In Figures 27 and 28, in the absence of knowledge of the concentrations of binding sites of the DOC, and hence, of the Ni / DOC mole ratio which should have been plotted, the Ni / DOC (mol / g) ratio have been plotted. Since the DOC concentrations of the water

samples are similar (Table 24), Ni / DOC (mol / g) ratio is a reasonable surrogate for Ni / DOC mole ratio.

Table 25 for the 1998 samples shows that the Ni / DOC (mol / g) ratio ranged from 9.8×10^{-5} to 7.3×10^{-3} and the fraction of labile nickel species varied from 50 to 97%. For sample No. 8, the Ni / DOC ratio (7.3×10^{-3} mol / g) was much higher than that (7.2×10^{-4} mol / g) of sample No. 1. Yet, both sample No. 1 and sample No. 8 have almost the same fraction of labile species ($C_1 = 95.0 \%$), and have almost the same dissociation rate coefficients ($k_1 = 6.0 \times 10^{-3} \text{ s}^{-1}$). It is also noteworthy that sample No. 1 has higher conductivity (1.98 mS/cm), which is probably due to Ca^{2+} and Mg^{2+} , than that of sample No. 8 (1.06 mS/cm).

Table 26 for the 1999 samples shows that the Ni / DOC (mol / g) ratio ranged from 9.3×10^{-5} to 1.0×10^{-3} and the fraction of labile nickel ranged from 31 to 88%. In general, the fraction of labile nickel and the corresponding rate coefficients is lower in samples with low Ni / DOC ratio. However, there are a few exceptions. For sample No. 1, (Ni / DOC = 3.5×10^{-4} mol/g) and sample No. 8, (Ni / DOC = 6.0×10^{-4} mol/g), the Ni / DOC ratios are lower than those of the following samples: sample No. 2 (Ni / DOC = 1.0×10^{-3} mol/g), sample No. 4 (Ni/DOC = 8.2×10^{-4} mol/g) and sample No. 3 (Ni/DOC = 6.8×10^{-4} mol/g). Yet, both sample No. 1 and sample No. 8 have higher dissociation rate coefficients (Figure 28b) than those of sample Nos. 2, 3 and 4. These results can be explained as follows.

Metal competition. As demonstrated in the previous Chapter, the presence of a massive excess of major cations, Ca^{2+} and Mg^{2+} , results in release of larger fractions of bound

nickel as free Ni^{2+} ion, as seen from Table 27: the higher concentrations of major cations, Ca^{2+} and Mg^{2+} , in sample No. 1 ($8.8 \times 10^{-3} \text{ M Ca}^{2+}$ and $1.6 \times 10^{-3} \text{ M Mg}^{2+}$), and sample No. 8 ($1.2 \times 10^{-2} \text{ M Ca}^{2+}$), results in release of larger fraction of free plus labile nickel compared to sample No. 2 ($1.4 \times 10^{-3} \text{ M Ca}^{2+}$ and $1.2 \times 10^{-3} \text{ M Mg}^{2+}$), sample No. 4 ($6.4 \times 10^{-4} \text{ M Ca}^{2+}$) and sample No. 3 ($1.8 \times 10^{-3} \text{ M Ca}^{2+}$). Such high concentrations of major cations are not normally found in the poorly buffered lakes and rivers of this region, and may have resulted from effluent treatment activities. Previous work [113] has shown that, Cu(II) and Co(II), at concentrations (Table 27) that were present in the water samples, would have no effect on the DOC-bound nickel, whereas the Fe and Al (Table 27) present in the samples do have an appreciable effect of releasing Ni^{2+} ions from DOC-bound nickel (but not reported here). However, the major cations, Ca^{2+} , Mg^{2+} , at the high concentrations that were present in the water samples, may have greater effect of releasing Ni^{2+} ions and other labile nickel species from the DOC-bound nickel. The result of sample No. 8 might be also partly due to the fact that the DOC for this sample was lower (3.51 mg/L) than that of the other samples (~6-7 mg/L, Table 24).

As Table 27 shows, the major anions present in the samples are NO_3^- and SO_4^{2-} . It is reported in the literature [108] that in freshwaters, inorganic species of nickel are NiCO_3^0 and Ni^{2+} . However, NiCO_3 was not determined for the water samples. Since NiCO_3 is labile, it contributes to the free Ni^{2+} ions and other nickel labile species determined by CLEM/GFAAS, and hence, its omission does not affect the main conclusions of this Chapter.

Polyelectrolyte effects of DOC [99]. When a cation reacts with an acidic functional group of a polyanion, the chemical free energy is augmented by the long-range coulombic attraction emanating from all the neighbouring, non-reacting, negative sites [99]. At relatively high pH, this polyelectrolyte effect can strengthen greatly the binding of metal ions by polyanions. The change in free energy of reaction (ΔG^0) between a metal M and the functional group L is the sum of the chemical free energy change (ΔG_{chem}^0) due to the reaction with the functional group itself and the coulombic free energy change (ΔG_{coul}^0) due to electrostatic interactions between M and all the charges on the polyanion [99]

$$\Delta G^0 = \Delta G_{\text{chem}}^0 + \Delta G_{\text{coul}}^0 \quad (15)$$

According to equation 15, the total binding energy of the Ni-DOC complex has two components: 1) chemical binding energy and, 2) coulombic binding energy. Ca^{2+} and Mg^{2+} could compete with Ni^{2+} for specific binding sites, and because of the competitive advantage of Ca^{2+} and Mg^{2+} (especially Ca^{2+}) over Ni^{2+} in terms of their rates of exchange of coordinated water [91] and their presence in overwhelming excess over the Ni^{2+} , could outcompete Ni^{2+} for specific binding sites, and also, preferentially bind to other sites for which they may have greater affinity for binding than Ni^{2+} . Moreover, the overwhelming excess of Ca^{2+} and Mg^{2+} would result in a large amount of Ca^{2+} and Mg^{2+} in the diffuse electrical double layer of the DOC polyanions, thereby decreasing significantly the total binding energy of the Ni-DOC by screening the charge on the DOC polyanions. Na^+ and K^+ , although not competing with nickel for the specific binding sites of DOC, would nevertheless provide strong screening of the DOC polyanionic charges by

their presence in large excess (Table 27). This would result in further weakening of the Ni-DOC binding, with the consequence that more free Ni^{2+} ions and other labile nickel species would be released from the Ni-DOC complex, as observed.

The WHAM predictions and the experimental results

The Windermere Humic Aqueous Model (WHAM), Model V, a chemical equilibrium model and a computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion binding by humic substances [102], was used to predict the nickel binding by DOC in the water samples and to compare its prediction with the above experimental results. As suggested by Tipping [116], the originator of WHAM, in the WHAM calculation only 50% of the DOC was assumed to be "active" fulvic acid. The WHAM predictions presented in Table 28 agree reasonably well with the experimental results ($r^2 = 0.965$) (Figure 29), even though the Model V (unlike the Model VI [101] is not optimized for the competition of major ions, Ca(II) and/or Mg(II) , and the ionic strength effects.

Bioavailability and ecotoxicological tests on the 1999 samples

Figure 30 shows satisfactory correlation between (a) % inhibition and Ni / DOC (mol/g) ratio ($r^2 = 0.732$), (b) % inhibition and free plus labile nickel concentration ($r^2 = 0.690$); and (c) % inhibition and dissociation rate coefficients ($r^2 = 0.752$). As explained earlier for Figures 25 and 26, the higher Ni / DOC mol/g ratio meant more Ni(II) was bound to the weaker sites, making the Ni(II)-DOC complex labile. According to Free-Ion Activity

Model [11], free metal-ion activity is a good predictor of bioavailability, and hence, of metal toxicity. In Figures 30(a) and 30(b), sample No.1 appeared to be an outlier, and as shown in Table 27, is high in copper concentration (9.0×10^{-7} M). Sample No. 2 also contained copper but at a lower concentration (6.0×10^{-7} M) and fitted with the other data better. All other samples were low in Cu.

Both the results of kinetic speciation and bioassay toxicity tests of the nickel species in the nickel-polluted water samples from the Sudbury (Canada) area shows that the release of free Ni^{2+} ion and other labile nickel species from the Ni(II)-DOC complex and the resulting toxicity, in general, depends on the Ni / DOC ratio. The kinetic lability of the nickel species varies in a reasonable way with the Ni / DOC (mol / g) ratio, suggesting that the kinetic speciation model gives chemically significant metal species. The results of both the kinetic speciation and bioassay toxicity tests agree reasonably well, suggesting that kinetic lability is a good predictor of nickel toxicity.

Figure 25. Effect of Ni / DOC (mol/g) ratio on the kinetic lability of the Ni(II)-DOC complexes in the Sudbury area water samples collected in October 1998. Symbols for samples are arranged in increasing order of free plus labile nickel concentrations. The error bar represents 95.5% confidence intervals.

▽, Sample # 10, (Ni / DOC 9.8×10^{-5} mol/g); ○, Sample # 7, (Ni / DOC 1.4×10^{-4} mol/g); □, Sample # 6, (Ni / DOC 5.9×10^{-4} mol/g); ◇, Sample # 3, (Ni / DOC 2.0×10^{-3} mol/g); ●, Sample # 8, (Ni / DOC 7.3×10^{-3} mol/g); △, Sample # 1, (Ni / DOC 7.2×10^{-4} mol/g).

Figure 26. Effect of Ni / DOC (mol/g) ratio on the kinetic lability of the Ni(II)-DOC complexes in the Sudbury area water samples collected in June 1999. Symbols for samples are arranged in increasing order of free plus labile nickel concentrations. The error bar represents 95.5% confidence intervals.

○, Sample # 10, (Ni / DOC 9.3×10^{-5} mol/g); □, Sample # 6, (Ni / DOC 1.8×10^{-4} mol/g); △, Sample # 3, (Ni / DOC 6.8×10^{-4} mol/g); ▽, Sample # 4, (Ni / DOC 8.2×10^{-4} mol/g); ●, Sample # 8, (Ni / DOC 6.0×10^{-4} mol/g); ◇, Sample # 1, (Ni / DOC 3.5×10^{-4} mol/g).

Figure 27. (a) Concentrations of free plus labile nickel versus Ni / DOC (mol / g) ratio, and (b) dissociation rate coefficients versus Ni / DOC (mol / g) ratio, for the Sudbury area water samples collected in October 1998.

Figure 28. (a) Concentrations of free plus labile nickel versus Ni / DOC (mol / g) ratio, and (b) dissociation rate coefficients versus Ni / DOC (mol / g) ratio, for the Sudbury area water samples collected in June 1999.

Figure 29. The WHAM vs. the experimental results for the Sudbury area water samples collected in June 1999.

Figure 30. Algal yield expressed as a fraction of maximum algal yield as a function of (a) Ni / DOC (mol / g) ratio, (b) free plus labile nickel concentration, and (c) dissociation rate coefficients, k_1 .

Table 25. Kinetic parameters of the Ni(II)-DOC complexes in the Sudbury area water samples collected in October 1998.

Sample No.	Ni / DOC (mol / g)	Kinetically distinguishable components		Dissociation rate coefficients	
		C ₁ (%)	C ₂ (%)	C ₁ k ₁ x 10 ³ (s ⁻¹)	C ₂ k ₂ x 10 ⁴ (s ⁻¹)
1	7.2 x 10 ⁻⁴	96.6 ± 5.0	-	5.9 ± 0.1	-
3	2.0 x 10 ⁻³	87.9 ± 4.8	12.2 ± 0.5	5.5 ± 0.1	1.6 ± 0.1
5	5.3 x 10 ⁻⁴	69.5 ± 4.0	30.4 ± 1.0	4.5 ± 0.2	1.4 ± 0.5
6	5.9 x 10 ⁻⁴	71.5 ± 5.0	26.1 ± 1.7	5.1 ± 0.2	1.5 ± 0.5
7	1.4 x 10 ⁻⁴	62.9 ± 1.5	37.1 ± 1.3	4.0 ± 0.2	0.7 ± 0.2
8	7.3 x 10 ⁻³	95.3 ± 1.5	4.9 ± 0.1	6.3 ± 0.2	5.7 ± 0.2
9	2.3 x 10 ⁻⁴	65.9 ± 2.5	33.8 ± 0.8	4.1 ± 0.1	0.9 ± 0.1
10	9.8 x 10 ⁻⁵	50.0 ± 1.3	49.5 ± 1.8	3.0 ± 0.0	0.5 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 26. Kinetic parameters of the Ni(II)-DOC complexes in the Sudbury area water samples collected in June 1999.

Sample No.	Ni / DOC (mol / g)	Kinetically distinguishable components		Dissociation rate coefficients	
		C ₁ (%)	C ₂ (%)	k ₁ x 10 ³ (s ⁻¹)	k ₂ x 10 ⁴ (s ⁻¹)
1	3.5 x 10 ⁻⁴	88.0 ± 5.5	11.5 ± 0.6	7.1 ± 0.1	2.7 ± 0.1
2	1.0 x 10 ⁻³	88.2 ± 4.8	12.2 ± 0.5	6.9 ± 0.2	1.8 ± 0.1
3	6.8 x 10 ⁻⁴	75.6 ± 4.5	24.4 ± 1.0	5.4 ± 0.1	1.3 ± 0.1
4	8.2 x 10 ⁻⁴	82.7 ± 5.0	16.6 ± 0.6	6.1 ± 0.2	1.7 ± 0.1
5	2.8 x 10 ⁻⁴	71.5 ± 5.0	28.4 ± 1.0	5.1 ± 0.2	1.4 ± 0.5
6	1.8 x 10 ⁻⁴	48.1 ± 2.5	50.6 ± 1.8	5.0 ± 0.1	1.2 ± 0.5
7	1.2 x 10 ⁻⁴	40.0 ± 1.5	59.3 ± 3.1	4.5 ± 0.2	1.0 ± 0.2
8	6.0 x 10 ⁻⁴	87.8 ± 4.8	11.9 ± 0.5	9.5 ± 0.1	3.3 ± 0.1
10	9.3 x 10 ⁻⁵	30.7 ± 1.3	68.4 ± 3.7	4.0 ± 0.0	0.7 ± 0.1

The uncertainty represents 95.5 % confidence interval from the non-linear regression analysis.

Table 27. Concentrations of total cations and anions of the Sudbury area water samples collected in June 1999.

Sample No.	[Ni], μM	[Fe], μM	[Al], μM	[Cu], μM	[Co], μM	[Ca], mM	[Na], mM	[K], mM	[Mg], mM	[NO ₃], μM	[F], μM	[Cl], mM	[Br], μM	[SO ₄ ²⁻], mM
1	2.5	0.4	1.5	0.9	0.1	8.8	13	1.4	1.6	131.6	N.D.	2.7	N.D.	20
2	7.3	2.4	4.1	0.6	0.6	1.4	2.1	0.06	1.2	36.3	N.D.	3.2	N.D.	1.6
3	4.7	1.7	1.3	0.04	0.3	1.8	8.6	0.5	1.3	69	N.D.	2.9	4.3	5.4
4	4.9	0.8	4.5	0.09	N.D.	0.6	1.7	0.5	0.07	3.9	5.5	0.1	N.D.	0.6
5	1.8	1.8	1.8	N.D.	N.D.	1.8	3.9	0.04	1.1	N.D.	18	0.8	N.D.	1.1
6	1.3	1.6	7.6	0.1	N.D.	1.3	2.8	N.D.	0.5	71	N.D.	0.04	13	7.8
7	0.9	0.2	7.2	0.1	0.1	7.5	7.0	1.0	N.D.	137	N.D.	0.04	16	7.3
8	2.1	0.5	3.1	0.06	N.D.	12	8.0	1.0	N.D.	1474	23	0.01	N.D.	0.08
10	0.6	1.9	2.4	N.D.	N.D.	1.0	1.0	N.D.	N.D.	5.5	84	1.2	N.D.	3.4

Table 28. WHAM computer calculations for the speciation of nickel in the Sudbury area water samples collected in June 1999.

Sample No.	Ni / DOC, (mol / g)	WHAM predictions			Experimental
		Free Ni ²⁺ ions plus other small nickel species (%)	Non-specific binding of nickel (%)	Intrinsic binding of nickel (%)	
1	3.5×10^{-4}	98.5	0.2	15.9	88.0
2	1.0×10^{-3}	81.9	0.1	15.6	88.2
3	6.8×10^{-4}	79.7	0.2	21.1	75.6
4	8.2×10^{-4}	82.8	0.3	16.5	82.7
5	2.8×10^{-4}	75.8	0.1	31.1	71.5
6	1.8×10^{-4}	55.9	0.2	46.4	48.1
7	1.2×10^{-4}	45.5	0.2	56.3	40.0
8	6.0×10^{-4}	90.2	0.1	11.3	87.8
10	9.3×10^{-5}	36.5	0.3	65.1	30.7

SECTION 3

EXPERIMENTAL RESULTS OF METAL COMPETITION FOR BINDING BY HUMIC SUBSTANCES USING THE EQUILIBRIUM APPROACH

CHAPTER 9

AN ELECTROCHEMICAL INVESTIGATION OF COMPLEXATION OF Pb(II) BY LAURENTIAN FULVIC ACID IN MODEL SYSTEMS – EFFECT OF COMPETITION WITH MAJOR CATIONS AND TRACE METALS

INTRODUCTION

In unpolluted natural waters, most metals are present at trace or ultratrace levels, typically $10^{-7} - 10^{-12}$ M [117], or below 10^{-12} M [118]. In the freshwaters and soils, it is often impossible to determine the identity and concentration of metal species, many of which are transient. It is therefore necessary to characterize these metal species by the speciation parameters of their metal complexes. Speciation parameters are quantitative measures of chemical characteristics of chemical species, which are independent of the analytical methods that are used to measure them. It often requires a number of speciation parameters to characterize a system fully. Some important speciation parameters are e.g. dissociation rate coefficients (which are a measure of their kinetic lability), diffusion coefficients (which are a measure of their mobility), and stability constants (which are a measure of the availability of free metal ion at equilibrium).

Information about the competition between trace metals for naturally-occurring organic complexants in freshwaters is scarce. The experimental results of previous Chapters have clearly demonstrated that in freshwaters, competition of other trace metals and major cations with the target trace metal for strong (~1-10% of the total sites) and weak (~99-90% of the total sites) binding sites of polyfunctional, polyelectrolytical, macromolecular, heterogeneous, organic complexants, such as humic substances, determines the release of bound metal (by organic complexants) as free metal ions. However, all these results were based on the kinetic approach. Since interpretation of metal complexation by naturally-occurring, heterogeneous, organic complexants, and assessment of their relationship to biogeochemical processes require combined equilibrium and kinetic approaches [18,45], here, the effect of trace and major metal competition on the stability of the Pb(II)-FA complexes in model systems has been investigated using the equilibrium approach. Xue et al. [88], Ghosh et al. [119] and Cao et al. [94] have reported on the importance of competitive complexation of trace metals with humic acids on the metal release. Donat et al. [120], Sedlak et al. [121], Choi et al. [122] have emphasized the effect of stability of complexes on the above competition. Choi et al. [122] have demonstrated the importance of binding sites where electrostatic interactions dominate.

Electrochemical methods such as polarography and voltammetry are widely used in trace element speciation in natural water systems, because of their metal specificity, high sensitivity and their ability for direct (i.e. without external preconcentration) determination [123-125]. Although the specific potentialities of voltammetric techniques for the characterization of all kinds of complexation reactions of electroactive metal ions

have been recognized for a long period [126], the application of voltammetry to metal speciation in freshwater systems is quite recent.

THEORY

The method of Hoop et al. [97] has been adapted and described as below. The complexation scheme in which an electroactive metal ion, M (charge omitted for simplicity), associates with a ligand or site, L, to form an electroinactive complex, ML, can be presented as follows:

where M^0 denotes the metal atom, and k_a and k_d are the association and dissociation rate coefficients, respectively. The complexation equilibrium in the bulk is expressed by the stability K :

$$K = \frac{C_{ML}}{C_M C_L} \quad (17)$$

where C_M , C_L and C_{ML} denote the bulk concentrations of free metal ions, free ligand and bound metal, respectively.

For the case of excess of ligand over metal and a voltammetrically labile system, meaning that the rates of the association / dissociation reaction (eq. 16) are sufficiently fast to maintain equilibrium for any relevant values of space and time, the average stability in the case of $D_{ML} < D_M$ can be obtained from the normalized current ϕ [127]

$$\phi = \frac{i_p}{i_0} \quad (18)$$

where i_p and i_0 are the current for the complex system and the reference current under conditions of no complexation, respectively. The normalized current, ϕ , is related to the stability of the complex by

$$\phi = \left(\frac{\bar{D}}{D_M} \right)^p = \left(\frac{1 + \epsilon K C_L}{1 + K C_L} \right)^p \quad (19)$$

where $\bar{D}$ is the mean diffusion coefficient given by

$$\bar{D} = (C_M / C_T) D_M + (C_{ML} / C_T) D_{ML} \quad (20)$$

$$C_T = C_M + C_{ML}$$

which actually incorporates the speciation of the available metal over the species M and ML. D_M and D_{ML} are the diffusion coefficients of the free metal ion and metal complex, respectively, and the power p is related to the nature of the mass transport during the preelectrolysis step, which varies with geometrical conditions and the mode of stirring, and it is $\frac{1}{2}$ for semi-infinite linear diffusion and $\frac{2}{3}$ for laminar convective diffusion [128]. Anyway, the value of p does not affect K very much [97]. The parameter ϵ is defined as the ratio of the diffusion coefficients of the bound and free metal (D_{ML} / D_M).

A suitable procedure to fit sets of experimental ϕ , C_L data to eq. (19) has been described by van den Hoop et al. [129]. The initial part of the ϕ , C_L curve is the most important part in obtaining K . ϕ is more sensitive to K in the regime in which $K C_L$ is of the order of unity. Eq. (19) shows that for systems with ϵ much smaller than unity, $\epsilon K C_L$ is a

relatively unimportant term. This illustrates that the precise value of ϵ , or for that matter D_{ML} , is not important in computing K .

EXPERIMENTAL

Reagents and materials

A 2M stock solution of sodium acetate was prepared by dissolving an appropriate quantity of sodium acetate trihydrate (ACS grade / BDH) in ultrapure water. The sodium acetate solution was purified of metals by electrolysis at -1.5 V vs. E_{SCE} for at least 48 h. A 2M stock solution of acetic acid was prepared by diluting glacial acetic acid (ACS grade / Anachemia) with ultrapure water. A stock solution of 2.0 M PIPES-NaOH buffer, at pH 6.8, was prepared by dissolving an appropriate quantity of PIPES {Piperazine-N,N'-bis(2-ethanesulfonic acid)} (ACS grade/Fisher Scientific) in 2.0 M sodium hydroxide (Fisher Scientific, ACS grade) 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.

A well-characterized FA, Laurentian FA, was obtained from Fredriks Research Products (Amsterdam, The Netherlands). The FA was characterized by potentiometric titration [130], and the total number of phenolic OH and carboxylic groups determined by potentiometric titration are 3.03 and 8.60 mmol /g [130] (Table 29). The bidentate complexing capacity was therefore approximately 5.8 mmol / g. A stock solution (1.000 g/L) of the Laurentian FA was prepared by dissolving appropriate amount of the freeze-dried FA in ultrapure water. The stock solution was stored in the dark at 4°C until use.

Apparatus

Voltammetric measurements were made with a computer-controlled Bioanalytical systems (BAS) 100 B/W with Controlled Growth Mercury Electrode (CGME-BAS) stand connected to a Pentium 166MMX personal computer (Gateway 2000), a small size (size 4) static mercury drop electrode (SMDE) as the working electrode, the reference electrode was a Ag/AgCl electrode (BAS), filled with 3 M NaCl, and the counter electrode was a Pt wire (BAS). Analysis of voltammetric peaks was done using the BAS 100W windows control software v 2.1 (BAS).

Method for voltammetric measurement

The effect of metal competition on the stability of the Pb(II)-FA complexes in model systems was investigated using Square Wave Anodic Stripping Voltammetric (SWASV) titration of metals by ligand addition. Experiments were done by adding the Laurentian FA to the model systems containing constant concentration of the target trace metal, Pb(II) (10^{-7} M), and varying concentrations of the competing trace metals, Cu(II), Ni(II), Zn(II), or major cations, Ca(II) and Mg(II).

To obtain SWASV titration data for metal complexation in model systems, a series of test solutions were spiked with different concentrations of the Laurentian FA. Aliquots of 20 mL were pipetted into a series of 50 mL Teflon beakers. The test solutions were buffered to the appropriate pH (pH 5.0 using AcOH/NaOAc or pH 6.8 using PIPES/NaOH), and were allowed to equilibrate overnight at 4 °C. KNO₃ was used to fix the ionic strength of all the test solutions at 0.02 M.

Table 29. Elemental and functional group analysis of Laurentian fulvic acid [130].

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

The test solutions were purged with extra-pure nitrogen gas (Extra-Dry, BOC Gases) for 10 minutes prior to the measurements; a nitrogen blanket was maintained thereafter. The electrode rotation rate was set at 2000 rpm. The electrochemical parameters used in the SWASV study of Pb(II) complexation were as follows: deposition time 120 s, equilibration time 15 s, initial potential – 0.6 V, end potential – 0.3 V, step potential 10 mV, square wave amplitude 30 mV, frequency 75 Hz.

By plotting the normalized re-oxidation current i_p / i_o against the FA concentration, titration curves were obtained, and the values of the average stability constants (K) for various Pb(II)-FA complexes were obtained from the curves.

RESULTS AND DISCUSSION

Effect of varying concentrations of Ca(II) and Mg(II) on the stability of the Pb(II)-FA complexes

Figures 31-33 represent the voltammetric complexation curves for the effect of competition of Ca(II) (Figure 31), Mg(II) (Figure 32), and Ca(II) and Mg(II) together (Figure 33) on the stability of the Pb(II)-FA complexes at pH 5.0 using AcOH/NaOAc and at ionic strength 0.02 M. At a fixed ratio of $[Pb(II)] / [FA]$, increasing concentrations of Ca(II) and/or Mg(II) result in higher i_p / i_o values, indicating decreasing strength of the Pb(II)-FA complexes. This suggests that the major cations, Ca(II) and Mg(II), compete with Pb(II) for binding sites of the FA. The data of the complexation curves in Figures 31-33 have been analyzed according to eq. (19) using $p = 2/3$, and the resulting K-values are presented in Table 30. In agreement with the qualitative information obtained from

the shape of the complexation curves, the average stability constants of the Pb(II)-FA complexes decrease with increasing Ca(II) and Mg(II) concentrations. By increasing the Ca(II) concentration from 0 to 2.5×10^{-3} M, the log K-values of the Pb(II)-FA complexes decrease from 5.5 to 4.7, whereas the log K-values for the Pb(II)-FA complexes decrease from 5.5 to 5.2 in the presence of increasing concentrations of Mg(II) from 0 to 2.5×10^{-3} M. The log K-values of the Pb(II)-FA complexes decrease from 5.5 to 4.6 in the presence of increasing concentrations of Ca(II) and Mg(II) together from 0 to 5×10^{-3} M.

Similar investigation were done on the effect of varying concentrations of Ca(II) and/or Mg(II) on the stability of the Pb(II)-FA complexes at pH 6.8 using PIPES / NaOH and at the same ionic strength as before, 0.02 M. Figures 34-36 show the voltammetric complexation curves for the effect of competition of Ca(II) (Figure 34), Mg(II) (Figure 35), and Ca(II) and Mg(II) together (Figure 36) on the stability of the Pb(II)-FA complexes at pH 6.8. The results show behaviour similar to that of the Figures 31-33. At a fixed ratio of $[\text{Pb(II)}] / [\text{FA}]$, increasing concentrations of Ca(II) and/or Mg(II) results in higher i_p / i_o values, indicating decreasing strength of the Pb(II)-FA complexes. This suggests that the major cations, Ca(II) and Mg(II), compete with Pb(II) for binding sites of the FA. The data of the complexation curves in Figures 34-36 have been analyzed according to eq. (19) using $p = 2/3$, and K-values are presented in Table 31. In agreement with the qualitative information obtained from the shape of the complexation curves, the average stability constants of the Pb(II)-FA complexes decrease with increasing Ca(II) and Mg(II) concentrations. By increasing the Ca(II) concentration from 0 to 2.5×10^{-3} M,

Figure 31. Effect of varying concentrations of Ca(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 5.0 ± 0.1 (AcOH / NaOAc), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Ca(II) nil]; (O), [Ca(II) 2.5×10^{-5} M]; (Δ), [Ca(II) 2.5×10^{-4} M]; (◇), [Ca(II) 2.5×10^{-3} M].

Figure 32. Effect of varying concentrations of Mg(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 5.0 ± 0.1 (AcOH / NaOAc), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Mg(II) nil]; (O), [Mg(II) 2.5×10^{-5} M]; (Δ), [Mg(II) 2.5×10^{-4} M]; (◇), [Mg(II) 2.5×10^{-3} M].

Figure 33. Effect of varying concentrations of Ca(II) and Mg(II) together on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 5.0 ± 0.1 (AcOH / NaOAc), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Ca(II) + Mg(II) nil]; (O), [Ca(II) + Mg(II) 5.0×10^{-5} M]; (Δ), [Ca(II) + Mg(II) 5.0×10^{-4} M]; (◇), [Ca(II) + Mg(II) 5.0×10^{-3} M].

Table 30. Average stability constants of various Pb(II)-FA complexes as a function of the concentrations of Ca(II) and/or Mg(II) using SWASV at a SMDE.

[Pb(II)] 3.4×10^{-7} M. pH 5.0 ± 0.1 . Ionic strength 0.02 M. Temp. 298 K.

[Ca(II)], M	Mg(II), M	log K	r^2
nil	nil	5.5 ± 0.01	0.987
2.5×10^{-5}	nil	5.4 ± 0.02	0.992
2.5×10^{-4}	nil	5.3 ± 0.01	0.990
2.5×10^{-3}	nil	4.7 ± 0.02	0.973
nil	nil	5.5 ± 0.01	0.980
nil	2.5×10^{-5}	5.4 ± 0.01	0.997
nil	2.5×10^{-4}	5.3 ± 0.01	0.992
nil	2.5×10^{-3}	5.2 ± 0.01	0.997
nil	nil	5.5 ± 0.01	0.985
2.5×10^{-5}	2.5×10^{-5}	5.4 ± 0.01	0.999
2.5×10^{-4}	2.5×10^{-4}	5.3 ± 0.01	0.998
2.5×10^{-3}	2.5×10^{-3}	4.6 ± 0.02	0.968

The values after $\pm$ represent two standard deviation of replicate determinations, $n = 4$.

the log K-values of the Pb(II)-FA complexes decrease from 7.2 to 6.3, whereas the log K-values for the Pb(II)-FA complexes decrease from 7.2 to 6.5 in the presence of increasing concentrations of Mg(II) from 0 to 2.5×10^{-3} M. The log K-values of the Pb(II)-FA complexes decrease from 7.2 to 6.1 in the presence of increasing concentrations of Ca(II) and Mg(II) together from 0 to 5×10^{-3} M.

It is clear from the above results that the major cations, Ca(II) and/or Mg(II), have significant effect on the binding of the Pb(II)-FA complexes. The co-presence of large excess of Ca(II) and Mg(II) ($10^{-5} - 10^{-3}$ M) than that of the target trace metal Pb(II) (10^{-7} M) decreased the stability of the Pb(II)-FA complexes, and results in the formation of weak Pb(II)-FA complexes that releases free Pb^{2+} ions, which are reported to be toxic.

As stated earlier, Ca^{2+} and Mg^{2+} could compete with Pb^{2+} for specific binding sites, and because of their presence in overwhelming excess over the Pb^{2+} , could outcompete Pb^{2+} for specific binding sites, and also, preferentially bind to other sites for which they may have greater affinity for binding than Pb^{2+} . Moreover, the overwhelming excess of Ca^{2+} and Mg^{2+} would result in a large amount of Ca^{2+} and Mg^{2+} in the diffuse (electrical) double layer of the fulvate polyanions, thereby decreasing significantly the total binding energy of the Pb(II)-FA by screening the charge on the fulvate polyanions (polyelectrolyte effect). This would result in weakening of the Pb(II)-FA binding, with the consequence that more free Pb^{2+} ions would be released from the Pb(II)-FA complex, as observed.

Figure 34. Effect of varying concentrations of Ca(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 6.8 ± 0.2 (PIPES / NaOH), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Ca(II) nil]; (O), [Ca(II) 2.5×10^{-5} M]; (Δ), [Ca(II) 2.5×10^{-4} M]; (▽), [Ca(II) 2.5×10^{-3} M].

Figure 35. Effect of varying concentrations of Mg(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. $[\text{Pb(II)}] = 3.4 \times 10^{-7} \text{ M}$, pH 6.8 ± 0.2 (PIPES / NaOH), ionic strength 0.02 M (KNO_3), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), $[\text{Mg(II)} \text{ nil}]$; (O), $[\text{Mg(II)} 2.5 \times 10^{-5} \text{ M}]$; (Δ), $[\text{Mg(II)} 2.5 \times 10^{-4} \text{ M}]$; (V), $[\text{Mg(II)} 2.5 \times 10^{-3} \text{ M}]$.

Figure 36. Effect of varying concentrations of Ca(II) and Mg(II) together on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 6.8 ± 0.2 (PIPES / NaOH), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Ca(II) + Mg(II) nil]; (O), [Ca(II) + Mg(II) 5.0×10^{-5} M]; (Δ), [Ca(II) + Mg(II) 5.0×10^{-4} M]; (∇), [Ca(II) + Mg(II) 5.0×10^{-3} M].

Table 31. Average stability constants of various Pb(II)-FA complexes as a function of the concentrations of Ca(II) and/or Mg(II) using SWASV at a SMDE. [Pb(II)] 3.4×10^{-7} M. pH 6.8 ± 0.2 (PIPES/NaOH buffer). Ionic strength 0.02 M. Temp. 298 K.

[Ca(II)], M	Mg(II), M	log K	r^2
nil	nil	7.2 ± 0.01	0.987
2.5×10^{-5}	nil	6.9 ± 0.01	0.973
2.5×10^{-4}	nil	6.6 ± 0.01	0.983
2.5×10^{-3}	nil	6.3 ± 0.02	0.970
nil	nil	7.2 ± 0.01	0.980
nil	2.5×10^{-5}	7.0 ± 0.01	0.987
nil	2.5×10^{-4}	6.6 ± 0.02	0.975
nil	2.5×10^{-3}	6.5 ± 0.01	0.997
nil	nil	7.2 ± 0.01	0.985
2.5×10^{-5}	2.5×10^{-5}	6.9 ± 0.01	0.998
2.5×10^{-4}	2.5×10^{-4}	6.4 ± 0.01	0.979
2.5×10^{-3}	2.5×10^{-3}	6.1 ± 0.01	0.969

The values after $\pm$ represent two standard deviation of replicate determinations, $n = 4$.

Effect of varying concentrations of other trace metals, Cu(II), Ni(II) and Zn(II), on the stability of the Pb(II)-FA complexes

The effect of competition of the three trace metals, Cu(II), Ni(II) and Zn(II), on the stability of the Pb(II)-FA complexes have been investigated at constant pH and ionic strength. Figure 37 shows the voltammetric complexation curves for the effect of varying concentrations of Cu(II) on the stability of the Pb(II)-FA complexes at pH 5 using AcOH/NaOAc and at ionic strength 0.02 M using KNO₃. Two varying concentrations of the competing metal, Cu(II), were used: one is the same concentration as that of the target trace metal Pb(II) (3.4×10^{-7} M), and the other concentration is one order of magnitude higher than that of the target metal (3.4×10^{-6} M). At a fixed ratio of [Pb(II)] / [FA], the increasing concentration of Cu(II) results in higher i_p / i_o values, indicating decreasing strength of the Pb(II)-FA complexes. This suggests that the trace metal, Cu(II), competes with Pb(II) for binding sites of the FA. The data of the complexation curves in Figure 37 have been analyzed according to eq. (19) using $p = 2/3$, and the resulting K-values for the Pb(II)-FA complexes are presented in Table 32. In agreement with the qualitative information obtained from the shape of the complexation curves, the K-values of the Pb(II)-FA complexes decrease with the same and increasing concentration of Cu(II). The log K-values of the Pb(II)-FA complexes decrease from 5.5 to 4.8 in the presence of increasing concentrations of Cu(II) from 0 to 3.4×10^{-6} M.

Similar studies were done on the effect of varying concentrations Ni(II) on the stability of the Pb(II)-FA complexes at pH 5 and at ionic strength 0.02 M. Figure 38 shows the voltammetric complexation curves for the above investigation. Figure 38 shows a

behaviour similar to that in Figure 37. At a fixed ratio of $[\text{Pb(II)}] / [\text{FA}]$, the increasing concentration of Ni(II) results in higher i_p / i_o values, indicating decreasing strength of the Pb(II) -FA complexes. This suggests that the trace metal, Ni(II) , competes with Pb(II) for binding sites of the FA. The data of the complexation curves in Figure 38 have been analyzed according to eq. (19) using $p = 2/3$, and the resulting K -values for the Pb(II) -FA complexes are presented in Table 32. In agreement with the qualitative information obtained from the shape of the complexation curves, the K -values of the Pb(II) -FA complexes decrease with the same and increasing concentration of Ni(II) . The average $\log K$ - values of the Pb(II) -FA complexes decrease from 5.5 to 5.0 in the presence of increasing concentrations of Ni(II) from 0 to $3.4 \times 10^{-6} \text{ M}$.

Figure 39 presents the effect of varying concentrations of Zn(II) on the stability of the Pb(II) -FA complexes at pH 5 and at ionic strength 0.02 M, and shows a behaviour similar to that in Figures 37 and 38. It is clear from the Figure 39 that, at a fixed ratio of $[\text{Pb(II)}] / [\text{FA}]$ only the presence of higher Zn (II) concentration than that of the target trace metal, Pb(II) , results in higher i_p / i_o values, indicating decreasing strength of the Pb(II) -FA complexes. This indicates that only at higher concentration, Zn(II) competes with Pb(II) for binding sites of the FA. The data of the complexation curves in Figure 39 have been analyzed according to eq. (19) using $p = 2/3$, and the resulting K -values for the Pb(II) -FA complexes are presented in Table 32. In agreement with the qualitative information obtained from the shape of the complexation curves, the average K -values of the Pb(II) -FA complexes decrease with increasing concentration of Zn(II) . The $\log K$ -values of the Pb(II) -FA complexes are 5.5, 5.4 and 5.1 in the presence of 0, $3.4 \times 10^{-7} \text{ M}$ and $3.4 \times 10^{-6} \text{ M}$ Zn(II) concentrations, respectively.

Figure 37. Effect of varying concentrations of Cu(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 5.0 ± 0.1 (AcOH / NaOAc), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Cu(II) nil]; (O), [Cu(II) 3.4×10^{-7} M]; (Δ), [Cu(II) 3.4×10^{-6} M].

Table 32. Average stability constants of various Pb(II)-FA complexes as a function of the concentrations of other competing trace metals using SWASV at a SMDE.

[Pb(II)] 3.4×10^{-7} M. pH 5.0 ± 0.1 . Ionic strength 0.02 M. Temp. 298 K.

Competing Metal, M(II)	[M(II)] x 10^7 M	log K	r^2
nil	nil	5.5 ± 0.02	0.987
Cu(II)	3.4	5.2 ± 0.02	0.998
Cu(II)	34	4.8 ± 0.03	0.989
Ni(II)	3.4	5.3 ± 0.01	0.997
Ni(II)	34	5.0 ± 0.02	0.992
Zn(II)	3.4	5.4 ± 0.02	0.999
Zn(II)	34	5.1 ± 0.01	0.998

The values after $\pm$ represent two standard deviation of replicate determinations, $n = 4$.

Figure 38. Effect of varying concentrations of Ni(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 5.0 ± 0.1 (AcOH / NaOAc), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Ni(II) nil]; (O), [Ni(II) 3.4×10^{-7} M]; (Δ), [Ni(II) 3.4×10^{-6} M].

Figure 39. Effect of varying concentrations of Zn(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 5.0 ± 0.1 (AcOH / NaOAc), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Zn(II) nil]; (O), [Zn(II) 3.4×10^{-7} M]; (Δ), [Zn(II) 3.4×10^{-6} M].

Similar investigations were done on the effect of varying concentrations trace metals, Cu(II), Ni(II) and Zn(II), on the stability of the Pb(II)-FA complexes at pH 6.8 using PIPES / NaOH and at the same ionic strength as before, 0.02 M. Figures 40-42 show the voltammetric complexation curves for the effect of competition of Cu(II), Ni(II) and Zn(II) on the stability of the Pb(II)-FA complexes. The results show behaviour similar to that of the Figures 37-39. At a fixed ratio of $[Pb(II)] / [FA]$, increasing concentrations of the competing trace metals result in higher i_p / i_o values, indicating decreasing strength of the Pb(II)-FA complexes. This suggests that the trace metals Cu(II), Ni(II) and Zn(II), compete with Pb(II) for binding sites of the FA. The data of the complexation curves in Figures 40-42 have been analyzed according to eq. (19) using $p = 2/3$, and the resulting K-values are presented in Table 33. In agreement with the qualitative information obtained from the shape of the complexation curves, the K-values of the Pb(II)-FA complexes decrease with increasing Cu(II), Ni(II) and Zn(II) concentrations. By increasing the Cu(II) concentration from 0 to 3.4×10^{-6} M, the log K-values of the Pb(II)-FA complexes decrease from 7.2 to 6.5, whereas the log K-values for the Pb(II)-FA complexes decrease from 7.2 to 6.7 in the presence of increasing concentrations of Ni(II) from 0 to 3.4×10^{-6} M. The log K-values of the Pb(II)-FA complexes decrease from 7.2 to 6.8 in the presence of increasing concentrations of Zn(II) from 0 to 3.4×10^{-6} M.

Figures 37-42 and Tables 32-33 show that trace metals, Cu(II), Ni(II) and Zn(II), compete with Pb(II) for binding sites of the FA. The increasing order of competing effects on the stability of the Pb(II)-FA complexes are as follows: $Cu(II) > Ni(II) > Zn(II)$. This observation can be interpreted as follows.

There are several factors that govern the binding affinity of the metal cations towards a polyfunctional complexant such as FA, and the stabilities of these metal complexes are determined by this binding affinity. In humic substances the most important donor atoms are oxygen, nitrogen and sulphur. In order to fully understand the stability 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 [131]. To estimate the degree of relative hardness of Ni(II), Cu(II) and Zn(II), the hardness, η , was calculated according to the Mulliken definition which is as follows:

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

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 atom will have high I.P. and low E.A. The hardness values of nickel, copper and zinc are 391.6, 292.1 and 411.9 kJ/mol, respectively. Copper is the softest of the above metals, Cu^{2+} has d^9 electronic configuration, and binds with the softest of the ligands, forming strong complexes which are inert [132].

Figure 40. Effect of Varying concentrations of Cu(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 6.8 ± 0.2 (PIPES / NaOH), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Cu(II) nil]; (O), [Cu(II) 3.4×10^{-7} M]; (Δ), [Cu(II) 3.4×10^{-6} M].

Figure 41. Effect of varying concentrations of Ni(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 6.8 ± 0.2 (PIPES / NaOH), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Ni(II) nil]; (○), [Ni(II) 3.4×10^{-7} M]; (Δ), [Ni(II) 3.4×10^{-6} M].

Figure 42. Effect of varying concentrations of Zn(II) on the stability of the Pb(II)-FA complexes in model systems, using SWASV at a SMDE. [Pb(II)] = 3.4×10^{-7} M, pH 6.8 ± 0.2 (PIPES / NaOH), ionic strength 0.02 M (KNO₃), temp. 298 K. Concentration of Pb(II) same for all curves. The error bar represents 95.5 % confidence intervals.

(□), [Zn(II) nil]; (O), [Zn(II) 3.4×10^{-7} M]; (Δ), [Zn(II) 3.4×10^{-6} M].

Table 33. Average stability constants of various Pb(II)-FA complexes as a function of the concentrations of other trace metals using SWASV at a SMDE.

[Pb(II)] 3.4×10^{-7} M. pH 6.8 ± 0.2 . Ionic strength 0.02 M. Temp. 298 K.

Competing Metal, M(II)	[M(II)] x 10^7 M	log K	r^2
nil	nil	7.2 ± 0.02	0.987
Cu(II)	3.4	6.9 ± 0.02	0.994
Cu(II)	34	6.5 ± 0.03	0.989
Ni(II)	3.4	7.0 ± 0.01	0.989
Ni(II)	34	6.7 ± 0.02	0.999
Zn(II)	3.4	7.0 ± 0.02	0.989
Zn(II)	34	6.8 ± 0.01	0.998

The values after $\pm$ represent two standard deviation of replicate determinations, $n = 4$.

Table 34. Ligand-Field Stabilization Energies for some 3d transition metal ions [133].

Metal Ion	Mn²⁺	Fe²⁺	Co²⁺	Ni²⁺	Cu²⁺	Zn²⁺ *
dⁿ	d⁵	d⁶	d⁷	d⁸	d⁹	d¹⁰
LFSE (Dq)	0	6	8	12	6	0

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

For 3d transition metals in octahedral substitution reactions, LFSE is also an important factor in the ability of the 3d transition metal complexes to undergo ligand substitution reactions. The inert behaviour of some metals is related to unfavourable changes in LFSE during complex formation. 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 in octahedral complexes may be expected to parallel the magnitude of the LFSE. The Ligand-Field Stabilization Energies for some 3d transition metals are presented in Table 34. Those metals that have high LFSE form strong complexes. The stability of Cu(II) complexes is enhanced by the Jahn Teller effect, which is responsible for the stability order of Cu(II) complex: $\text{Cu(II)} > \text{Ni(II)} > \text{Zn(II)}$. Because of the Jahn Teller effect, in the tetragonally distorted Cu(II)L_6 complex, the four equatorial bonds are shortened and strengthened, and two axial bonds are lengthened and weakened compared with the undistorted octahedral Cu(II)L_6 complex.

Effect of pH on the stability of the Pb(II)-FA complexes

Figures 31-42 and Tables 30-33 show that, as expected, the change of pH from 5.0 to 6.8 enhance the stability of the Pb(II)-FA complexes. This is due to the fact that more functional groups are being ionized at higher pH and become available as binding sites. Increasing pH also has an effect on the size and/or aggregation on the polymeric ligand molecules because of their polyelectrolyte effect. Increasing pH increases ionization of the functional groups, and the resulting negative charges repel other negative charges on the macromolecule, resulting in uncoiling of the polymer chain, which makes previously

inaccessible binding sites now accessible for binding. As a result, the stability of the Pb(II)-FA complexes increase with increasing pH.

Effect of ionic strength on the stability of the Pb(II)-FA complexes

Figure 43 shows the voltammetric complexation curves for the effect of ionic strength on the stability of the Pb(II)-FA complexes using various concentrations of KNO₃. At a fixed ratio of [Pb(II)] / [FA], decreasing ionic strength result in lower i_p / i_o values, indicating increasing strength of the Pb(II)-FA complexes. The data of the complexation curves in Figure 43 have been analyzed according to eq. (19) using $p = 2/3$, and the resulting K-values are presented in Table 35. In agreement with the qualitative information obtained from the shape of the complexation curves, the average stability constants of the Pb(II)-FA complexes increase with decreasing ionic strength. By increasing the KNO₃ concentration from 0.002 to 0.2 M the log K-values of the Pb(II)-FA complexes decrease from 6.0 to 5.5. This is due to the fact that increasing ionic strength increases shielding, resulting in an decrease in the electrostatic attraction (polyelectrolyte effect), which weaken the Pb(II)-FA bonding and decrease its stability.

Figure 43. Effect of ionic strength on the stability of the Pb(II)-FA complexes in model systems containing constant concentration of Pb(II) and varying concentrations of KNO₃, using SWASV at a SMDE. The error bar represents 95.5 % confidence intervals.

[Pb(II) 3.4×10^{-7} M]. pH 5.0 ± 0.1 . Temp. 298 K.

(□), [KNO₃ 0.002 M]; (O), [KNO₃ 0.02 M]; (Δ), [KNO₃ 0.2 M].

Table 35. Average stability constants of various Pb(II)-FA complexes as a function of ionic strength using different concentrations of KNO₃.

[Pb(II)] 3.4×10^{-7} M. pH 5.0 ± 0.1 . Temp. 298 K.

[KNO ₃], M	log K	r ²
0.002	6.0 ± 0.03	0.998
0.02	5.6 ± 0.02	0.995
0.2	5.5 ± 0.01	0.996

The values after $\pm$ represent two standard deviation of replicate determinations, n = 4.

CHAPTER 10

VOLTAMMETRIC STUDY OF THE COMPLEXATION OF Pb(II), Cu(II), Cd(II) AND Zn(II) BY HUMIC SUBSTANCES IN FRESHWATERS (RIDEAU RIVER SURFACE WATERS)

INTRODUCTION

Two voltammetric titration methodologies can be employed to study metal complexation by humic substances using the equilibrium approach: (1) addition of humic substance to a solution containing fixed concentration of trace metal; and (2) addition of metal to a solution containing fixed concentration of humic substance. The latter method is mainly applicable to freshwater systems where the total concentration of ligands is constant (but unknown). The results of metal competition using voltammetric titration of metals by ligand addition are presented in the previous Chapter. This Chapter presents the results of complexation of trace metals, Pb(II), Cu(II), Cd(II) and Zn(II), by naturally-occurring organic complexants in freshwaters using voltammetric titration of ligands by metal addition.

THEORY

One of the ways to study metal complexation in freshwaters by heterogeneous complexants (humic substances) having both polyfunctional and polelectrolytical properties, is to create a model using simplifying assumptions. The model created using such simplifying assumptions for heterogeneous complexants gives an average stability constant, and not a true thermodynamic stability constant. However, such average stability constants are useful for practical purposes, and are acceptable as long as they can predict the complexation behaviour of the water samples. However, when the simplifying assumptions depart substantially from the realities of the water samples, the results obtained from the model become less useful.

For simplicity, in the present study, it was assumed that the organic ligands in the water sample can be regarded as a single ligand L species forming 1:1 complex, ML, with the metal ion, M [134,135]. If M is added to water samples in increments, and the equilibrium free metal ion concentrations present in water sample are determined initially, and after each addition, allowing a suitable equilibration period to elapse, a titration curve of the type shown in Figure 44 is obtained. The concentration of ML increases as a result of metal complexation with the free ligand. A marked change in the gradient of the titration curve occurs when the metal concentration is equivalent to that of the ligand (the equivalence point). Beyond this point, the metal complexation capacity of the ligand is saturated, and the slope of the upper portion of the titration curve is identical with the slope of the titration curve obtained when there is no metal complexation. The slope of the straight line in the upper region of the curve equals the sensitivity of the

technique.

The stability constant, K_{ML} , relates to the following equilibrium:

$$K_{ML} = \frac{[ML]}{[M][L]} \quad (21)$$

where

$[M]$ = concentration of uncomplexed metal ion

$[ML]$ = concentration of the metal complexed by organic complexing ligand L

$[L]$ = concentration of the uncomplexed organic ligand

K_{ML} = stability constant for metal organic ligand complex

C_L , total concentration of the organic ligand L, is given by

$$C_L = [ML] + [L] \quad (22)$$

Substitution in eq. (21) for $[L]$ using eq. (22), and rearrangement yields the following relationship:

$$\frac{[M]}{[ML]} = \frac{[M]}{C_L} + \frac{1}{K_{ML} C_L} \quad (23)$$

The concentration of uncomplexed metal, $[M]$, present in the original sample and after each addition is calculated as the ratio of the relevant peak current, i_p , to the sensitivity, S , of the technique.

$$[M] = \frac{i_p}{S} \quad (24)$$

The concentration of complexed metal, $[ML]$, is given by

$$[ML] = C_M - [M] \quad (25)$$

where C_M represents the overall total metal concentration (the amount initially present plus that added).

Equation (23) predicts that, on the assumption of a 1:1 stoichiometry and establishment of equilibrium, the ratio of free to bound metal concentration is linearly related to the free metal concentration. A plot of $[M] / [ML]$ as a function of $[M]$ gives a straight line, and C_L and K_{ML} can be obtained from the slope and the intercept, respectively.

EXPERIMENTAL

Samples

A 4.4 L sample of Rideau River surface water (RRSW) was collected in September 2000 from a site at Carleton University using pre-cleaned Teflon bottles. The pH, conductivity and DOC values of the RRSW sample were 8.1, 444 $\mu\text{S}/\text{cm}$ and 7.0 mg/L, respectively. The sample temperature was 298 K. The sample was filtered through 0.45 μm membrane filters (GN-6 Metrical, 47 mm diameter, Gelman Sciences, Ann Arbor, MI) in our laboratory within 24 h of the sample collection to filter out the particulate matter. These filters, made of mixed cellulose esters, had the advantage of providing filters having low blanks for the metals. Each 0.45 μm filter was able to filter ~ 1.1 L water sample without clogging. The filtered sample was stored in the dark at 4 $^{\circ}\text{C}$ and was used for voltammetric investigation within 72 h of filtration.

Dissolved Organic Carbon

The dissolved organic carbon (DOC) of the filtered water sample was measured using O I Analytical Model 1010 TOC Analyzer.

Method for voltammetric measurement

The procedure adopted for the study of metal complexation by naturally-occurring organic complexants in the RRSW sample is based on Square Wave Anodic Stripping Voltammetric (SWASV) titration of ligands by metal addition. The SWASV peak current was measured for the metal, initially and after each addition, allowing a suitable equilibration time to elapse. When the water sample was titrated with the metal, the concentration of free metal $[M]$ present in the original sample and after each addition was calculated as the ratio of the relevant peak current to the sensitivity of the technique. From the titration data, a plot of $[M] / [ML]$ as a function of $[M]$ (eq. 23) gives a straight line, and C_L and K_{ML} can be obtained from the slope and the intercept, respectively.

To obtain SWASV titration data for metal complexation in the RRSW sample, a series of sub-samples were spiked with different concentrations of standard metal solutions. Aliquots of 20 mL were pipetted into a series of 50 mL Teflon beakers. The sub-samples were buffered to pH 6.8 using PIPES/NaOH, and were allowed to equilibrate overnight at 4 °C.

Apparatus

Instrumentation for experimental voltammetric measurements has been described in the previous Chapter. The electrochemical parameters used in the SWASV determination of Pb(II), Cu(II), Cd(II) and Zn(II) were as follows: deposition time 120 s, equilibration time 15 s, step potential 10 mV, square wave amplitude 30 mV, frequency 75 Hz., initial potential (Pb) – 0.6 V, end potential (Pb) – 0.3 V, initial potential (Cu) – 0.5 V, end

potential (Cu) + 0.1 V, initial potential (Cd) – 0.9 V, end potential (Cd) – 0.3 V, initial potential (Zn) – 1.2 V, end potential (Zn) – 0.7 V.

RESULTS AND DISCUSSION

Complexation of Pb(II), Cu(II), Cd(II) and Zn(II) by DOC in the RRSW sample

The stripping voltammetric complexation curves $i_p = f(C_M)$ obtained for (a) Pb(II), (b) Cu(II), (c) Cd(II) and (d) Zn(II) in the RRSW sample at pH 6.8 are shown in Figure 44. All the curves obtained with the investigated metals have similar shapes which have two linear portions. The first linear segment of the representation of $i_p = f(C_M)$ has a low slope which reflects an important complexation phenomenon ($[M] \ll C_M$) before the saturation point of the binding sites of the DOC is reached. For the metals of interest, the values of $[M]$ and $[ML]$ were estimated from the curves $i_p = f(C_M)$. The slopes of the straight lines in the upper region of the curves (a), (b), (c) and (d) in Figure 44 (the ligand completely saturated) equals the sensitivity of the technique. The concentration of free metal, $[M]$, present in the original sample and after each addition was calculated as the ratio of the relevant peak current to the sensitivity.

Figure 45 shows the plots of $[M]/[ML] = f[M]$ obtained for (a) Pb(II), (b) Cu(II), (c) Cd(II) and (d) Zn(II) in the RRSW sample at pH 6.8. The values of $\log K_{ML}$ were calculated from the intercept of the linear regressions applied to these plots. The $\log K_{ML}$ -values for the metal (Pb, Cu, Cd and Zn)-DOC complexes in the RRSW sample at pH 6.8 are presented in Table 36. The good agreement between the experimental data points and the plot of the linear regression applied to these points (indicated by a high value of the correlation coefficient r^2) showed that the method proposed by Ruzic and van den Berg

[134,135] could be successfully used for the systems of all the investigated metal complexes.

Table 36 shows that the stability of the metal-DOC complexes in the RRSW samples increases in the following order: Cu(II) > Pb(II) > Cd(II) > Zn(II). The binding strength in the M(II)-DOC complexation arises from several factors: the intrinsic acidity of the M^{2+} ion, dependent mainly on charge to radius ratio Z^2 / r (called charge density or ionic potential), where Z is the number of units of cationic charge and r is the ionic radius; the other factors are the basicity of the DOC, the Hard-Soft Acid-Base (HSAB) interactions between the soft acid (M^{2+}) and the soft bases (e.g. S^{2-}), resulting in polarization of the anion (e.g. S^{2-}) and cation-anion mutual polarization, and the Ligand Field Stabilization Energy (LFSE) for d^n metal complexes. As stated earlier, those metals that have high LFSE form strong complexes (Table 34). The experimental results show that the relative stability of the investigated metal complexes in the RRSW sample follows the same trend as predicted from the LFSE of the metals. The stability of the Cu(II) complexes is enhanced by the Jahn Teller effect, which is responsible for the stability order of the Cu(II) complex. Zn(II) and Cd(II) with their d^{10} electronic configuration cannot have any LFSE contribution to their bond energy. For Pb(II), the Z^2 / r is a contributing factor for the stability of its Pb(II)-DOC complexes. Also, the large polarizable Pb^{2+} cation is expected to be preferentially complexed by soft (minor) binding sites (e.g. S^{2-}), forming strong complexes [136]. Cleven and van Leeuwen [137] have reported on their results of a set of conductometric experiments which show that the binding of Cd(II) to HA has a significant electrostatic contribution, whereas for Pb(II) there is a higher level of covalence in the binding.

Figure 44. Stripping voltammetric complexation curves for (a) Pb(II), (b) Cu(II), (c) Cd(II) and (d) Zn(II) in the RRSW sample.

[DOC] = 7.0 mg/L. pH 6.8 ± 0.2 (PIPES/NaOH). Ionic strength 0.02M. Temperature 298 K.

Figure 45. Plots of $[M] / [ML]$ vs. $[M]$ obtained for (a) Pb(II), (b) Cu(II), (c) Cd(II), and (d) Zn(II) in the RRSW sample.

Table 36. Average stability constants for various metal-DOC complexes in the Rideau River surface water samples using SWASV at a SMDE. [DOC] = 7.0 mg/L. pH 6.8 ± 0.2 (PIPES/NaOH). Ionic strength 0.02 M. Temperature 298 K.

Metals	$\log K_{ML}$	r^2
Cu(II)	8.0 ± 0.03	0.999
Pb(II)	7.7 ± 0.04	0.926
Cd(II)	6.9 ± 0.03	0.959
Zn(II)	6.7 ± 0.05	0.954

The values after $\pm$ represent two standard deviation of replicate determinations, $n = 4$.

CHAPTER 11

CONCLUSIONS

This thesis has examined the competition of trace metals and major cations (Ca^{2+} and Mg^{2+}) with the target trace metals for binding by humic substances in laboratory model systems and in freshwaters using both the kinetic and the equilibrium approach. The hypothesis (which has been accepted) is that the above competition of trace metal and major cations determines the release of target trace metal ion bound by humic substances in freshwaters.

Competing Ligand Exchange Method with Chelex-100 as the competing ligand was used in the kinetic speciation of Ni(II). The effect of competition of Cu(II) and Co(II) on the lability of the Ni(II)-FA complexes in the laboratory model systems was studied using a well-characterized soil FA from Armadale, Prince Edward Island, and Graphite Furnace Atomic Absorption Spectrometry to follow the kinetics.

Prior to this work, it was widely held among researchers that trace metals do not compete with one another and therefore the trace metal competition did not have any effect on the target trace metal speciation. This work has demolished the above myth and has demonstrated that trace metals do compete and have profound effect on the target trace metal speciation. It was found that even at relatively low Ni(II) loadings, trace metals,

Cu(II) and Co(II), which were usually associated with nickel in nickel ores, could successfully compete with target trace metal, Ni(II), for the few (~ 1-10% of the total) strong binding sites of fulvic acid, resulting in the Ni(II) being bound to much larger (~99-90% of the total) weak binding sites, forming labile nickel complexes, with the consequence that Ni(II) existed as free Ni^{2+} ion (Ni(II)-aqua complex) which was reported to be toxic. This situation would be further exacerbated by the co-presence of other metals having high water-loss rate coefficients, such as Pb(II) and Hg(II), in freshwaters and in an organic-complexant-limited freshwater environment.

The above study was then extended to freshwaters (Rideau River surface waters) using the same analytical techniques as the above. It was found that in the absence of Cu(II) and Co(II), Ni(II) was bonded to strong binding sites of the DOC (mainly humic substances), forming strong complexes which were inert. At sufficiently high concentrations of Cu(II) and Co(II), all the strong binding sites of the DOC were occupied by Cu(II) and Co(II), with the result that Ni(II) was bound to weak binding sites of the DOC, forming weak complexes that were labile (free Ni^{2+} ion). The environmental significance of this work is that even at relatively low nickel loadings in freshwaters, copper and cobalt which are usually associated with nickel ores, will successfully compete with Ni(II) for binding sites of DOC, and will release Ni(II) as free Ni^{2+} ions which are toxic.

The competition of major cations, Ca(II) and Mg(II), with the target trace metal, Ni(II), was studied using the same analytical techniques as the above. Prior to this study, little knowledge existed on the effect of competition of major cations, Ca(II) and Mg(II), on

the binding of target trace metal Ni(II) by DOC in freshwaters. Laboratory model systems containing Ni(II) and a well-characterized FA from Armadale, Prince Edward Island, were used in the study of the effects of competition of major cations, Ca^{2+} and Mg^{2+} , on the nickel binding by the FA. It was found that free Ni^{2+} ions (which are toxic) were released from the Ni(II)-FA complexes by the competition of Ca(II) and Mg(II) added to the model solutions in large excess (in concentrations typically found in freshwaters). The type of FA discrete binding sites which dominated the metal binding under the above experimental conditions were the sites where electrostatic attractions dominated; for these sites there were also direct competition between Ca^{2+} , Mg^{2+} , and Ni^{2+} . Also, there was competition for counterion accumulation in the diffuse part of the electrical double layer (which screened the fulvate anionic charge), and reduction in the net electric charge on the fulvate anions by Ca^{2+} and Mg^{2+} binding to discrete sites, which diminished the electrostatic contribution to the Ni^{2+} binding at other sites. The overall result was formation of weak Ni(II)-FA complexes which released free Ni^{2+} ions (which are toxic). The significance of this work is that it has demonstrated the importance of the competition of major cations, Ca^{2+} and Mg^{2+} , on the release of DOC-bound Ni(II) as free Ni^{2+} ions (which are toxic).

The above results led to a hypothesis that there exists a mechanistic link as follows: presence of large excess of Ca(II) and Mg(II) $\rightarrow$ release of DOC-bound Ni(II) as free Ni^{2+} ion $\rightarrow$ results in toxicity. When this hypothesis was first formulated in 1998, the idea was novel – no studies in natural waters had addressed this research area. Hence, this work is one of the ground-breaking studies in this field. The hypothesis was tested by extending the above study to nickel-polluted freshwaters and applying bioassay toxicity

tests to chemically-speciated water samples, and the results proved the hypothesis. Free metal ion availability of the target trace metals is dependent on the concentration of naturally-occurring organic matter in freshwaters and the competition by Ca^{2+} and Mg^{2+} and other trace metals. The bioassay toxicity tests using the alga *Selenastrum capricornutum* and the zooplankter *Daphnia magna* showed that the released free Ni^{2+} ion was bioavailable and toxic. The significance of this work is that the relationship between metal speciation and metal bioavailability and toxicity has been demonstrated. This observation has important implications for the regulation of metal loading to freshwaters.

One of the unusual consequences of the above study is that it calls into question the assertion of the proponents [138] of Biotic Ligand Model (BLM) that Free-Ion Activity Model (FIAM) fails to predict the effects of the competition of major cations, Ca^{2+} and Mg^{2+} , on the DOC-bonded nickel in freshwaters. The BLM proponents [138] are incorrect in their assertion that the FIAM has failed, whereas, in fact, it has not. What has occurred is that the competition of major cations, Ca^{2+} and Mg^{2+} , on the DOC-bound nickel has released nickel as free-nickel ions, thereby increasing the free-nickel ion activity, which is responsible for toxicity in freshwaters.

The effect of metal competition was also studied using the equilibrium approach involving measurement of average stability constants of metal complexes, which gave a measure of the availability of free metal ions at equilibrium. Square wave anodic stripping voltammetry (SWASV) at a static mercury drop electrode was employed to determine the average stability constants of metal complexes.

The results of both the kinetic and the equilibrium studies have shown that the trace metals compete with the target trace metals for binding by humic substances even when the competing trace metals are present at the same concentration as that of the target trace metals, whereas the major cations, Ca^{2+} and Mg^{2+} , compete with the target trace metals, only when they are present at concentrations massive excess over those of the target trace metals.

The environmental significance of this research work for freshwaters is that it has demonstrated the importance of competition of trace metals and major cations in release of DOC-bonded target trace metal as free metal ions (toxic).

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