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**A MULTI-TECHNIQUE APPROACH FOR THE STUDY OF
SPECIATION OF Ni, Cd, Cu, Pb, AND Zn IN FRESHWATERS**

by

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A thesis submitted to the Faculty of Science
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

Department of Chemistry

Carleton University

Ottawa, Ontario

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Abstract

Several techniques for the determination of metal speciation in freshwaters were employed. Model solutions of nickel and Humic Acid (HA) were studied using the kinetics-based Competing Ligand Exchange Method (CLEM) to determine the dissociation rate coefficients of Ni(II)-HA complexes as a function of pH and [Ni]/[HA] mole ratio. These results were compared with the previous results of Ni(II)-complexes with a well-characterized Fulvic Acid (FA). It was found that Ni(II)-HA complexes were stronger than Ni(II)-FA complexes and had smaller dissociation rate coefficients than Ni(II)-FA complexes. Kinetics-based CLEM and equilibrium-based speciation methods employing Cathodic or Anodic Stripping Voltammetry were used to determine the free-metal ion and labile metal-complex concentrations of Ni, Zn, Cd, and Cu in some metal-impacted lake surface waters from Rouyn-Noranda, Québec. The measured free-metal ion and labile metal-complex concentrations were compared with the predictions of a widely-used computer speciation code, the Windermere Humic Aqueous Model (WHAM), versions V and VI, to determine the capability of WHAM in modelling the above metals in freshwaters. WHAM was also used to model the effect of the presence or absence of Al and Fe on the free-metal ion concentration. Both versions of WHAM were more successful in modelling Ni, Cd, and Zn in the freshwaters, but were not as successful in modelling Cu. WHAM VI was generally better or equal to WHAM V for predicting the free-metal ion concentrations. The modelled competition by Al and Fe in the lake surface waters showed that in most cases, copper was most affected by this competition. WHAM VI predicts a larger effect from the Al and Fe competition than

does WHAM V, probably because WHAM VI allows tridentate binding to occur. Rotating Disk Electrode Voltammetry (RDEV) was employed for the study of speciation parameters such as diffusion coefficients and free-metal ion concentrations of model solutions of Zn- Cd- and Cu-HA and of freshwaters as a function of pH. The RDEV study showed that increasing pH resulted in smaller diffusion coefficients in both the model solutions and the freshwater samples, suggesting increased aggregation of the metal complexes.

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-- Chapter 1 --

Introduction

Humic Substances in the Freshwater Environment

The freshwater environment is an extremely complex chemical system. Within it, one can find most, if not all, of the elements in the Periodic Table, as well as a vast number of natural organic components, most of which are poorly characterized and whose interactions with the surrounding environment are largely unknown [1]. 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. Some of these are trace metal ions, which within narrow concentration limits, are essential nutrients, but outside these narrow limits, toxicity can occur. Humic substances (HS) help to control the metal ecotoxicity by providing a suite of binding sites which act cooperatively, complexing metals, some beginning to be occupied, some near the half titration point, and some close to saturation. As a consequence of this property, HS are able to “absorb” potentially toxic metals over a large concentration range (several orders of magnitude) with only a gradual increase in the biologically critical free-metal ion concentration ($[M^{n+}]$) (i.e. no sharp concentration jumps are observed). This property of the minor background sites is important ecologically since it helps living organisms to adapt to gradual changes in the concentrations of toxic metal species in the freshwater environment [2].

At present, environmental regulatory measures are based on total metal concentrations. However, the importance of the specific physicochemical form of a metal (i.e. chemical speciation), and not the total concentration (except in the rare and unlikely event when the total concentration is present as the bioavailable form) in influencing transport, fate

and bioavailability of metals in the aquatic environment is now recognized [3]. Although the overall importance of the metal speciation in the bioavailability and ecotoxicity is recognized, this recognition has not been translated in our ability to identify and quantify the actual chemical species that are bioavailable. An understanding of the distribution of a metal among its different chemical forms (metal speciation) and the bioavailability of the metal species is essential for predicting the ecotoxicity of metal species [4]. This is a formidable task by itself, which is further compounded by the fact that our understanding of the metal speciation in freshwaters is severely limited by the inherent extreme complexity of naturally-occurring complexants, of which the ubiquitous HS are predominant.

Metal Ion Binding to Humic Substances in Freshwaters

At very low concentrations of metals in freshwaters, the metal-binding mainly occurs at binding sites which have a high and specific affinity, e.g., nitrogen or sulphur containing functional groups [1,5,6]. Because of the existence of different types of functional groups in HS, they are *chemically heterogeneous*. HS are polydisperse (a mixture of molecules of various dimensions and molar masses). Because of this, they are *physically heterogeneous*, often containing a mixture of components of different molecular weights even after exhaustive fractionation; these components are different from one another in physical characteristics. Other important factors in metal-ion binding by HS in freshwaters are competition between different ions and electrostatic interactions between metals and major cations such as Ca and Mg [1]. HS have a pH-dependent charge arising

from the dissociation of the functional groups. This is the polyelectrolyte effect described below.

Complexation Properties of Humic Substances

Compared to metal complexation by simple complexants, such as ethylenediaminetetraacetic acid (EDTA), metal complexation by HS involves several additional effects (herein called *secondary*), described below, which can influence the overall complexation process [1]. In HS, the coordinating sites are groups of atoms (e.g. -COOH) which *directly* participate in bonding. However, the environment (steric, electric) of the coordinating sites can exert a considerable influence on the stability of the complexes, through *secondary* effects, which can be divided into three categories based on the following properties of HS.

- (a) **Polyfunctional Properties.** Each macromolecule bears a large number and a wide variety of coordinating sites with different intrinsic binding properties. Examples include carboxylic-, phenolic-, salicylic-, and phthalic acids, and nitrogen and sulphur bearing functional groups. Even within a particular group of complexants, such as fulvic acids, the functional groups are similar, but not identical.
- (b) **Polyelectrolytic Properties.** Since most of the coordinating sites have acid-base properties, they undergo dissociation at appropriate pH values, producing anionic moieties with negative charges. At a given temperature, the total charge depends on the pH, the degree of site occupation by metals [1,7-11], and on the ionic strength of the solution. However, in the development of

surface charges, HS are in between simple complexants (e.g. EDTA) with a few charges on them and true **polyelectrolytes** like proteins with numerous charges on them, and are more correctly classified as **oligoelectrolytes** [12].

- (c) **Conformational Properties.** The conformational changes depend on hydration-dehydration processes (HS are colloidal, forming water-swollen gels), and the formation of hydrogen bonds (both intra- and intermolecular – some metals forming bridges between hydrodynamically separate macromolecules). Conformational changes include electrostatic interactions among various functional groups on a single molecule, and formation of metallic bridges between neighbouring molecules. The intensity of electrostatic interactions themselves depends on the degree of site occupation [1,7-11]. The relative importance of these properties varies with pH, ionic strength and the degree of site occupation.

Conformational changes in water-swollen-HS-gels, resulting from coiling and uncoiling, can change the shape of the molecule drastically, turning spheres into ellipsoids, rods, sheets, and vice versa, dramatically changing the proximity of the coordinating site to the metal ion. Because of coiling and uncoiling of macromolecular, colloidal polymers such as HS, the binding sites on the HS may be located in the interior of the water-swollen gel of HS, and the metal ion bound to such sites must diffuse out of the cavernous gel of HS by a process called **hindered** diffusion, instead of the normal process of **free** diffusion [13]. Hence, transport of the metal ion from the reaction sites in the

interior of the cavernous humic gel to its exterior becomes the **limiting factor** in metal-complex formation and dissociation. The effect of this slow diffusion of the metal ion to and from the coordinating sites is to make the overall process slow, resulting in a seeming paradox: thermodynamically equivalent sites appear to be kinetically different [14,15].

Free Ion Activity Model (FIAM)

As has been stated earlier, it is now recognized that it is the specific chemical form of the metal (i.e. chemical speciation), rather than its total concentration that controls its bioavailability, and potential toxicity, to aquatic organisms. Symptoms of intracellular toxicity include ultrastructural deformities, and reductions in cell division rate, respiration, photosynthesis, mobility, electron transport activity, and ATP production. In order for a metal to penetrate the cell and elicit a toxic response, the complex in which the metal is present must be small enough to be directly incorporated into the cell, or it must first dissociate and enter through transporter ligands or protein channels as illustrated in Fig. 1.1 [1,7,16]; hence, as explained below, the free-metal ion is crucial for determining the uptake of metals [17]. This concept was developed by Morel [18], who proposed the FIAM as a predictor for metal bioavailability in the aquatic environment.

The importance of the free-metal ion, i.e. the hydrated ion $M(H_2O)_x^{n+}$, in linking the reactions between the aquatic environment and living organisms is illustrated in Fig. 1.2 [19]. Because of the wide prevalence of the misconception that the FIAM implies that the free-metal ion is the chemical species that is actually taken up by the aquatic organisms [18], it is important to emphasize that the FIAM does not imply that the free-

Figure 1.1 Schematic diagram of metal assimilation by aquatic organisms [20]. M is the free metal ion, L_1 is a naturally occurring organic ligand such as humic substances, ML_1 is the metal-ligand complex, D_M and D_{ML} are the diffusion coefficients of M and ML , respectively, L_2 is a transporter ligand, P is a protein channel, and k_f and k_d are the formation and dissociation rate coefficients, respectively.

Figure 1.2 Schematic representation of the reaction of a metal ion with different types of aquatic components [19].

metal ion is the chemical species actually taken up by aquatic organisms. It merely reflects the fact that free-metal ion activity is a measure of the chemical reactivity of a metal, and that it provides a measure of the extent of the reactions with the sites of a biological membrane that is independent of the mechanisms of the reactions, regardless of the particular chemical species taken up by the organism [18].

Weakness of the Free-Ion Activity Model

Many exceptions to the FIAM have been reported [17]. One of the key assumptions of the FIAM is equilibrium (or pseudoequilibrium) at the surface of the biological membrane. Such equilibrium requires that the formation and dissociation kinetics and transport of the metal species towards the biological membrane is rapid relative to the uptake of metal by the organism (i.e. the flux of free-metal ions must be much greater than the biouptake flux). Only under these conditions, the surface of the biological membrane is in equilibrium with the various metal species in the bulk solution. For natural systems with a high degree of complexation, this condition may not be fulfilled.

Current State of Knowledge

Humic substances are macromolecular, polymeric, polydisperse systems, which are ubiquitous in the freshwater environment. They arise from chemical and biological degradation of plant materials, and from microbial respiration, or from photochemical degradation. HS constitute about 10 to 30% of Dissolved Organic Carbon (DOC) in seawater, 70 to 90% in wetland waters, and 40-80% in surface freshwaters [21]. In ground water, the humic fraction of the DOC is largely determined by the origin of the recharge water and varies from 10 to 90% [5,6]. The term humic acid (HA) is normally

used to indicate the fraction (ca 10%) of DOC that precipitates at $\text{pH} \leq 2$. The term fulvic acid (FA) refers to the acid-soluble fraction (ca 40% of DOC), usually of lower molecular weight. Both of these fractions are generally defined operationally by the method of extraction, most often by what is retained on a particular type of chromatographic column. For most FA, the molecular weight ranges from 500 to 5000 g mol^{-1} . The molecular weight of HA is larger than that of FA, and ranges from 1500 up to $>10^5$ g mol^{-1} [1,5,6,22,23]. Figure 1.3 shows a proposed structure of HA. It is the most comprehensive structure of HA proposed in the last decade and is based on extensive pyrolysis, spectroscopic, chemical, oxidative/reductive degradation, and electron microscope studies [24].

Humic substances abound in freshwaters and may have significant effects on metal speciation, mobility, transport and bioavailability, especially in waters with high levels of DOC. At present, our ability to predict these effects using equilibrium models is quite limited. A generally accepted picture is that metal ions form complexes with the functional groups of HS [1,6]. One characteristic of metal ion binding is *stoichiometry* of the binding to the sites. The metal ions can be bound in various ways; for example, by forming monodentate complexes to a single site, or multidentate complexes to structures in which several functional groups are coordinated, forming chelate rings which confer much greater thermodynamic stability to the metal complexes compared to metal-binding by monodentate binding sites. With divalent metal ions such as Cu^{2+} , salicylic-acid-type functional groups form six-membered-ring chelates, and phthalic-acid-type functional groups form seven-membered-ring chelates [25]. The reactivity of a functional group

Figure 1.3 Schematic representation of HA proposed by Schulten and Schnitzer [24].

depends on its type and its environment [26]. For the bulk of metal-binding, the -COOH and the -COH functional groups are of importance.

An important characteristic of HS is the type and content of functional groups where ion binding occurs [1,5,6,22]. Humic substances contain mainly carboxylic and phenolic type functional groups, which make up 90-99% of the total binding sites on HS, and are weak metal-binding groups. The other 1-10% of the binding sites include nitrogen and sulphur containing functional groups, and are strong metal-binding groups. The functional group content of HS ranges from 1 to 10 eq. kg^{-1} ; the functional group content of FA is larger than that of HA. However, in spite of the above difference in the functional groups, neither a systematic study of the differences between HA and FA in metal-complexation properties in freshwaters, nor a systematic study on the validity of the measured speciation parameters of metal-complexes of HA and FA has been reported. In particular, a comparative study of the measured concentrations of free-metal ions with the predictions of speciation models is lacking, in spite of the ready availability of several speciation models, including the widely-used Windermere Humic Aqueous Model (WHAM).

Techniques for Metal Speciation in Freshwaters

The only analytical technique capable of direct (i.e. without pre-concentration) determination of free-metal ion activity is ion-selective electrode potentiometry [27]—other analytical techniques, such as Inductively-Coupled Plasma Mass Spectrometry, measure total metal concentration, not the activity of the free-metal ion. However, the limit of detection of ion-selective electrode potentiometry, in particular for Cu^{2+} , is

reported to be 10^{-8} M under only the most ideal conditions, and is prone to interferences from other ions and the chemical matrix [14]. A detection limit of 10^{-8} M is still inadequate for the determination of environmentally relevant free-metal ion concentrations, which can be lower than 10^{-9} M. Traditional techniques for chemical speciation provide results that are operationally-defined, e.g.: “ASV-labile”, which is the amount of metal that produces current in Anodic Stripping Voltammetry (ASV) [28], and “Chelex-labile” [29], which is the amount of metal that is taken up by Chelex 100 chelating resin. While the significance of all analytical results is limited by the analytical procedures used, it is precisely the degree to which the results are independent of the analytical methods that gives them meaning and usefulness. The major limitation of operationally-defined methods is that the results are inherently ill-defined. Consequently, the results cannot be related to naturally-occurring processes in the environment. Hence, new concepts and approaches are required to investigate metal speciation and bioavailability in freshwaters. One such approach is the use of speciation models, which have recently emerged as a new method for determining metal speciation in freshwaters and soils. Until recently, speciation models have been too primitive to be useful for modeling freshwaters; however, recent years have seen them develop dramatically to a point where rigorous testing is required to determine their capability.

Hypothesis

Within the framework outlined above, the overall objective of this work is to test the **hypothesis:** (i) that HA is so different from FA in its functional groups, size and conformational properties, as to form metal-complexes which are significantly different

in their thermodynamic stability and kinetic lability; (ii) that a computer-based speciation code, WHAM, can satisfactorily model the $[M^{2+}]$ and other speciation parameters; (iii) that aggregation of HS is influenced by the pH of the sample, which drastically affects the metal-binding properties of HS.

Objectives

The objectives of this work are

1. To determine the speciation parameters of Ni(II), Zn(II), Cd(II), and Cu(II) complexes of HS in freshwaters using several different speciation techniques.
2. To determine the metal-binding characteristics of a well-characterised HA – a specific, operationally-defined fraction of HS – using two different techniques, and compare the results with those of a previous study of a well-characterised FA to determine the differences in the binding characteristics between HA and FA.
3. To measure the $[M^{2+}]$, and labile metal-DOC complexes of Ni(II), Cu(II), Zn(II), and Cd(II) in a few selected metal-impacted freshwater lakes, and to compare the experimental results with the predictions of a widely-used, computer-based speciation code, WHAM, versions V and VI.
4. To determine $[M^{2+}]$, diffusion coefficients, conditional stability constants, and the lability parameter for Zn(II)-, Cd(II)-, and Pb(II)-complexes in model solution of HA, and in freshwaters, at various pH values.

Outline of the Thesis

This thesis contains 8 chapters. Chapter 1 provides an introduction and the rationale of this research. It also outlines the current knowledge and the importance of HS in the freshwater environment. Chapter 2 outlines the scientific background of this thesis. Chapter 3 provides the theoretical background behind the experimental methods used in this work, including kinetics-, and equilibrium-based approaches. The experimental methods used in this work are outlined in Chapter 4. Chapter 5 presents a new concept: kinetic speciation of Ni(II)-HA complexes in water: the application of the kinetics-based Competing Ligand Exchange Method (CLEM) to model solutions of Ni(II) and a well-characterized HA, using two different techniques: CLEM with Graphite Furnace Atomic Absorption Spectrometry (GFAAS), and CLEM with Adsorptive Cathodic Stripping Voltammetry (AdCSV). The results of this study are also compared with those of a previous study of a well-characterized FA to determine the difference in the characteristic Ni(II)-binding properties of HA and FA. Chapter 5 also presents dissociation rate coefficients which are important speciation parameters of the metal complexes. Chapter 6 presents a computer-based speciation code, WHAM, versions V and VI, and presents a critical comparison of the WHAM-predicted $[\text{Ni}^{2+}]$, $[\text{Zn}^{2+}]$, $[\text{Cd}^{2+}]$, and $[\text{Cu}^{2+}]$ with the experimentally measured concentrations of the free-metal ions. Chapter 6 also presents the effects of inclusion (or exclusion) of Al and Fe in the WHAM modelling on the competition of Al and Fe with the above target trace metals. Chapter 7 presents the following experimental results of voltammetric determination of metal speciation parameters with a Thin Mercury-Film Rotating Disk Electrode: diffusion coefficients, conditional stability constants, free-metal ion concentrations and the lability parameters

of Cd(II), Pb(II), and Zn(II) complexes in model solutions of HA and in freshwaters from a few metal-impacted lakes from Rouyn-Noranda, Québec, and from the Grand River, Ontario. Chapter 8 presents concluding comments and major findings of this thesis.

-- Chapter 2 --

Scientific Background

Complex Formation by Metals

In freshwaters, metals are present as either ML complexes (where M is the metal ion and L is any ligand except water) or as free-metal ions, which have been reported to be good predictors of metal ecotoxicity. Free-metals ions; however, do not exist simply as uncomplexed species, since they are always associated (bonded) with the coordinating water, which can be represented as ' $M[H_2O]_x^{n+}$ ', where x is the number of coordinated H_2O molecules and n is the charge on the metal ion. Because of this, all complexation reactions in water are ligand exchange reactions.

Bonding Theory

Bonding can occur in one of three ways: it can either be electrostatic, covalent, or both. In most cases, bonds have some electrostatic, and some covalent character, the amount of each depends on electronegativity differences in the bonding atoms. If the bonding in a complex is primarily electrostatic, the product is called either an ion-pair or an outer-sphere complex [30].

When metals are bonding to complexants such as natural organic matter, or other ligands that are multidentate (having more than one binding site), the metal may bind to more than one site and form a chelate (from Greek, *chelos* = crab – has two binding claws) (see Table 2.1 [30]). For this to occur, the coordination number of the metal must be larger than one. Transition metals commonly have coordination numbers of 4 and 6, corresponding to tetrahedral or square planar geometry for 4-coordinate complexes, and octahedral geometry for 6-coordinate complexes. The more sites the metal is bound to,

Table 2.1: Complexants and the structure of complexes [30].

	Complexant	Cation	Example of complex
simple inorganic ligands	OH^-	M	$\text{M}(\text{OH})_2^0$
	Cl^-	M	$\text{M}-\text{Cl}^+$
bidentate organic ligand	(Alanine)	M	(chelate)
complexing site of a fulvic compound		M
complexing site of a polysaccharide	(Unidentate site of a uronic acid)	M
surface complexing sites of Fe(III) hydrous oxides	(Bidentate site)	M
fluoride	F^-

the stronger the complex [31]. Chelation is also dependent on the metal radius and its charge. A metal that is too large or too small will result in a non-ideal bite angle (the separation of the two donor atoms in a chelate ring) which will increase the ring strain and decrease the strength of the bond. The strengthening of complexes that are chelated is known as the chelate effect, which is mostly an entropy effect. This is demonstrated by the following reactions [31].

The chelating reaction (Eq. 2.1, where EN is the bidentate complexing ligand, ethylenediamine) results in an increase in the number of molecules in solution, whereas the non-chelating reaction (Eq. 2.2) produces no net change. The former has a more positive change in entropy and is the more favourable process.

Kinetics of Complex Formation

Reaction kinetics are important when considering the role of metals in freshwaters since the rates of formation of metal complexes with HS and the dissociation of these complexes may determine the speciation of a metal species which is related to its bioavailability. Factors that affect the speciation of metals in freshwaters are pH, ionic strength, and major ion concentrations (e.g. Ca^{2+} and Mg^{2+}). In freshwaters, these factors are mainly controlled by the surrounding geology and by biological processes such as respiration. Anthropogenic activities can alter these conditions and result in a change in the chemical speciation.

The rates of metal-ligand reactions in freshwaters are generally consistent with the Eigen mechanism, which describes the rapid formation of an outer-sphere complex between a metal and a ligand, followed by the rate-limiting loss of water from the inner hydration sphere of the metal ion:

where K_{os} is the outer-sphere stability constant, and k_{-w} is the water loss rate coefficient. K_{os} is dependent on the charges of the reacting species and the ionic strength of the medium, and k_{-w} is characteristic of the reacting metal (see Table 2.2). Values for K_{os} range from approximately 10 M^{-1} for ions with a single negative (-1) charge to $3 \times 10^3 \text{ M}^{-1}$ for ions with a triply negative (-3) charge. The rate determining step is the loss of water since the complex-formation reaction is usually fast. The rate coefficient for the complex formation, k_f , can be written as:

$$k_f [\text{M}^{2+}] [\text{L}^{n-}] = \frac{d[\text{ML}^{(2-n)+}]}{dt} \quad (2.5)$$

where

$$k_f = K_{\text{os}} k_{-w} \quad (2.6)$$

Table 2.2: Water loss rate constants (k_w) [18] and crystal radii [32] of various metals.

Metal Ion	k_w (s^{-1})	*Crystal Radii for Coordination
		Number 6 (pm)
Al^{3+}	1	67.5
Fe^{3+}	2×10^2	69 (78.5)
Ni^{2+}	3×10^4	83
Co^{2+}	2×10^6	79 (88.5)
Fe^{2+}	4×10^6	75 (92)
Cr^{3+}	5×10^7	75.5
Zn^{2+}	7×10^7	88
Cd^{2+}	3×10^8	109
Ca^{2+}	6×10^8	114
Cu^{2+}	1×10^9	87
Hg^{2+}	2×10^9	116
Pb^{2+}	7×10^9	133

* Ionic radii are for low-spin complexes. Values for high-spin complexes are shown in parentheses.

The magnitude of the rate of water loss constant, k_{-w} , can be explained by the electrostatic interactions between the metal cation and the coordinated water molecules. Note that k_{-w} is related to the charge of the cation, and inversely related to the size of the metal. Most metals have very fast water loss rate constants; however, for metals such as Ni^{2+} and Fe^{3+} the water loss rate constant is slow. The difference in these rate constants will have a large effect on how quickly the metal ions are able to react with the natural ligands.

The overall reaction for metal and ligand exchange reactions can be expressed as the following:

The rate expression for this reaction can be expressed as

$$-\frac{d[\text{ML}]}{dt} = k_{\text{ex}} [\text{ML}][\text{X}] \quad (2.8)$$

This reaction can either proceed via a disjunctive mechanism (similar to the dissociative and $\text{S}_{\text{N}}2$ mechanisms), where the complex first dissociates before the second metal or ligand reacts, or by an adjunctive mechanism (similar to the associative and $\text{S}_{\text{N}}1$ mechanisms), in which the initial complex goes through a transition state where the second metal or ligand coordinates to the initial complex before dissociation occurs. These two pathways can be summarized by the following equations for a metal exchange reaction; analogous equations can be written for ligand exchange reactions.

The adjunctive pathway is dependent on the ligand structure since the formation of the $\text{M}^* \text{ML}$ complex or the dissociation of the transition state can be rate limiting, and the disjunctive pathway is dependent on pH and ligand structure.

Since all exchange reactions are double-exchange reactions (i.e. all metals are initially bound to 6 coordinated water molecules), the kinetics must take this into account. A double exchange reaction which accounts for simultaneous metal- and ligand-exchange reactions can be expressed as

and can be either ligand- or metal-initiated. The reaction is disjunctive with respect the ML complex, and can be either adjunctive or disjunctive with respect to the incoming ligand or metal. This condition will depend on whether the initial $\text{M}^* \text{L}^*$ complex is strong or weak. If the initial $\text{M}^* \text{L}^*$ complex is strong, the pathway will likely be adjunctive, whereas if the complex is weak, the pathway will likely be disjunctive. In many cases, a ligand-initiated pathway which results in the replacement of one ligand with another often involves chain reactions, where the initial ligand reacts further with the initial ML complex. This may strongly affect the rates as these interactions may promote or terminate the mechanism.

Reactions in the Bulk Solution

The following discussion is mainly based on the work by de Jong et al and Davison [33-37]. Consider a metal complex that undergoes a first-order dissociation to give the dissociation products, M and L (charges have been omitted for simplicity).

where the formation and dissociation rate coefficients, k_f and k_d , are related to the conditional stability constant, K , by the principle of microscopic reversibility [30]. K is given by Eq. 2.13,

$$K = \frac{c_{ML}}{c_M c_L} = \frac{k_f}{k_d} \quad (2.13)$$

where c_{ML} , c_M and c_L are the concentrations of ML, M and L in the bulk solution, respectively.

In the presence of excess ligand,

$$k'_f = k_f c_L \text{ and } K' = K c_L = c_{ML}/c_M \quad (2.14)$$

The metal species in reaction 2.12 have characteristic lifetimes (the capability of the system to attain equilibrium) related to their formation and dissociation rate coefficients (e.g. $t = 1/k'_f$ and $t = 1/k_d$). When the lifetimes of M and ML are much shorter than the timescale of measurement (i.e. the interconversion between M and ML in the bulk solution is rapid), the system is in *dynamic* equilibrium as shown in Eq. 2.15.

$$k'_f t \text{ and } k_d t \gg 1 \quad (2.15)$$

When the lifetimes of M and ML are much larger than the timescale of measurement, equilibrium is not maintained. This is the *static* case and the metal complexes, ML, are *inert* (Eq. 2.16).

$$k'_f t \text{ and } k_d t \ll 1 \quad (2.16)$$

Conditions 2.15 and 2.16 define *dynamic* and *inert* metal complexes. *Dynamic* complexes require rapid reactions to maintain dynamic equilibrium, whereas *inert* complexes are essentially frozen in time (static) relative to the analytical timescale of measurement.

Coupled Diffusion of M and ML in the Diffusion Layer

Even when the formation and dissociation kinetics in the bulk solution are rapid (i.e. condition 2.15 is fulfilled), the system may still be limited by the supply of free-metal ions to the interface.

In a strongly complexed system, the purely kinetic flux, J_{kin} , [36] is described by,

$$J_{kin} = k_d c_{ML} \mu \quad (2.17)$$

where μ is the thickness of the **reaction layer** [38,39], a thin layer adjacent to the electrode surface in which the chemical reaction of interest (i.e. dissociation of ML) is significant for the flux of the electroactive species towards the electrode/solution interface as shown in the following equation,

$$\mu = (D_M/k'_f)^{1/2} \quad (2.18)$$

where D is the diffusion coefficient. The purely diffusion-controlled flux, J_{diff} , is described by Eq. 2.19 [36]

$$J_{\text{diff}} = D_{\text{ML}} c_{\text{ML}} / \delta \quad (2.19)$$

where δ is the thickness of the diffusion layer. Hence, the definition of *lability* requires that the kinetic flux from the dissociation of ML is much greater than the purely diffusional flux ($J_{\text{kin}}/J_{\text{diff}} \gg 1$). Combining Eqs 2.17, 2.18 and 2.19 gives the following:

$$J_{\text{kin}}/J_{\text{diff}} = (k_d/\varepsilon D_{\text{ML}} K')^{1/2} \delta_{\text{ML}} \gg 1 \quad (2.20)$$

where $\varepsilon = D_{\text{ML}}/D_{\text{M}}$ is the ratio of the diffusion coefficients of the metal complex ML and the metal ion, M, respectively.

The time, t , is related to thickness of the diffusion layer, δ , and the diffusion coefficient, D , by Eq. 2.21 [33].

$$t = \delta^2 / \pi D \quad (2.21)$$

The lability condition may be obtained by combining Eqs. 2.18 and 2.19,

$$\Lambda t^{1/2} \gg 1 \quad (2.22)$$

where the lability parameter, Λ , is given by,

$$\Lambda = \frac{k_d^{1/2}}{D_{\text{ML}} (\varepsilon K')^{1/2}} t^{1/2} \quad (2.23)$$

Hence, *lability* (Eq. 2.22) corresponds to the limiting condition where the formation and dissociation kinetics are rapid enough to ensure the coupled diffusion of M and ML to the interface, which occurs with a mean (weighted average) diffusion coefficient, $\bar{D}$, and diffusion layer thickness, $\bar{\delta}$.

Eq. 2.23 has been derived for the condition of strong complexation ($\epsilon K' \gg 1$). The general form for the lability parameter has been derived by de Jong et al. [34,35].

$$\Lambda = \frac{k_d^{1/2} \epsilon^{-1/2} (\epsilon^{-1} + K')}{K' (1 + K')^{1/2}} \quad (2.24)$$

The *non-labile* case is defined by condition 2.25.

$$\Lambda t^{1/2} \ll 1 \quad (2.25)$$

The distinction between *labile* and *non-labile* systems is related to the nature of the flux towards the interface, not the dynamics in the bulk solution. If the kinetics are rapid relative to the diffusional transport, the flux is purely diffusion-controlled and the system is *labile*; if the kinetics are slow relative to the diffusional transport, the flux is controlled by the kinetics and the system is *non-labile*. The fundamental distinction between *static (inert)* systems and *non-labile* systems is that static systems obey condition 2.16, whereas non-labile systems obey conditions 2.15 and 2.25. A summary of the classification of kinetic regimes for metal complexes is presented in Fig. 2.1.

The preceding discussion illustrates the importance of investigating both 1) thermodynamic parameters, such as equilibrium constants, and 2) kinetic parameters,

such as diffusion coefficients and the formation/dissociation kinetics of metal complexes. Although the determination of diffusion coefficients, conditional stability constants and dissociation rate coefficients are relatively straightforward for simple, homogeneous chemical systems, the investigation of natural systems is complicated by the structural and physicochemical complexity and heterogeneity of naturally-occurring complexants, such as HA and FA. As a consequence, the equilibrium constants and rate coefficients do **not** represent characteristic constants for the system, but must be described by some distribution that is a function of the degree of occupation of the binding sites of the complexants.

Figure 2.1 Kinetic regimes for metal complexes defined by the timescale of measurement (adapted from van Leeuwen [37]).

-- Chapter 3 --

Theoretical Background

Theory

Competing Ligand Exchange Method (CLEM): The Kinetic Model

The kinetic model proposed by Olson et al. [40] was adapted [41,42] to measure the dissociation kinetics of complex ML, where M is a metal ion, and L is a complexant such as HA. The kinetics of metal complex dissociation in freshwaters can be modelled by employing the CLEM using Chelex 100 resin or dimethylglyoxime (DMG) as the competing ligands.

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_f and k_d are the rate coefficients for the complex formation and dissociation, respectively. The charges on M and L have been neglected in this general statement of the reaction.

In CLEM the competing ligand, P, reacts with M as follows:

The model assumes that

- (a) reaction 3.2 is much faster than reaction 3.1,

- (b) the MP complex is thermodynamically much stronger than ML complex,
- (c) $[P] \gg [M]$, and
- (d) P does not bind with the ML complex.

Because $[P] \gg [M]$, reaction 3.2 is pseudo-first-order. Since k'_f is large, as has been determined using a Perkin Elmer Elan 6000 Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) [43,44], and P is in sufficiently large excess, the condition $k'_f[P] \gg k'_d[L]$ is valid. Hence, the overall reaction (Eq. 3.3) is irreversible.

The rate of formation of the MP complex is determined by the rate of dissociation of ML_i in Eq. 3.3, and the rate expression is simply as follows:

$$\frac{d[MP]}{dt} = -\frac{d[ML_i]}{dt} = k_{d,i} [ML_i] \quad (3.4)$$

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 , $C_{ML}(t)$, decreases exponentially as:

$$C_{ML}(t) = \sum_{i=1}^n C_i^0 \exp(-k_{d,i}t) \quad (3.5)$$

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, $C_{ML}(t)$, is measured as a function of time.

The time taken for completion of reaction in Eq. 3.2, (i.e. its rate) will establish the theoretical upper limit of the dissociation reaction that can be observed with this technique.

In the CLEM/AdCSV, the concentration of “free” metal ion produced by the dissociation of ML at any time t , $C_M(t)$, is described by an exponential function that increases to a limiting value given by Eq. 3.6:

$$C_M(t) = \sum_{i=1}^n C_i^0 [1 - \exp(-k_{d,i}t)] \quad (3.6)$$

CLEM/AdCSV for nickel using DMG as the competing ligand has also been reported by other workers [45-47].

Anodic Stripping Voltammetry at a Thin Mercury Film Rotating Disk Electrode (TMF-RDE)

The general solution of the three-dimensional convective-diffusion equation to describe the limiting flux, J , of a metal complex system at the electrode surface was derived by de Jong and van Leeuwen [34,35]. The solution is fully rigorous with respect to the differences in the diffusion coefficients between the free-metal ion, M , and the metal complexes, ML , and to the values of the formation and dissociation rate coefficients, thus including the conditional stability constant of the complex:

$$J(t) = D_M^{1/2} k_d^{1/2} c_{M,T} \frac{(\varepsilon^{-1} + K')^{3/2}}{K'(1 + K')} \exp(\Lambda^2 t) \operatorname{erfc}(\Lambda t^{1/2}) \quad (3.7)$$

where $c_{M,T}$ is the total metal concentration which equals the concentration of the free metal ion plus the concentration of the metal-ligand complex ($c_{M,T} = c_M + c_{ML}$), $K' = Kc_L$, D is the diffusion coefficient, $\varepsilon = D_{ML}/D_M$ and Λ is the lability parameter as defined by Eq. 2.23.

Under the totally mass-transfer-limited conditions at a Rotating Disk Electrode (RDE), the Levich equation (Eq. 3.8) predicts that the limiting current, i_{lim} , is proportional to the total metal concentration, $c_{M,T}$, and the angular velocity of the RDE, $\omega^{1/2}$ (rad s⁻¹).

$$i_{lim} = 0.62nFAD^{2/3}\omega^{1/2}\nu^{-1/6}c_{M,T} \quad (3.8)$$

where n is the number of electrons involved in the electrode reaction, F is the Faraday constant, A is the surface area of the electrode, and ν is the kinematic viscosity (viscosity/density). A typical value of ν for an aqueous solution is 10^{-6} m s⁻¹, which has been adopted for this work [48]. A simple steady-state diffusion layer yields,

$$i_{lim} = nFA\left(\frac{D}{\delta}\right)c_{M,T} \quad (3.9)$$

where δ is the thickness of the diffusion layer. Thus, for an RDE

$$\left(\frac{D}{\delta}\right) = 0.62D^{2/3}\omega^{1/2}\nu^{-1/6} \quad (3.10)$$

The timescale of measurement is defined by the thickness of the Nernstian diffusion layer. At an RDE, δ , is given by [49]:

$$\delta = 1.61D^{1/3}\omega^{-1/2}\nu^{1/6} \quad (3.11)$$

Combining Eqs. 3.7 and 3.11 gives,

$$J(t) = D_M^{1/2} k_d^{1/2} c_{M,T} \frac{(\varepsilon^{-1} + K')^{3/2}}{K'(1 + K')} \exp(\Lambda_{ss}^2 \delta^2) \operatorname{erfc}(\Lambda_{ss} \delta) \quad (3.12)$$

where Λ_{ss} is given by,

$$\Lambda_{ss} = \frac{k_d^{1/2}(1 + \varepsilon K')^{1/2}}{D_{ML}^{1/2} \varepsilon K'} \quad (3.13)$$

Combining Eqs 3.13 and 2.21 for the relationship between time and the diffusion layer thickness, the lability condition given by Eq. 2.22 may be expressed equivalently as,

$$\Lambda_{ss} \delta \gg 1 \quad (3.14)$$

The limiting current is related to the flux by Eq. 3.15:

$$i_{lim} = nFAJ(t) \quad (3.15)$$

Combining Eqs. 3.12 and 3.15 gives the limiting current at an RDE.

$$i_{lim} = nFAD_M^{1/2} k_d^{1/2} c_{M,T} \frac{(\varepsilon^{-1} + K')^{3/2}}{K'(1 + K')} \exp(\Lambda_{ss}^2 \delta^2) \operatorname{erfc}(\Lambda_{ss} \delta) \quad (3.16)$$

Conditional Stability Constant of Zn(II)-DOC Complexes by the van den Berg and Ružić Method [50,51].

Titration curves for Zn(II) were plotted by using a method developed by van den Berg and Ružić, which is based on the Scatchard Plot. Scatchard plots are obtained from the Scatchard equation which was derived for investigating the equivalence of binding sites on macromolecular substances [52]. Increments of Zn(II) were added to a water sample and, following equilibration, the complexing capacity and conditional stability constant were derived from the titration curve. The concentration of ML increases as a result of the metal complexation with free ligand. A marked change in the gradient of the titration curve occurs when the metal concentration is equivalent to that of the ligand. Beyond this point, the metal complexation capacity of the ligand is saturated, and the slope of the titration curve is identical to the slope of the titration curve observed in the absence of complexing ligands. Extrapolation of the linear portion of the curve down to the x -axis gives a crude measure of the ligand concentration (see Fig. 3.1). By considering the metal ligand equilibrium in solution, the conditional stability constant, K' , is given by the following equation:

$$K' = [\text{ML}]/[\text{M}^{2+}][\text{L}] \quad (3.17)$$

where the total ligand concentration, C_{lx} , is given by Eq. 3.18.

$$C_{\text{lx}} = [\text{ML}] + [\text{L}] \quad (3.18)$$

Figure 3.1 Schematic representation of a metal-ligand titration curve. The dotted line represents the titration curve in the absence of ligand. Extrapolation of the linear portion of the titration curve to the x -axis gives a crude estimate of the ligand concentration, C_{lx} .

In the above equations, $[ML]$ is the concentration of the metal-ligand complex, $[M^{2+}]$ is the free-metal ion concentration, and $[L]$ is the free ligand concentration.

Substitution and rearrangement of Eqs. 3.17 and 3.18 gives Eq. 3.19,

$$[ML]/[M^{2+}] = K'C_{lx} - K'[ML] \quad (3.19)$$

which provides the equation for the Scatchard plot. Rearrangement of the Scatchard equation gives the van den Berg/Ružić equation:

$$[M^{2+}]/[ML] = [M^{2+}]/C_{lx} + 1/K'C_{lx} \quad (3.20)$$

By plotting $[M^{2+}]/[ML]$ vs. $[M^{2+}]$, a straight line is obtained with a slope of $1/C_{lx}$ and an intercept of $1/K'C_{lx}$. This method, as well as the Scatchard plot, assumes that metal complexation in natural waters can be represented by a single ligand and complexes having 1:1 stoichiometry. Curvature in either of the plots is an indication of the presence of two or more ligands which form metal complexes having different conditional stability constants.

Xue and Sigg's Equilibrium-Based Ligand Exchange Method for Cu [53,54] and Cd [55]

This method has been described by Xue et al. and is based on ligand exchange equilibrium between natural ligands in water samples and an added, well-characterized ligand (e.g. catechol for $[Cu^{2+}]$ [53,54] and ethylenediamine (EN) for $[Cd^{2+}]$ [55]). The samples are first titrated with metal in the presence of an added competing ligand.

Following the equilibration of the sample, the Cd(II)- and Cu(II)-complexes were determined using AdCSV for Cu(II), or ASV for Cd(II).

The total dissolved metal distributes between labile and inert species. With the above assumption, the peak current i_p (A) is proportional to the concentration of labile species:

$$i_p / S = [M_{lab}] = [M^{2+}] + [M_{in}] + \sum [MR_i] = [M^{2+}](1 + \alpha_{in} + \alpha_R) \quad (3.21)$$

where S stands for to the sensitivity in the $A M^{-1}$; $[M_{lab}]$, $[M_{in}]$, and $[MR_i]$ represent the labile metal, inorganic metal, and metal complexed to the competing ligand, and α_{in} and α_R are the inorganic, and added ligand complexing coefficients which are equal to the ratios of inorganic, and added ligands to the $[M^{2+}]$, respectively. In most cases, the term $(1 + \alpha_{in})$ is negligible in Eq. 3.18, therefore the $[M^{2+}]$ in the presence of added ligand can be estimated from Eq. 3.22:

$$[M^{2+}] = \sum [MR_i] / \alpha_R = i_p / S \alpha_R \quad (3.22)$$

With the assumption of a 1:1 stoichiometry of ML, where L is a natural ligand (omitting charges for simplicity):

$$[M^{2+}] + L = ML, \quad K = [ML] / [M^{2+}][L] \quad (3.23)$$

the total Cd or Cu is distributed as follows:

$$[M_t] = [M_{nat}] + \sum [MR_i] = [M^{2+}] + [M_{in}] + [ML_i] + \sum [MR_i] \quad (3.24)$$

where $[M_t]$ is the total Cd or Cu concentration, $[ML_i]$ is the concentrations of metal complexes with natural organic ligands, and $[M_{nat}]$ is determined from the difference between $[M_t]$ and $\sum [MR_i]$.

The addition of the competing ligand binds a portion of the metal in the sample and thereby reduces both $[M_{nat}]$ and $[M^{2+}]$. Thus, to determine the ambient $[M^{2+}]$ of the sample, one must be able to correct back to the $[M^{2+}]$ that was present before the addition of the competing ligand. In cases where there were complete titration curves, the initial ambient $[M^{2+}]$ was determined by interpolation of the plot of $[M^{2+}]$ versus $[M_{nat}]$.

-- Chapter 4 --

Experimental Methods

Materials and Methods

Ultrapure water of resistivity 18.2 M Ω -cm, obtained direct from a Milli-Q-Plus water purification system (Millipore Corporation), was used to prepare all standards and test solutions. A 2 M stock solution of NaOH was purified by electrodeposition at -1.4 V vs. SCE for at least 72 hours immediately prior to use. A 2 M stock solution of pure KNO₃ was prepared by dissolving an appropriate quantity of pure KNO₃ (Aldrich) in ultrapure water. For formation of a Thin Mercury Film (TMF), a stock solution of 5000 $\mu\text{g/mL}$ Hg(NO₃)₂ was prepared by dissolving pure mercury in ultrapure nitric acid (Seastar, Canada) and diluting with ultrapure water to contain 1% (v/v) nitric acid.

Teflon bottles used for storing filtered samples were pre-cleaned by soaking in 10% nitric acid (Reagent Grade) for one week at room temperature, followed by rinsing five times with ultrapure water. All containers were filled with ultrapure water and stored until they were used. Teflon containers used for sample preparation were pre-cleaned by soaking in 10% (v/v) nitric acid (Reagent grade) for a minimum of 24 hours and rinsed five times with ultrapure water prior to use.

Metal Solutions

The solutions containing 10 $\mu\text{g/mL}$ of Cd, Cu, Ni, Pb and Zn were prepared separately for each metal by diluting the appropriate quantity of a 1000 $\mu\text{g/mL}$ standard solution (Fisher, A.C.S. reagent) in ultrapure water; the final solution was made to contain 1% (v/v) HNO₃ in order to prevent metal loss by adsorption on the container walls.

Buffers

A stock solution of 2 M HEPES-NaOH buffer (pK_a 7.55) was prepared by dissolving an appropriate quantity of HEPES (N-2-Hydroxyethylpiperazine-N'-3-ethanesulfonic acid, BDH 99%) in ultrapure water and adjusting the pH of the solution to 7.5 using NaOH (ANACHEMIA). A stock solution of PIPES (Piperazine-N,N'-bis[2-ethanesulfonic acid] SIGMA, ACS Grade) was prepared by dissolving an appropriate amount of PIPES in ultrapure water and adding NaOH to the solution. HEPES and PIPES are widely used as buffers because, being very weak binders of metals, they do not measurably interfere in the metal-complexation reactions in which they are used as buffers [56]. A stock solution of 2.0 M acetic acid was prepared by diluting ultrapure glacial acetic acid (Aristar grade) with ultrapure water. A stock solution of 2.0 M sodium acetate solution (to be used as an electrolyte) was prepared by dissolving an appropriate quantity of sodium acetate trihydrate (ACS grade/BDH) in ultrapure water. This solution was then purified by electrodeposition at -1.4 V vs. SCE for at least 72 h immediately prior to its use.

Complexing Ligands

A solution of 2 M ethylenediamine (EN) was prepared by diluting an appropriate quantity of EN (Aldrich) in ultrapure water. A solution of catechol (Aldrich Chemical Company, 99+% pure) was freshly prepared (as catechol is degraded by O_2 oxidation) prior to each experiment, by dissolving an appropriate quantity of solid catechol in ultrapure water.

For the kinetic speciation experiments, a 0.1 M dimethylglyoxime (DMG) (Fisher Scientific) stock solution was prepared by dissolving an appropriate mass of solid DMG in pure methanol (Caledon, Spectro Grade).

Chelex-100 (Biorad) resin was prepared by washing with 150 mL of ultrapure water. Chelex-100 is a styrene divinylbenzene copolymer containing paired iminodiacetate ions which act as chelating groups in binding nickel. These groups have a high attraction for transition metals. The effectiveness of Chelex as a chelating agent is pH dependent since at low pH values, the cheleation groups are protonated and are not available to bind nickel. At the pH values used for the kinetic study (6-8), both iminodiacetic acid groups are ionized and form chelates with nickel. This ligand is used in the CLEM/GFAAS technique.

In CLEM/AdCSV, two DMG molecules bind to each nickel ion. The Ni-DMG complex is then adsorbed at the surface of the Hg drop and the stripping current is measured. Both ligands are strong chelators, and, as required by the kinetic model, are many orders of magnitude stronger than HA for binding nickel [57]. Figure 4.1 shows the structures of the above two competing ligands used in the kinetic speciation experiments.

For Cu speciation studies by CLEM and AdCSV, catechol, tropolone, 8-hydroxyquinoline (oxine), salicylaldoxime [58] and benzoylacetone [59] are commonly used competing ligands. Catechol is one of the most commonly used and well known competing ligands for copper and was applied for this work.

The formation of the Cu-catechol complex is illustrated in Eq. 4.1.

Figure 4.1 Competing ligands:

(a) DMG-Ni complex.

(b) Forms of Chelex 100 resin at different pH values. The form at pH 7.41 was used in this work.

The use of catechol as the competing ligand is of particular interest, since catechol is a diol, containing similar functional groups to those found on the natural organic ligands (alcoholic and phenolic functional groups). The surface-active Cu-catechol complex then adsorbs at the surface of the Hg electrode. During the stripping step, the cathodic peak is due to the reduction of Cu(II) in the adsorbed complex to Cu(0) [60].

Humic Acid

The soil HA was supplied by Dr. Les Evans [61] (University of Guelph, Guelph, Ontario), who characterized and purified the HA according to the procedure recommended by the International Humic Substances Society. Elemental analysis of the HA was as follows: 53.15% C, 4.81% H, 3.35% N, 0.46% S, and 37.99% O. Solid state ^{13}C NMR was used to estimate the concentration of carboxylic and phenolic groups to be 6.21 and 3.55 mmol/g, respectively [61]. The bidentate complexing capacity, which is defined as the average concentration of the carboxylic and phenolic sites which are involved in the nickel binding, was calculated to be 4.88 mmol/g. A stock solution of approximately 1.00 g L^{-1} HA was prepared by dissolving approximately 0.100 g of the above HA in 100 mL of ultrapure water and adding NaOH – the pH of the HA solution was initially 10, and was then adjusted to 8.0 with ultrapure HNO_3 . The HA solution was stored in the dark at 4°C . Since the molecular weight of the HA was not known, the metal to HA mole ratios for the work presented in Chapters 5 and 7 were calculated based on the bidentate complexing capacity of the HA.

Field-Study Area and Water Samples

Figures 4.2 and 4.3 show maps of the sampling area of Rouyn-Noranda, Québec, and the Grand River, Ontario, from where the freshwater samples were collected. The lakes in the Rouyn-Noranda area were impacted by acids and metals from current metal mining operations, abandoned mines, and atmospheric deposition from a large, nearby copper smelter that had been in operation since 1927 [62]. Lakes Opasatica and Vaudray were upwind from the smelter, and were the least affected by atmospheric fallout from the smelter. Lakes Dufault and Osisko were the most highly contaminated lakes based on data from previous studies [62-64]. Lake Osisko also received liquid effluents from the smelting operations in the past. Samples of surface waters were collected in 2.2 L Teflon bottles (screw-capped) in June of 2001 from lakes Opasatica, Dufault, Osisko, and Vaudray, and in July and August of 2002 from lakes Opasatica and Dufault.

The Grand River is a Canadian heritage river and originates in the highlands of Dufferin County, and flows through the heartland of southern Ontario. The watershed consists of all the land that drains into the Grand River through tributary creeks and rivers such as the Conestogo, Speed, Eramosa and Nith. Part of the Grand River's course flows through the Carolinian life zone, which contains a southern type of forest that is found only in this area of Canada. Many plants and animals that are rare in our country are found here. However, because of human activities, its water quality deteriorated to the point where it was a public health concern. The sampling station was located at coordinates 44° 05' 48 N, 80° 22' 27 W.

Figure 4.2 Map of the sampling area of Rouyn-Noranda, Québec and the lakes investigated. The filled circle is the location of the smelter. Adapted from J.C. Brodeur [37].

Figure 4.3 Map of the sampling area of the Grand River, Ontario. The star shows the location of the sampling site.

The water samples were brought to our laboratories for treatment and analysis, which were done without any delay.

Filtration of Water Samples

Immediately after the receipt of the water samples at our laboratories, they were filtered through 0.45 μm filters (Gelman Material No. 63077) to remove suspended particulate matter. An aliquot of the filtered sample was immediately transferred to a 500 mL Teflon bottle and acidified to contain 1% (v/v) ultrapure HNO_3 to preserve the sample integrity (i.e. to prevent loss of metals to the container walls); this aliquot was used for the determination of total dissolved metals by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS). The remaining filtered (unacidified) water samples were poured into pre-cleaned Teflon bottles which were filled to the top, closed with screw caps, and stored in the dark at 4°C until the water samples were subjected to further tests.

Determination of pH, Total Metals, Anions, Cations, and DOC

The pH and conductivity of the water samples were measured by an Accumet 20 pH/conductivity meter, fitted with an Accuplast combination glass electrode. Total dissolved metals were determined by ICP-MS using the SCP Science ICP Standards for Multi-Element Analysis, for calibration. The limits of detection for each metal were determined from 1% (v/v) nitric acid blanks and were as follows: Ni, 9 pg/mL; Cu, 9 pg/mL; Cd, 3 pg/mL; Zn, 36 pg/mL; Pb, 9 pg/mL. DOC was measured by OI Analytical Model 1010 TOC Analyzer, anions by a Dionex Series 4000i Ion Chromatograph, and major cations by a Thermo Jarrel Ash IRIS Radial Inductively Coupled Plasma Optical

Emission Spectrometer (ICP-OES). The experimental results for these analyses are presented in Tables 4.1, 4.2a and 4.2b.

Apparatus

In the CLEM/GFAAS technique, a Zeeman GFAAS Varian model SpectrAA 880 was used to determine the concentration of nickel that remained bound to HA. In the CLEM/AdCSV, Differential Pulse ASV (DPASV), and RDE-ASV voltammetric measurements were carried out using a computer-controlled Autolab PGSTAT 30 potentiostat/galvanostat (Eco Chemie BV, The Netherlands), equipped with a Metrohm 663 VA stand (Metrohm, Switzerland). The working electrode was a static mercury drop electrode or a TMF-RDE (Metrohm, Switzerland), and the reference electrode was a Ag/AgCl electrode, held in a glass tube filled with a 3 M KCl solution and fitted with a porous Vycor tip (Bioanalytical Systems, Inc., USA). A platinum rod (Metrohm, Switzerland) was used as the counter electrode. Analysis of voltammetric peaks was carried out using the General Purpose Electrochemical Software V4.9 (Eco Chemie BV, The Netherlands). The data were transferred to and saved on a computer for subsequent analysis.

Analytical Methods

Optimization of the Analytical Procedure

Before the start of the CLEM/GFAAS experiments, the ashing and atomization temperatures were optimized for maximum recovery of nickel. For the test, a Ni(II)-HA solution with a [Ni] of 2.6×10^{-7} M was used to determine the optimum experimental

Table 4.1: Concentrations of dissolved metals and DOC in the freshwater samples, collected from the Grand River, Ontario in June of 2002 and the lakes in Rouyn-Noranda, Québec, in June of 2003. Uncertainties were less than 5% RSD unless stated otherwise.

	Lake Vaudray	Lake Dufault	Grand River
pH (± 0.2)	6.1	7.0	7.5
DOC (± 0.2 mg/L)	11	16	15
[M] (M)^a			
Pb	6.7×10^{-10}	2.9×10^{-9}	2.4×10^{-10}
Cd	7.1×10^{-10}	4.9×10^{-9}	1.8×10^{-10}
Zn	6.9×10^{-8}	9.6×10^{-7}	N/D
Cu	4.2×10^{-8}	1.9×10^{-7}	2.0×10^{-8}
Al	2.9×10^{-6}	1.6×10^{-7}	3.9×10^{-7}
Fe	6.8×10^{-6}	3.7×10^{-5}	9.8×10^{-7}
Ni	1.5×10^{-8}	1.2×10^{-8}	4.0×10^{-8}
Co	9.5×10^{-10}	1.6×10^{-9}	N/D
Cr	3.5×10^{-8}	4.4×10^{-8}	N/D
Major Cations (M)^b			
Ca	1.1×10^{-4}	4.9×10^{-4}	1.3×10^{-3}
Mg	1.2×10^{-5}	1.3×10^{-4}	6.3×10^{-4}
K	7.7×10^{-6}	5.4×10^{-5}	1.1×10^{-5}
Na	9.3×10^{-8}	1.4×10^{-8}	2.2×10^{-7}
Major Anions (M)^c			
F	9.0×10^{-6}	7.1×10^{-6}	7.3×10^{-6}
Cl	1.7×10^{-5}	1.4×10^{-4}	4.0×10^{-4}
NO₃	5.7×10^{-6}	7.0×10^{-6}	4.3×10^{-5}
SO₄	4.3×10^{-5}	3.7×10^{-4}	6.7×10^{-5}

^a Determined by ICP-MS

^b Determined by ICP-OES

^c Determined by Ion Chromatography

N/D: Not Detectable

Table 4.2a: Input parameters used for calculating free-metal ion concentrations in the four lakes using WHAM. Blank spaces refer to metals that were not determined.

	Samples Collected in June of 2001				Samples Collected in July of 2002				Samples Collected in August of 2002			
	Lake Dufault	Lake Osisko	Lake Vaudray	Lake Opasatica	Lake Dufault	Lake Opasatica	Lake Dufault	Lake Opasatica	Lake Dufault	Lake Opasatica	Lake Dufault	Lake Opasatica
pH of samples	7.1	9	6.9	7.2	7.2	7.7	7.3	7.6	7.3	7.6	7.3	7.6
Experimental pH ^a	7.3	7.8	7.3	7.3	7.6	7.6	7.3	7.3	7.3	7.3	7.3	7.3
DOC (± 0.2 mg/L)	6.9	3.4	10	6.4	10	11	6.7	6.9	6.7	6.9	6.7	6.9
[M] ^b (M)												
Ni	2.0×10^{-8}	2.7×10^{-8}	1.7×10^{-8}	2.6×10^{-8}	1.6×10^{-8}	1.4×10^{-8}	3.1×10^{-8}	1.5×10^{-8}	3.1×10^{-8}	1.5×10^{-8}	3.1×10^{-8}	1.5×10^{-8}
Zn	1.3×10^{-6}	2.6×10^{-7}	6.1×10^{-8}	4.6×10^{-8}	8.3×10^{-7}	5.7×10^{-8}	5.8×10^{-7}	4.6×10^{-8}	5.8×10^{-7}	4.6×10^{-8}	5.8×10^{-7}	4.6×10^{-8}
Cd	8.0×10^{-9}	1.8×10^{-9}	8.9×10^{-10}	8.9×10^{-10}	3.1×10^{-9}	1.8×10^{-10}	2.6×10^{-9}	4.5×10^{-11}	2.6×10^{-9}	4.5×10^{-11}	2.6×10^{-9}	4.5×10^{-11}
Cu	2.1×10^{-7}	9.6×10^{-8}	3.8×10^{-8}	3.8×10^{-8}	1.5×10^{-7}	4.2×10^{-8}	2.4×10^{-7}	4.6×10^{-8}	2.4×10^{-7}	4.6×10^{-8}	2.4×10^{-7}	4.6×10^{-8}
Fe	2.9×10^{-6}	5.4×10^{-7}	1.2×10^{-6}	5.6×10^{-7}	3.5×10^{-7}	3.5×10^{-7}	1.5×10^{-6}	1.1×10^{-6}	1.5×10^{-6}	1.1×10^{-6}	1.5×10^{-6}	1.1×10^{-6}
Al	2.6×10^{-7}	3.7×10^{-7}	2.9×10^{-6}	2.4×10^{-6}	2.3×10^{-7}	1.2×10^{-6}	5.6×10^{-7}	1.1×10^{-7}	5.6×10^{-7}	1.1×10^{-7}	5.6×10^{-7}	1.1×10^{-7}
Co	5.1×10^{-9}	1.7×10^{-9}	1.2×10^{-8}	1.0×10^{-9}	N/D	N/D	N/D	8.5×10^{-10}	N/D	8.5×10^{-10}	N/D	8.5×10^{-10}
Pb	2.8×10^{-4}	4.8×10^{-5}	6.3×10^{-4}	5.3×10^{-4}	1.4×10^{-9}	7.2×10^{-10}	2.3×10^{-10}	1.9×10^{-10}	2.3×10^{-10}	1.9×10^{-10}	2.3×10^{-10}	1.9×10^{-10}
Mn	9.5×10^{-7}	9.7×10^{-9}	2.4×10^{-8}	1.5×10^{-8}	N/D	N/D	3.6×10^{-9}	6.7×10^{-9}	3.6×10^{-9}	6.7×10^{-9}	3.6×10^{-9}	6.7×10^{-9}
Sr	4.1×10^{-7}	8.6×10^{-7}	1.8×10^{-7}	1.6×10^{-6}								
Ba	6.1×10^{-8}	1.8×10^{-7}	4.7×10^{-8}	5.5×10^{-8}								

^a The experimental pH was fixed by the pH of HEPES buffer

^b Determined by ICP-MS

N/D: Not Detectable

Uncertainties in pH values were ± 0.2 . All other uncertainties were less than 5% RSD unless stated otherwise.

Table 4.2b: Input parameters used for calculating free-metal ion concentrations in the four lakes using WHAM. Blank spaces refer to metals that were not determined..

Inputs	Samples Collected in June of 2001				Samples Collected in July of 2002				Samples Collected in August of 2002	
	Lake Dufault	Lake Osisko	Lake Vaudray	Lake Opasatica	Lake Dufault	Lake Opasatica	Lake Dufault	Lake Opasatica	Lake Dufault	Lake Opasatica
Major Cations^a (M)										
Mg	1.3×10^{-4}	2.1×10^{-4}	4.0×10^{-5}	1.2×10^{-4}	1.4×10^{-4}	1.4×10^{-4}	1.4×10^{-4}	1.6×10^{-4}	1.6×10^{-4}	1.3×10^{-4}
Ca	4.2×10^{-4}	6.2×10^{-4}	9.0×10^{-5}	2.5×10^{-4}	4.9×10^{-4}	3.5×10^{-4}	3.5×10^{-4}	5.1×10^{-4}	5.1×10^{-4}	2.9×10^{-4}
Na	1.8×10^{-4}	1.3×10^{-3}	3.8×10^{-5}	1.4×10^{-4}	1.7×10^{-4}	2.2×10^{-4}	2.2×10^{-4}	1.7×10^{-4}	1.7×10^{-4}	1.6×10^{-4}
K	1.9×10^{-5}	3.8×10^{-5}	1.0×10^{-5}	2.8×10^{-5}	1.7×10^{-5}	2.0×10^{-5}	2.0×10^{-5}	1.6×10^{-5}	1.6×10^{-5}	1.9×10^{-5}
NH ₄ ⁺ ^b	2.2×10^{-6}	N/D	N/D	8.2×10^{-7}						
Major Anions^b (M)										
F ⁻	2.9×10^{-6}	7.2×10^{-6}	1.9×10^{-6}	2.0×10^{-6}	N/D	N/D	N/D	N/D	N/D	1.1×10^{-6}
Cl ⁻	1.3×10^{-4}	1.6×10^{-3}	5.5×10^{-6}	9.8×10^{-5}	1.1×10^{-4}	1.2×10^{-4}	1.2×10^{-4}	1.2×10^{-4}	1.2×10^{-4}	1.1×10^{-4}
SO ₄ ²⁻	4.8×10^{-4}	5.0×10^{-4}	5.5×10^{-6}	9.3×10^{-5}	3.8×10^{-4}	1.6×10^{-4}	1.6×10^{-4}	3.8×10^{-4}	3.8×10^{-4}	1.0×10^{-4}
Br ⁻	N/D	3.6×10^{-6}	N/D	N/D						
NO ₃ ⁻	N/D	N/D	N/D	2.8×10^{-6}	8.4×10^{-3}	1.3×10^{-3}	1.3×10^{-3}	4.7×10^{-6}	4.7×10^{-6}	1.1×10^{-6}

^a Determined by ICP-OES

^b Determined by Ion Chromatography

N/D: Not Detectable

Uncertainties in all measurements were less than 5% RSD.

parameters. The ashing temperature was optimized first by keeping the atomization temperature constant at 2400°C (see Fig. 4.4). After optimization of the ashing temperature, the atomization temperature was optimized by keeping the ashing temperature constant at the optimum ashing temperature and varying the atomization temperature (see Fig. 4.5). The results summarized below were used throughout the study. The electrochemical parameters for AdCSV had been previously optimized [65] and were kept the same to facilitate comparison of the results of HA and FA.

CLEM/GFAAS for Model Solutions of Ni and HA

A series of model solutions for the CLEM/GFAAS experiment were prepared by adding known amounts of the HA stock solution to a 2.6×10^{-7} M nickel solution to make the [Ni]/[HA] mole ratios 0.1, 0.01, and 0.001. The [Ni]/[HA] mole ratios were based on the concentration of binding sites (carboxylic and phenolic) on the HA as determined by Dr. Evans [61]. The [HA] in each solution was 0.50, 5.0, and 50 mg/L, respectively, which was chosen to model a wide range of freshwater conditions. The [Ni]/[HA] mole ratio for saturation of the strong sites was estimated to be 0.01; but, to be on the safe side, a mole ratio of 0.001 was used as the starting point. The pH values of the model solutions were adjusted to 6.0, 7.0, or 8.0 by adding NaOH. The solutions were equilibrated for 24 hours in the dark at room temperature. The pH of the above model solutions were again determined immediately prior to analysis.

100 mL of the above model solution was placed in a Teflon Reactor (see Fig. 4.6), and 1% (w/v) of the pre-treated Chelex 100 resin was added to the solution. Chelex 100 resin was chosen because it binds Ni(II) very rapidly and forms strong complexes with Ni^{2+} ; it

Figure 4.4 Loss of atomic vapour as a function of ashing temperature, using an atomization temperature of 2400°C. Error bars represent the 95.5% confidence interval of 3 replicate measurements.

Figure 4.5 The atomization as a function of the atomization temperature, using the optimum ashing temperature of 800°C. Error bars represent the 95.5% confidence interval of 3 replicate measurements.

Figure 4.6 Teflon reactor used in the CLEM/GFAAS study.

had been demonstrated earlier [66] that the uptake of Ni(II) by Chelex 100 in the absence of other complexants is fast – much faster than the dissociation of the Ni(II)-FA complex, and also, of the Ni(II)-HA complex (because the dissociation of the Ni(II)-HA complex is slower).

The above solution was then stirred with a Teflon-coated magnetic stirring bar. Sample aliquots were collected in the sample cups and transferred to the GFAAS. The first aliquot was taken before the Chelex 100 resin was added to the sample to verify that the total concentration of nickel in the model solution was equal to the amount of nickel added before equilibration to account for any losses to the container walls. The concentrations of the nickel complexes remaining undissociated, $C_{ML}(t)$, (Eq. 3.5) were measured by GFAAS as a function of time. For the determination of nickel concentration by GFAAS, 20 μL of the test solution was injected into the GFAAS. The signal was measured in peak area mode using the pre-established analytical calibration curve. The GFAAS program used Ni analysis line of 232.0 nm, and the optimum values for ashing and atomization temperatures. Each completed determination was followed by a 2-second clean-up cycle of the graphite tube at 2500°C. Every third sample was a blank.

Quality control for Ni analysis included repeated injections of samples (typically $\leq 5\%$ relative standard deviation among Ni concentrations determined in replicate injections) and periodic (two in every two-hour operation of the equipment) analysis of the NIST Standard Reference Material 1640, Trace Elements in Natural Water (a prior set of samples was re-run if analyzed value differed from the Reference Standard's certified

value by $> 20\%$). The certified value for nickel in NIST-SRM 1640 was $(4.7 \pm 0.1) \times 10^{-7}$ M.

The pH change in the test solution was found to be < 0.5 pH units between the beginning and the end of the experiment.

CLEM/AdCSV for Model Solutions of Ni and HA

Table 4.3 presents the parameters for the electrochemical techniques used except for the RDE-ASV technique, which is reported in the RDE section on page 68. The model solutions containing the HA and the 2.6×10^{-7} M nickel solution for the CLEM/AdCSV study were prepared in a manner similar to that for the CLEM/GFAAS; however, they were buffered using HEPES for pH values 7.0 and 8.0, or PIPES for pH 6.0, and adding NaOH or HNO₃, depending on the pH required. The nickel speciation could not be studied above pH 8.0 because of the hydrolysis of nickel forming Ni(OH)₂ and its precipitation, or, much below pH 6.0 because the CLEM/AdCSV technique was not sensitive at such low pH values. In order to get the most reproducible and accurate results from the CLEM/AdCSV experiment, a 25.00 g aliquot of the model solution was weighed out and used as the test solution. Since the complexation kinetics of nickel had been reported to be slow [56,67,68], the solutions were equilibrated in the dark at room temperature for at least 24 hours prior to analysis. An equilibrium time study of a 2.6×10^{-7} M nickel solution with a [Ni]/[HA] mole ratio of 0.01 and a pH of 7.0 was done using CLEM/AdCSV – the results were recorded every 4 hours for 48 hours. This study showed that equilibration between nickel and HA under the above experimental conditions was complete in about 24 hours (see Fig. 4.7). The pH of the model solution

Table 4.3: Parameters of the electrochemical techniques used for the determination of metal speciation: nickel, copper, cadmium, and zinc, in model solutions and surface waters of the lakes in Rouyn-Noranda, Québec.

Parameter	Nickel	Copper ^a	Copper ^b	Cadmium	Zinc
Method	CLEM/ AdCSV	CLEM/ AdCSV	CLEM/AdCSV	DPASV	DPASV
Deposition Potential (V)	0.60	-0.3	-0.05	-0.8	-1.2
Deposition Time (s)	30	10	30 (Dufault) 120 (Opasatica)	300	180
Equilibration Time (s)	5	5	15	30	15
Initial Potential (V)	-0.60	0.10	0.10	-0.80	-1.2
Final Potential (V)	-1.30	-0.40	-0.70	-0.40	-0.9
Pulse Height (mV)				50	50
Scan rate (mV/s)		375	375	10	10
Step Potential (mV)	5	10	0.5		
Frequency (Hz)	50	75	75		
Amplitude (mV)	20	20	20		

^a Using kinetics-based CLEM/AdCSV with 8-hydroxy-quinoline as the competing ligand.

^b Using Xue and Sigg's equilibrium-based CLEM/AdCSV method [53].

Figure 4.7 Equilibration time study for Ni(II)-HA complexes. pH: 7.0, [Ni]/[HA] mole ratio: 0.01. Error bars represent the 95.5% confidence interval of 3 replicate measurements.

was again determined immediately prior to analysis.

The reference solutions of no complexation, i.e. without HA, were prepared immediately prior to the test. As had been demonstrated earlier [69], the uptake of nickel by DMG in the reference solution was sufficiently fast to complex all the nickel within the first few minutes of the addition of DMG.

For the kinetic study by CLEM/AdCSV, all test solutions were purged for 10 minutes with ultra-high-purity nitrogen gas prior to analysis. Just before the stirring of the test solution was initiated, enough DMG was added to ensure a large excess (at least 500-fold excess) over [Ni(II)]. The limit of detection was approximately 1×10^{-10} M.

Determination of Nickel [41,42,45,65] and Copper by Kinetics-Based CLEM/AdCSV in the Samples from Rouyn-Noranda, Québec

The test solutions were buffered at the natural water pH (~7) with HEPES and NaOH. The test solutions were then allowed to equilibrate at $23.0 \pm 1.0^\circ\text{C}$ for 24 h prior to the test. The reference solutions for no metal-complexation, i.e. without HS, were prepared immediately prior to the test. Just before the stirring of the test solution was initiated, enough competing ligand (DMG for Ni, 8-hydroxy-quinoline for Cu) was added to ensure a large excess over [Ni(II)] or [Cu(II)] (at least 500-fold excess). The signal was measured in the peak height mode. The limit of detection was approximately 1×10^{-10} M.

Cadmium Titrations for the Determination of $[Cd^{2+}]$ by Xue and Sigg's Ligand Exchange Method

A series of 10 mL sub-samples from the filtered water samples of each lake were transferred to 50 mL screw-top, Teflon cups (Savalex, USA), then buffered to the natural water pH (~7) with HEPES and NaOH. The sub-samples were spiked to give a series of 10 to 12 spiked sub-samples containing 10^{-9} to 10^{-7} M of cadmium. EN was added to each of the test solutions which were equilibrated in the dark at 23.0 ± 1.0 °C for 24 h prior to the test. The limit of detection was approximately 1×10^{-11} M.

Copper Titrations for the Determination of $[Cu^{2+}]$ by Xue and Sigg's Ligand Exchange Method

The sample was buffered to the natural water pH (~7) of the lake water sample with HEPES and NaOH. The ionic strength of the solution was adjusted to 0.01 M using KNO_3 ; 15 mL of the buffered sample was then added to each of the 14 Teflon sample jars. All but one of the sub-samples were spiked with increasing concentrations of copper to give an added copper concentration range between 0 and 6.5×10^{-7} M, and the pH of each sample was readjusted to the natural water pH (~7) using NaOH. The test solutions were then allowed to equilibrate at 23.0 ± 1.0 °C for 24 h prior to the analysis.

Prior to the analysis, the pH of each sample was measured and, if necessary, readjusted to pH ~7. After purging, catechol was pipetted into the sample cell to give a catechol concentration of 1.0×10^{-4} M. The test solution was then allowed to equilibrate for 5 minutes (stirring during the first minute, followed by a 4-minute quiescent period),

followed by measurement by Square Wave AdCSV. The limit of detection was approximately 1×10^{-11} M.

Data Analysis for Cd and Cu Titration Experiments using Equilibrium-Based CLEM/AdCSV or CLEM/ASV

Titration curves for each lake water sample were obtained by plotting peak current (i_p) vs. total cadmium or copper concentration ($[Cd]_T$, $[Cu]_T$). The sensitivity of the method was calibrated for each titration experiment and was determined from the slope of the titration curves at high metal concentrations, where all natural ligands were saturated and measured concentrations of copper-catechol or cadmium-EN complexes were essentially proportional to the added concentration of copper (i.e. total metal concentrations exceed concentrations of natural organic ligands). Free cadmium or cupric ion concentration ($[Cd^{2+}]$, $[Cu^{2+}]$) and the total concentration of natural copper species ($[Cd_{nat}]$, $[Cu_{nat}]$) (i.e. metal species other than those complexed to the catechol or EN) were calculated at all titration points using mass-balance and equilibrium calculations. A data set of $\log [M^{2+}]$ values were plotted as a function of $\log [M_{nat}]$ to obtain a second type of titration curve which was fitted with the equilibrium modelling program FITEQL [70] using a one-ligand model, which assumes formation of discrete 1:1 complexes, to obtain the ambient free cupric or cadmium ion concentration ($[Cu^{2+}]$ or $[Cd^{2+}]$), ligand concentration ($[L]$) and conditional stability constants ($\log K$).

Zinc Titrations for the Determination of $[Zn^{2+}]$ by the van den Berg and Ružić Method

Samples were buffered to the natural water pH (~ 7) of the lakes with HEPES and NaOH, and the ionic strength was adjusted to 0.1 M using KNO_3 . 15 mL of the buffered sample

was then added to each of 14 Teflon sample jars. All but one of the sub-samples were spiked with increasing concentrations of zinc to give an added zinc concentration ranging from 0 to 1.0×10^{-6} M. The pH of each sample was readjusted to the natural water pH (~7) using NaOH. The test solutions were then allowed to equilibrate for 24 hours at $23.0 \pm 1.0^\circ\text{C}$. Prior to determination by ASV, the pH of the sample was checked and readjusted, if necessary. The ASV labile fraction was then measured by DPASV. The limit of detection was approximately 1×10^{-11} M.

Formation of the Thin Mercury Film for the Rotating Disk Electrode (TMF-RDE)

The glassy carbon electrode was prepared by polishing with 3 μm monocrystalline diamond paste (DP-Paste, M, Struers) for 10 minutes prior to use. The TMF was formed on the RDE by applying a potential of -1.0 V for 15 minutes at a rotation rate of 1000 rpm [71] in a plating solution containing 50 mL ultrapure water, 0.12 M nitric acid and 20 $\mu\text{g/mL}$ Hg^{2+} , that had been deoxygenated for 10 minutes using pure nitrogen gas immediately prior to this operation. After deposition of the TMF, a 5-second quiescent period was initiated, after which the potential was ramped from -0.3 V to 0 V using a square wave waveform (pulse height 20 mV, frequency 50 Hz) to strip any impurities from the TMF. The plating solution was then removed and the TMF visually inspected. Non-uniform films and films containing bubbles were wiped off and the TMF was reformed.

TMF-RDE Measurements

Model solutions were prepared by dissolving 5 mg/L of HA in ultrapure water with Pb, Zn, or Cd. Each sample was buffered to pH 5.0, 6.0 or 7.0 with NaOAc/HOAc for pH

5.0, PIPES for pH 6.0 or HEPES for pH 7.0, and kept at an ionic strength of 0.1 M by adding an appropriate amount of 2.5 M KNO_3 . Since the concentrations of Pb and Cd were too low to be detected using this method, the lake water samples were treated by spiking them with the appropriate quantity of Pb or Cd to match the model solutions; the sample from lake Dufault already contained a high concentration of Zn so it was not spiked. These samples were also buffered at pH 5.0, 6.0, or 7.0 with NaOAc/HOAc, PIPES or HEPES and kept at a constant ionic strength of 0.1 M with KNO_3 . All samples were left to equilibrate at room temperature in the dark for at least 24 hours prior to analysis.

Prior to analysis, the pH was rechecked and readjusted to the appropriate pH if necessary. Each sample was purged with nitrogen gas for 10 minutes prior to the test. The electrode rotation rate was programmed to take measurements at 100, 200...500, 750, 1000, 2000...and 7000 rpm. Linear Scan ASV was used for the measurements. The deposition potential for each metal was as follows: Zn, -1.20 V; Cd, -0.80 V; and Pb, -0.80 V. The metals were deposited at each rotation rate for 180 seconds, after which there was 15-second equilibration time before the metal was stripped from the Hg film by scanning in a positive direction starting from the deposition potential. The peaks were measured in peak height mode at a step potential of 2.5 mV. The limit of detection was approximately 1×10^{-11} M.

-- Chapter 5 --

**Kinetic Studies of Nickel Speciation in Model Solutions of a
Well-Characterized Humic Acid using the Competing Ligand
Exchange Method**

Introduction

Humic substances represent typically 40-90% of the DOC [18] and control the speciation, bioavailability, and transport of trace metals in freshwaters [72-74]. The knowledge of metal-binding to HS comes mainly from studies on the binding of metals to FA [56,74]; relatively much less is known about the binding of metals to HA. Since both FA and HA are main constituents of HS which are ubiquitous in freshwaters, it is important to know the role of HA as distinct from FA in the binding of metals, including nickel; the latter is the subject of this study.

Many studies of metal complexation with HS have been done using equilibrium models. Xue et al. [56] have studied nickel speciation and complexation kinetics in freshwaters by ligand exchange with differential pulse cathodic stripping voltammetry and have found that more than 99.9 % of the dissolved nickel was strongly bound to organic ligands ($\log K = 12.1 - 14.9$). So far, local equilibrium assumptions have been the dominant tool for metal speciation in freshwaters. One problem with the equilibrium assumption is that freshwater environments are often non-equilibrium systems, requiring kinetic approaches to determine the chemical speciation of metals [15,40,75-78] more realistically than the metal speciation based on local equilibrium approximations. For freshwater systems, kinetic studies of metal speciation can not only differentiate chemical species according to their kinetic parameters, but can also give information on the distribution of the chemical species in the system at any time during biogeochemical processes it may undergo. The results obtained can be used to estimate the metal bioavailability if a kinetic model can be constructed to represent the process of biological uptake.

Langford et al. [14] have reported on kinetic speciation of metal ions using ligand-exchange kinetics: the speciation is controlled by the history of the sample prior to the initiation of the ligand exchange reaction, but the rate coefficients are determined by the reagent concentrations, pH, ionic strength, and temperature of the specific kinetic run. This experimental limitation is; however, not as serious for metal complexes as it might be in other circumstances since six-coordinate metal complexes (which are characteristic of 3d transition metals including nickel) tend to follow the disjunctive mechanism (Eq. 2.9) and to have reaction rates controlled by the rate of the original metal-complex dissociation. As a result, extrapolation from one condition to another is fairly simple and reliable; in particular, relative rates tend to remain the same [15].

The environmental significance of this work for freshwaters is that it elucidates the Ni(II) binding characteristics of HA. Since HA is ubiquitous in the aquatic environment and nickel is one of the serious potential metal pollutants in Canadian freshwaters, and $[\text{Ni}^{2+}]$ has been reported to be a good predictor of nickel bioavailability and ecotoxicity [11,14,79], the study of Ni(II)-HA complexes as the progenitor of free Ni^{2+} ion in freshwaters, is environmentally significant.

Results and Discussion

Although these data were fitted to a simplified equation, it is necessary to note here that the conditions of the system – even a simple model solution – are extremely complex. Nickel complexation is known to be slow [67,68] and it has been shown by Xue et al. [56] that ligand exchange between natural ligands and DMG for Ni(II)-complexation takes days and has half-lives of 5-95 h, the range of half-lives reflecting different kinetics

for different complexes of naturally occurring complexants. There are many factors that can influence the complexation kinetics of Ni(II); for example, the rates of the reactions may be affected by the uncomplexed sites of HA, the deprotonation of HA at different pH values, the functional groups of the competing ligand and the kinetics of Ni(II) hydrolysis at different pH values. In this work, we have simplified the problem by making a reasonable assumption that the dissociation of the Ni(II)-HA complex is the rate determining step, and therefore, all other reactions do not contribute to the measured rate coefficients. We have also assumed that the reaction of Ni(II) with the competing ligands, DMG and Chelex, is fast and essentially irreversible; this assumption is justified by the published literature [14,15,40,66,76].

The total time that the experiment is run is an important factor to consider since longer times will allow more Ni(II) to dissociate from the HA. In order to obtain the optimum time, the CLEM/GFAAS experiment was first run for a total time of 24 hours (see Fig. 5.1). The data from these curves were then truncated to 12, 8, 4, and 2 hours to determine the minimum time required to adequately model the system. It was decided that the optimum time for the experiment was 8 hours, since at longer times, no additional information about the system was resolved from non-linear regression analysis, and at shorter times, the curves did not level off sufficiently enough to provide an adequate description of the system. For CLEM/AdCSV, 2 hours was chosen as the optimum time.

The data in Figs. 5.2 and 5.3 were fitted to the kinetic model (Eqs. 3.5 and 3.6, respectively) to obtain the numerical values of the kinetically distinguishable components

Figure 5.1 Ligand exchange kinetics of Ni(II)-HA complexes in model solutions at pH 8.0, with the [Ni]/[HA] mole ratio as a parameter, studied by CLEM/GFAAS over a period of 24 hours, using Chelex 100 resin as the competing ligand. [Ni(II)] was held constant at 2.6×10^{-7} M for all solutions and [HA] was varied. Curve A (O) mole ratio 0.1, [HA] = 2.6×10^{-6} M, Curve B (□) mole ratio 0.01, [HA] = 2.6×10^{-5} M, Curve C (△) mole ratio 0.001, [HA] = 2.6×10^{-4} M. Error bars represent the 95.5% confidence interval of at least 3 replicate measurements.

Figure 5.2 Ligand exchange kinetics of Ni(II)-HA complexes in model solutions at pH 8.0, with the $[\text{Ni}]/[\text{HA}]$ mole ratio as a parameter, studied by CLEM/GFAAS over a period of 8 hours, using Chelex 100 resin as the competing ligand. $[\text{Ni(II)}]$ was held constant at 2.6×10^{-7} M for all solutions and $[\text{HA}]$ was varied. Curve A (O) mole ratio 0.1, $[\text{HA}] = 2.6 \times 10^{-6}$ M, Curve B (Δ) mole ratio 0.01, $[\text{HA}] = 2.6 \times 10^{-5}$ M, Curve C ($\square$) mole ratio 0.001, $[\text{HA}] = 2.6 \times 10^{-4}$ M. Error bars represent the 95.5% confidence interval of at least 3 replicate measurements.

Figure 5.3 Ligand exchange kinetics of Ni(II)-HA complexes in model solutions at pH 7.0, with the $[\text{Ni}]/[\text{HA}]$ mole ratio as a parameter, studied by CLEM/AdCSV, using DMG as the competing ligand. $[\text{Ni(II)}]$ was held constant at 2.6×10^{-7} M for all solutions and $[\text{HA}]$ was varied. i_o is the signal from the reference solution of Ni^{2+} in ultrapure water (no complexation), i_p is the signal from the sample. Curve A (O) mole ratio 0.1, $[\text{HA}] = 2.6 \times 10^{-6}$ M, Curve B (Δ) mole ratio 0.01, $[\text{HA}] = 2.6 \times 10^{-5}$ M, Curve C ($\square$) mole ratio 0.001, $[\text{HA}] = 2.6 \times 10^{-4}$ M. Error bars represent the 95.5% confidence interval of at least 3 replicate measurements.

and the dissociation rate coefficients by non-linear regression analysis using the Marquardt-Levenberg algorithm [80] (see Tables 5.1 and 5.2). The Marquardt-Levenberg algorithm is an advanced non-linear regression algorithm specifically designed to minimize the sum-of-squares error function. The calculations were done using the SigmaPlot 5 computer program (SPSS Science). It is important to note that the two rate coefficients given do not refer to specific molecular species. There may still be some diversity of the individual sites, but they group into recognizable sets which may be adequately approximated by a small set of stable rate coefficients. The kinetic curves (see Figs. 5.1-5.7) of the model solutions can be separated into sections representing broadly the major kinetically distinguishable components. The initial portion of the curve (rapidly falling for CLEM/GFAAS, or rapidly rising for CLEM/AdCSV) represents the faster-kinetically distinguishable component (C_1). The slower-descending portion of the curve (for CLEM/GFAAS), and the slower-ascending portion of the curve (for CLEM/AdCSV) represent the slower-kinetically distinguishable component, including the plateau portion of the curve representing a very slowly labile, or an almost inert component (C_2).

Effect of the [Ni]/[HA] Mole Ratio on the Rate of Dissociation of Ni(II)-HA Complexes

CLEM/GFAAS

In Fig. 5.2, the fastest dissociation kinetics were shown by [Ni]/[HA] mole ratio 0.1, and the slowest by [Ni]/[HA] mole ratio 0.001. Table 5.1 shows the kinetic parameters obtained by non-linear regression analysis of Fig. 5.2. The concentration of nickel bound to stronger binding sites of the HA increased with the decreasing [Ni]/[HA] mole ratio, as expected. The rate coefficients varied greatly with [Ni]/[HA] mole ratio,

Table 5.1: Ligand exchange kinetics of Ni(II)-HA in model solutions at 3 different pH values, determined by CLEM/GFAAS over a period of 8 hours using Chelex 100 as the competing ligand. The uncertainty represents variations in the experimental data obtained on different days at 95.5% confidence interval.

[Ni]/[HA] mole ratio	pH (± 0.2)	Kinetically distinguishable components		Dissociation rate coefficients	
		C_1 (%)	C_2 (%)	k_1 (10^{-4} s^{-1})	k_2 (10^{-6} s^{-1})
0.1	8.0	76 ± 2.5	18 ± 4.5	2.0 ± 0.2	$< 10^{-6}$ (Inert)
0.01	8.0	100 ± 5.8		0.29 ± 0.05	
0.001	8.0		98 ± 0.6		$< 10^{-6}$ (Inert)
0.1	7.0	38 ± 2.4	57 ± 1.2	2.8 ± 0.7	$< 10^{-6}$ (Inert)
0.01	7.0	52 ± 5.0	44 ± 4.2	1.1 ± 0.2	$< 10^{-6}$ (Inert)
0.001	7.0		91 ± 1.3		$< 10^{-6}$ (Inert)
0.1	6.0	61 ± 5.0	42 ± 4.2	3.2 ± 0.7	$< 10^{-6}$ (Inert)
0.01	6.0	62 ± 2.9	41 ± 3.0	1.1 ± 0.2	$< 10^{-6}$ (Inert)
0.001	6.0		96 ± 1.2		$< 10^{-6}$ (Inert)

Table 5.2: Ligand exchange kinetics of Ni(II)-HA in model solutions at 3 different pH values, determined by CLEM/AdCSV, using DMG as the competing ligand. The uncertainty represents variations in the experimental data obtained on different days at 95.5% confidence interval.

[Ni]/[HA] mole ratio	pH (± 0.2)	Kinetically distinguishable components		Dissociation rate coefficients	
		C_1 (%)	C_2 (%)	k_1 (10^{-2} s^{-1})	k_2 (10^{-4} s^{-1})
0.1	8.0	62 ± 12	36 ± 11	1.2 ± 0.2	1.9 ± 1.3
0.01	8.0	66 ± 2.4	31 ± 2.0	0.9 ± 0.4	1.8 ± 1.6
0.001	8.0	43 ± 0.3	61 ± 0.4	0.7 ± 0.2	1.8 ± 0.6
0.1	7.0	67 ± 14	32 ± 15	1.7 ± 0.7	0.9 ± 0.4
0.01	7.0	63 ± 8.7	36 ± 11	0.6 ± 0.2	1.2 ± 0.6
0.001	7.0	48 ± 1.6	44 ± 0.9	0.4 ± 0.2	0.9 ± 0.4
0.1	6.0	71 ± 0.7	25 ± 1.1	1.1 ± 0.4	1.4 ± 0.8
0.01	6.0	66 ± 1.3	33 ± 0.7	1.1 ± 0.1	1.5 ± 0.4

probably because the nickel was bound to stronger sites at lower $[\text{Ni}]/[\text{HA}]$ mole ratios.

The data of curve B (see Fig. 5.2) are compatible with the hypothesis that there are no more than 1% strong binding sites in the HA, with the remaining 99% of the binding sites being weak. Curves A and B are also consistent with the hypothesis that with the increasing $[\text{Ni}]/[\text{HA}]$ mole ratio, the strong binding sites are first occupied and the excess Ni^{2+} ions then bind to the weaker sites, forming weak complexes, which are labile.

For all the $[\text{Ni}]/[\text{HA}]$ mole ratios studied, there were two kinetically distinguishable components comprising the major fraction of the Ni(II)-HA complexes, which were slowly labile to inert ($k \sim 10^{-4} \text{ s}^{-1}$ to $< 10^{-6} \text{ s}^{-1}$). Examination of the data (see Table 5.1) shows that the relatively long measurement time involved in the GFAAS precludes any reaction that occurs faster than the measurement time from being studied, i.e. precludes fast components such as free Ni^{2+} ion, i.e. $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$, nor any other fast, labile, nickel species, from being resolved – this very labile component was experimentally indistinguishable from the free Ni^{2+} ion (aqua complex).

CLEM/AdCSV

Previous work from this laboratory [81] showed that the reaction mechanisms of Ni(II)-FA complexes, whether disjunctive or adjunctive (Eqs. 2.9 and 2.10), depended on the initial $[\text{Ni}]/[\text{FA}]$ mole ratio and the $[\text{DMG}]$. It was also reported that the disjunctive pathway is the primary mechanism at high $[\text{DMG}]/[\text{FA}]$ ratios ($> 0.05 \text{ mM DMG/mg FA}$). In this work, the $[\text{DMG}]/[\text{HA}]$ ratio was kept at or above $0.25 \text{ mM DMG/mg HA}$, so the contribution of the disjunctive pathway predominated; the dissociation kinetics were

independent of the [DMG], and the observed rate coefficients represented the average dissociation rate coefficients of the Ni(II)-HA complexes.

Figure 5.3 presents the dissociation kinetics of Ni(II)-HA in model solutions, determined by CLEM/AdCSV, using DMG as the competing ligand, at the stated three [Ni]/[HA] mole ratios at pH 7.0. The symbol, i_o , stands for the current observed for Ni(II) with the reference solutions of Ni(II) with no complexation, whereas i_p stands for the current observed with the sample containing Ni(II) complexed partly or fully with HA. The concentration of Ni(II) bound to the strong binding sites of the HA increased with the decreasing [Ni]/[HA] mole ratio, as expected. The differences between the mole ratios are most striking in the initial slopes of the three curves, which are in marked contrast with Fig. 5.2, where these differences are muted. The rapidly rising portion for all curves reflected the dissociation of weak nickel complexes, which could not be observed in the CLEM/GFAAS curves because the measurement time of the CLEM/GFAAS was longer than the reaction time. Generally, Fig. 5.3 confirms the trends of Fig. 5.2; however, there are significant differences.

The main difference between Figs. 5.2 and 5.3 is that at longer times, all the three mole ratios in Fig. 5.3 become much less distinguishable from one another and show similar lability. This is probably because the ionic strength of the solutions is higher in the CLEM/AdCSV experiment, because of the addition of the buffer. Increasing ionic strength has been shown (in previous work from this laboratory [41] and by others [82]) to result in increased electrostatic screening of humate polyanionic charges, resulting in the formation of weak metal-humate complexes that are labile. Another way of

explaining this phenomenon is that increased ionic strength results in a decrease in the activity coefficient, and hence, in weaker binding, with the same results as above. Also, the measurement time in CLEM/AdCSV is significantly shorter than that in the CLEM/GFAAS, which, added to the factor stated in the preceding paragraph, may contribute to the differences, resulting in the CLEM/AdCSV being able to measure faster kinetics than the CLEM/GFAAS, revealing the presence of a fast component such as free Ni^{2+} ion ($[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$) plus other labile nickel species that could not be revealed by the slower measurement time of CLEM/GFAAS.

This advantage of the CLEM/AdCSV over the CLEM/GFAAS exists only for relatively fast kinetics ($k \gtrsim 10^{-2} \text{ s}^{-1}$); CLEM/AdCSV and CLEM/GFAAS are equal in revealing slower kinetics. These two techniques are, however, complementary rather than competitive because of their different analytical windows.

With the CLEM/AdCSV, two kinetically distinguishable components were resolved for all the three mole ratios studied (see Table 5.2). The dissociation rate coefficients of $(1.0 \pm 0.3) \times 10^{-2} \text{ s}^{-1}$ and $(1.4 \pm 0.8) \times 10^{-4} \text{ s}^{-1}$ represent the first (labile) component and the second (slow) component, respectively. However, the rate coefficients of $(1.0 \pm 0.3) \times 10^{-2} \text{ s}^{-1}$ and $(1.4 \pm 0.8) \times 10^{-4} \text{ s}^{-1}$ are not expected to correspond to two precisely defined and homogeneous site types. Rather, they probably represent averages over a distribution of similar sites which remain kinetically defined over the full range of conditions; the former representing labile complexes, the latter representing weakly labile complexes. In the system under study, the first (labile) component must be $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$, the nickel aqua complex, the dissociation rate coefficient of which has been found to be

0.62 s^{-1} by Lavigne et al. [83]. The dissociation rate coefficient of 0.62 s^{-1} for $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$ indicates that almost 99% of this component has reacted within the first 7 seconds, and its contribution beyond this time would be negligible.

The Effect of pH on the Rate of Dissociation of Ni(II)-HA Complexes

Figure 5.4 presents the results of the ligand exchange kinetics of the Ni(II)-HA complexes measured by CLEM/GFAAS. At pH 8.0 (curve A), there was a steady decrease in the curve over time which shows less lability than curves B and C (pH 7.0 and 6.0, respectively, but are considered as one group for the purpose of lability). The results probably reflect the differences in binding of Ni(II) by the dicarboxylate groups of the HA deprotonated at pH 6.0 and 7.0, resulting in the nickel being bound in a phthalic-acid-like structure (forming a seven-membered chelate ring [25]), and the phenolic hydrogen also being deprotonated at pH 8.0, thereby resulting in the Ni(II) being bound in a salicylic-acid-like structure (forming a six-membered chelate ring [25]) The observed difference in the lability is probably a reflection of their differences in bonding involved in these structures.

A similar study was done for CLEM/AdCSV and the results (see Table 5.2) show trends similar to those for CLEM/GFAAS (see Table 5.1). As the pH is decreased from 8.0 - 6.0, there is an increase in the percentage of the faster (C_1) component with a corresponding decrease in the slower (C_2) component even though both the dissociation rate coefficients remain unchanged, indicating that the identity of the clusters of the Ni(II)-complexes represented collectively by C_1 and C_2 probably remain unchanged.

Figure 5.4 Ligand exchange kinetics of Ni(II)-HA complexes for [Ni]/[HA] mole ratio 0.01 with pH as a parameter, studied by CLEM/GFAAS over a period of 8 hours, using Chelex 100 resin as the competing ligand. [Ni(II)] was held constant at 2.6×10^{-7} and [HA] was 2.6×10^{-5} M for all solutions. Curve A (O) pH 8.0, Curve B (Δ) pH 7.0, Curve C ($\square$) pH 6.0. Error bars represent the 95.5% confidence interval of at least 3 replicate measurements.

Comparison of Cane Soil Humic Acid with Armadale Soil Fulvic Acid for Binding of Ni(II)

The Armadale FA which had been studied earlier in this laboratory in a manner similar to this study using CLEM/GFAAS, CLEM/AdCSV, and CLEM/ICP-MS [41,84,85] is here compared with the Cane Soil HA regarding their Ni(II)-complexation properties. Figure 5.5 shows the effect of the [Ni]/[HA] or [Ni]/[FA] mole ratio on the rates of dissociation of the Ni(II)-FA and Ni(II)-HA complexes. For the purpose of this comparison, the Ni-HA plot done by CLEM/GFAAS was truncated to 2 hours in order to match the Ni-FA plot, also done by CLEM/GFAAS. In Fig. 5.5, the pH values used (5.0 and 6.0) were slightly different, so also were the [Ni]/[HA] or [FA] mole ratios – the studies with the FA had been done some time ago. Assuming that the above slight differences in the pH values and the mole ratios does not significantly affect general trends in the Ni(II)-complexation behaviour of HA and FA, the only significant difference between their kinetic behaviour is that Ni(II)-HA complexes dissociate more slowly than Ni(II)-FA complexes. Table 5.3 presents the results of non-linear regression analysis of the kinetic data of Ni(II)-FA by CLEM/GFAAS using Chelex 100 resin as the competing ligand; the results of Table 5.3 confirm the general trends of Fig. 5.5 described above.

Fig. 5.6 presents the dissociation kinetics of Ni(II)-FA in model solutions determined by CLEM/AdCSV with DMG as the competing ligand using [Ni]/[HA] mole ratios of 0.1, 0.01, and 0.001 at pH 8.0. Comparison with Fig. 5.3 shows that the Ni(II)-HA complexes for all the mole ratios at pH 8.0 differ from Ni(II)-FA complexes at pH 7.0 (see Fig. 5.6). The Ni(II)-FA complexes show a much sharper increase in the initial

Figure 5.5 Ligand exchange kinetics of Ni(II)-HA or Ni(II)-FA complexes with $[\text{Ni}]/[\text{HA}]$ or $[\text{Ni}]/[\text{FA}]$ mole ratios as a parameter, studied by CLEM/GFAAS over a period of 2 hours, using Chelex 100 resin as the competing ligand. (a): Ni(II)-FA at pH 5.0. $[\text{Ni}(\text{II})]$ was 1×10^{-8} M for all solutions and $[\text{FA}]$ was varied. Curve A (O) mole ratio 0.18, $[\text{FA}] = 1 \times 10^{-6}$ M, Curve B (△) mole ratio 0.018, $[\text{FA}] = 1 \times 10^{-5}$ M, Curve C (□) mole ratio 0.0018, $[\text{FA}] = 1 \times 10^{-4}$ M. (b): Ni(II)-HA at pH 6.0. $[\text{Ni}(\text{II})]$ was held constant at 2.6×10^{-7} M for all solutions and $[\text{HA}]$ was varied. Curve A (O) mole ratio 0.1, $[\text{HA}] = 2.6 \times 10^{-6}$ M, Curve B (△) mole ratio 0.01, $[\text{HA}] = 2.6 \times 10^{-5}$ M, Curve C (□) mole ratio 0.001, $[\text{HA}] = 2.6 \times 10^{-4}$ M. Error bars represent the 95.5% confidence interval of at least 3 replicate measurements.

Table 5.3: Ligand exchange kinetics of Ni(II)-FA in model solutions at pH 5.0, determined by CLEM/GFAAS over a period of 2 hours, using Chelex 100 as the competing ligand. The uncertainty represents variations in the experimental data obtained on different days at 95.5% confidence interval.

[Ni]/[FA] mole ratio	Kinetically distinguishable components		Dissociation rate coefficients	
	C_1 (%)	C_2 (%)	k_1 (10^{-4} s^{-1})	k_2 (10^{-5} s^{-1})
0.18	40 ± 2	60 ± 1	10.2 ± 3	23 ± 1
0.018	15 ± 1	85 ± 1	0.4 ± 0.1	0.6 ± 0.5
0.0018		100 ± 2		0.2 ± 0.1

Figure 5.6 Ligand exchange kinetics of Ni(II)-FA complexes in model solutions at pH 8.0, with $[\text{Ni}]/[\text{FA}]$ mole ratio as a parameter, studied by CLEM/AdCSV, using DMG as the competing ligand. $[\text{Ni(II)}]$ was held constant at 1×10^{-7} M for all solutions and $[\text{FA}]$ was varied. i_o is the signal from the reference solution of Ni^{2+} in ultrapure water (no complexation), i_p is the signal from the sample. Curve A (O) mole ratio 0.1, $[\text{FA}] = 1 \times 10^{-6}$ M, Curve B (Δ) mole ratio 0.01, $[\text{FA}] = 1 \times 10^{-5}$ M, Curve C ($\square$) mole ratio 0.001, $[\text{FA}] = 1 \times 10^{-4}$ M. Error bars represent the 95.5% confidence interval of at least 3 replicate measurements.

part of Fig. 5.3 associated with labile complexes including Ni(II) aqua complex (free-nickel ion) and other labile complexes of Ni(II) than do the Ni(II)-HA complexes. There is also a marked difference in the plateau part of the curves; the Ni(II)-FA curves levelled off at higher values of i_p/i_o than did the Ni(II)-HA curves. Tables 5.2 and 5.4 present the dissociation rate coefficients obtained from non-linear regression analysis of the results of both the Ni(II)-HA study (see Fig. 5.3) and of the Ni(II)-FA study (see Fig. 5.6), respectively. The Ni(II)-FA (see Table 5.4) complexes consistently show a larger percentage of the first kinetically distinguishable component having a faster dissociation rate coefficient than the Ni(II)-HA complexes (see Table 5.2).

The results of the CLEM/AdCSV study agree with those of the CLEM/GFAAS study, confirming that the Ni(II)-FA complexes are more labile than the Ni(II)-HA complexes, i.e. the nickel bound to FA produces free Ni^{2+} ion more rapidly than the nickel bound to HA.

The Effect of the [Ni]/[HA] Mole Ratio on the Lability of Ni(II)-Complexes in Model Solutions

A second study was conducted using CLEM/AdCSV where the concentration of HA was held constant at 5 mg/L and the concentration of nickel was varied from 8.5×10^{-9} to 4.3×10^{-7} M, which resulted in [Ni]/[HA] mole ratios ranging from 0.00035 to 0.018. Table 5.5 shows the results of the non-linear regression analysis of Fig. 5.7. The fastest dissociation rate coefficient was for [Ni]/[HA] mole ratio 0.018 and the slowest was from [Ni]/[HA] mole ratio 0.00035, which was expected because the highest mole ratio (0.018) had the highest concentration of nickel and the lowest mole ratio (0.00035) had the

Table 5.4: Ligand exchange kinetics of Ni(II)-FA in model solutions at pH 8.0, determined by CLEM/AdCSV, with DMG as the competing ligand. The uncertainty represents variations in the experimental data obtained on different days at 95.5% confidence interval.

[Ni]/[FA] mole ratio	Kinetically distinguishable components		Dissociation rate coefficients	
	C_1 (%)	C_2 (%)	k_1 (10^{-4} s^{-1})	k_2 (10^{-5} s^{-1})
0.1	85 ± 1.0	15 ± 0.6	3.2 ± 0.1	11 ± 0.3
0.01	82 ± 2.0	18 ± 0.5	4.2 ± 0.3	2.1 ± 0.3
0.001	60 ± 1.0	40 ± 0.1	0.6 ± 0.0	2.2 ± 0.1

Table 5.5: Ligand exchange kinetics of Ni(II)-HA in model solutions at pH 8.0, determined by CLEM/AdCSV over a period of 2 hours, using DMG as the competing ligand. The uncertainty represents variations in the experimental data obtained on different days at 95.5% confidence interval.

[Ni]/[HA] mole ratio	Kinetically distinguishable components			Dissociation rate coefficients	
	[Ni] (M)	C ₁ (%)	C ₂ (%)	k ₁ (10 ⁻² s ⁻¹)	k ₂ (10 ⁻⁴ s ⁻¹)
0.018	4.3×10^{-7}	80 ± 4.4	20 ± 5	2.0 ± 0.6	1.4 ± 0.5
0.014	3.4×10^{-7}	78 ± 5.4	21 ± 5	1.9 ± 0.4	2.3 ± 1.6
0.011	2.6×10^{-7}	75 ± 0.1	24 ± 0.8	1.5 ± 0.3	2.9 ± 0.3
0.0070	1.7×10^{-7}	60 ± 10	38 ± 9.3	1.0 ± 0.3	1.2 ± 0.4
0.0035	8.5×10^{-8}	52 ± 5.1	46 ± 2.9	0.8 ± 0.3	1.1 ± 0.4
0.00070	1.7×10^{-8}	30 ± 1.2	68 ± 0.5	0.7 ± 0.1	1.2 ± 0.1
0.00035	8.5×10^{-9}	25 ± 0.2	73 ± 0.5	0.6 ± 0.1	1.2 ± 0.4

Figure 5.7 Ligand exchange kinetics of Ni(II)-HA complexes in model solutions at pH 8.0, with $[\text{Ni}]/[\text{HA}]$ mole ratio as a parameter, studied by CLEM/AdCSV, using DMG as the competing ligand. $[\text{HA}]$ was held constant at 2.6×10^{-5} M for all solutions and $[\text{Ni(II)}]$ was varied. i_0 is the signal from the reference solution of Ni^{2+} in ultrapure water (no complexation), i_p is the signal from the sample. Curve A ($\diamond$) mole ratio 0.00035, $[\text{Ni(II)}] = 8.5 \times 10^{-9}$ M, Curve B ($+$) mole ratio 0.0070, $[\text{Ni(II)}] = 1.70 \times 10^{-8}$ M, Curve C (O) mole ratio 0.0035, $[\text{Ni(II)}] = 8.5 \times 10^{-8}$ M, Curve D ($\square$) mole ratio 0.0070, $[\text{HA}] = 1.70 \times 10^{-7}$, Curve E ($\triangle$) mole ratio 0.011, $[\text{Ni(II)}] = 2.55 \times 10^{-7}$, Curve F ($\diamond$) mole ratio 0.014, $[\text{Ni(II)}] = 3.40 \times 10^{-7}$ M, Curve G (∇) mole ratio 0.018, $[\text{HA}] = 4.26 \times 10^{-7}$. Error bars represent the 95.5% confidence interval of at least 3 replicate measurements.

lowest concentration of nickel. At the lowest [Ni]/[HA] mole ratio of 0.00035, most of the nickel was bound to the strong sites and only a small proportion (25%) was able to dissociate with a moderately labile dissociation rate coefficient of $6.0 \times 10^{-3} \text{ s}^{-1}$. As can be seen in Fig. 5.7, the change in the [Ni]/[HA] mole ratio is relatively constant, increasing by about 0.004 between ratios; however, the highest three [Ni]/[HA] mole ratios share similar kinetic parameters, with both of their kinetically distinguishable components (ranging from 75-80%) and first dissociation rate coefficients (ranging from $1.5 - 2.0 \times 10^{-2} \text{ s}^{-1}$) falling within the analytical uncertainty of the experiment. The [Ni]/[HA] mole ratios of 0.0070 and 0.0035; however, have distinctly different kinetically distinguishable components and dissociation rate coefficients, even though the change in the [Ni]/[HA] mole ratio was the same as it was for the highest three mole ratios. The same can be said for the lowest two [Ni]/[HA] mole ratios compared to the middle two mole ratios. This might be due to the different binding sites on the HA. As the concentration of nickel increases, the strong sites are first saturated; then the moderately strong sites are occupied, and finally, the weak sites are occupied.

Figure 5.8 shows the component distributions as a function of the [Ni]/[HA] mole ratio. A decrease in component 1 and an increase in component 2 are observed with increasing [Ni]/[HA] mole ratio.

Chemical Significance of the Components

A key concern in studies of natural systems is that polymeric, heterogeneous, colloidal complexants such as HA may have a distribution of binding sites [14]. It is therefore

Figure 5.8 Relative contribution of components in the Ni(II)-HA system. (●) component 1, (□) component 2. Results are for solutions at pH 8. Uncertainties represent a 95.5% confidence interval of 3 replicate measurements.

important to make sure that the fitting of a small number of kinetically distinguishable components has chemical significance. Two criteria must be satisfied before the number of kinetically distinguishable components and the rate coefficients obtained from the kinetic analysis can be judged to have any chemical significance since there is always a danger that real data with noise are simply successfully archived by a set of fitting constants not described by genuine chemical parameters. The first of these criteria is that the rate coefficients must be stable and reproducible as pH and metal:ligand ratios are varied, and the second is that the changes in concentrations of components must follow mass action law considerations. This step is important. Unless the rate coefficients are stable and reproducible as pH and concentrations are varied and concentrations evolve according to mass actions law considerations, there is little assurance of the chemical significance of the components.

Stability of the Rate Coefficients

The results of ligand exchange kinetics of Ni(II)-HA in model solutions by CLEM/GFAAS shown in Table 5.1 (only the results obtained with a given [Ni]/[HA] mole ratio, for example 0.1, at the three pH values are comparable) illustrate the degree of stability of the rate coefficients. Two kinetically distinguishable components were consistently identified. The variations over the full concentration ratio and pH range are small enough that they cannot be said to differ in a statistically significant way. Both rate coefficients appear to be rugged with average values of $(2.7 \pm 0.5) \times 10^{-4} \text{ s}^{-1}$ and $< 10^{-6} \text{ s}^{-1}$. Similar conclusions can be drawn also for the other two mole ratios, 0.01, and 0.001. The results of ligand exchange kinetics of Ni(II)-HA in model solutions by

CLEM/AdCSV shown in Table 5.2 also illustrate the same degree of stability of the rate coefficients for a given $[\text{Ni}]/[\text{HA}]$ mole ratio.

Validation of the Kinetic Model

Tables 5.1-5.4 show that the above two criteria are satisfied. The components represented in Tables 5.1-5.4 are really averages of several binding sites with similar labilities that cannot be resolved with the present techniques. In most cases, under any specific set of chemical conditions, the rate coefficients were reproducible to within 10% and the sum of the component concentrations yielded mass balances, which were reproducible to within 5%. As the $[\text{Ni}]/[\text{HA}]$ mole ratio was increased, the proportion of free Ni^{2+} ion (aqua complex) and/or other very labile Ni(II) complexes increased and the less labile components decreased at all the three pH values. Hence, the Kinetic Model seems to be valid.

Conclusions

The results from the CLEM/GFAAS give somewhat different results from those of the CLEM/AdCSV. The measurement time of GFAAS is significantly longer than that of AdCSV, with the consequence that unlike CLEM/AdCSV, CLEM/GFAAS cannot measure fast kinetics. However, even allowing for these differences, it is reasonable to conclude that the binding characteristics of HA are different from those of FA: dissociation of Ni(II)-HA complexes is significantly slower than that of Ni(II)-FA. Both HA and FA are polymeric colloids and are water-swollen gels [86], but HA is generally much larger than FA [1]. The above difference in the lability of their Ni(II) complexes can be explained by hypothesizing differential diffusional accessibility to similar binding

sites in the interior of the water-swollen gels. Hence, mass transport is more rate limiting for Ni(II)-HA complexes than for Ni(II)-FA complexes, and the former appears kinetically less labile than the latter. There could also be significant differences in the binding functional groups, since HA has, on average, higher concentration of N and S containing functional groups than FA. This could result in the formation of stronger Ni(II)-HA complexes which have slower dissociation rates than Ni(II)-FA complexes. Both HA and FA are polydisperse systems and this polydispersity and the difference in size between HA and FA, as well as their conformational properties could also affect binding [1,26]. As explained earlier, the differences in the conformational properties of HA and FA is particularly important. HA is generally much larger in size than FA, which makes the water-swollen HA gels present **more** binding sites hidden in the cavernous interior of HA compared to FA, resulting in the differential accessibility to thermodynamically equivalent binding sites. The result is thermodynamically equivalent binding sites act as kinetically different [14,15].

It is important to recognize the differences between FA and HA because they are chief constituents of HS which are ubiquitous in freshwaters. HS dominate complexation and transport mechanism of trace metals in freshwaters; however, their effects will be different depending upon the ratio of HA and FA that make up the HS. Hence, differentiation between the complexation characteristics of HA and FA becomes important in predicting nickel speciation in freshwaters.

-- Chapter 6 --

**Complexation of Ni, Cu, Zn, and Cd by DOC in Some Metal-
Impacted Freshwater Lakes: A Comparison of the Results of
Electrochemical Measurement Techniques with those of the
Computer Speciation Code: Windermere Humic Aqueous
Model (WHAM), V and VI**

Introduction

It is well known that the free-metal ion concentration, $[M^{n+}]$, is often a good predictor of ecotoxicity in freshwaters [17,18]; however, determination of the $[M^{n+}]$ in freshwaters is a difficult task to accomplish at environmentally relevant concentrations, which can be lower than 10^{-9} M. Few analytical techniques are available to determine the $[M^{n+}]$ at this low concentration level, and the reliability of methods based on available techniques such as ion-selective electrode potentiometry, is often questionable. However; one approach which has recently received increasing acceptance is the use of equilibrium-based, computer speciation codes [87-93].

Modelling chemical speciation is an important step in predicting metal speciation in freshwaters, especially in circumstances when direct measurement is difficult or prohibitively expensive. Many models such as MINTEQA2 [87], GEOCHEM [88], and more recently, WHAM V and VI [89,90], and the Consistent Non-Ideal Competitive Adsorption (NICCA)-Donnan Model [93], have been used to model the metal speciation in freshwater systems. In modelling freshwaters for metal speciation, HS, which are ubiquitous in the aquatic environment and which represent predominant fractions ($\geq 60\%$) of the DOC, have played an important role as naturally-occurring organic complexants in binding metals in freshwaters. The most important constituents of HS are FA which is soluble in acid, and HA which is insoluble in acid. The earlier models, GEOCHEM and MINTEQA2, do not correctly describe the fulvic and humic acids, and are further hampered by their limited databases on which to base their predictions. The models that have been more widely used are WHAM V and VI, and the NICCA-Donnan

model. These models have been tested on numerous data sets that have been compiled by various authors. Of these two models, WHAM is the most widely used and the most comprehensive with respect to modelling ion binding in freshwaters, and was employed in this work.

WHAM, developed by Tipping et al. [87,90-92], is an equilibrium-based, computer speciation code that assumes discrete-site electrostatic binding. It draws on a number of sub-models which have been calibrated against the literature data. Many studies using WHAM have been done with the objective of parameterizing the model [90,94]. One approach of those studies was to conduct acid/base titrations of isolated HS over a range of pH values and ionic strengths to determine the proton binding characteristics of particular HS [95]. The results of those studies were used to optimize the parameter values in the WHAM model. Those studies were important in the development of WHAM; however, they do not tell much about how WHAM performs when modelling freshwater samples. In order to appraise the applicability of WHAM for freshwater samples, WHAM predictions must be compared with measured concentrations. One important parameter to consider is the $[M^{n+}]$. WHAM is able to predict the $[M^{n+}]$ as long as the input data for pH and concentrations of total metals, dissolved anions and cations, and DOC are available. Accurate determination of free-metal ion concentrations in freshwaters is an important requirement for studying the interactions of metals with natural organic ligands in freshwaters.

One major problem in modelling by WHAM is that it requires an input datum for the “active” DOC concentration, i.e. the DOC concentration that behaves like isolated HS

and is actively involved in the binding of metals. This is the most important parameter since it determines the number of binding sites available to the metals. Only a portion of the DOC concentration is “active” with respect to metal-binding [96-102] since the DOC is not entirely composed of HA and FA – the main metal-binding agents; DOC also contains amino acids, sugars and peptides which have varying metal-binding properties [1]. Since the value of the “active” DOC concentration, although critical, was not known, WHAM was run assuming that 50, 65, and 80% of the total DOC concentration as “active” to determine the “active” DOC concentration that best modelled the measured free-metal ion concentrations. WHAM V was compared with WHAM VI in order to determine which version (V or VI) of WHAM was better suited for modelling the above lake surface waters.

Another important consideration is the effect of competition by Al and Fe on the binding of the trace metals. Al and Fe are important metals present as acid minerals in soils and rocks, and leaching of these metals into freshwaters, especially by acid rain or acid mine drainage, is a common occurrence. Since Al and Fe are usually trivalent, they may compete differently from divalent trace metals for the binding sites on HS. This may be important in freshwaters containing high concentrations of Al and Fe. In order to study this effect, WHAM was run both with and without Al and Fe as concomitants.

Description of WHAM

WHAM is designed to calculate equilibrium speciation of metals in complex systems such as surface and ground waters, sediments, and soils. It is reported to be very useful for systems in which HS, such as FA and HA, are the main complexants that control the

speciation of metals. Both WHAM V and its improved version VI, are composite models which combine Humic Ion Binding Models V and VI (hereafter referred to as Models V and VI, respectively) with models of inorganic solution chemistry, precipitation of aluminum and iron oxyhydroxides, cation exchange on a representative clay, and adsorption, desorption reactions of FA. A more detailed description of WHAM V and VI can be found in the literature [89,90].

Model V [89]

Model V was developed by Tipping and Hurley [92] using the work of Marinsky et. al. [103] as the basis for the Model. Model V makes the following assumptions. It is a discrete-site binding model in which binding is modified by electrostatic interactions. The accumulation of counterions in the diffuse layer is described by a simple Donnan-type expression. HS are assumed to be rigid spheres of uniform size with ion binding groups on the surface. The proton binding groups on HS are heterogeneous, having a range of intrinsic pK values. Two types of acid groups, which are represented by COOH and phenolic OH groups, respectively, are considered. Binding can take place at both monodentate (single) and bidentate (consisting of 2 proton-dissociating groups) sites. A proximity factor is used to control the extent of bidentate binding, by defining the likelihood of pairs of proton-binding groups being close enough to form bidentate sites. Model V permits the binding of the first hydrolysis product (e.g., CuOH^+ in the case of Cu^{2+}) as well as the parent species and assumes that they bind to HS with the same affinity. Model V also accounts for competition and ionic strength effects, and for proton-metal exchange.

The overall success of Model V has been reported to be good; its main advantage is its ability to use small data sets to estimate the intrinsic equilibrium constants, thereby allowing comparisons of results conducted under different conditions with different HS, and by different techniques. Model V has been applied by other workers [99,104-108] to a range of metals including transition metals, the alkaline earths, actinides and lanthanides, and aluminum; it has also been used to model field data for freshwaters, soils and groundwaters.

Model VI [90]

Model VI is an improved version of the Model. The principal difference between Model V and VI is that Model VI includes provisions for high-affinity tridentate sites for metals present in small amounts. This is an important change because freshwaters contain Fe and Al, both from crustal and anthropogenic sources; the leaching of the earth's crust by acid rain constitutes an important anthropogenic source of Al. In addition, the distributions of proton affinity and metal affinity are no longer fully correlated in Model VI as they were in Model V. This allows Model VI to employ a more elaborate scheme which generates a wider range of metal-binding strengths.

Results and Discussion

The “active” DOC concentration was modelled entirely as FA, since FA is the major component of HS in freshwaters. Table 6.1 shows the default values for the constant log K_{MA} values of FA in both WHAM V [89] and VI [109]. These parameters, which control the strength of metal-binding to FA, were determined by fitting the model to data from titrations of isolated HS.

Table 6.1: Default values of the constant $\log K_{MA}$ in Model V [89] and VI [109]. Smaller values for $\log K_{MA}$ represent stronger metal-binding to FA in WHAM V, whereas larger values represent stronger metal-binding in WHAM VI. Standard deviations are for the $\log K_{MA}$ values for WHAM VI, which are based on the number of measurements shown in parentheses.

Metal	Log K_{MA} for FA	Log K_{MA} for FA	Standard Deviation
	WHAM V	WHAM VI	
Ni	1.4	1.4	0.3 (4)
Zn	1.3	1.6	0.2 (4)
Cd	1.5	1.6	0.1 (3)
Cu	0.8	2.1	0.2 (9)

Table 6.2 presents the lake water chemistries of the four lakes in Rouyn-Noranda, Quebec. Tables 6.3-6.5 present the WHAM-predicted free-metal ion concentrations based on 65% of the [DOC] as “active”, and the measured $[M^{n+}]$, and labile inorganic- and organic-metal complexes in the surface waters of the four lakes in Rouyn-Noranda, Québec.

The samples of the lake surface waters contained varying concentrations of metals. One possible reason for the differences was their different locations in relation the copper smelter (see Fig. 4.2). Lakes Opasatica and Vaudray were the least polluted, probably because they were upwind from the prevailing south-westerly wind direction, and did not receive large amounts of airborne deposits from the flue-stack. Lakes Dufault and Osisko were directly downwind and much closer to the flue-stacks, so they were much more likely to be polluted by airborne deposits. Lake Osisko also received liquid effluents direct from the smelter in the past since it is contiguous with the smelter. Lakes Vaudray and Opasatica had the highest DOC concentrations and were expected to have the lowest $[M^{n+}]$ since the metals were probably bound to the DOC. Lakes Osisko and Dufault had much lower DOC concentrations, and much higher metal concentrations, and were expected to have higher $[M^{n+}]$ than the other two lakes since the binding sites on the DOC were all probably occupied by the metals, and the remaining metals were present as metal-aquo complexes. However, the experimental results did not always conform to this expectation probably because of competition by other metals, such as Al, Fe, Ca, and Mg which would result in increased free-metal ion concentrations. There was also a

Table 6.2: Chemistry of the Lake Waters for samples collected from Rouyn-Noranda in 2001 and 2002.

Inputs	Samples Collected in June of 2001				Samples Collected in July of 2002				Samples Collected in August of 2002			
	Lake Dufault	Lake Osisko	Lake Vaudray	Lake Opasatic ^a	Lake Dufault	Lake Opasatic ^a	Lake Dufault	Lake Opasatic ^a	Lake Dufault	Lake Opasatic ^a	Lake Dufault	Lake Opasatic ^a
pH of samples ^a	7.1	9.0	6.9	7.2	7.2	7.2	7.2	7.7	7.3	7.3	7.6	7.6
Experimental pH	7.3	7.8	7.3	7.3	7.6	7.3	7.6	7.6	7.3	7.3	7.3	7.3
[DOC] (± 0.2 mg/L)	6.9	3.4	10	6.4	10	6.4	10	11	6.7	6.7	6.9	6.9
[Ca] ^b (M)	4.2×10^{-4}	6.2×10^{-4}	9.0×10^{-5}	2.5×10^{-4}	4.9×10^{-4}	2.5×10^{-4}	4.9×10^{-4}	3.5×10^{-4}	5.1×10^{-4}	5.1×10^{-4}	2.9×10^{-4}	2.9×10^{-4}
[Mg] ^b (M)	1.3×10^{-4}	2.1×10^{-4}	4.0×10^{-5}	1.2×10^{-4}	1.4×10^{-4}	1.2×10^{-4}	1.4×10^{-4}	1.4×10^{-4}	1.6×10^{-4}	1.6×10^{-4}	1.3×10^{-4}	1.3×10^{-4}
[Al] ^c (M)	2.6×10^{-7}	3.7×10^{-7}	2.9×10^{-6}	2.4×10^{-6}	2.3×10^{-7}	2.4×10^{-6}	2.3×10^{-7}	1.2×10^{-6}	5.6×10^{-7}	5.6×10^{-7}	1.1×10^{-7}	1.1×10^{-7}
[Fe] ^c (M)	2.9×10^{-6}	5.4×10^{-7}	1.2×10^{-6}	5.6×10^{-7}	3.5×10^{-7}	5.6×10^{-7}	3.5×10^{-7}	3.5×10^{-7}	1.5×10^{-6}	1.5×10^{-6}	1.1×10^{-6}	1.1×10^{-6}

^a The pH of the water samples as received

^b Determined by ICP-OES

^c Determined by ICP-MS

Uncertainties in Ca, Mg, Al and Fe are less than 5% RSD

Table 6.3: WHAM predictions based on 65% of the DOC as “active”, and measured labile-Ni(II) and free-Zn ion concentrations of the Lake Waters collected in June of 2001.

Nickel					
Lake	[Ni] ^a (M)	[Ni] ^a /[DOC] ratio (mol/mg)	WHAM V [Ni ²⁺] (M)	WHAM VI [Ni ²⁺] (M)	Measured concentration of labile Ni-complexes by CLEM/AdCSV (M)
Opasatica	2.6×10^{-8}	4.1×10^{-9}	1.8×10^{-8}	1.9×10^{-8}	2.5×10^{-8}
Vaudray	1.7×10^{-8}	1.7×10^{-9}	1.1×10^{-8}	1.2×10^{-8}	1.0×10^{-8}
Osisko	2.7×10^{-8}	7.9×10^{-9}	1.1×10^{-8}	2.1×10^{-8}	2.7×10^{-8}
Zinc					
Lake	[Zn] ^a (M)	[Zn] ^a /[DOC] ratio (mol/mg)	WHAM V [Zn ²⁺] (M)	WHAM VI [Zn ²⁺] (M)	Measured [Zn ²⁺] (M)
Opasatica	4.6×10^{-8}	7.2×10^{-9}	2.5×10^{-8}	2.8×10^{-8}	2.3×10^{-8}
Vaudray	6.1×10^{-8}	6.1×10^{-9}	3.3×10^{-8}	4.0×10^{-8}	3.6×10^{-8}
Osisko	2.6×10^{-7}	7.6×10^{-8}	1.6×10^{-7}	2.0×10^{-7}	3.2×10^{-8}
Dufault	1.3×10^{-6}	1.9×10^{-7}	9.9×10^{-7}	9.8×10^{-7}	9.1×10^{-7}

^a Determined by ICP-MS

Uncertainties in [Ni] and [Zn] are less than 5% RSD

Uncertainties in WHAM-predicted and experimentally determined [Ni²⁺] and [Zn²⁺] are a factor of 2

Table 6.4: WHAM predictions based on 65% of the DOC as “active”, and measured free-Cd ion concentrations of the Lake Waters collected in June of 2001.

Lake	[Cd] ^a (M)	[Cd] ³ /[DOC] ratio (mol/mg)	WHAM V [Cd ²⁺] (M)	WHAM VI [Cd ²⁺] (M)	Measured [Cd ²⁺] (M)
Opasatica	8.9×10^{-10}	1.4×10^{-10}	7.1×10^{-10}	4.3×10^{-10}	4.0×10^{-11}
Vaudray	8.9×10^{-10}	8.9×10^{-11}	6.2×10^{-10}	4.8×10^{-10}	5.8×10^{-11}
Osisko	1.8×10^{-9}	5.3×10^{-10}	1.0×10^{-9}	1.1×10^{-9}	6.0×10^{-10}
Dufault	8.0×10^{-9}	1.2×10^{-9}	6.8×10^{-9}	5.9×10^{-9}	2.7×10^{-9}

^a Determined by ICP-MS

Uncertainties in [Cd] are less than 5% RSD.

Uncertainties in measured [Cd²⁺] and WHAM-predicted [Cd²⁺] are a factor of 2.

Table 6.5: WHAM predictions based on 65% of the DOC as “active”, and measured free-Cu ion concentrations of the Lake Waters collected in July and August of 2002.

Lake	[Cu] ^a (M)	[Cu] ^a /[DOC] ratio (mol/mg)	WHAM V [Cu ²⁺] (M)	WHAM VI [Cu ²⁺] (M)	Measured [Cu ²⁺] by Xue and Sigg's CLEM/AdCSV Method [53] (M)	Measured labile Cu-complexes by the kinetics-based CLEM/AdCSV Method [65] (M)
Opasatica July 2002	4.2×10^{-8}	4.0×10^{-9}	1.9×10^{-11}	2.0×10^{-12}	4.8×10^{-10}	2.8×10^{-8}
Opasatica August 2002	4.4×10^{-8}	6.9×10^{-8}	1.1×10^{-10}	4.2×10^{-12}		3.9×10^{-8}
Dufault July 2002	1.5×10^{-7}	1.4×10^{-8}	3.6×10^{-10}	1.1×10^{-10}	3.9×10^{-9}	7.9×10^{-8}
Dufault August 2002	1.9×10^{-7}	2.7×10^{-8}	5.2×10^{-9}	8.8×10^{-10}		1.5×10^{-7}

^a Determined by ICP-MS

Uncertainties in [Cu] are less than 5% RSD.

Uncertainties in measured [Cu²⁺] are a factor of 2

Uncertainties in and WHAM-predicted [Cu²⁺] are an order of magnitude

significant difference in the initial pH of one lake water (lake Osisko, sampled in June of 2001), which was considerably higher (pH 9) than the pH values of the other lake-waters, which had about neutral pH. The experimental pH of all the lake-waters; however, was set to near natural pH (~7) by the buffer HEPES ($pK_a = 7.55$); this made the ionization of the acid groups of the DOC in all lake waters roughly the same and thereby facilitated direct comparison of the results of all the four lakes. WHAM calculations were based on the experimental pH in order to compare WHAM-predicted free-metal ion concentrations to the experimentally measured values.

Nickel Modelling

Figures 6.1 and 6.2 show the results of the modelling and experimental results for nickel. It is important to note here that for the nickel speciation, the technique employed, CLEM/AdCSV [41,42,45-47,65,110], gives an analytical signal which represents the total concentration of all labile complexes including $[Ni^{2+}]$. CLEM/AdCSV yields the following experimental results: kinetically distinguishable components of the nickel complexes and their dissociation rate coefficients. Since a kinetically distinguishable component represents an average of several metal complexes with similar binding energies, the dissociation rate coefficient also represents an average of similar rate coefficients of the above metal complexes. The labile (i.e. rapidly-dissociating) complexes are distinguished from the non-labile (or very slowly-dissociating) complexes by their dissociation rate coefficients. The percentage of the labile fraction is multiplied by the total metal concentration of the sample, yielding a value that consists of a mixture of very labile complexes, e.g. for nickel small organic and inorganic complexes of nickel

Figure 6.1 Comparison of WHAM-V-predicted pNi ($-\log_{10}[\text{Ni}^{2+}]$) with the measured molar concentrations of labile Ni(II)-complexes in water samples collected from Rouyn-Noranda, Québec, in June 2001. Al and Fe were included in WHAM. Each lake was modelled using 3 different active DOC concentrations. (---) 1:1 line, (O) 50% active DOC, ($\square$) 65% active DOC, ($\triangle$) 80% active DOC. Uncertainty in WHAM predicted values represents 1 standard deviation of the $\log K_{MA}$ values. Experimental uncertainty represents a 95.5% confidence interval of 3 replicate measurements.

Figure 6.2 Comparison of WHAM-VI-predicted pNi ($-\log_{10}[\text{Ni}^{2+}]$) with the measured molar concentrations of labile Ni(II)-complexes in water samples collected from Rouyn-Noranda, Québec in June 2001. Al and Fe were included in WHAM. Each lake was modelled using 3 different active DOC concentrations. (---) 1:1 line, (O) 50% active DOC, ($\square$) 65% active DOC, ($\triangle$) 80% active DOC. Uncertainty in WHAM predicted values represents 1 standard deviation of the log K_{MA} values. Experimental uncertainty represents a 95.5% confidence interval 3 replicate measurements.

as well as the free-nickel ion (i.e. $\text{Ni}(\text{H}_2\text{O})_6^{2+}$). Since WHAM is able to predict concentrations of small, inorganic species, the experimentally determined labile-nickel concentrations were compared with the sum of the WHAM-predicted concentrations of the free-nickel ion and inorganic Ni(II) species (such as NiSO_4 , NiCl , and $\text{Ni}(\text{NO}_3)_2$).

The $[\text{Ni}^{2+}]$ depends on the $[\text{Ni}]/[\text{DOC}]$ ratio (see Table 6.3). The general expectation was for lake Osisko to have the highest $[\text{Ni}(\text{II})]$ since it had the highest $[\text{Ni}]/[\text{DOC}]$ ratio, and lake Vaudray to have the lowest $[\text{Ni}(\text{II})]$. Figures 6.1 and 6.2 show that the results predicted by WHAM V and VI compared reasonably well with the measured concentrations. The predictions of both WHAM V and VI were nearly equal for all the lakes except for Lake Osisko, which WHAM V predicted, would have the lowest $[\text{Ni}(\text{II})]$, whereas WHAM VI predicted would have the highest $[\text{Ni}(\text{II})]$. The predictions of WHAM VI are in closer agreement with the measured concentrations for lake Osisko. The ability of WHAM VI to better model lake Osisko showed that there were significant differences between WHAM V and VI. This difference was probably due to the differences in the modelled metal-binding to HS; in WHAM V, the binding strictly followed proton dissociation, whereas WHAM VI relaxed this condition to allow a wider range of binding to occur, which was more realistic and resulted in better agreement between the WHAM-predicted and the measured nickel concentrations. WHAM VI predicted a slightly higher $[\text{Ni}(\text{II})]$ than WHAM V for lakes Opasatica and Vaudray.

Zinc Modelling

Figures 6.3 and 6.4 show the results of the modelled and the measured concentrations of $[\text{Zn}^{2+}]$. The measured results were obtained using a modified Scatchard Plot method to

estimate the equilibrium constant by the electrochemical method. A simple calculation then allowed an estimate of the $[\text{Zn}^{2+}]$ to be determined.

The results for total $[\text{Zn}]$ were much more variable between the lakes than were those for total $[\text{Ni}]$. The results for lakes Osisko and Dufault differed by an order of magnitude and were 1 and 2 orders higher than those for lakes Vaudray and Opasatica, respectively.

The $[\text{Zn}^{2+}]$ depends on the $[\text{Zn}]/[\text{DOC}]$ ratio (see Table 6.3). The water sample from lake Dufault had a much higher $[\text{Zn}]/[\text{DOC}]$ ratio than those from the other three lakes, which probably resulted in lake Dufault having the highest measured $[\text{Zn}^{2+}]$; the measured results agreed with the WHAM predictions for $[\text{Zn}^{2+}]$, except for lake Osisko, which was predicted to have a higher $[\text{Zn}^{2+}]$ than what was determined experimentally. This was probably a result of the high experimental pH, being close to the pH of Zn hydrolysis, which would result in a lower measured $[\text{Zn}^{2+}]$. Lake Vaudray had the lowest $[\text{Zn}]/[\text{DOC}]$ ratio; hence, it was expected to have the lowest measured $[\text{Zn}^{2+}]$. WHAM V and VI were successful at modelling the measured $[\text{Zn}^{2+}]$. In most cases, assuming that 65-80% of the DOC was “active” resulted in good predictions of the measured $[\text{Zn}^{2+}]$.

Cadmium Modelling

The $[\text{Cd}^{2+}]$ was determined by a method proposed by Xue et al. [55]. It is an extension of the van den Berg and Ružić method which was used in this study for the determination of zinc speciation in the lake waters. This method employs ASV and is based on a simple ligand model that assumes formation of discrete 1:1 complexes with organic ligands. Although ASV measures $[\text{Cd}^{2+}]$, the measured concentrations may be overestimated owing to the electrochemical availability of some labile Cd(II)-organic complexes during

Figure 6.3 Comparison of WHAM-V-predicted pZn ($-\log_{10}[\text{Zn}^{2+}]$) with the measured pZn in water samples collected from Rouyn-Noranda, Québec, in June 2001. Al and Fe were included in WHAM. Each lake was modelled using 3 different active DOC concentrations. (---) 1:1 line, (O) 50% active DOC, ($\square$) 65% active DOC, ($\triangle$) 80% active DOC. Uncertainty in WHAM predicted values represents 1 standard deviation of the $\log K_{\text{MA}}$ values. Experimental uncertainty represents a 95.5% confidence interval 3 replicate measurements.

Figure 6.4 Comparison of WHAM-VI-predicted pZn ($-\log_{10}[\text{Zn}^{2+}]$) with the measured pZn in water samples collected from Rouyn-Noranda, Québec in June 2001. Al and Fe were included in WHAM. Each lake was modelled using 3 different active DOC concentrations. (---) 1:1 line, (O) 50% active DOC, (□) 65% active DOC, (△) 80% active DOC. Uncertainty in WHAM predicted values represents 1 standard deviation of the log K_{MA} values. Experimental uncertainty represents a 95.5% confidence interval 3 replicate measurements.

the effective time of measurement [55], which, for typical ASV conditions (diffusion layer = 10 μm , diffusion coefficient = $5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), was about 0.1 s [107].

Figures 6.5 and 6.6 show the results of the modelled and the measured concentrations of $[\text{Cd}^{2+}]$. The $[\text{Cd}^{2+}]$ depends on the $[\text{Cd}]/[\text{DOC}]$ ratio. Table 6.4 shows that the highest $[\text{Cd}]/[\text{DOC}]$ ratio was for the water sample of lake Dufault, and the lowest ratio was for the water sample of lake Vaudray. The measured $[\text{Cd}^{2+}]$ did not follow the trend expected from the $[\text{Cd}]/[\text{DOC}]$ ratio and showed a reversal in the order for lakes Opasatica and Vaudray (see Figs. 6.5 and 6.6). However, the measured $[\text{Cd}^{2+}]$ agreed with the predictions of WHAM – both versions V and VI. For cadmium, the two polluted lakes, lakes Osisko and Dufault, agreed better with the predictions of WHAM, whereas for the other two lakes the WHAM predictions did not agree that well, being almost one order of magnitude higher than the measured $[\text{Cd}^{2+}]$. Both lakes Opasatica and Vaudray had low total metal concentrations. WHAM had been parameterized mainly in high metal loading conditions, which may result in the model being unable to adequately model lakes under low metal loading conditions. WHAM VI had a slightly better agreement with the measured $[\text{Cd}^{2+}]$ than WHAM V.

Copper Modelling

It has been reported in the literature [111] and also by our laboratories [112] that Cu(II) forms strong complexes with HS, which are ubiquitous in freshwaters. Copper also effectively competes with other trace metals for the binding sites on the HS, resulting in most of the copper being bound to HS (~95-99%) and freeing up other, weaker bound trace metals such as Ni(II) , Zn(II) , and Cd(II) . This is reflected in WHAM – the $\log K_{\text{MA}}$

Figure 6.5 Comparison of WHAM-V-predicted pCd ($-\log_{10}[\text{Cd}^{2+}]$) with the measured pCd in water samples collected from Rouyn-Noranda, Québec, in June 2001. Al and Fe were included in WHAM. Each lake was modelled using 3 different active DOC concentrations. (---) 1:1 line, (O) 50% active DOC, (□) 65% active DOC, (△) 80% active DOC. Uncertainty in WHAM predicted values represents 1 standard deviation of the log K_{MA} values. Experimental uncertainty represents a 95.5% confidence interval 3 replicate measurements.

Figure 6.6 Comparison of WHAM-VI-predicted pCd ($-\log_{10}[\text{Cd}^{2+}]$) with the measured pCd in water samples collected from Rouyn-Noranda, Québec, in June 2001. Al and Fe were included in WHAM. Each lake was modelled using 3 different active DOC concentrations. (---) 1:1 line, (O) 50% active DOC, ($\square$) 65% active DOC, ($\triangle$) 80% active DOC. Uncertainty in WHAM predicted values represents 1 standard deviation of the $\log K_{\text{MA}}$ values. Experimental uncertainty represents a 95.5% confidence interval 3 replicate measurements.

value for Cu reflects the strongest modelled metal-binding (Table 6.1) of the four metals investigated here. Figures 6.7 and 6.8 show the results of the measured concentrations of $[\text{Cu}^{2+}]$ using the Xue and Sigg method [53], and those predicted by WHAM V and VI. Measurements of the $[\text{Cu}^{2+}]$ were not done for lakes Vaudray and Osisko. The lowest $[\text{Cu}]$ was in lake Opasatica, and the highest $[\text{Cu}]$ was in lake Dufault. Since the $[\text{Cu}^{2+}]$ depends on $[\text{Cu}]/[\text{DOC}]$ ratios (see Table 6.5), and the $[\text{Cu}]/[\text{DOC}]$ ratio of lake Dufault was much higher than that of lake Opasatica, it was expected that lake Dufault would have the highest $[\text{Cu}^{2+}]$ concentration. Both the measured $[\text{Cu}^{2+}]$ and the WHAM predictions agree with the expectation; however, the agreement between the WHAM-predicted and the experimental $[\text{Cu}^{2+}]$ was poor. The values of $\log K_{\text{MA}}$ (see Table 6.1) are simple averages of values obtained from different data sets. Variations in $\log K_{\text{MA}}$ among data sets may be due to errors in the data, and to errors in the model, arising from its application to data covering different experimental conditions. However, the main reason for the variation is probably that differences among the samples of HS used to obtain the data. In turn, such differences may arise from differences in isolation procedures, or they may reflect variability in the natural materials. It is informative to consider errors in the prediction of binding that might arise from the use of the model with its default parameter set. Table 6.1 shows standard deviations of the $\log K_{\text{MA}}$ value for each metal. The effect on the prediction of the $[\text{M}^{n+}]$ by adding or subtracting one standard deviation of $\log K_{\text{MA}}$ is shown as the uncertainty in Figs. 6.1-6.8. For Cu, the WHAM-predicted values can vary by about 1.5 log units. WHAM V was better for predicting the $[\text{Cu}^{2+}]$ in lake Opasatica, whereas WHAM VI was slightly better at modelling lake Dufault. The WHAM predicted values of $[\text{Cu}^{2+}]$ were only 1 order of

Figure 6.7 Comparison of WHAM-V-predicted pCu ($-\log_{10}[\text{Cu}^{2+}]$) with the measured pCu in water samples collected from Rouyn-Noranda, Québec, in July 2002. Al and Fe were included in WHAM. Each lake was modelled using 3 different active DOC concentrations. (---) 1:1 line, (O) 50% active DOC, (□) 65% active DOC, (△) 80% active DOC. Uncertainty in WHAM predicted values represents 1 standard deviation of the log K_{MA} values. Experimental uncertainty represents a 95.5% confidence interval of 3 replicate measurements.

Figure 6.8 Comparison of WHAM-VI-predicted pCu ($-\log_{10}[\text{Cu}^{2+}]$) with the measured pCu in water samples collected from Rouyn-Noranda, Québec, in July 2002. Al and Fe were included in WHAM. Each lake was modelled using 3 different active DOC concentrations. (---) 1:1 line, (O) 50% active DOC, (□) 65% active DOC, (△) 80% active DOC. Uncertainty in WHAM predicted values represents 1 standard deviation of the log K_{MA} values. Experimental uncertainty represents a 95.5% confidence interval of 3 replicate measurements.

magnitude lower than the measured $[\text{Cu}^{2+}]$, except for the WHAM VI prediction for lake Opasatica, which was 2 orders of magnitude lower than the measured $[\text{Cu}^{2+}]$.

The kinetics-based CLEM/AdCSV method for copper [65] yielded concentrations which were 2 to 4 orders of magnitude greater than the WHAM predicted values (see Table 6.5). This was because the results of the CLEM/AdCSV method represents the sum of labile Cu(II) complexes including the Cu-aqua complex ($[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$), as $[\text{Cu}^{2+}]$; hence, the measured results are expected to be equal to or greater than those predicted by WHAM.

There have been several other studies that have used WHAM to try and model the copper binding to DOC, and the results have shown a varying degree of success. Nolan et al. [99] conducted a study similar to the present one, but for soil pore waters. The Nolan et al. study compared the measured Cu^{2+} activity to the WHAM-VI-predicted values and found that the WHAM-predicted $[\text{Cu}^{2+}]$ were in poor agreement with their measured concentrations; the WHAM-predicted free- Cu^{2+} activities were 1 to 4 orders of magnitude lower than their measured concentrations. The authors noted that the measured free- Cu^{2+} activities were close to the detection limit of the analytical technique, ion-selective electrode potentiometry, which might have accounted for the poor agreement. Xue and Sigg used the competitive ligand/DPASV technique to determine the $[\text{Cu}^{2+}]$, $[\text{Zn}^{2+}]$ and $[\text{Cd}^{2+}]$ in surface water samples [55,113,114]. Tipping [109] applied WHAM VI globally, i.e. adjustments were made for the concentration of “active” FA and HA to optimize for the entire data set. It was found that $[\text{Cu}^{2+}]$ was generally overestimated in rivers and high-alkalinity lakes, and underestimated in low-alkalinity lakes. Both the

WHAM-predicted $[\text{Cd}^{2+}]$ and $[\text{Zn}^{2+}]$ showed better agreement with the experimental results. It was concluded that on average, the observed and WHAM-predicted values differed by ca 1 log unit, and that there was a reasonable correlation between the observations and the model. Dwane and Tipping [96] reported that the interactions of copper with DOC in copper amended natural waters were modelled well by WHAM V; however, the $[\text{Cu}^{2+}]$ was measured by them [96] at higher concentrations (10^{-6} to 10^{-5} M) than the present study; these high copper concentrations were required to detect the copper in solution using ion-selective electrode potentiometry. The ion-selective electrode potentiometry may not correctly measure the free- Cu^{2+} activity at much lower concentrations of copper found in natural freshwaters. An important distinction to note is the above and other studies added copper to the samples in concentrations that were higher than those present in natural freshwaters. In order to improve WHAM speciation so that it can correctly predict the low $[\text{Cu}^{2+}]$ usually present in freshwaters, the data sets to be used for improvement of the WHAM must be made with the correctly measured $[\text{Cu}^{2+}]$. Only such measurements will enable more correct descriptions of metal-binding to be incorporated into the WHAM, resulting in its improvement.

Modelling the Competition between Trace Metal Species Including Al and Fe, and also, of Ca^{2+} and Mg^{2+} for Binding by HS.

Ca^{2+} and Mg^{2+} are usually present in freshwaters at concentration levels which are several orders of magnitude higher than those of the trace metals, and hence, the competition of Ca^{2+} and Mg^{2+} with the target trace metals for the binding of the HS deserves special consideration. Several authors have reported the effects of Ca and Mg competition on the

binding of trace metals by HS. Recently, Cao et al. [115] have reported that Ca^{2+} has a measurable effect on the binding of trace metals such as Cd(II) , Pb(II) and Cu(II) by HA. Van den Hoop et al. [79] have observed a decreased association of Cd^{2+} and Zn^{2+} with the humate polyanion at pH 5 and constant ionic strength, suggesting that Ca^{2+} ions compete with the trace metal ions for binding by humic material. Ephraim et al. [116] suggested that Ca^{2+} has a minimal effect of competition for binding sites of FA with strongly complexing metals like Cu^{2+} , but weakens the binding of weak complexing metals like Cd^{2+} and Zn^{2+} .

There have been a few reports on the effect of Al and Fe competition with Cd. Penheiro et al. [117] have reported a significant competition effect between Cd and Al with a purified peat HA at pH 4 and 4.5, although the competition seems to lessen at higher pH values. For this study, the results for the WHAM-predicted effects of competition by Al and Fe are presented below.

Modelled Competition of the Target Trace Metals, Ni(II), Zn(II), Cd(II) and Cu(II), with Al and Fe

Nickel

The modelled competition between Ni(II) and Al and Fe is shown in Fig. 6.9. Table 6.2 shows the total concentrations of Al and Fe for each of the lakes. The competition for the modelled binding sites is very small as can be seen in Fig. 6.9. For lakes Opasatica and Vaudray, the effect was equal for both WHAM V and VI; however, there were slight differences between WHAM V and VI for lakes Osisko and Dufault. WHAM VI always predicted a higher $[\text{Ni}^{2+}]$ regardless of the competition effects.

Figure 6.9 Comparison of the pNi predicted by WHAM V and WHAM VI, with and without modelled Al and Fe for the lakes collected from Rouyn-Noranda, Québec; water samples collected in June 2001. (■) WHAM VI with Al and Fe, (■) WHAM VI without Al and Fe, (□) WHAM V with Al and Fe, (□) WHAM V without Al and Fe.

Zinc

The effect of competition for the binding sites between Zn(II) and Al and Fe was minimal just as it was with Ni(II) (see Fig. 6.10). There was a slightly larger effect predicted by WHAM V than WHAM VI; however, the effect of competition was very small for Zn(II).

Cadmium

For Cd(II), the effect of competition by Al and Fe was slightly larger than for Zn(II) and Ni(II), especially for lakes Opasatica and Vaudray, as can be seen from Fig. 6.11. There was little effect for the other two lakes. This was probably be due to the fact that lakes Opasatica and Vaudray had higher concentrations of Al than lakes Dufault and Osisko; the higher concentrations of Al might compete more favourably than Fe with Cd(II). In contrast to Ni(II) and Zn(II), WHAM VI showed a larger effect for Cd(II) than WHAM V.

Copper

Modelled competition by Al and Fe was much greater for Cu(II) than for Ni(II), Zn(II) and Cd(II) (see Fig. 6.12). In some cases, the difference in the effects of competition between “with” and “without” Al and Fe was 1 order of magnitude. Copper is most affected by Al(III) and Fe(III) for the following reasons. First, Al(III) binds strongly to HS, and may compete directly with copper for the same binding sites. The binding strength of Fe(III) is still unknown. Fe present as Fe(III) it might bind strongly; however, the Fe(III) might be reduced upon binding to HS to form the weaker-binding species, Fe(II). Secondly, and probably most importantly, the percent of copper bound to HS in the lakes (95-99%) is far greater than Ni, Zn, and Cd (~40%). Even a small amount of

Figure 6.10 Comparison of the pZn predicted by WHAM V and WHAM VI, with and without modelled Al and Fe for the lakes collected from Rouyn-Noranda, Québec; water samples collected in June 2001. (■) WHAM VI with Al and Fe, (■) WHAM VI without Al and Fe, (□) WHAM V with Al and Fe, (▨) WHAM V without Al and Fe.

Figure 6.11 Comparison of the pCd predicted by WHAM V and WHAM VI, with and without modelled Al and Fe for the lakes collected from Rouyn-Noranda, Québec; water samples collected in June 2001. (■) WHAM VI with Al and Fe, (■) WHAM VI without Al and Fe, (□) WHAM V with Al and Fe, (□) WHAM V without Al and Fe.

Figure 6.12 Comparison of the pCu predicted by WHAM V and WHAM VI, with and without modelled Al and Fe for the lakes in the area of Rouyn-Noranda, Québec; water samples collected in June 2001. (■) WHAM VI with Al and Fe, (■) WHAM VI without Al and Fe, (□) WHAM V with Al and Fe, (▨) WHAM V without Al and Fe.

competition by Al(III) and Fe(III) with Cu will result in a large increase in the proportion of $[\text{Cu}^{2+}]$, since the $[\text{Cu}^{2+}]$ is initially very low. The effect was the least for lake Osisko, probably because it had the lowest concentration of both Al and Fe. Both WHAM V and VI were equal in the degree of competition predicted; however, WHAM VI predicted a lower $[\text{Cu}^{2+}]$ than WHAM V regardless of the competition. It has been reported by Tipping [90] that Al and Fe competition causes a much larger increase in $[\text{Cu}^{2+}]$ than the competition of [Ca] and [Mg], unless [Ca] and [Mg] are of the order of 10^{-3} M, which is not the case in any of these lakes ([Ca] and [Mg] were $\lesssim 10^{-4}$ M)

Conclusions

The results of the WHAM comparison have confirmed the usual assumption that if the mechanistic base of WHAM adequately describes the differences in the solution composition such as pH, ionic strength and DOC, WHAM effectively acts as a vehicle for comparing the past data on metal-binding with the results and conditions of this work. Our results showed that for most metals, WHAM V and VI provided reasonable estimates for $[\text{Ni}^{2+}]$, $[\text{Zn}^{2+}]$, $[\text{Cd}^{2+}]$ and $[\text{Cu}^{2+}]$ in lake surface waters having different chemical conditions, such as pH and DOC concentrations.

In comparing the measured free-metal ion concentrations with the WHAM predictions, one should bear in mind the metal species measured by the speciation technique employed. In the case of nickel and copper (measured by kinetics-based CLEM/AdCSV), the speciation techniques employed provided the concentration of labile inorganic and organic metal complexes including metal-aqua complexes whereas the WHAM-predicted concentrations were only those of the free-metal ion, M^{2+} , and labile inorganic

complexes. Hence, in the case of copper, it was not unreasonable to expect that the measured concentrations were greater than the WHAM-predicted $[\text{Cu}^{2+}]$. Indeed, the measured concentrations of the labile Cu(II) complexes were larger than the WHAM-predicted concentrations. However, for zinc, the measured $[\text{Zn}^{2+}]$ were in good agreement with the WHAM-predicted $[\text{Zn}^{2+}]$. For nickel, the agreement between the measured concentrations and the WHAM-predicted concentrations were satisfactory. This was probably because the measured concentrations represented all the labile Ni species that produced the analytical signal. The measured $[\text{Cd}^{2+}]$ by the Xue and Sigg method and the WHAM-predicted $[\text{Cd}^{2+}]$ were different; however, the difference was not large enough to impugn the validity of the WHAM modelling. The WHAM-predicted results for $[\text{Cu}^{2+}]$ were at least an order of magnitude lower than the measured $[\text{Cu}^{2+}]$, probably because the default parameters in WHAM did not correctly describe the binding of Cu to the binding sites on the DOC. The general conclusions are that WHAM provided reasonable estimates of free-metal ion fractions in comparison with the measured fractions for the four metals in the surface waters of the four lakes.

Optimization of the “active” DOC concentration gave better fits to the experimental data; however, empirical modification done by this optimization proved to be unavailing when WHAM was applied to field samples. This study has shown that an active DOC concentration of between 65% and 80% is adequate to model the lake waters studied. The WHAM has shown that there is little competition from Al and Fe with Ni(II), Cd(II), and Zn(II); however, there is a significant effect on the binding of Cu(II). Comparing WHAM V with WHAM VI, our study has found that the models are very similar; however,

WHAM VI is equal to or better than WHAM V for most of the lake waters studied. Both WHAM V and WHAM VI have been tested extensively by others against several data sets and have been found to perform adequately when modelling freshwaters. WHAM is not always able to match the measured concentrations, yet, the information it gives often provides a good estimate of the $[M^{2+}]$, especially when good estimates of $[M^{2+}]$ are not otherwise readily available.

-- Chapter 7 --

**Speciation of Zn, Cd and Pb in Model Solutions and
Freshwater Samples from the Grand River, Ontario, and
Rouyn-Noranda, Québec, using Thin Mercury Film-Rotating
Disk Electrode Voltammetry**

Introduction

Characterization of the physical and chemical properties of HS is fundamental to understanding its role in the fate, reactivity, and transport of trace metals in freshwaters. This is difficult because of the complexity of freshwaters which contain mixtures of several polymers of HS that may or may not undergo aggregation, depending on the chemical conditions of the freshwater.

The diffusion coefficient is an important characteristic of metal-HS complexes in freshwaters, and can be strongly affected by the size distribution of HS. Several techniques have been employed for the size determination of HS, such as gel permeation chromatography/size exclusion chromatography [118,119], ultrafiltration [120-123], viscometry [124], diffusion through an activated carbon column [125], dynamic and static light scattering [126-129], small-angle neutron scattering [130], vapour-phase osmometry [131], field-flow fractionation [132], transmission electron microscopy [120,128], low angle X-ray scattering [133], and voltammetry [134]. The results obtained by these techniques usually are not in agreement. Some of the differences can be attributed to the origin of the sample and/or the purification procedures used. However, the same sample studied by different techniques also yields discrepant results, which is probably because of the specificity of each technique i.e., each technique responds to a specific property, and of the different sample preparation procedures required for the individual technique, as reported earlier in Chapter 5. Some of the observed differences result from the HS in the sample having different physical properties arising from aggregation/disaggregation.

It has been reported in dialysis experiments that aggregates of HS larger than the molar mass cut-off of the dialysis membrane pass through the membrane and are present in the dialyzed solution [138]. This has led to the hypothesis that HS can undergo disaggregation as it passes through the membrane, and then reaggregate on the other side. HS may therefore respond to changes in pH and/or ionic strength by adopting an optimal conformation (or size) depending on the experimental conditions used; however, this does not always proceed in the same way for samples of different origins and/or different preparation procedures [135].

Pinheiro et al. [136] have reported that the size of a peat HA decreased as the pH decreased from 4 to 6 with no further variation in size to pH 9, whereas others [127,128] have found no variation in particle dimensions in the same pH range, or an increase in size with increasing pH [137,138]. These discrepancies are again probably due to differences in the nature of the HA being studied and the experimental conditions employed.

Thin Mercury Film – Rotating Disk Electrode (TMF-RDE) for Determination of Speciation Parameters of Metal-HS Complexes

The application of a TMF-RDE and Linear Scan Anodic Stripping Voltammetry (LSASV) to determine the dissociation kinetics of metal complexes in natural waters was reported by Shuman and Michael [28,139] using the method of Hale [140]. Shuman and Michael [28] reported a first-order dissociation rate coefficient, $k_d \approx 2 \text{ s}^{-1}$, for a copper complex in a spiked marine coastal water sample. In our laboratories, a TMF-RDE with Differential Pulse ASV and Square Wave ASV was used to determine dissociation rate

coefficients and diffusion coefficients of metal complexes in samples of a river surface water [141], model solutions and snow [48,142]. However, the previous investigations were based on the assumption of equal diffusion coefficients for the free-metal ion, M^{2+} , and the complex, ML. This assumption is not valid in the case when the ML is much larger than M, and consequently has a much smaller diffusion coefficient than M^{2+} alone.

A Thin Mercury Film-Rotating Disk Electrode (TMF-RDE)

A schematic diagram of a TMF-RDE is presented in Fig. 7.1. In ASV, the timescale of measurement is defined by the thickness of the diffusion layer [143]. In conventional ASV using a hanging mercury drop, the diffusion layer is largely undefined and irreproducible [33], although an average diffusion layer thickness can be estimated. At an RDE, the diffusion layer is precisely controlled by the rotation rate of the electrode [49]. At extremely high rotation rates, the rotating motion of the electrode acts as a suction pump, drawing the bulk solution towards the electrode surface by laminar convection. As the solution approaches the electrode surface, it is thrown radially outwards by the centrifugal motion of the RDE, resulting in the formation of a well-defined and reproducible diffusion layer immediately adjacent to the electrode surface, known as the Nernstian diffusion layer, in which diffusion is the form of mass transport (see Fig. 7.1). When the RDE is rotated at a high velocity, a steady-state is rapidly established, typically within 0.25 s [144]. High rotation rates result in thin diffusion layers, in which only labile species (i.e. those which are able to rapidly dissociate) are measured since they are able to dissociate before reaching the electrode surface, whereas non-labile and quasi-labile species are not. The current is completely mass-transport-controlled. It is a

Figure 7.1 Schematic diagram of a Thin Mercury Film - Rotating Disk Electrode.

convective-diffusive system of mass transport. The contribution from charging the electrical double layer current is nil, which results in the absence of the non-faradaic current. Therefore, under the steady-state condition, the recorded current may be unequivocally equated with the faradaic current.

Another advantage of the RDE is that the entire surface of the electrode is uniformly accessible to the entire sample since the thickness of the diffusion layer is constant over the entire electrode surface. Regardless of the distance from the axis of rotation, the conditions for transport of the analyte to any point on the disk surface are identical. This makes RDE ideally suited for investigating kinetic reactions such as the dissociation of metal complexes.

The Nature of 'ASV-Lability'

The application of ASV to chemical speciation is based on the assumption that ASV can distinguish between labile and non-labile complexes. In the traditional sense, lability refers to the ability of a metal complex in solution to make and break bonds rapidly and to rapidly exchange a ligand within the metal coordination sphere for ligands outside the sphere. In ASV, lability has an operational definition related to the observed stripping current. During the pre-electrolysis step, the concentration of the metal near the mercury film is decreased, which results in perturbation of the equilibrium between the metal complex and its components. For a complex to be labile, it must be able to dissociate rapidly enough to maintain equilibrium and have a dissociation rate at least as rapid as the rate of mass transfer of metal to the electrode surface. The measured current in ASV results from the reduction of metal ions supplied by dissociation of labile complexes. If a

complex dissociates very slowly, it is non-labile and does not produce a current. Complexes that lie between the operational definitions of labile and non-labile complexes contribute to the ASV current to an extent that depends on their dissociation rate and concentration [28], as shown in Fig. 7.2. The timescale of the electrochemical measurement defines the metal species that can be measured and those that can be called labile [28,33,143].

Results and Discussion

Linear Scan ASV is much less sensitive than the pulse techniques such as Differential Pulse ASV and Square Wave ASV, but has been employed in this work in preference to the much more sensitive pulse techniques named above because the pulse techniques suffer from a number of factors that may influence the peak height (and peak area), such as adsorption of organic surfactants, and secondary effects during the stripping step, when pulse techniques are applied to freshwaters containing organic surfactants.

Peak height is almost universally used as the measure of the amount of metal oxidized during the stripping step in ASV. However, a broadening and flattening of the stripping peak in the presence of FA has been reported by Pinheiro et al. [145]. This effect has been attributed to changes in the effective metal-to-ligand ratio, and hence, the conditional stability constants of metal complexes at the electrode surface. Although peak height may decrease significantly in the presence of heterogeneous complexants, the reduction in peak area has been reported to be significantly less [146].

Figure 7.2 Concentration profile of ML at the electrode-solution interface for labile, quasi-labile and non-labile metal complexes. δ is the thickness of the Nernstian diffusion layer.

Model Solution of Pb(II)- Zn(II)- and Cd(II)-HA Complexes

Metal complexes of natural complexants are significantly larger than the metal-aqua complex, thus have smaller diffusion coefficients. As a rough approximation, the metal complex of individual molecules of a given group of heterogeneous complexants (e.g. HS) are often assumed to have similar sizes and a single diffusion coefficient [147]. However, heterogeneous complexants such as HS are polydisperse, and the diffusion coefficients of the metal-HS complexes reported are only averages over a wide range of HS molecules.

Figures 7.3 and 7.4 present the Levich plot and the modified Cottrell plot (current-time response), respectively, which are commonly used in pulse polarography to study electrochemical processes [148]. The Cottrell equation (Eq. 7.1) indicates that there is a linear relationship between the current and $t^{-1/2}$,

$$i_{\text{lim}} = \frac{nFAD^{1/2}c_{\text{M,T}}}{\pi^{1/2}t^{1/2}} \quad (7.1)$$

where i_{lim} is the limiting current, n is the number of electrons involved in the reaction, F is the Faraday constant, A is the surface area of the electrode, D is the diffusion coefficient, and $c_{\text{M,T}}$ is the total metal concentration. A plot of i_{lim} vs. $t^{-1/2}$ is often referred to as the Cottrell plot. Figures 7.3 and 7.4 have been adapted for ASV, where $t^{-1/2}$ ($\text{s}^{-1/2}$) along the x -axis of a Cottrell plot has been replaced with its analogue for RDE-ASV, the square root of the angular velocity, $\omega^{1/2}$ ($\text{s}^{-1/2}$), which defines the thickness of the diffusion layer, and hence, the analytical timescale of measurement in ASV. The data in Fig. 7.4 were fitted to Eq. 3.16 to determine the dissociation rate coefficients, k_d ,

Figure 7.3 Levich plot of Cd(II)-HA complexes in a model solution of HA. $\omega^{1/2}$ represents the angular velocity of the RDE. PIPES buffer, pH 6.0, $I = 0.1$ M, $T = 23^\circ\text{C}$. (○) Solution containing only Cd^{2+} , $c_{\text{Cd},T} = 4.45 \times 10^{-7}$ mol/L; (□) Solution containing Cd^{2+} and HA, $c_{\text{Cd},T} = 4.45 \times 10^{-7}$ mol/L, $c_{\text{HA}} = 2.44 \times 10^{-5}$ mol/L. The solid lines represent data that were fitted to the Levich equation (Eq. 3.8). Error bars represent the 95.5% confidence interval of 3 replicate measurements.

Figure 7.4 Modified Cottrell plot of Cd(II)-HA complexes in a model solution of HA. $\omega^{1/2}$ represents the angular velocity of the RDE. PIPES buffer, pH 6.0, $I = 0.1$ M, $T = 23^{\circ}\text{C}$, $c_{\text{Cd,T}} = 4.45 \times 10^{-7}$ mol/L, $c_{\text{HA}} = 2.44 \times 10^{-5}$ mol/L. The solid curve represents data that were fitted to Eq. 3.15. Error bars represent the 95.5% confidence interval of 3 replicate measurements.

of the metal complexes, which can then be used to determine the lability condition, Λ , from Eqs. 3.13 and 3.14, and the $[M^{n+}]$.

Diffusion coefficients at different pH values for the Zn(II)-, Cd(II)- and Pb(II)-HA complexes presented in Table 7.1 were determined by fitting the experimental data to the Levich equation [49] (Eq. 3.8) by linear regression analysis. Pb could only be studied at pH 5.0, since Pb hydrolyzes significantly at higher pH values. The diffusion coefficients of the Zn(II)- Cd(II)- and Pb(II)-HA complexes were much lower than those for the uncomplexed ions ($D_{Zn} = 6.38 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, $D_{Cd} = 6.90 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ and $D_{Pb} = 8.28 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [144]), which suggests that the Zn(II), Cd(II), and Pb(II), are complexed as large, metal-HA complexes. As the pH was increased from 5.0 to 7.0, the diffusion coefficients for the Cd(II)- and Zn(II)-HA complexes decreased further, suggesting that the increase in pH resulted in binding to larger HA molecules, which was probably due to an increase in the hydrogen bonding between deprotonated carboxylic groups and phenolic groups and/or to the swelling of the aggregates due to the repulsion between the negative charges on the HA [136]. The decrease in the diffusion coefficient from pH 5.0 to 7.0 was more remarkable for the Cd(II)-HA complexes than the Zn(II)-HA complexes. The diffusion coefficient is mainly affected by the HS, due to its large dimension, and not by the metal ion (which is relatively much smaller in size). The effect of increasing pH from 5.0 on Zn is greater than on Cd because of greater hydrolysis of Zn, producing hydrolyzed zinc species at the higher pH, which resulted in the HA complex of the hydrolyzed zinc being different in size from the HA complex of unhydrolyzed cadmium at the same pH.

Table 7.1: Diffusion coefficients and dissociation rate coefficients for Zn(II)-, Cd(II)- and Pb(II)-HA complexes in model solutions of HA. $c_{M,T}$ is the total metal concentration, c_{HA} is the concentration of HA, $c_{M,T}/c_{HA}$ is the metal:HA mole ratio, $\bar{D}$ is the average diffusion coefficient, and k_d is the dissociation rate coefficient. pH 5.0, 6.0 and 7.0, $I = 0.10$ M, $T = 23^\circ\text{C}$.

$c_{M,T}$ (mol/L)	pH (± 0.2)	c_{HA} (mol/L)	$c_{M,T}/c_{HA}$	$\bar{D}$ (cm^2/s)	k_d (s^{-1})
Zn(II)					
9.5×10^{-7}	5.0	2.4×10^{-5}	0.039	4.7×10^{-6}	3.8×10^1
9.5×10^{-7}	6.0	2.4×10^{-5}	0.039	4.5×10^{-6}	9.9×10^1
9.5×10^{-7}	7.0	2.4×10^{-5}	0.039	3.5×10^{-6}	1.1×10^2
Cd(II)					
4.5×10^{-7}	5.0	2.4×10^{-5}	0.018	5.1×10^{-6}	1.2×10^2
4.5×10^{-7}	6.0	2.4×10^{-5}	0.018	3.4×10^{-6}	9.9×10^1
4.5×10^{-7}	7.0	2.4×10^{-5}	0.018	3.1×10^{-6}	1.1×10^2
Pb(II)					
2.4×10^{-7}	5.0	2.4×10^{-5}	0.010	1.6×10^{-6}	2.5×10^2

Figure 7.5 Diffusion layer thickness as a function of the rotation rate of the RDE for the free Cd^{2+} ion. $D = 6.90 \times 10^{-6} \text{ cm}^2/\text{s}$. Error bars represent the 95.5% confidence interval of 3 replicate measurements.

A straight line passing through the origin of both the Levich plot (see Fig. 7.3) and the modified Cottrell plot (see Fig. 7.4) indicates a labile complex. When the rotation rate of the electrode is extremely low, the diffusion layer around the electrode is infinitely large, which allows infinite time for all the complexes to dissociate [35]. As the rotation rate increases, the diffusion layer decreases exponentially (see Fig. 7.5), and deviations from linearity (i.e. curvature) may occur, since there is less time available for complex dissociation..

A better indication of the lability of the Zn(II)-, Cd(II)- and Pb(II)-HA complex system; however, can be obtained by the lability parameter, which is presented in Table 7.2. The data were fitted to Eq. 3.16 by non-linear regression analysis using the commercial software program NLREG v5.1 (Philip H. Sherrod). Following the criterion set by condition 2.22, the metal species are defined as labile if $\Lambda t^{1/2} \gg 1$, or equivalently $\Lambda_{ss}\delta \gg 1$ (Eq. 3.14). Table 7.2 shows that all the complexes of Cd(II)-, Zn(II)- and Pb(II)-HA are quasi-labile to labile ($\Lambda_{ss}\delta \approx 1$ quasi-labile; $\Lambda_{ss}\delta \gg 1$ labile). For Zn(II)-HA complexes, an increase in pH results in an increase in the lability of the system (see Table 7.2). These results are reinforced by the fact that the k_d and $[M^{2+}]$ increase as the lability of the system increases. This, however, is the opposite of what is expected. As pH increases from 5.0 to 7.0, there are more sites on the HA that are ionized and available for binding of the Zn. This should result in a decrease in lability as more metal is complexed. This trend could probably be a result of increased aggregation of the HS, as explained above. If the deprotonated sites of the HS are involved in hydrogen bonding, they will not be available for complexation by the metal, resulting in more labile complexes.

Table 7.2: Dynamic characteristics and labilities of metal complexes in model solutions of HA. $c_{M,T}/c_{HA}$ is the metal:HA mole ratio, K is the conditional stability constant, k_f is the formation rate coefficient, $\bar{\delta}$ is the average diffusion layer thickness, k_d is the dissociation rate coefficient, $[M^{2+}]$ is the free metal ion concentration, and $\Lambda_{ss}\bar{\delta}$ is the lability parameter. pH 5.0, 6.0 and 7.0, $I = 0.10$ M, $T = 23^\circ\text{C}$.

$c_{M,T}/c_{HA}$	pH (± 0.2)	K (L mol^{-1})	$k_f^\dagger$ ($\text{M}^{-1} \text{s}^{-1}$)	k_d (s^{-1})	$\bar{\delta}^\ddagger$ (m)	$[M^{2+}]$ (M)	$\Lambda_{ss}\bar{\delta}$ ($\Lambda t^{1/2}$)
Zn(II)							
0.039	5	2.0×10^5	7.6×10^6	3.8×10^1	5.7×10^{-5}	1.6×10^{-7}	9.7
0.039	6	7.7×10^4	7.6×10^6	9.9×10^1	5.6×10^{-5}	3.3×10^{-7}	31
0.039	7	7.0×10^4	7.6×10^6	1.1×10^2	5.2×10^{-5}	3.5×10^{-7}	43
Cd(II)							
0.018	5	2.7×10^5	3.3×10^7	1.2×10^2	5.9×10^{-5}	5.8×10^{-8}	14
0.018	6	3.3×10^5	3.3×10^7	9.9×10^1	5.1×10^{-5}	4.9×10^{-8}	16
0.018	7	3.1×10^5	3.3×10^7	1.1×10^2	5.0×10^{-5}	5.2×10^{-8}	18
Pb(II)							
0.010	5	3.5×10^6	7.6×10^8	2.2×10^2	4.0×10^{-5}	2.8×10^{-9}	12

† calculated as 6/55 times the first-order rate coefficient for water exchange, k_w [18,30].

‡ $\bar{\delta}$ calculated from Eq. 3.11 for an angular velocity of 1000 rpm.

Uncertainties in $[M^{2+}]$ are a factor of 2.

Freshwater Samples of Pb(II)- Zn(II)- and Cd(II)-DOC Complexes

The dissociation rate coefficient, conditional stability constant, and lability parameter were similar for the Zn(II)-DOC and Zn(II)-HA complexes at pH 7.0, suggesting that Zn(II)-HA complexes in the model solution were nearly the same with respect to their complexation as the Zn(II)-DOC complexes in lake Dufault. The results for Cd(II)-DOC and Pb(II)- DOC were different (for the lake Dufault freshwater sample) from the model solution, probably because of the different concentration and type of DOC in the freshwater sample, as well as other competing ligands that were present in the lake Dufault freshwater sample, but were not in the model solutions.

Tables 7.3 and 7.4 present the results from the freshwater samples collected from Rouyn-Noranda, Québec, and the Grand River, Ontario. The samples were buffered at their natural pH of ~7 for the study of Zn(II)- and Cd(II)-DOC complexes; however, Pb(II)-DOC complexes could only be studied at pH 5.0, for the same reason discussed earlier. Lake Dufault was the most metal-impacted lake and had a high initial concentration of Zn (see Table. 4.1). Of the complexes studied, the Zn(II)-DOC complexes in lake Dufault had the lowest diffusion coefficient, and the highest lability. The low diffusion coefficient is probably a result of the higher pH value, which results in repulsion of the negatively charged functional groups on the DOC. The metal complexes in the other 2 freshwater samples had smaller diffusion coefficients than the Zn(II)-DOC complexes in lake Dufault, suggesting that the Zn(II)-DOC complexes were larger. Since lake Dufault was the most metal-impacted, the binding sites on the DOC were probably saturated, resulting in the formation of weak Zn(II)-DOC complexes which were more labile than the

Table 7.3: Diffusion coefficients and dissociation rate coefficients for Zn(II)-, Cd(II)- and Pb(II)-DOC complexes in freshwater samples from the Grand River, Ontario, and Rouyn-Noranda, Québec. pH 5.0, 6.0 and 7.0, I = 0.10 M, T = 23°C. $c_{M,T}$ is the total metal concentration, c_{DOC} is the concentration of DOC, assuming a bidentate complexing capacity of 5.43 mmol/g, $c_{M,T}/c_{DOC}$ is the metal:HA mole ratio, $\bar{D}$ is the average diffusion coefficient, and k_d is the dissociation rate coefficient.

$c_{M,T}$ (mol/L)	pH (± 0.2)	c_{DOC} (mol/L)	$c_{M,T}/c_{DOC}$	$\bar{D}$ (cm ² /s)	k_d (s ⁻¹)
Zn(II), lake Dufault					
9.5×10^{-7}	7.0	8.5×10^{-5}	0.011	1.5×10^{-6}	1.1×10^2
Cd(II), lake Vaudray					
4.5×10^{-7}	6.0	6.0×10^{-5}	0.0075	5.3×10^{-6}	2.0×10^2
Pb(II), lake Vaudray					
2.4×10^{-7}	5.0	6.0×10^{-5}	0.0040	5.4×10^{-6}	7.8×10^2
Pb(II), Grand River					
8.5×10^{-7}	5.0	8.2×10^{-5}	0.010	5.2×10^{-6}	6.4×10^2

Table 7.4: Dynamic characteristics and labilities of metal complexes in samples collected from the Grand River, Ontario, and Rouyn-Noranda, Québec. pH 5.0, 6.0 and 7.0, $I = 0.10 \text{ M}$, $T = 23^\circ\text{C}$. $c_{\text{M,T}}/c_{\text{DOC}}$ is the metal:DOC mole ratio, K is the conditional stability constant, $\bar{\delta}$ is the average diffusion layer thickness, $\Lambda_{\text{ss}}\bar{\delta}$ is the lability parameter, and $[\text{M}^{2+}]$ is the free metal ion concentration.

$c_{\text{M,T}}/c_{\text{DOC}}$	pH (± 0.2)	K (L mol^{-1})	$\bar{\delta}^\dagger$ (m)	$\Lambda_{\text{ss}}\bar{\delta}$ ($\text{M}^{1/2}$)	$[\text{M}^{2+}]$ (M)	WHAM V §	WHAM VI §
Zn(II), Lake Dufault							
0.011	7.0	6.9×10^4	3.9×10^{-5}	38	1.4×10^{-7}	7.4×10^{-7}	6.6×10^{-7}
Cd(II), Lake Vaudray							
0.0075	6.0	1.7×10^5	6.0×10^{-5}	14	4.1×10^{-8}	4.1×10^{-7}	3.8×10^{-7}
Pb(II), Lake Vaudray							
0.0040	5.0	9.8×10^5	6.0×10^{-5}	12	4.1×10^{-9}	4.1×10^{-7}	4.2×10^{-7}
						$^*3.5 \times 10^{-8}$	$^*1.4 \times 10^{-7}$
Pb(II), Grand River							
0.010	5.0	1.5×10^6	6.0×10^{-5}	8.5	8.3×10^{-9}	2.6×10^{-7}	4.8×10^{-7}
						$^*2.1 \times 10^{-9}$	$^*5.4 \times 10^{-9}$

$^\dagger \bar{\delta}$ calculated from Eq. 3.11 for an angular velocity of 1000 rpm.

§ WHAM predictions based on 65% “active” DOC and spiked Cd and Pb concentrations.

* Values calculated to reflect the natural pH of the freshwater sample (pH 6.1 for lake Vaudray, pH 7.5 for the Grand River).

Uncertainties in measured and WHAM-predicted $[\text{M}^{2+}]$ are a factor of 2.

metal-complexes in lake Vaudray and the Grand River, as shown in Table 7.4 by the larger lability parameter for the Zn(II)-DOC complexes. Both the Cd(II)- and the Pb(II)-DOC complexes studied in lake Vaudray showed similar diffusion coefficients and labilities.

The [Zn]/[DOC] mole ratio for the freshwater sample was approximately 3 times less than the [Zn]/[HA] mole ratio in the model solutions. The diffusion coefficients for the Zn(II)-DOC complexes in the lake Dufault freshwater sample were about 3 times less than the Zn(II)-HA sample at the same pH of 7.0. This suggests that the Zn(II)-DOC complexes in lake Dufault were larger than the Zn(II)-HA complexes. The opposite is true for the Pb(II)- and Cd(II)-DOC complexes. The Pb(II)- or Cd(II)-HA complexes have lower diffusion coefficients, suggesting larger complexes.

Figures 7.6 and 7.7 show the Levich and modified Cortell plots for the Pb(II)-DOC complexes from the Grand River. Unlike the results of the model solutions, in Fig. 7.6, the Pb(II)-DOC sample showed a deviation from linearity at high rates of rotation. This was probably a result of a loss in lability with increasing rates of rotation as the thickness of the diffusion layer was decreased, suggesting that the Pb(II)-DOC in the Grand River freshwater sample was *quasi-labile*. At very high rotation rates, the diffusion layer became extremely thin, providing almost no time for the complexes to dissociate and the system approached the *static* limit (Eq. 2.16).

The measured diffusion coefficients shown in Tables 7.1 and 7.3 are weighted averages of all metal species diffusing toward the electrode surface, as defined by Eq. 7.2 [149].

Figure 7.6 Levich plot of Pb(II)-DOC complexes in the Grand River. $\omega^{1/2}$ represents the angular velocity of the RDE. HOAc/NaOAc buffer, pH 5.0, $I = 0.1$ M, $T = 23^\circ C$. ($\circ$) Solution containing only Pb^{2+} , $c_{Pb,T} = 8.5 \times 10^{-7}$ mol/L; ($\square$) Solution containing Pb^{2+} and DOC, $c_{Pb,T} = 8.5 \times 10^{-7}$ mol/L, $c_{DOC} = 8.2 \times 10^{-5}$ mol/L. The solid lines represent data that were fitted to the Levich equation (Eq. 3.8).

Figure 7.7 Modified Cottrell plot of Pb(II)-DOC complexes in the Grand River. $\omega^{1/2}$ represents the angular velocity of the RDE. HOAc/NaOAc buffer, pH 5.0, $I = 0.1$ M, $T = 23^\circ\text{C}$, $c_{\text{pb},T} = 8.5 \times 10^{-7}$ mol/L, $c_{\text{DOC}} = 8.2 \times 10^{-5}$ mol/L. The solid line represents data that were fitted to Eq. 3.12.

$$\bar{D} = \frac{D_M c_M + D_{ML} \sum_i c_{ML_i}}{c_M + \sum_i c_{ML_i}} \quad (7.2)$$

Hence, the mean diffusion layer thickness, $\bar{\delta}$, is defined jointly by M and ML as shown in Fig. 7.8.

Comparison of WHAM Predicted Free-Metal Ion Concentrations with those Measured Using the TMF-RDE

Table 7.4 shows the results of the WHAM V and VI predicted $[M^{2+}]$ compared with those determined by the TMF-RDE. The results for Pb are shown both at the natural pH of the lake water sample as well as at the experimental pH of 5.0. Both WHAM V and VI compared well with the $[Zn^{2+}]$ determined by the TMF-RDE. In both this study and the one presented in Chapter 6, the $[Cd^{2+}]$ was not modelled as well as $[Zn^{2+}]$ by WHAM. The modelled $[Pb^{2+}]$ was predicted to be higher than the experimentally determined $[Pb^{2+}]$ at the experimental pH of 5.0 for both lake Vaudray and the Grand River. At the natural pH of 6.1 for lake Vaudray, and pH 7.5 for the Grand River, the difference between the experimentally determined $[Pb^{2+}]$ and the WHAM-predicted value was much smaller. Higher pH should have a large effect on the $[Pb^{2+}]$ because of the formation of Pb-hydroxides at higher pH values. Both WHAM V and WHAM VI were nearly equal in predicting the $[M^{2+}]$ for all the metals; WHAM VI was slightly better for all the samples except for the lake Vaudray sample.

Figure 7.8 Concentration profile for a metal complex system under limiting conditions [28]. Dashed lines: M and ML are fully independent of each other (e.g. ML is an electroactive, fully inert complex) and diffuse separately, with its specific diffusion layer thickness, δ_{ML} and δ_M . Solid lines: ML is a labile complex in equilibrium with M and L . M and ML diffuse simultaneously, under conditions where $\bar{D}$ is operative (and $D_{ML} < D_M$), so that the resulting diffusion layers have the corresponding mean thickness, $\bar{\delta}$.

Conclusions

The results of the TMF-RDE study show that aggregation of the Zn(II)- and Cd(II)-HA complexes increases with increasing pH as suggested by the decreasing diffusion coefficients. This was rather unexpected, since increasing pH should result in increased ionization (more negative charges on the HS) that should repel one another and promote peptization. However, the result was not unusual. The lability parameter generally followed the dissociation rate coefficient and conditional stability constant, as expected. The Zn(II)-HA complexes were more labile, and had a larger range in the lability between each pH compared with the Cd(II)-HA complexes. The Pb(II)-HA complexes were equally as labile as the Zn(II)- and Cd(II)-HA complexes at pH 5.0. However, the lowest $[M^{2+}]$ was given by Pb, which suggested that although the Pb(II)-DOC complexes might be just as labile as Zn(II)- and Cd(II)-DOC complexes, most of the Pb might be still bound as weak Pb(II)-HA complexes.

The freshwater samples showed similar results. The largest $[M^{2+}]$ was for Zn, and the lowest was for Pb in all of the freshwater samples. The diffusion coefficient was similar for both freshwaters studied with Pb, even though the concentration of Pb in the Grand River was about 4 times greater than in lake Vaudray. The Pb(II)-DOC mole ratio was about three times greater for the Grand River sample than lake Vaudray, but the $[M^{2+}]$ was only 2 times higher in the Grand River.

The diffusion coefficients were similar for the Cd(II)- and Pb(II)-DOC complexes from lake Vaudray and the Grand River; however, that of the Zn(II)-DOC complex was much

lower, suggesting that the Zn(II)-DOC complexes in lake Dufault were larger than the Pb(II)- and Cd(II)- DOC complexes in lake Vaudray and the Grand River.

The TMF-RDE is a powerful tool for the determination of speciation parameters in freshwaters. The formation of a precisely controlled and very thin diffusion layer in a very short period of time (typically within 0.25 s [144]) is akin to the diffusion layer formed around a microelectrode (or a micro-biological surface of a biota). Under the condition of an infinitely small diffusion layer, only free-metal ions are measured; free-metal ions have been reported to be usually a good predictor of ecotoxicity, TMF-RDE serves an extremely useful purpose of providing the only, unequivocal, experimentally-measured $[M^{2+}]$, at the concentration levels of freshwaters.

-- Chapter 8 --

Concluding Comments

Concluding Comments

The $[M^{n+}]$ has been reported to be usually a good indicator of toxicity in freshwaters; however, it is extremely difficult to measure the $[M^{n+}]$ at environmentally relevant concentrations, speciation parameters, which include $[M^{n+}]$, are used to describe the chemical properties of the metal-DOC complexes that can be related to metal ecotoxicity. This thesis has examined the application of speciation parameters in model solutions and freshwaters in an attempt to better understand the interactions between the metals and the natural organic ligands.

A major finding of this thesis is the importance of HA on nickel binding in freshwaters. Previously, FA was used as the model for DOC since it comprises a much larger portion of the DOC than does HA. In both model solutions and computer speciation models, these two operationally-defined fractions (HA and FA) of HS can be studied separately. However, in freshwaters, their size distribution is continuous, covering a range of compounds with molecular weights from a few hundred to tens of thousands of Da; hence, it is extremely difficult to study them separately in freshwaters.

The binding properties of a particular freshwater sample depend on several factors, not the least of which is the composition of the humic fraction; the ratio of HA to FA, and the metal characteristics of HA and FA. Nickel was found to bind to HA more strongly than to FA, suggesting that HA had a larger fraction of stronger binding sites, and probably the larger size of HA resulted in smaller differential diffusional accessibility of the nickel from the inside to the outside of the HA which resulted in stronger Ni(II)-HA complexes. This finding shows that HA might be as important as FA when modelling freshwaters,

since even a small fraction of HA might still have a large effect on the overall nickel binding properties of the DOC.

Computer-based speciation codes, such as WHAM, are now becoming sophisticated enough to be applied and tested in real freshwater samples. One of the major difficulties in modelling freshwaters is the determination of the “active” concentration DOC, that is the DOC that is actively involved in the binding of metals. The determination of “active” DOC is subject to debate, and may be different for different freshwater samples. The ratio of HA to FA is also an important factor to consider in WHAM modelling. As was stated above and in Chapter 5, HA forms stronger complexes with nickel than does FA. Since these two parameters are rarely known for natural freshwaters, many results, including the ones presented in this thesis, were compiled by assuming a certain proportion of DOC as “active”, which was modelled entirely as FA. The literature has shown that the proportion of “active” DOC ranges from 40-80% [96-102]. Despite this and the other assumptions of WHAM, the model was reasonably successful in modelling the free-metal ion concentrations for Ni, Zn, Cd, and Cu in the freshwater samples studied.

Computer modelling also allows us to model systems that may be impossible to do experimentally, such as to determine how the free-metal ion concentrations of Ni, Cd, Zn, and Cu would be affected in a freshwater sample in the presence or absence of Al or Fe. These two metals are important because they are easily leached from the surrounding rocks by acid rain. They are also trivalent, and may compete with other trace metals which are divalent for the binding sites on DOC. In the freshwater lakes studied, it was

predicted from modelling that the presence of Al and Fe would have a profound effect on the free-Cu ion concentration, but little effect on the free-Ni, Zn, and Cd concentrations.

Freshwaters are difficult to study because of the numerous reactions and interactions that are occurring simultaneously. Modelling freshwaters with computer-based speciation models such as WHAM, likewise, is also difficult, since every freshwater is different, and there may not be chemical equilibrium in freshwaters. For WHAM to be more useful; however, it must be able to model all freshwaters of different chemical compositions. To do this, WHAM must make a compromise between complexity and simplicity; it must be complex enough to adequately describe the freshwater system of interest, and it must be simple enough to allow interpretation of the results. This leads to many assumptions in WHAM about freshwaters that may not be valid, but are necessary for the model to be useful in a variety of real applications. Further development of WHAM can only be accomplished if it is rigorously tested under many different experimental conditions. This was one of the goals of this thesis (Chapter 6), and although WHAM was shown to adequately predict free-metal ion concentrations in a small number of freshwater lakes, there are still problems that need to be addressed, such as the poor modelling of Cu, if WHAM is to be more widely acceptable as a useful tool for modelling freshwaters.

For metals such as Cd, Zn, and Pb that dissolve in Hg, electrochemical methods employing ASV are easy and convenient since they require little sample pre-treatment. The most important factor to control in ASV is the thickness of the Nernstian diffusion layer, since it determines time allowed for metal complexes to dissociate, and hence, the measurement time. In freshwaters, metals are only bioavailable if they are able to

dissociate from the natural complexant and become incorporated into the cells of aquatic organisms. For this to occur there must be adequate time for the metal-complex to dissociate before it reaches the surface of the cellular membrane. For small organisms such as algae, the diffusion layer is extremely small. The RDE is able to approximate the effects of this small diffusion layer at high rotation rates, when the diffusion layer is extremely thin. Because of this, the RDE is akin to the diffusion layer at the surface of a small aquatic organism, and the results of the TMF-RDE study can be directly related to bioavailability.

Although ASV measures an operationally defined quantity (ASV lability), the application of the RDE with ASV allows the labile species to be defined based on the diffusion layer thickness. This is unlike ASV with a hanging mercury drop, where the diffusion layer is much thicker and undefined. The RDE can differentiate between labile and non-labile complexes kinetically, which is consistent with the way they are defined [28].

Our understanding of the complex interactions that occur between metals and naturally-occurring organic ligands in freshwaters is still limited. Through the further development of speciation parameters and computer-based speciation codes, and the concurrent application of these techniques, further insight into these complex interactions, as well as the elusive free-metal ion concentration, may be achieved.

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Glossary

Symbol	Definition	Symbol	Definition
A	Exposed surface area of the electrode (m^2)	k_f	Formation rate coefficient ($\text{L mol}^{-1} \text{s}^{-1}$)
c_L	Concentration of ligand, L (mol L^{-1})	k'_f	$= k_f c_L$ (s^{-1})
c_{HA}	Concentration of humic acid (mol L^{-1})	k_d	Dissociation rate coefficient (s^{-1})
c_M	Free-metal ion concentration (mol L^{-1})	K_{os}	Stability constant for an outer-sphere complex (L mol^{-1})
$c_{\text{M,T}}$	Total metal concentration (mol L^{-1})	$k_{\text{-w}}$	Water loss rate constant (s^{-1})
c_{ML}	Concentration of complex ML (mol L^{-1})	$\text{M}^{\text{n+}}$	Free-metal ion (metal aqua complex)
$C_{\text{ML}}(t)$	Concentration of complex ML at any time, t (mol L^{-1})	ML	Metal complex formed by M with ligand, L
C_i^0	Concentration of complex formed by site i (mol L^{-1})	ML_i	Metal complex formed by M with site type i
C_{Lx}	Total ligand concentration (mol L^{-1})	M_{nat}	Concentration of naturally occurring free-metal ions (mol L^{-1})
D	Diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$)	S	Sensitivity (M^{-1})
$\bar{D}$	Mean diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$)	t	time (s)
D_M	Diffusion coefficient of free-metal ion, M ($\text{cm}^2 \text{s}^{-1}$)	δ	Thickness of the Nernstian diffusion layer
D_{ML}	Diffusion coefficient of complex, ML ($\text{cm}^2 \text{s}^{-1}$)	$\bar{\delta}$	Mean thickness of the Nernstian diffusion layer (m)
i_{lim}	Limiting current (A)	ε	$= D_{\text{ML}}/D_M$
i_p	Peak current (A)	Λ	lability parameter
J	Flux ($\text{cm}^{-2} \text{s}^{-1}$)	Λ_{ss}	Steady state lability parameter
J_{kin}	Kinetically controlled flux ($\text{cm}^{-2} \text{s}^{-1}$)	μ	Reaction layer thickness (units)
J_{diff}	Diffusion-controlled flux ($\text{cm}^{-2} \text{s}^{-1}$)	α_{in}	Inorganic ligand complexing coefficient (units)
M_{lab}	Concentration of labile metal complexes (mol L^{-1})	α_{R}	Competing ligand complexing coefficient (units)
M_{in}	Concentration of inorganic metal complexes (mol L^{-1})	ν	Kinematic viscosity (viscosity/density) ($\text{cm}^2 \text{s}^{-1}$)
MR_i	Concentration of metal complexed to the competing ligand (mol L^{-1})	ω	Angular velocity (rad s^{-1})
K	Thermodynamic equilibrium constant (L mol^{-1})		

Abbreviation	Definition	Abbreviation	Definition
AdCSV	Adsorptive Cathodic Stripping Voltammetry	GFAAS	Graphite Furnace Atomic Absorption Spectrometry
ASV	Anodic Stripping Voltammetry	HA	Humic Acid
CLEM	Competing Ligand Exchange Method	HS	Humic Substances
DMG	Dimethylglyoxime	HEPES	N-2-Hydroxyethylpiperazine-N'-3-ethanesulfonic acid
DOC	Dissolved Organic Carbon	ICP-MS	Inductively-Coupled Plasma Mass-Spectrometry
DPASV	Differential Pulse Anodic Stripping Voltammetry	ICP-OES	Inductively-Coupled Plasma Optical Emission - Spectrometry
EDTA	Ethylenediaminetetraacetic acid	PIPES	Piperazine-N,N'-bis[2-ethanesulfonic acid
EN	Ethylenediamine	RDE	Rotating Disk Electrode
FA	Fulvic Acid	TMF	Thin Mercury Film
FIAM	Free Ion Activity Model	WHAM	Windermere Humic Aqueous Model