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**SPECIATION DYNAMICS IN THE FRESHWATER ENVIRONMENT:
UNIFYING CONCEPTS IN METAL SPECIATION AND
BIOAVAILABILITY**

by

John David Murimboh

B.Sc. (McMaster University)

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

Department of Chemistry

Carleton University

Ottawa, Ontario

October 2001

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acceptance of the thesis.

**“Speciation dynamics in the freshwater environment: unifying concepts in metal
speciation and bioavailability”**

submitted by

John David Murimboh

B.Sc. (McMaster University)

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

Chair, Department of Chemistry

Thesis Supervisor

External Examiner

2001, Carleton University

Dedicated to mummy and deddy

Abstract

The objective of this research was to achieve a better understanding of the chemical speciation and bioavailability in freshwaters. The Free Ion Activity Model (FIAM) has been held by its proponents to be a good indicator of metal bioavailability. However, many exceptions to FIAM have been reported. FIAM was accepted as a working hypothesis. Free metal ion activity (and hence, bioavailability) was found to be influenced by two kinetically-linked processes: 1) reaction kinetics in the bulk solution, and 2) transport kinetics at a consuming interface (e.g. an electrode or a cell membrane). Both the equilibria (stability constants) and kinetics (diffusion coefficients and association/dissociation rate coefficients) of metal complexes in model solutions of a well-characterized fulvic acid (Laurentian fulvic acid) and in freshwater samples from Sudbury, Ontario, were investigated. Anodic Stripping Voltammetry with a Rotating Disk Electrode was used to investigate the coupled diffusion of M and ML. Competing ligand exchange methods along with Adsorptive Cathodic Stripping Voltammetry or Graphite Furnace Atomic Absorption Spectrometry were used to investigate reaction kinetics in the bulk solution. The analytical timescale of measurement was identified as the key factor for defining metal species and predicting bioavailability. The timescale for an electrode or a biological membrane was related to the thickness of the Nernstian diffusion layer. Under conditions of excess ligand, such as those usually encountered in freshwaters, Pb(II)-, Cd(II)- and Zn(II)-fulvic acid complexes were found to be non-labile. The Differential Equilibrium Function (DEF) and the Differential Kinetic Function (DKF) were developed and applied to describe the influence of the heterogeneity of naturally-occurring complexants on the thermodynamic stability and kinetic lability of

metal complexes. The main advantage of the DEF and DKF is that they are independent of the experimental conditions. The chemostatic property of naturally-occurring complexants was found to be related to the cooperative action of the binding sites which enables them to buffer metals over a large concentration range with only gradual changes in free metal ion concentration. This effect probably helps organisms to adapt to large changes in the natural environment.

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Chapter 1 Introduction

1.1 Introduction

“The elucidation of the chemical speciation of trace elements in natural waters is probably the greatest challenge to analytical chemists; the objective is to demonstrate and quantify the existence of fractions of chemical constituents as picomolar concentrations of perhaps ephemeral species.” [1] I accepted the challenge, and thus began an exploration of the metal-complexation properties of freshwaters. This exploration led me to forge unity in the diversity by integrating a number of new concepts. My objective was to undertake a scientific investigation of the metal-complexation properties of freshwaters and to present an in-depth study of speciation dynamics in the freshwater environment, by formulating unifying concepts in metal speciation and bioavailability. The emphasis would be the development of predictive models to replace the existing empirical studies of chemical speciation and bioavailability.

1.2 Statement of the problem

Trace metals are natural components of the aquatic environment. Although many metals are essential nutrients for life at very low concentrations, they may be toxic (toxicants) at relatively high concentrations. Trace metals exist in a variety of forms: free metal ions, oxyhydroxides, metal-organic and organometallic complexes – each with its own unique properties. As persistent pollutants in the environment, environmental regulatory measures are based on total metal concentration. 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) has been well established in influencing the transport,

fate and bioavailability of metals in the aquatic environment [2]. An understanding of the metal distribution among different chemical forms (i.e. chemical speciation) is therefore essential for predicting the potential beneficial or adverse environmental impacts [3]. However, our understanding of metal speciation in natural systems is severely limited by the inherent complexity of naturally-occurring complexants. The impact assessment of elements in the natural environment is ultimately concerned with how these elements directly and indirectly affect biota. The overall **goal** of environmental speciation studies is to provide the information required for environmental impact assessment of elements by determining their distributions, properties and fluxes.

The concept of bioavailability has different interpretations in biology and chemistry. Whereas biology is concerned with the assimilation properties of the organism, in chemistry bioavailability is defined by the supplying potential of the medium [4]—the latter category is the definition adopted for this work.

1.3 Current state of knowledge

Metal speciation in freshwaters is largely controlled by natural organic matter (NOM). Naturally-occurring complexants, such as humic and fulvic acids, are ubiquitous in freshwaters. Unlike the simple homogeneous ligands (low molecular weight ligands with well-defined structures), humic substances are both chemically and physically heterogeneous [5-8]. Hence, naturally-occurring complexants **cannot** be isolated from environmental samples in a form that consists of a single molecular structure. Metal speciation in natural waters is complicated because the complexing agents are

heterogeneous, and possess the following unusual properties that make their metal-complexation reactions quite different from those that metals undergo with simple, well-defined (homogeneous) ligands such as citric acid.

Figure 1.1 Proposed structure of humic acid [1].

Polydispersity. Humic substances represent a variety of different molecules with a wide range of molecular masses.

Polyfunctional properties. Each macromolecule bears a large number and variety of complexing sites with different intrinsic binding properties. Examples include carboxylic, phenolic, salicylic and nitrogen-bearing functional groups. Even within a particular group of complexants, such as fulvics, the individual molecules are similar but not identical.

Oligoelectrolytic properties. Since most of the complexing sites have acid-base properties, they undergo dissociation at appropriate pHs, producing anionic moieties with negative charge. At a given temperature, the total charge depends on the pH and degree of site occupation by the metals [6,8-12], and on the ionic strength of the medium.

Conformational properties. The electric charge and degree of hydration of the macromolecules also influences their conformational properties, and for steric reasons, this may also affect the stability of metal complexes. The conformation depends on the hydration-dehydration process, the formation of hydrogen bonds (both intramolecular and intermolecular), the formation of electrostatic interactions among the various functional groups on a single molecule, and the formation of metallic bridges between neighbouring molecules; the formation of electrostatic interactions themselves depend on the degree of occupation of the complexing sites [6,13]. The relative importance of these properties varies with pH, ionic strength and the degree of occupation of the complexing sites.

A complete complexing system such as a soil is composed of several subsystems (e.g. organisms and nonliving components). Nonliving components include “simple” compounds and groups of homologous compounds (clays, humics, polysaccharides), which are themselves operationally defined. In Figure 1.2, the heterogeneity increases from right to left and is a basic property of naturally-occurring complexants [14].

Figure 1.2 Classification of naturally-occurring complexants according to their degree of heterogeneity.

Buffle et al. [8,15-17] and others [18] have developed a model for metal complexation in which the complexing sites of natural heterogeneous complexants consist of major and minor site types. The major site types are large in number (≈ 90 -99% of the total sites present), but small in chemical heterogeneity, and are weak Lewis bases. They can be considered to be chemically homogeneous. Examples of major sites include the carboxylate groups of polysaccharides, proteins and humic compounds, the phenolate groups of humic compounds and the $\equiv\text{Fe-OH}$ surface groups of iron oxyhydroxides [6,16,17]. By contrast, minor sites comprise a small fraction of the total sites (≈ 1 -10% of the total sites present), but a large number of the site types, with a broad range of associated complexation constants that span several orders of magnitude. They can be

considered to be chemically heterogeneous. Although minor sites comprise only a small fraction of the total number of sites, they are strong Lewis bases and may be the most important sites for controlling the extent of metal complexation in aquatic systems. Examples of minor sites include the CN^- and S^{2-} complexing functional groups of fulvic/humic acids, the $-\text{SH}$ groups of proteins, and the $\equiv\text{Mn}-\text{OH}$ groups of iron oxyhydroxides containing Mn impurities [6,16,17]. Two important consequences result: 1) L is poorly characterized and understood, 2) ML is a complex mixture of different metal complexes; it is often not possible to isolate ML as a single, pure compound.

1.3.1 Free Ion Activity Model (FIAM)

Exceeding the capacity of a cell to detoxify accumulated metals leads to damage of the cytoplasmic constituents. This is the toxic effect. Symptoms of intracellular toxicity include ultrastructural deformities, reductions in cell division rate, respiration, photosynthesis, mobility, electron transport activity and ATP production. Since only specific metal species can penetrate cell membranes, total metal concentrations cannot be used to predict metal toxicity. Two general pathways of metal assimilation by microorganisms have been suggested [19]. Although some complexes, such as diethyldithiocarbamate [20] and xanthates [21], may cross cell membranes directly due to their hydrophobicity and size, most metal complexes (e.g. large and hydrophilic complexes) must first dissociate prior to transfer into the cell by transporter ligands or protein channels [6].

Figure 1.3 Schematic representation of the reaction of a metal ion with different types of aquatic components [22].

Free metal ion activity has been proposed [23,24] as an indicator for metal uptake, nutrition and toxicity for organisms in the aquatic environment. The importance of the free metal ion in linking the reactions between the aquatic environment and living organisms is illustrated Figure 1.3. The Free Ion Activity Model (FIAM) does not imply

that the free metal ion (i.e. the metal aqua complex) 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 the physiological effects of a metal are mediated by chemical reactions between the metal and the various cellular ligands. Consequently, free metal ion activity 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 and regardless of the particular species taken up by the organism [23], provided that the following assumptions are satisfied [24].

1. The cell membrane is the primary site for the interaction of metals with living organisms.
2. The interaction between metals and the cell membrane can be described as a surface complexation reaction.
3. Metal transport in solution, towards the membrane, and the subsequent surface complexation reaction occur rapidly, such that a pseudo-first-order equilibrium is established between metal species in the bulk solution and those at the biological surface.
4. The biological response, whether it is metal uptake, nutrition, or toxicity, strictly depends on the concentration of metal complexed at the cell surface.
5. In the range of metal concentrations of toxicological interest, the concentration of free sites remains virtually constant and variations in the metal complexed at the cell surface follow changes in the free metal ion activities in the bulk solution.

6. During exposure to the metal of interest, the nature of the biological surface remains constant (i.e. the metal does not induce any changes in the nature of the cell membrane).

1.4 Weakness

Despite its successes, many exceptions to the Free Ion Activity Model (FIAM) have been reported [24]. A critical assumption of FIAM is equilibrium (or pseudoequilibrium) between the metal species in the bulk solution and the various sites on the surface of the biological membrane. This requires that the association and dissociation kinetics, and the transport of the metal species towards the biological membrane are 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). 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 satisfied.

Figure 1.4 Schematic diagram of metal assimilation by aquatic organisms [4].

1.5 Hypothesis

The **hypothesis** to be tested is that free metal ion availability (and hence, bioavailability) is influenced by two kinetically-linked processes: 1) the ability of the free metal ion, M, and a metal complex, ML, to maintain dynamic equilibrium (i.e. rapid association and dissociation kinetics) in the bulk solution, and 2) the ability of metal complexes to rapidly supply free-metal ions to a consuming interface, such as a biological membrane or electrode, by rapid association and dissociation kinetics, and rapid transport.

1.6 Theoretical Background

1.6.1 Reactions in the bulk solution (volume reactions)

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 association and dissociation rate coefficients, (k_a and k_d) are related to the stability constant, K , by the principle of microscopic reversibility [1].

$$K = \frac{c_{\text{ML}}}{c_{\text{M}}c_{\text{L}}} = \frac{k_a}{k_d} \quad (1.2)$$

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,

$$k'_a = k_a c_L \text{ and } K' = K c_L = c_{ML}/c_M \quad (1.3)$$

The metal species in reaction (1.1) have characteristic lifetimes related to their association and dissociation rate coefficients (e.g. $\tau = 1/k'_a$ and $\tau = 1/k_d$). 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.

$$k'_a \tau \text{ and } k_d \tau \gg 1 \quad (1.4)$$

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*.

$$k'_a \tau \text{ and } k_d \tau \ll 1 \quad (1.5)$$

Conditions (1.4) and (1.5) 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.

1.6.2 Coupled diffusion of M and ML in the diffusion layer (flux reactions)

However, even when the association and dissociation kinetics in the bulk solution are rapid (i.e. condition 1.4 is fulfilled), the system still may be limited by the supply of free metal ions to the interface.

In a strongly complexed system (i.e. $\epsilon K' \gg 1$, where $\epsilon = D_{ML}/D_M$ is the ratio of the diffusion coefficients of ML and M, respectively), the purely kinetic flux, J_{kin} , is [25]

$$J_{kin} = k_d c_{ML} \mu \quad (1.6)$$

where μ is the thickness of the **reaction layer** [26,27], 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.

$$\mu = (D_M/k'_a)^{1/2} \quad (1.7)$$

The purely diffusion-controlled flux, J_{diff} , is [25]

$$J_{diff} = D_{ML} c_{ML} / \delta \quad (1.8)$$

where δ is the thickness of the Nernstian 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 ($J_{kin}/J_{diff} \gg 1$). Combining equations (1.6), (1.7) and (1.8),

$$J_{kin}/J_{diff} = (k_d/\epsilon D_{ML} K')^{1/2} \delta_{ML} \gg 1 \quad (1.9)$$

The time, t , is related to thickness of the diffusion layer, δ , and the diffusion coefficient, D , by equation (1.10).

$$t = \delta^2 / D \quad (1.10)$$

The lability condition may be obtained by combining equations (1.9) and (1.10),

$$\Lambda t^{1/2} \gg 1 \quad (1.11)$$

where the lability parameter, Λ , is given by

$$\Lambda = \frac{k_d^{1/2}}{D_{ML}(\epsilon K')^{1/2}} t^{1/2} \quad (1.12)$$

Hence, *lability* (condition 1.11) corresponds to the limiting condition where the association 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 a diffusion layer thickness, δ .

Equation (1.12) was 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. [28,29].

$$\Lambda = \frac{k_d^{1/2}(\epsilon^{-1} + K')}{\epsilon^{-1} K' (1 + K')} \quad (1.13)$$

The *non-labile* case is defined by condition (1.14).

$$\Lambda t^{1/2} \ll 1 \quad (1.14)$$

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 (1.5), whereas non-labile systems obey conditions (1.4) and (1.14). A summary of the classification of kinetic regimes for metal complexes is presented in Figure (1.5).

Figure 1.5 Kinetic regimes for metal complexes defined by the timescale of measurement (adapted from van Leeuwen [30]).

The preceding discussion illustrates the importance of investigating the equilibria (e.g. thermodynamic stability constants) and kinetics (e.g. association/dissociation rate coefficients and diffusion coefficients) of metal complexation. Although the determination of stability constants, dissociation coefficients and diffusion coefficients is 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 humic and fulvic acids. 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 site occupation and distribution of binding energies of the binding sites.

1.6.3 The Differential Equilibrium Function

In the presence of heterogeneous complexants, the *apparent* equilibrium constant, $\bar{K}$, corresponds to an average over all the sites, L_i .

$$\bar{K} = \frac{\sum_i c_{ML_i}}{c_M \sum_i c_{L_i}} \quad (1.15)$$

where c represents the concentration of the different species, and $\bar{K}$ is an arithmetic mean of the true equilibrium constants, K_i , weighted by the corresponding free site concentrations.

$$\bar{K} = \frac{\sum_i K_i c_{L_i}}{\sum_i c_{L_i}} \quad (1.16)$$

The value of $\bar{K}$ varies with the degree of site occupation, θ . Hence, $\bar{K}$ decreases with increasing θ because $\bar{K}$ is averaged over an increasing number of weak sites.

Under conditions of a large excess of binding sites, L_i , relative to the concentration of metal complexes, ML_i (i.e. $\theta \ll 1$), the degree of site occupation has been found to follow a Freundlich isotherm [31].

$$\theta = Ac_M^\Gamma \quad (1.17)$$

where A is a constant and Γ is a measure of the heterogeneity of the system. Allowed values for Γ range between $0 < \Gamma \leq 1$, where $\Gamma = 1$ corresponds to the case for a simple, homogeneous complexant.

Equation (1.17) is analogous to the Differential Equilibrium Function (DEF) [15,32,33]. The DEF was first introduced by Gamble and Langford [32,33], and was further developed by Filella et al. [15] to describe the complexing properties of heterogeneous complexants, such as humic substances.

$$\log \theta = \Gamma \log K_o^* - \Gamma \log K^* \quad (1.18)$$

where K^* is the differential equilibrium parameter and K_o^* is a constant. K_o^* has no special significance other than the fact that it is the value of K^* when all the sites are fully occupied (i.e. when $\theta = 1$). Equation (1.18) suggests a log-log relationship between the degree of site occupation by the metals, θ , and the differential equilibrium parameter, K^* [15].

As shown in equation (1.19), K^* is a well-defined, weighted average of equilibrium constants, K_i , for all the sites in the system [34].

$$K^* = \frac{\sum_i K_i \theta_i (1 - \theta_i) c_{L_i}}{\sum_i \theta_i (1 - \theta_i) c_{L_i}} \quad (1.19)$$

where c_{L_i} is the concentration of ligand i in a mixture, and θ_i is the degree of occupation of each site ($\theta_i = c_{ML_i} / c_{L_i}$). K^* is analogous to a conditional equilibrium constant (i.e. valid at a given pH, temperature and ionic strength) [31]. The degree of occupation of each site determines the weighting factor, $\theta_i(1-\theta_i)$, in equation (1.19). Thus, K^* decreases as θ increases because K^* is averaged over an increasing number of weak sites (because the strongest sites are saturated first).

1.6.4 The Differential Kinetic Function

The differential equilibrium parameter, K^* , can be related to the association rate coefficient, k_a , and the differential kinetic parameter, k_d^* , by analogy with equation (1.2).

$$K^* = \frac{k_a}{k_d^*} \quad (1.20)$$

Combining equations (1.18) and (1.20) yields the Differential Kinetic Function (DKF).

$$\log \theta = \Gamma \log K_o^* - \Gamma \log k_a + \Gamma \log k_d^* \quad (1.21)$$

As shown in equation (1.21), the Differential Kinetic Function also represents a log-log relationship between the degree of site occupation by the metals, θ , and the differential kinetic parameter, k_d^* .

1.6.5 Complex formation

The observed rates of complex formation are generally consistent with the Eigen-Wilkins mechanism [1,35,36], in which formation of an outer-sphere complex between a metal and a ligand is followed by rate-limiting loss of water from the inner coordination sphere of the metal as shown in reactions (1.22) and (1.23).

The stability constant for the outer-sphere complex, K_{os} , primarily depends on the reacting species and the ionic strength of the medium. The rate coefficient for water loss, k_{-w} , is characteristic of the reacting metal. Water loss from the ligand is considered to be fast relative to the water loss from the metal and to precede formation of the outer-sphere complex. Hence, the rate of complex formation is independent of the type of ligand and depends only on the nature of M.

1.6.6 Techniques for metal speciation

The only analytical technique capable of **direct** (i.e. without pre-concentration) determination of free metal ion activity is ion selective electrode potentiometry [37]—all

other analytical techniques measure concentration, not activity. However, the limit of detection of ion-selective electrode potentiometry is typically 10^{-5} mol/L, which is insufficient for environmentally relevant concentrations. Traditional techniques for chemical speciation provide results that are **operationally-defined**. Examples include: “ASV-labile”, which is the amount of metal that is available by Anodic Stripping Voltammetry [38] and “Chelex-labile”, which is the amount of metal that can be taken up by Chelex 100 chelating resin. Another example is the operationally-defined fractions obtained by Sequential Solvent Extractions [39].

Table 1.1 Techniques for metal speciation (adapted from Campbell and Tessier [40]).

	Examples
Physical properties	
size	dialysis, ultrafiltration, gel permeation, chromatography
solubility	solvent extraction
electronic structure	electron spin resonance
Chemical reactivity	
electrochemical	anodic stripping voltammetry, adsorptive cathodic stripping voltammetry, potentiometry
ion exchange	cation-exchange resins (e.g. Chelex 100)
adsorption	macroreticular hydrophobic resins (e.g. XAD), glass-bonded organic phases (e.g. Sep-pak cartridges)
complexiometric determination	Chelation-extraction (e.g. oxine / MIBK), colorimetry

While the significance of all analytical results is necessarily 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. In a critique of operationally-defined sequential extraction methods, Nirel and Morel [41] argued that the “ultimate defence against all criticisms of Sequential Solvent Extractions is that the results are operationally-defined. To put it another way, what is measured is defined by what is measured, a safe if not illuminating tautology.” 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.

1.7 Outline of thesis

In this thesis, three independent concepts: reactions in the bulk solution (volume reactions), the coupled diffusion of M and ML in the diffusion layer, and the heterogeneous properties of natural systems are integrated in a **unified** approach to predicting metal bioavailability in the freshwater environment. The theory and main concepts of the thesis are introduced in Chapter 1. The organization of this thesis has been divided into two parts.

1.7.1 Electrochemical techniques to investigate the coupled diffusion of M and ML in the diffusion layer (flux reactions)

Electrochemical stripping techniques provide advantages of very low detection limits, extremely high analytical sensitivity and the ability to differentiate between different physicochemical forms of metals. Chapter 2 describes pseudopolarography (stripping polarography) to investigate the stability constants of metal complexes in heterogeneous systems, such as fulvic acid model solutions and freshwaters. The Differential Equilibrium Function (DEF) has been introduced to describe the influence of naturally-occurring heterogeneous complexants on changes in stability constants. Anodic Stripping Voltammetry (ASV) with a Rotating Disk Electrode has been evaluated for metal speciation in Chapter 3. Chapter 4 deals with the dissociation kinetics of metal complexes in heterogeneous systems, such as model solutions of a well-characterized fulvic acid, by Anodic Stripping Voltammetry with a Rotating Disk Electrode.

1.7.2 Kinetic studies in the bulk solution (volume reactions)

Chapter 5 presents the Competing Ligand Exchange Method (CLEM) using Chelex 100 as the competing ligand and Graphite Furnace Atomic Absorption Spectrometry to measure the dissociation kinetics of Cu(II), Pb(II) and Cd(II) complexes in model solutions of a well-characterized fulvic acid. Although CLEM is limited to kinetic studies in the bulk solution, it can be applied to investigate the speciation of virtually any element [42]. The Differential Kinetic Function has been used to describe changes in the dissociation rate coefficients with variations in the metal loading of the system. Chapter 6 presents a study of the kinetic speciation of Ni(II) using Adsorptive Cathodic Stripping

Voltammetry (AdCSV) and dimethylglyoxime as the competing ligand. In Chapter 6, some of the earlier concepts as well as the new concepts of adjunctive and disjunctive pathways in mechanistic studies have been applied to freshwaters.

1.7.3 Achievements and impact

The conclusions and major findings of this thesis are presented in Chapter 7. The key feature of the experimental approach of this thesis is the explicit consideration of the **timescale of measurement** to precisely define the metal species and interpret the results, which eliminates the operationally-defined nature of the measured signal. Furthermore, novel concepts such as the DEF and DKF have been integrated with each experimental technique to **predict** the influence of metal loading on the equilibrium and kinetic properties of natural systems. The overall impact of this work is that the concepts that have been developed in this thesis have provided the long-sought linkage between metal speciation and bioavailability in the freshwater environment.

1.8 Analytical considerations of Anodic Stripping Voltammetry

The main advantage of Anodic Stripping Voltammetry is the ability to investigate both thermodynamic and kinetic properties of metal complexes with a single technique. However, its application to freshwaters has been limited to only four metals: copper, lead, cadmium and zinc. This limitation is due to a combination of two factors. The first factor concerns the mercury electrode, in which the metals must be soluble in order to be determinable by ASV (copper, lead, cadmium and zinc are all soluble in mercury). The

practical higher limit of internal pre-concentration of the metal in the plating step of ASV is limited by the need to avoid longer periods (exceeding a few minutes) of plating in order to minimize adsorption of organic surfactants on the mercury electrode. The second factor concerns the analytical sensitivity of ASV for a particular metal *vis-à-vis* its typical concentration in unpolluted freshwaters. This consideration places a practical lower limit on the metal concentrations in freshwaters which can be determined by ASV. Only the above four metals have typical concentrations ($\approx 10^{-8}$ mol/L) in unpolluted freshwaters and high enough limits of determination by ASV to satisfy the second criterion (factor).

When a mercury drop is used as an electrode, the reduced metal diffuses into the mercury drop. In pulsed polarography when the anodic cycle returns for the re-oxidation of the amalgamated metal, the metal must diffuse from the interior of the mercury drop to the surface for re-oxidation (anodic stripping). However, the diffusion of the amalgamated metal from inside the mercury drop to its surface is a slow process, which limits the scan-rate to low frequencies, and hence, to slower data acquisition processes, militating against the great potential offered by high-frequency, pulsed voltammetry, such as Square Wave Voltammetry. In the last 15 years, such new possibilities of faster data acquisition have been realized by replacing the traditional hanging mercury drop with thin mercury film electrodes, which has eliminated the diffusional limitation of ASV and has greatly facilitated high frequency pulsed-techniques to be employed for faster data acquisition. This has allowed the full potential of Stripping Voltammetry to be exploited, as in Adsorptive Cathodic Stripping Voltammetry.

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Part 1:

***Flux Reactions – Electrochemical techniques
to investigate the coupled diffusion of M and
ML in the diffusion layer***

Chapter 2 Pseudopolarography and the Differential Equilibrium Function

2.1 Introduction

Interactions between transition metal ions and a wide and complex variety of organic ligands, inorganic anions, reducible and oxidizable chemical species, surfaces and organisms in natural water systems, play a significant role in the hydrogeochemical and biogeochemical cycle of these metals in the aquatic environment and are of great importance in understanding transport, toxicity and bioavailability of metal species in natural waters. Both metal speciation and bioavailability are functions of the tendency of the metal ion to react, as quantified by the free metal ion activity, with organic matter under pseudoequilibrium conditions [1]. Among the physical and chemical factors affecting the bioavailability of trace metals in the aquatic environment, complexation by natural organic matter (NOM) is important.

The main components of NOM in the aquatic environment are humic substances. Buffle [2] has reported that soil fulvic acids comprise up to 75% of the NOM content in rivers and streams. Humic and fulvic substances are highly complex mixtures of polyelectrolytes with a wide range of functional moieties and molecular weight distributions [3]. Binding of potentially toxic metals by humic and fulvic acids strongly affects their availability to living organisms [4].

Electrochemical stripping techniques provide advantages of very low detection limits, extremely high sensitivity and the ability to differentiate between different physicochemical forms of metals. The high sensitivity enables **direct** (i.e. without external preconcentration) determination of trace metals in natural waters with minimum

sample treatment so that the chemical equilibrium in the bulk solution is not perceptibly disturbed.

2.2 Theory

Reduction of the metal ion during pre-electrolysis at the mercury electrode surface lowers its concentration near the electrode surface, disturbing the equilibrium between the complex and its components, as shown in reaction (2.1), where the charges have been omitted for simplicity. The magnitude of the applied potential required to reduce M depends on the stability of ML.

As discussed in equation (1.2), the association and dissociation rate coefficients are coupled by the stability constant, K.

2.2.1 Pseudopolarography

Metal speciation studies analogous to those performed by polarography at a dropping mercury electrode can be carried out at much lower concentrations ($10^{-9} - 10^{-7}$ M) by pseudopolarography (stripping polarography) [5-7]. As shown in Figure 2.1, the method involves a series of Anodic Stripping Voltammetry (ASV) cycles that are plotted as a function of the deposition potential. The relationship between the stripping (oxidation)

current and the corresponding reduction potential for the metal of interest is an s-shaped, polarogram-like curve, known as a pseudopolarogram. It has been shown that for simple, labile complexes, the half-wave potential for the pseudo-polarographic curve is shifted to more negative potentials relative to that for the free metal ion, in a way that is analogous to classical dc-polarography [8,9]. Hence, stability constants for complexes can be calculated from pseudopolarographic data using methods developed for conventional dc-polarography. The advantages of pseudopolarography over polarography are its increased sensitivity and much lower limits of detection, which enable electrochemical investigations at environmentally relevant conditions.

The polarographic wave for a solution containing “simple” ligands is described by the De Ford and Hume equation [10,11].

$$E = E^{\circ} + \frac{RT}{nF} \ln \left[\frac{1}{\alpha} \left(\frac{D_R}{D_{ML}} \right)^{1/2} \right] + \frac{RT}{nF} \ln \left(\frac{1 - i/i_{lim}}{i/i_{lim}} \right) \quad (2.2)$$

where E° is the standard electrode potential (V) of the redox couple, R is the gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), T is the absolute temperature (K), n is the number of electrons exchanged, F is Faraday constant ($96,485 \text{ C mol}^{-1}$), α is the degree of complexation of a metal, D_R is the diffusion coefficient of reduced M in mercury, D_{ML} is the diffusion coefficient of ML_i , i_{lim} is the limiting current and i is the measured current.

Pseudopolarography has been applied to simple, reversible systems such as cadmium and lead chloride complexes [8,12-17], labile zinc complexes with inorganic ligands [18], lead carbonate in seawater [20], and cadmium with L-aspartic acid [19].

Figure 2.1 Pseudopolarography (a) a series of ASV cycles at different deposition potentials, (b) voltammograms corresponding to various deposition potentials, and (c) the resulting polarographic curve. [20]

Heterogeneous complexants have a continuous distribution of binding energies, which results in a spreading of the polarographic wave. With heterogeneous, organic complexants (such as fulvic acid) stronger sites become active in controlling the free metal ion's concentration as M is progressively depleted. Increasingly negative applied potentials are required to drive the dissociation of the remaining metal complexes, and the polarographic wave becomes asymmetrical, elongated and shifted towards negative potentials [24] as shown in Figure 2.2. An important consequence is that the Deford and Hume equation cannot be applied to determine conditional stability constants of metal complexes of heterogeneous complexants based on shifts in the half-wave potential.

Figure 2.2 Simulated polarographic curves for the reduction of M a) under non-complexing conditions, b) in the presence of a simple, homogeneous ligand (equation 2.2), $\Gamma = 1$, and c) in the presence of a heterogeneous complexant (equation 2.3), $\Gamma = 0.3$. [21]

An equation to describe the shape of a polarographic wave in the presence of heterogeneous complexants was derived by Filella et al. [24].

$$E = E_0 + \frac{RT}{nF} \ln \left[\frac{1}{A^{1/\Gamma} c_L} \left(\frac{c_M}{c_L} \right)^{(1-\Gamma)/\Gamma} \left(\frac{D_R}{D_{ML}} \right)^{1/2} \right] + \frac{RT}{nF} \ln \left[\frac{(1 - i/i_{lim})^{1/\Gamma}}{i/i_{lim}} \right] \quad (2.3)$$

where A is the Freundlich constant, c_M and c_L are the concentrations of M and L in the bulk solution, respectively, and all other terms are as defined previously.

The advantage of equation (2.3) is that the heterogeneous properties of the sample (i.e. Γ) is explicitly considered. Comparison of equations (2.2) and (2.3) reveals that the Deford and Hume equation is simply a special case of equation (2.3) for the condition of a homogeneous, simple complexant (i.e. $\Gamma = 1$). Although the concepts outlined above (equations 2.2 and 2.3) are based on conventional dc-polarography, they are completely analogous to those for Anodic Stripping Voltammetry provided that the same hydrodynamic conditions are used (i.e. semi-infinite linear diffusion) and that there are no secondary effects during the stripping step.

Using equation (2.3), the polarographic wave can be used to define the Differential Equilibrium Function (DEF) [22-24], and hence, the thermodynamic binding properties of naturally-occurring heterogeneous complexants such as humic and fulvic acids.

$$\log \theta = \Gamma \log K_o^* - \Gamma \log K^* \quad (1.18)$$

The relationship between the limiting current and the diffusion coefficient for a hanging mercury drop electrode is given by equation (2.4) [2].

$$i_{\text{lim}} = \frac{nFAD^{1/2}c_{\text{M,T}}\omega^{1/2}}{g} \quad (2.4)$$

where A is the electrode surface area, D is the weighted average diffusion coefficient, ω is the angular velocity (rad s^{-1}), $c_{\text{M,T}}$ is the total metal concentration in the bulk solution, g is a constant that depends on the cell geometry [2], and all other terms are as defined previously.

2.3 Experimental

2.3.1 Reagents and materials

Standard solutions (1000 $\mu\text{g/mL}$) of Zn(II), Cd(II), Pb(II) and Cu(II) were purchased from SCP Science. Ultrapure water of resistivity 18.2 $\text{M}\Omega\cdot\text{cm}$ was obtained direct from a Milli-Q-Plus water purification system (Millipore Corporation). A 2 M sodium acetate stock solution was prepared by dissolving an appropriate quantity of sodium acetate trihydrate (ACS grade/BDH) in ultrapure water. The sodium acetate solution was purified of metals by electrolysis at -1.5 V vs. E_{SCE} for at least 48 h. A 2 M stock solution of acetic acid was prepared by diluting glacial acetic acid (ACS grade/Anachemia) with ultrapure water. The Laurentian fulvic acid was obtained from Fredriks Research Products (Amsterdam, The Netherlands). The concentration of carboxylic and phenolic groups in the Laurentian fulvic acid is 11.6 mmol/g [25,26]. The stock solution of Laurentian fulvic acid was prepared by dissolving 1.0000 g of freeze-dried Laurentian fulvic acid into 1.00 L of ultrapure water. All test solutions were prepared by serial dilution of the above stock solutions with ultrapure water immediately prior to

determination unless otherwise specified. Ultrapure water ($18.2 \text{ M}\Omega\cdot\text{cm}$) was obtained direct from a Milli-Q Academic water purification system (Millipore Corporation, USA), fitted with an organic purification column to remove organic matter.

2.3.2 *Apparatus*

Voltammetric measurements were made with a computer-controlled Autolab PGSTAT30 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 (Metrohm, Switzerland). A Ag/AgCl electrode in a glass tube filled with 4 M KCl and fitted with a porous Vycor tip (Bioanalytical Systems, Inc., USA) was used as the reference electrode. The counter electrode was made of a platinum rod (Metrohm, Switzerland). Analysis of voltammetric peaks was done using the General Purpose Electrochemical Software v4.8 (Eco Chemie BV, The Netherlands). The data was transferred to a Pentium computer and saved for processing.

2.3.3 *Electrochemical parameters*

Each sample was purged with nitrogen gas for 10 min. prior to use. The electrode stirring rate was set at 2000 rpm. LSASV: deposition time 180 s, equilibration time 15 s, initial potential -1.2 V, end potential 0 V, step potential 2.5 mV. DPASV: deposition time 120 s, equilibration time 15 s, initial potential -1.2 V, end potential 0 V, step potential 2.5 mV, modulation amplitude 25 mV, modulation time 50 ms, interval time 50 ms. SWASV: deposition time 120 s, equilibration time 15 s, initial potential -1.2 V, end potential 0 V, step potential 2.5 mV, modulation amplitude 20 mV, frequency 75 Hz.

2.4 Results and Discussion

Typical pseudopolarograms for two solutions of Pb(II) are presented in Figure 2.3. The top curve is that for a solution of lead buffered in HOAc/NaOAc, which is defined as the pseudopolarogram for the Pb^{2+} aqua complex. A sharp, well-defined wave with a flat plateau was observed for the Pb^{2+} pseudopolarograms, indicating a fully reversible electrode process. The lower curve is the same concentration of Pb(II) equilibrated overnight in a model solution of Laurentian fulvic acid. Laurentian fulvic acid is a well-characterized fulvic acid that has been stoichiometrically defined for the total concentration of binding sites, 11.6 mmol/g [25,26]. Equation (2.4) indicates that the observed reduction in the limiting current of the Pb(II)-FA complexes relative to that for the Pb^{2+} aqua complex is a direct result of a decrease in the mean diffusion coefficient of the Pb(II)-FA complexes. In addition, the wave was elongated (broadened and flattened), reflecting a decrease in Γ . The spreading and flattening of the polarographic wave in the presence of heterogeneous complexants results from the fact that stronger sites become free and therefore active in controlling the free metal ion concentration as the total metal concentration is depleted at the electrode surface. The overall complexation energy therefore increases at the electrode surface relative to the bulk solution. This effect increases as the polarographic wave becomes more and more negative.

The parameters Γ , K_0^* and i_{lim} (and hence $\bar{D}_{\text{ML}}$) can be determined by fitting the experimental data to equation (2.3) [24]. The difficulty in applying equation (2.3) is that there is no closed-form solution for the measured current, i (the independent variable). Equation (2.5) has been proposed for the determination of Γ and i_{lim} [24].

$$\frac{1}{(d\chi_E/d\ln i) + 1} = \Gamma - \Gamma \frac{i_{lim}}{i} \quad (2.5)$$

where $\chi_E = nF(E - E^\circ)/RT$. However, numerous attempts to apply equation (2.11) using both real and simulated pseudopolarograms were unsuccessful. The experimental data was fitted to equation (2.3) using a commercial software program, NLREG v5.2 (Phillip H. Sherrod, Brentwood, TN, USA).

Figure 2.3 Typical pseudopolarogram for Pb(II)-FA in model solutions of Laurentian fulvic acid. $c_{Pb,T} = 7.87 \times 10^{-7}$ mol/L, ● no fulvic acid, ■ $c_{FA} = 63.6$ mmol/L, □ data omitted from the regression analysis. LSASV, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.

Table 2.1 Comparison of the experimental parameters ($E_{1/2}$, i_{lim} and Γ) for solutions of Zn^{2+} , Cd^{2+} and Pb^{2+} aqua complexes. $c_{M,T} = 7.87 \times 10^{-7}$ mol/L, LSASV, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ C$.

	$E_{1/2}$, V	i_{lim} , A	Γ
Zn aqua complex			
Filelal et al. (equation 2.3)	-1.02	3.29×10^{-8}	1.04
Deford and Hume (equation 2.2)	-1.02	3.29×10^{-8}	Not applicable
Cd aqua complex			
Filelal et al. (equation 2.3)	-0.602	1.27×10^{-8}	1.02
Deford and Hume (equation 2.2)	-0.602	1.28×10^{-8}	Not applicable
Pb aqua complex			
Filelal et al. (equation 2.3)	-0.420	4.23×10^{-8}	1.02
Deford and Hume (equation 2.2)	-0.420	4.23×10^{-8}	Not applicable

The solid lines in Figure 2.3 represent the experimental data that was fitted to equation (2.3). The open squares represent the experimental data that was not included in the regression analysis. Experimental data points at the baseline and at the plateau of the polarographic wave cause equation (2.3) to “blow up”. Consequently, the regression analyses typically rely on data points from the rising portion of the polarographic wave only. Since only a small section (the rising portion) of the polarographic wave is utilized in the regression analysis, it is important to ensure that a sufficient number of measurements (e.g. 10 – 15) are made in the rising section of the polarographic wave.

The effectiveness of applying equation (2.3) to a simple, homogeneous system is shown in Table 2.1, where the experimental parameters for solutions of Zn^{2+} , Cd^{2+} and Pb^{2+} aqua complexes were in good agreement with those obtained from the Deford and Hume equation, which has been rearranged as shown in equation (2.6). In addition, values of Γ obtained by applying equation (2.3) were close to the theoretical value of $\Gamma = 1$ expected for homogeneous systems such as metal aqua complexes.

$$i = \frac{i_{lim}}{1 + \frac{nF}{RT} \exp(E - E_{1/2})} \quad (2.6)$$

The constant, K_o^* , was determined from the shift of the polarographic wave relative to the pseudopolarogram of the metal aqua complex using equations (2.7) [10] and (2.8) [24].

$$\ln \alpha = \frac{nF}{RT} (E_{1/2} - E_{1/2}^L) + \ln \left(\frac{i_{lim}}{i_{lim}^L} \right) \quad (2.7)$$

where $E_{1/2}$ is the half-wave potential in the absence of ligand, $E_{1/2}^L$ is the half-wave potential in the presence of ligand, i_{lim} is the limiting current in the absence of ligand, i_{lim}^L is the limiting current in the presence of ligand, and α is related to K_o^* by equation (2.13) [24].

$$\alpha = c_L \frac{K_o^* \Gamma}{1 - \Gamma} \left(\frac{c_L}{c_M} \right)^{(1-\Gamma)/\Gamma} \quad (2.8)$$

The currents presented in Figures (2.3) – (2.7) and the limiting currents presented in Tables (2.2) – (2.6) were normalized so that the results from different days and stripping techniques could be compared. A comparison of the Differential Equilibrium Functions (equation 1.18) for Zn(II), Cd(II), Pb(II) and Cu(II) complexes in the model solutions of Laurentian fulvic acid (Table 2.2) shows that $\Gamma_{Zn} \cong \Gamma_{Cd} > \Gamma_{Pb} > \Gamma_{Cu}$, suggesting that Laurentian fulvic acid behaves as a relatively homogeneous complexant towards zinc and cadmium, whereas it behaves as a relatively heterogeneous complexant towards lead ($\Gamma_{Pb} = 0.44$) and copper ($\Gamma_{Cu} = 0.39$). The results are in agreement with those of Town and Filella [27], who have reported that Pb(II) complexes with Suwannee River fulvic acid (SRFA) are less heterogeneous than Cu(II)-SRFA complexes, and also with those of Pinheiro et al. [28], who reported that Pb(II) complexation by a peat-derived humic acid is more heterogeneous than the corresponding Cd(II) complexes.

Table 2.2 Differential Equilibrium Functions for Zn(II), Cd(II), Pb(II) and Cu(II) in model solutions of Laurentian fulvic acid. $c_{M,T} = 7.87 \times 10^{-7}$ mol/L, $c_{LFA} = 1.27 \times 10^{-4}$ mol/L, LSASV, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.

	Γ	$\log K_0^\circ$	i_{lim} (normalized)
Zn(II)	0.80	3.3	0.65
Cd(II)	0.99	2.4	0.71
Pb(II)	0.44	2.4	0.34
Cu(II)	0.39	1.4	0.31

Values for $\Gamma < 1$ reflect the range and change in the stabilities of different complexes as a function of the effective metal-to-ligand ratio at the electrode surface. Hence, the results suggest that bindings sites of Zn(II) and Cd(II) are thermodynamically similar, while those of Pb(II) and Cd(II) are thermodynamically more diverse. The results demonstrate the importance of the chosen probe metal in defining the physicochemical heterogeneity, Γ , of a system. Furthermore, the results stress the importance of reporting the metal used for the determination of Γ .

2.4.1 Influence of metal loading

Since the Differential Equilibrium Function explicitly accounts for the degree of site occupation by the metals, θ , it is relatively independent of the experimental conditions.

However, the determination of the experimental parameters by anodic stripping voltammetry, Γ and K_0^* , still may be influenced by the limitations of the technique used.

The effects of various metal loading conditions on the determination of Γ and K_0^* are presented in Tables 2.3 – 2.6 for Zn(II), Cd(II), Pb(II) and Cu(II), respectively.

Table 2.3 Differential Equilibrium Functions for Zn(II) in model solutions of Laurentian fulvic acid. LSASV, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.

$c_{\text{Zn,T}}/c_{\text{FA}}$	Γ	$\log K_0^*$	i_{lim} (normalized)	$\bar{D}_{\text{ML}}$ ($\text{m}^2 \text{s}^{-1}$)
1.35×10^{-2}	0.99	2.2	0.84	4.5×10^{-10}
6.77×10^{-2}	0.97	2.5	0.82	4.3×10^{-10}
1.13×10^{-3}	0.80	3.3	0.65	2.7×10^{-10}
7.64×10^{-4}	0.75	3.3	0.60	2.3×10^{-10}

Table 2.4 Differential Equilibrium Functions for Cd(II) in model solutions of Laurentian fulvic acid. LSASV, HOAc/NaOAc buffer, pH 5.0, I = 0.03 M, T = 23°C.

$c_{Cd,T}/c_{FA}$	Γ	$\log K_0^*$	i_{lim} (normalized)	$\bar{D}_{ML}$ ($m^2 s^{-1}$)
1.35×10^{-2}	0.99	2.1	0.96	6.4×10^{-10}
6.77×10^{-3}	0.99	1.9	0.83	4.8×10^{-10}
1.13×10^{-3}	0.99	2.4	0.71	3.5×10^{-10}
7.65×10^{-4}	0.95	2.8	0.85	5.0×10^{-10}

The results for Zn(II) in model solutions of Laurentian fulvic acid at various metal-to-ligand ratios are presented in Table 2.3. A decrease in the limiting current relative to the limiting current for the free metal ion was observed for all metal loading conditions, suggesting a decrease in the diffusion coefficient from the presence of labile Zn(II)-LFA complexes. At the high metal-to-fulvic acid ratios (i.e. $c_{M,T}/c_{FA} = 1.35 \times 10^{-2}$, 6.77×10^{-3}) the value of Γ was indistinguishable from that for a homogeneous system, whereas $\Gamma \approx 0.80$ was lower and independent of metal loading conditions at sufficiently low metal-to-fulvic acid ratios (i.e. $c_{M,T}/c_{FA} = 1.13 \times 10^{-3}$, 7.64×10^{-4}). The extremely high sensitivity of Anodic Stripping Voltammetry is based on *in-situ* preconcentration. Hence, an important requirement for applying equations (2.8) and (2.9) to ASV is that the ligand, L, must be present in large excess (1000-fold excess) relative to the total metal concentration [2] to ensure that L is not saturated at the electrode surface during the

stripping step. The relatively high values of Γ observed at high metal loading conditions probably results from the fact that the fulvic acid was not present in sufficient excess to satisfy the above condition.

It is interesting to note that the values of Γ for Cd(II) in model solutions of Laurentian fulvic acid were virtually indistinguishable from a homogeneous complexant ($\Gamma \approx 1$) for all metal loading conditions. The results suggest that Laurentian fulvic acid behaves as a relatively homogeneous complexant towards Cd(II). Cadmium(II) is known to form weak complexes. Consequently, the binding sites probably have similar (homogeneous) binding energies for Cd(II).

Table 2.5 Differential Equilibrium Functions for Pb(II) in model solutions of Laurentian fulvic acid. LSASV, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.

$c_{\text{Pb,T}}/c_{\text{FA}}$	Γ	$\log K_0^*$	i_{lim} (normalized)	$\bar{D}_{\text{ML}}$ ($\text{m}^2 \text{s}^{-1}$)
6.76×10^{-3}	0.81	3.2	0.52	2.2×10^{-10}
1.13×10^{-3}	0.44	2.4	0.34	9.6×10^{-11}
7.64×10^{-4}	0.54	2.2	0.40	1.3×10^{-10}

Table 2.6 Differential Equilibrium Functions for Cu(II) in model solutions of Laurentian fulvic acid. LSASV, HOAc/NaOAc buffer, pH 5.0, I = 0.03 M, T = 23°C.

$c_{\text{Cu,T}}/c_{\text{FA}}$	Γ	$\log K_0^*$	i_{lim} (normalized)	$\bar{D}_{\text{ML}}$ ($\text{m}^2 \text{s}^{-1}$)
6.77×10^{-3}	0.39	1.4	0.31	7.0×10^{-11}
1.13×10^{-3}	0.20	-2.6	0.19	2.7×10^{-11}
7.65×10^{-4}	Not applicable	Not applicable	Not applicable	Not applicable

The results for Pb(II) complexes in model solutions of Laurentian fulvic acid (Table 2.5) roughly paralleled those for Zn(II). Hence, the relatively high value of Γ for $c_{\text{Pb,T}}/c_{\text{FA}} = 6.76 \times 10^{-3}$ probably resulted from an insufficient excess complexant (Laurentian fulvic acid). The main difference is the lower value of Γ for Pb(II)-FA complexes ($\Gamma \approx 0.5$), which indicates that Laurentian fulvic acid is a heterogeneous complexant for Pb(II).

The results for Cu(II) in model solutions of Laurentian fulvic acid are presented in Table 2.6. The most important observation is the very low values of Γ for the Cu(II)-FA system. The negative value for $\log K_0^*$ is a direct result of the very low value of Γ . The results indicate that equations (2.7) and (2.8) cannot be applied and hence, the Differential Equilibrium Function cannot be defined for very heterogeneous systems (i.e. $\Gamma < 0.3$). Linear Scan Anodic Stripping Voltammetry was not sufficiently sensitive to detect $8.89 \times$

10^{-8} mol/L Cu(II) in the presence of Laurentian fulvic acid under the experimental conditions used.

At low metal concentrations ($c_{\text{Cu,T}}/c_{\text{FA}} = 7.65 \times 10^{-4}$), the Cu(II)-FA system was close to the limit of detection of the instrument and the pseudopolarograms suffered from a significant amount of scatter. This effect is reflected in the relatively low limiting currents observed for the Cu(II)-FA systems. Consequently, the results from this experiment are subject to a greater degree of uncertainty than the experiments conducted at higher metal concentrations.

In the presence of a sufficient excess of complexing ligand, L, the Γ values were independent of the metal-to-ligand ratio. The polarographic waves are asymmetrical and consequently shifts in the half-wave potential cannot be directly related to differences in stability constants as is the case for simple labile ligands. The site occupation by the metals at the electrode surface depends on the applied potential. Hence, the spreading of the polarographic wave is due to a change in the metal-to-ligand ratio in the diffusion layer and is specific for heterogeneous complexants. As a consequence, the lability of metal complexes may also vary along the polarographic wave.

2.4.2 *Influence of lability*

The exceptionally large reduction in the limiting currents of the Cu(II)-FA complexes presented in Table 2.6 is much greater than the effect expected from diffusion coefficients alone. A commonly used estimate for the kinetic behaviour of metal

complexes is the rate coefficient for water exchange, which is a measure of the simplest substitution reaction—exchange of coordinated water with bulk solvent (in the absence of other ligands). Although the very high rate coefficient for water exchange, $k_w = 1 \times 10^9 \text{ s}^{-1}$ for Cu^{2+} [29] suggests that Cu(II) should form complexes that are labile, recent work using competing ligand exchange techniques has shown that the relatively large ligand field stabilization energy and the Jahn-Teller effect results in Cu(II)-FA complexes and Cu(II) complexes in freshwaters that are inert [30]. Similar results were observed by Town and Filella [27] for Cu(II) in model solutions of Suwannee River fulvic acid.

The significant reduction in the limiting current and the significantly lower values of Γ for the Cu(II)-FA systems (Table 2.6 and Figure 2.4) relative to the Zn(II)-, Cd(II)- and Pb(II)-FA systems suggests a considerable loss in lability of the system, and indicate that there may be conditions in which the metal complex, ML, is not labile over the entire range of potentials. Under the conditions of large ligand excess, Cu(II) forms strong fulvic acid complexes that have a lower lability.

In natural systems, interpretation of the voltammetric signal is often further complicated by the loss of lability at some stage of the polarographic wave. As increasingly negative potentials are applied, the complexes become more stable and less labile until the polarographic wave attains a partially kinetic limiting current—the surface concentration of ML is still finite, but it does not decrease as increasingly negative potentials are applied because of the limitations imposed by the very low dissociation rate coefficients. Numerical simulations have been developed by Filella et al. [31] to investigate the

influence of non-labile complexes on the shape of the polarographic wave (Figure 2.5). Although, a detailed investigation of lability using the numerical methods of Filella et al. is beyond the scope of this work, the lability of metal complexes can be studied by varying the analytical timescale of measurement. In Anodic Stripping Voltammetry, in which a convective-diffusive mass transport regime is employed using forced convection, this may be achieved by varying the thickness of the diffusion layer.

Figure 2.4 Pseudopolarograms for Cu(II) in model solutions of Laurentian fulvic acid.

$c_{Pb,T} = 7.87 \times 10^{-7}$ mol/L, $\bullet$ no fulvic acid, $\square$ $c_{FA} = 12.7$ mmol/L. HOAc-NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$, LSASV.

Figure 2.5 Numerical simulations on the influence of lability on the shape of the polarographic wave) a labile system, and b) and c) non-labile systems. (Adapted from Filella et al. [24]).

Figure 2.6 shows the effect of the stirring rate on the shape of the pseudopolarograms. As will be shown in Chapter 4, the stirring rate directly affects the analytical timescale of measurement by influencing the thickness of the diffusion layer. The top curve is that for a solution of Pb(II) buffered in NaOAc/HOAc, which can be attributed to the Pb^{2+} aqua complex. Figure 2.6 shows how the limiting current decreases as the rotation rate is increased (and hence, the timescale of measurement is decreased). This suggests that the

complexes move from the labile state to the non-labile state as the thickness of the diffusion layer is decreased. The influence of lability on the shape and height of the polarographic wave shown in Figures 2.4 and 2.6 is in general agreement with the trend predicted by Fillela et al. [24] (Figure 2.5).

Figure 2.6 The influence of lability on the shape and height of the polarographic wave of Pb(II). SWASV, HOAc-NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$. • no fulvic acid, $\square$ 500 rpm, $\blacklozenge$ 1500 rpm, $\triangle$ 3000 rpm.

At the foot of the polarographic wave the interfacial kinetic flux of M that results from the dissociation of ML is much larger than the purely diffusion-controlled flux. Consequently, the metal complexes are labile and the deviation from the response of the fully labile system is negligible at the foot of the polarographic wave. However, as M is consumed at the electrode surface, concentration of bound metal (and consequently θ) decreases, resulting a progressive reduction in k_d . The deviation from the labile state becomes significant near the plateau of the wave. This effect is related to a loss of lability of the system, and increases as M is consumed at the electrode surface. The polarographic wave eventually approaches a current which has a partially kinetic nature in which the surface concentration is still finite, but it does not decrease with more negative potentials because the current is kinetically limited by the coupled diffusion of M and ML. The problem is that a loss in lability leads to a reduction in the limiting current and an elongation of the polarographic wave that is similar and difficult to distinguish from the effects due to **labile**, heterogeneous complexants (equation 2.3).

2.4.3 *Diffusion coefficients*

Metal complexes of naturally-occurring complexants are significantly larger than the metal aqua complex, and hence, have smaller diffusion coefficients. As a rough approximation, the metal complexes of individual molecules of a given group of heterogeneous complexants (e.g. fulvic compounds) are often assumed to have similar sizes and a single diffusion coefficient [31]. The experimentally determined diffusion coefficients are obtained by fitting the measured current to equation (2.4). The most significant observation was an approximately 50% reduction in the diffusion coefficients

relative to those of the Zn^{2+} , Cd^{2+} , Pb^{2+} and Cu^{2+} aqua ions ($D_{\text{Zn}} = 6.38 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, $D_{\text{Cd}} = 6.90 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, $D_{\text{Pb}} = 8.28 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ [32], and $D_{\text{Cu}} = 7.33 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ [33]). The large decrease in the diffusion coefficients suggests that Zn(II) , Cd(II) , Pb(II) and Cu(II) were bound as large, metal-fulvic acid complexes.

2.5 Conclusions

This work established the validity and applicability of the pseudopolarographic approach for the investigation of heterogeneous systems. An important factor for interpreting experimental data obtained by Anodic Stripping Voltammetry is the metal-to-ligand ratio at the surface of the electrode [34]. An advantage of pseudopolarography (stripping polarography) is that problems associated with changes in the individual peak potentials and peak widths due to the metal-to-ligand ratio at the electrode surface are avoided. This work illustrates the importance of accounting for the heterogeneity of naturally-occurring systems for interpreting metal speciation. The main advantage of this work is that the polarographic wave can be used to completely define the differential equilibrium function in a single experiment.

The Differential Equilibrium Function is a useful predictive tool to describe the thermodynamic binding properties that is independent of the experimental conditions. The main advantage of the Differential Equilibrium Function is that it can be applied to **predict** the stability constants for a wide range of metal binding conditions. When the spreading of the polarographic wave is only due to the presence of labile, heterogeneous

complexes, equation (2.9) can be used to describe the shape of the polarographic wave. Nevertheless, valid interpretation requires careful consideration of lability effects.

2.6 References

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**Chapter 3 A study of nafion-coated and uncoated thin mercury
film-rotating disk electrodes**

3.1 Introduction

A serious limitation of the voltammetric determination of trace metals in freshwaters is adsorption of organic, surface-active compounds [1-8], which are ubiquitous in freshwaters [9,10], onto the surface of the mercury electrode [11]. Adsorption of surface-active organic matter onto the electrode surface [12,13,14] arises from the presence of hydrophobic aromatic and aliphatic functional groups of natural organic matter [15], and the favourable gain in entropy that results from the displacement of a large number of ordered water molecules adjacent to the electrode interface [16]. Electrode contact time has a significant effect on adsorption [17,18]; however, adsorption is reported to be not greatly influenced by the nature of the measuring technique or the sample origin [18]. Adsorption is especially a concern in ASV [17], where the electrode contact time (electrolysis time) ≥ 60 s is much longer than in polarography (drop time) ≤ 10 s. Adsorption can affect the observed peak current (i_p) and shift the peak potential (E_p) toward more negative values [17]. One promising approach has been the use of chemically modified electrodes, such as the Nafion-coated Thin Mercury Film – Rotating Disk Electrode (TMF-RDE), to prevent adsorption of surface-active compounds onto the surface of the thin mercury film [1]. The Nafion coating has been reported to prevent the organic interferents from reaching the interface at which the electrolysis and stripping processes take place [19].

3.1.1 Nafion-coated thin mercury film electrodes

Nafion polymers are a family of non-cross-linked, perfluorosulphonate cation-exchange polymers that have been widely used to prevent electrode fouling [20,21] by organic,

surface-active compounds. They are chemically and thermally inert, non-electroactive, hydrophobic and insoluble in water. The polymers consist of hydrophobic, negatively charged ionic clusters, distributed throughout a backbone of tetrafluoroethylene at approximately 5.0 nm intervals [22]. The ionic clusters consist of negatively-charged sulphonate groups, their counter ions, sorbed water molecules and the polymer material. Hoyer *et al.* [19] reported that in a NCTMFE, the mercury film is most likely attached to the glassy carbon surface, and that the Nafion coating (200 nm thick) is considerably thicker than the mercury film (6.6 nm thick), suggesting that the mercury film is located well within the Nafion coating.

Nafion has been reported to have a size- and charge-dependent permselectivity for metal species [23-28] that results from the negatively-charged sulphonate groups of the polymer's pendant chain. The Nafion coating creates a second diffusion layer that the metal complexes must traverse with an apparent diffusion coefficient, D_{app} [29], before the metal is reduced at the electrode surface. However, the thickness of the Nernstian diffusion layer is approximately one hundred times that of the Nafion film [1], suggesting that the Nafion film is relatively thin and is located well within the Nernstian diffusion layer, as shown in Figure 3.1. Hence, its permselectivity is not effective since the metal complex (ML) is dissociated within the Nernstian diffusion layer and the concentration of the metal aqua complex decreases linearly from the outside boundary of the diffusion

layer to the mercury surface where it falls to zero (Figure 3.1) at the deposition potential of the analyte metal species.

Nafion contains negatively-charged sulphonate groups ($\text{pK}_a \approx 1$) [30]. At the pH ranges (pH 8 – 9) of samples, the sulphonate groups should be negatively charged, and therefore act as a cation-exchange polymer, giving non-faradaic preconcentration of cationic electroactive species [30,31]. This factor may lead to incorrect measurements of dissociation rate coefficients and diffusion coefficients of metal complexes, which rely on well-defined equations. However, conditional stability constants obtained by polarograms or pseudopolarograms do not rely on strictly-defined equations for the analyte transport. Hence, the measurement of conditional stability constants is expected to benefit from the advantages of a protective Nafion coating on the electrode surface.

Other advantages of Nafion-coated electrodes are enhanced sensitivity, which results from more efficient replating of metal ions during the stripping step, enhanced mechanical stability [19,32,33], and improved resistance to interference from the negatively charged, surface-active compounds. The above properties make Nafion polymers attractive for preparation of modified electrodes [19,34] for studies of metal speciation in freshwaters.

Figure 3.1 Concentration profile of ML at a Nafion-coated thin mercury film – rotating disk electrode.

3.1.2 Theory

Equations to describe the shape of the polarographic wave in the presence of heterogeneous complexants analogous to equations (2.2) and (2.3) in Chapter 2 may also be applied to laminar convective-diffusion (i.e. Anodic Stripping Voltammetry at a Rotating Disk Electrode).

Hence,

$$E = E_0 + \frac{RT}{nF} \ln \left[\frac{1}{\alpha} \left(\frac{D_R}{D_{ML}} \right)^{2/3} \right] + \frac{RT}{nF} \ln \left[\frac{(1 - i/i_{lim})^{1/\Gamma}}{i/i_{lim}} \right] \quad (3.1)$$

Similarly, the Levich equation [35] for Rotating Disk Electrode is:

$$i_{lim} = \frac{nFAD^{2/3}c_{M,T}\omega^{1/2}}{1.61\nu^{1/6}} \quad (3.2)$$

where the exponential factor 2/3 in equations (3.1) and (3.2) is for the case of laminar convective diffusion mode of mass transport.

3.2 Experimental

3.2.1 Reagents and materials

Standard solutions (1000 µg/mL) of Cd(II) and Pb(II) were purchased from SCP Science. A 2 M sodium acetate stock solution was prepared by dissolving an appropriate quantity of sodium acetate trihydrate (ACS grade/BDH) in ultrapure water. The sodium acetate solution was purified of metals by electrolysis at -1.5 V vs. E_{SCE} for at least 48 h. A 2 M

stock solution of acetic acid was prepared by diluting glacial acetic acid (ACS grade/Anachemia) with ultrapure water. The Laurentian fulvic acid was obtained from Fredriks Research Products (Amsterdam, The Netherlands). The concentration of carboxylic and phenolic groups in the Laurentian fulvic acid is 11.6 mmol/g [36,37]. The stock solution of Laurentian fulvic acid was prepared by dissolving 1.0000 g of freeze-dried Laurentian fulvic acid into 1.00 L of ultrapure water. All test solutions were prepared by serial dilution of the above stock solutions with ultrapure water immediately prior to determination unless otherwise specified. Ultrapure water (18.2 M Ω ·cm) was obtained direct from a Milli-Q Academic water purification system (Millipore Corporation, USA), fitted with an organic purification column to remove organic matter.

Nafion solutions were prepared from dilutions of a 5% (w/w) Nafion solution (Aldrich) with absolute ethanol (99%) to give a 0.5% (w/w) Nafion solution for direct application onto the electrode surface. A casting solvent of *N,N*-dimethylacetamide (DMAA) was used with the Nafion solution to form the Nafion coating.

3.2.2 Apparatus

Voltammetric measurements were made with a computer-controlled Autolab PGSTAT30 potentiostat/galvanostat (Eco Chemie BV, The Netherlands) equipped with a Metrohm 663 VA stand (Metrohm, Switzerland). The working electrode was a 2 mm diameter glassy carbon electrode surrounded by a PEEK jacket (Metrohm, Switzerland). A Ag/AgCl electrode in a glass tube filled with 4 M KCl and fitted with a porous Vycor tip (Bioanalytical Systems, Inc., USA) was used as the reference electrode. The counter

electrode was made of a platinum rod (Metrohm, Switzerland). Analysis of voltammetric peaks was done using the General Purpose Electrochemical Software v4.6 (Eco Chemie BV, The Netherlands). The data was transferred to a Pentium computer and saved for processing.

3.2.3 *Electrochemical parameters*

Each sample was purged with nitrogen gas for 10 min. prior to use. The electrode rotation rate was set at 2000 rpm. LSASV: deposition potential -1.2 V deposition time 180 s, equilibration time 15 s, step potential 2.5 mV. DPASV: deposition potential -1.2 V , deposition time 120 s, equilibration time 15 s, step potential 2.5 mV, modulation amplitude 25 mV, modulation time 50 ms, interval time 50 ms. SWASV: deposition potential -1.2 V , deposition time 120 s, equilibration time 15 s, step potential 2.5 mV, modulation amplitude 20 mV, frequency 75 Hz.

3.2.4 *Formation of the thin mercury film electrode*

The glassy carbon electrode was prepared by polishing with 3 μm monocrystalline diamond paste (DP-Paste, M, Struers) prior to use. The mercury film was formed on the rotating disc electrode (RDE) by applying a potential of -1.0 V for 15 min., rotating the RDE at 2000 rpm [38] in a plating solution containing 50 mL ultrapure water, 0.12 M HNO_3 and $\approx 5000\text{ }\mu\text{g/mL}$ Hg^{2+} that had been deoxygenated for 15 min. using pure nitrogen gas immediately prior to this operation. A 10 min. deposition period was used, followed by a 5 s quiescent period, after which the potential was ramped from -0.3 V to 0 V using a square-wave waveform (20 mV pulse height, 50 Hz frequency) to strip any

impurities from the TMFE. The plating solution was then removed and the TMFE visually inspected. Non-uniform films and films containing bubbles were wiped off and the TMFE reformed.

3.2.5 *Nafion coating*

NCTMFEs were formed using the method of Hoyer et al. [39]. The Nafion coating was applied to the bare glassy carbon electrode surface. 1 μL of the DMAA casting solution was deposited on the surface of the RDE, followed by 1 mL of 0.5% (w/w) Nafion solution, with a 10 μL Hamilton syringe. The electrode was rotated at 2000 rpm, then removed and covered with a test tube and heated with a heat gun. After the evaporation was complete, the Nafion coating was cured in an air stream at $\approx 90^\circ\text{C}$ for 1 min. The electrode was then air-cooled to room temperature and the mercury film was formed as described above.

3.3 *Results and Discussion*

3.3.1 *Thin Mercury Film-Rotating Disk Electrode*

Figure 3.2 shows the pseudopolarograms for Pb^{2+} spiked into a sample of ultrapure water, buffered with the acetic acid-sodium acetate buffer solution, with no FA present. A sharp, well-defined, polarographic wave with a flat plateau and $\Gamma = 1$ was observed for the pseudopolarograms obtained by LSASV, DPASV and SWASV; the observed polarographic waves can be attributed to the Pb^{2+} -aqua complex. The increase in limiting current ($i_{\text{lim,LSASV}} < i_{\text{lim,DPASV}} < i_{\text{lim,SWASV}}$) reflects the order of increasing sensitivity of DPASV and SWASV, respectively, over LSASV. Similar results were obtained for the

pseudopolarograms of Cd^{2+} spiked into a sample of ultrapure water, buffered with the acetic acid-sodium acetate buffer solution, with no fulvic acid present. The results indicate that the type of waveform used in the stripping step does not have a significant effect of the shape of the polarographic wave for the Pb^{2+} and Cd^{2+} aqua complexes, other than to enhance the analytical sensitivity.

Figure 3.3 and Table 3.1 show the results for Pb(II)-FA complexes in model solutions of Laurentian fulvic acid obtained using LSASV, DPASV and SWASV. The results for the Pb(II)-FA complexes indicate an enhancement in the normalized limiting currents ($i_{\text{lim,LSASV}} / i_0 < i_{\text{lim,DPASV}} / i_0 < i_{\text{lim,SWASV}} / i_0$). The results for Cd(II)-FA complexes in model solutions of Laurentian fulvic acid are presented in Figure 3.4 and Table 3.2. Little change was observed for the limiting currents of pseudopolarograms obtained by LSASV and DPASV, but a very large enhancement in the limiting current ($i_{\text{lim,SWASV}} / i_0 > 1$) was observed for the Cd(II)-FA pseudopolarogram obtained by SWASV (e.g. $i_{\text{lim,LSASV}} / i_0 \approx i_{\text{lim,DPASV}} / i_0 < i_{\text{lim,SWASV}} / i_0$). In ASV, the measured current can be affected by i) diffusion of M and ML towards the electrode surface, ii) the degree of lability of ML and iii) adsorption of organic surfactants onto the electrode surface. Because the thickness of the diffusion layer determines the time required by the diffusing species to cross the diffusion layer, the lability of ML is defined by the thickness of the diffusion layer [9,40]. All experiments were conducted at a rotation rate of 3000 rpm, which corresponds to a diffusion layer thickness of ≈ 8500 nm for the Pb^{2+} and Cd^{2+} aqua complexes. Since both the kinetic effects and the diffusional effects were kept constant by precise and reproducible control of the diffusion layer thickness (regardless of the waveform used in

the stripping step), the observed increase in the limiting currents (Tables 3.1 and 3.2) was probably due to adsorption. Induced adsorption of metal ions was demonstrated in systems containing adsorbing ligands such as fulvic and humic acids [41]. The mechanism of adsorption is complex and beyond the scope of this work. However, weak to intermediate adsorption has been reported to cause a maximum at the onset of the polarographic wave in normal pulse polarography and an increase in the peak height in differential pulse polarography [42].

Figure 3.2 Pseudopolarograms for lead in the absence of complexants using an uncoated TMF-RDE. $c_{Pb,T} = 4.83 \times 10^{-8}$ mol/L, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$. ■ SWASV, ◆ DPASV, ▼ LSASV.

The normalized currents presented in Figures 3.3 – 3.6 and the limiting currents presented in Tables 3.1 – 3.2 were normalized so that the results of the different stripping techniques and electrodes (e.g. TMF-RDE and Nafion coated TMF-RDE) could be compared.

Figure 3.3 Pseudopolarograms for Pb(II)-FA complexes in model solutions of Laurentian fulvic acid using an uncoated TMF-RDE. $c_{\text{Pb,T}} = 4.83 \times 10^{-8}$ mol/L, $c_{\text{FA}} = 9.41 \times 10^{-5}$ mol/L, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$. ● $c_{\text{Pb}} = 4.83 \times 10^{-8}$ mol/L without fulvic acid, ■ SWASV, ◆ DPASV, ▼ LSASV.

However, Tables 3.3 and 3.4 show that the differential equilibrium function (which is defined by Γ and K_o^*) is relatively independent of the type of waveform used in the stripping step. This suggests that the nature of the stripping waveform does not affect either the shape of the polarographic wave or the shift in the half-wave potential relative to that of the free metal ion. Since LSASV was the least susceptible to adsorption, the results suggest that it is probably the most suitable technique for speciation studies with a TMF-RDE in aqueous samples containing naturally-occurring complexants, such as fulvic acid.

Table 3.1 Average diffusion coefficients of Pb(II)-FA complexes in model solutions of Laurentian fulvic acid. $c_{\text{Pb,T}} = 4.83 \times 10^{-8}$ mol/L, $c_{\text{FA}} = 9.41 \times 10^{-5}$ mol/L, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.

	Uncoated		Nafion coated	
	i_{lim} (normalized)	$\bar{D}$ ($\text{cm}^2 \text{s}^{-1}$)	i_{lim} (normalized)	$\bar{D}$ ($\text{cm}^2 \text{s}^{-1}$)
SWASV	0.62	4.3×10^{-6}	0.60	4.0×10^{-6}
DPASV	0.28	1.3×10^{-6}	0.30	1.4×10^{-6}
LSASV	0.15	5.0×10^{-7}	0.13	4.1×10^{-7}

Table 3.2 Average diffusion coefficients of Cd(II)-FA complexes in model solutions of Laurentian fulvic acid. $c_{\text{Cd,T}} = 4.45 \times 10^{-8}$ mol/L, $c_{\text{FA}} = 9.41 \times 10^{-5}$ mol/L, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.

	Uncoated TMF-RDE		Nafion coated TMF-RDE	
	i_{lim} (normalized)	$\bar{D}$ ($\text{cm}^2 \text{s}^{-1}$)	i_{lim} (normalized)	$\bar{D}$ ($\text{cm}^2 \text{s}^{-1}$)
SWASV	1.3	Not applicable	1.3	Not applicable
DPASV	0.38	1.7×10^{-6}	0.51	2.6×10^{-6}
LSASV	0.44	2.1×10^{-5}	0.34	1.4×10^{-6}

Pulsed stripping techniques such as DPASV and SWASV have become the electrochemical techniques of choice due to their greatly enhanced analytical sensitivity. However, the observed enhancement of the (normalized) limiting currents has an important effect on the determination of diffusion coefficients of the metal complexes using the Levich equation (equation 3.2). Weighted average diffusion coefficients of Pb(II)-FA and Cd(II)-FA complexes are presented in Table 3.1 and 3.2, respectively, for the three different waveforms. The diffusion coefficients for the Pb^{2+} and Cd^{2+} aqua complexes reported in the literature [43] are $D_{\text{Pb}} = 8.67 \times 10^{-10} \text{ m}^2 \text{s}^{-1}$ and $D_{\text{Cd}} = 7.15 \times 10^{-10} \text{ m}^2 \text{s}^{-1}$, respectively. The results indicate that the weighted average diffusion coefficient of the Pb(II)-FA complexes may be overestimated by > 400% when SWASV is used. Furthermore, the results from SWASV give the improbable result that the diffusion coefficient of the Cd(II)-FA complexes is higher than the diffusion coefficient

of the free metal ion ($D_{ML} > D_M$), suggesting that SWASV is strongly susceptible to adsorption.

Although the pulse techniques are favoured for their enhanced sensitivity, the results presented in Tables 3.1 and 3.2 suggest that SWASV is very susceptible to adsorption, DPASV is moderately susceptible and LSASV is the least susceptible to adsorption of fulvic acid on the electrode surface. Multi-step pulsed techniques such as DPASV and SWASV are more complex than LSASV; hence, peak height (or peak area) cannot be assumed to have a direct relationship with the bulk concentration. Enhanced limiting currents (normalized) given by SWASV and DPASV probably result from secondary effects during the stripping step. Cleven et al. [44] have reported that adsorption impacts also may depend on scan rate and frequency. Other workers [45,46] have reported that normal pulse polarography is susceptible to adsorption.

3.3.2 *Nafion-Coated Thin Mercury Film-Rotating Disk Electrode*

The top curve in each of Figures 3.2 and 3.3 is that of Pb^{2+} spiked into a sample of buffered ultrapure water, with no fulvic acid present. A sharp, well-defined, polarographic wave with $\Gamma = 1$ was observed with both the Nafion-coated and the uncoated TMF-RDE; the wave can be attributed to the Pb^{2+} aqua complex. A comparison of the top curve in each Figure shows that the Nafion coating had no effect on the pseudopolarogram of the lead aqua complex, except for an increase in the limiting current, which was probably due to non-Faradaic preconcentration of Pb^{2+} by the ionic clusters of negatively-charged sulfonic acid groups within the Nafion coating [47].

Similar results were obtained for both the Pb^{2+} aqua complex and the Cd^{2+} aqua complex using all three waveforms.

Figure 3.4 Pseudopolarograms for Pb(II)-FA complexes in model solutions of Laurentian fulvic acid using a Nafion-coated TMF-RDE. $c_{\text{Pb,T}} = 4.83 \times 10^{-8}$ mol/L, $c_{\text{FA}} = 9.41 \times 10^{-5}$ mol/L, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$. ● $c_{\text{Pb}} = 4.83 \times 10^{-8}$ mol/L without fulvic acid, ■ SWASV, ◆ DPASV, ▼ LSASV.

Figure 3.5 Pseudopolarograms for Cd(II)-FA complexes in model solutions of Laurentian fulvic acid using an uncoated TMF-RDE. $c_{\text{Cd,T}} = 4.45 \times 10^{-8}$ mol/L, $c_{\text{FA}} = 9.41 \times 10^{-5}$ mol/L, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$. $\bullet$ $c_{\text{Cd}} = 4.45 \times 10^{-8}$ mol/L without fulvic acid, $\blacksquare$ SWASV, $\blacklozenge$ DPASV, $\blacktriangledown$ LSASV.

Table 3.3 Differential Equilibrium Functions for Pb(II)-FA complexes in model solutions of Laurentian fulvic acid. $c_{\text{Pb},T} = 4.83 \times 10^{-8}$ M, $c_{\text{FA}} = 9.41 \times 10^{-5}$ M, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.

	$\log \theta = \Gamma \log K_0^* - \Gamma \log K^*$	
	Uncoated TMF-RDE	Nafion coated TMF-RDE
SWASV	$\log \theta = 2.5 - 0.74 \log K^*$	$\log \theta = 2.7 - 0.73 \log K^*$
DPASV	$\log \theta = 2.6 - 0.68 \log K^*$	$\log \theta = 2.4 - 0.68 \log K^*$
LSASV	$\log \theta = 2.4 - 0.65 \log K^*$	$\log \theta = 3.4 - 0.73 \log K^*$

Figure 3.4 and Table 3.1 show the effect of LSASV, DPASV and SWASV using a Nafion-coated TMF-RDE for the Pb(II)-FA model solutions. As with the experiments conducted using the uncoated TMF-RDE, both kinetic effects and diffusional effects were kept constant by precise control of the diffusion layer thickness. Thus, changes in the normalized limiting current were probably due to adsorption of fulvic acid on the electrode surface. The increase in limiting current with the type of waveform used in the stripping step ($i_{\text{lim,LSASV}} / i_0 < i_{\text{lim,DPASV}} / i_0 < i_{\text{lim,SWASV}} / i_0$) suggests that SWASV is extremely susceptible, DPASV is moderately susceptible, and LSASV is the least susceptible to adsorption by fulvic acid, which agree with the results obtained using the uncoated TMF-RDE. The same trend was observed for Cd(II)-FA complexes in model solutions of Laurentian fulvic acid (Table 3.2). Weighted average diffusion coefficients for Pb(II) and Cd(II) complexes in the model solutions of Laurentian fulvic acid are

presented in Tables 3.1 and 3.2, which show that the diffusion coefficients can be overestimated by over one order of magnitude when SWASV is used. As was found previously in the case of the uncoated TMF-RDE, the weighted average diffusion coefficient of the Cd(II)-FA complexes obtained by SWASV was higher than that for the Cd²⁺ aqua complex, suggesting that SWASV is strongly affected by adsorption.

Table 3.4 Differential Equilibrium Functions for Cd(II)-FA complexes in model solutions of Laurentian fulvic acid. $c_{\text{Cd,T}} = 4.45 \times 10^{-8}$ M, $c_{\text{FA}} = 9.41 \times 10^{-5}$ M, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.

$\log \theta = \Gamma \log K_0^* - \Gamma \log K^*$		
	Uncoated TMF-RDE	Nafion coated TMF-RDE
SWASV	Not applicable	Not applicable
DPASV	$\log \theta = 3.2 - 0.93 \log K^*$	$\log \theta = 2.6 - 0.86 \log K^*$
LSASV	$\log \theta = 2.8 - 0.98 \log K^*$	$\log \theta = 3.2 - 0.96 \log K^*$

A comparison of the results obtained using the uncoated TMF-RDE and the Nafion-coated TMF-RDE show a significant increase in the limiting current when SWASV is used. This effect was probably due to non-faradaic preconcentration of the analyte at the electrode surface, which results from the presence of negatively-charged ionic (SO₃⁻) clusters within the Nafion coating. The results suggest that the Nafion coating greatly complicates interpretation of the analytical signal in the metal speciation studies. As a

result, the Nafion-coated TMF-RDE is not suitable for metal speciation studies when pulse techniques such as SWASV are used. However, Tables 3.1 and 3.2 show that the use of Nafion-coated TMF-RDE resulted in a slight reduction in the limiting current of both the Pb(II)-FA and Cd(II)-FA pseudopolarograms obtained by LSASV, suggesting that the Nafion-coated TMF-RDE may prevent adsorption of fulvic acid when LSASV is used.

Figure 3.6 Pseudopolarograms for Cd(II)-FA complexes in model solutions of Laurentian fulvic acid using a Nafion-coated TMF-RDE. HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$. ● $c_{Cd} = 4.45 \times 10^{-8}$ mol/L without fulvic acid, ■ SWASV, ◆ DPASV, ▼ LSASV.

3.3.3 Baseline effects

A positive shift was observed in the baselines of all pseudopolarograms obtained using a hanging mercury drop electrode as shown in Figures 2.3 and 2.4 in Chapter 2. As shown in Figure 2.6, the shift in the baseline increased significantly when pulsed techniques such as DPASV and SWASV were employed. The vast majority of the published literature neither provide any details about the method used to correct for the shift in the baseline, nor any explanation of the shift in the baseline. A few workers have inferred that the positive shift in the baseline is due to the kinetically slow adsorption of metal-FA complexes [48,49] on the surface of the mercury electrode during ASV measurement. However, Figures 3.2 – 3.6 clearly do not show any significant shift in the baseline in any of the pseudopolarograms (uncorrected for any shift in the baseline) obtained with a Thin Mercury Film – Rotating Disk Electrode, irrespective of the waveform used in the stripping step.

3.4 Conclusions

The Nafion-coated TMF-RDE was not particularly effective in preventing adsorption when pulsed techniques such as DPASV and SWASV were used; however, it may be effective in preventing adsorption when LSASV is used. Furthermore, the results suggested that although pulsed techniques such as DPASV and SWASV benefit from a significant increase in sensitivity, interpretation based on LSASV measurements is less complex and more straightforward.

Laurentian fulvic acid exhibits a wide range of complexation energies, which arises from its intrinsic chemical heterogeneity: polyfunctionality, polyelectrolytic character and conformational properties. It can therefore be expected to buffer metal ion concentrations over a wide range of concentrations and pH. In the event of a large increase in the total metal concentration due to environmental change, the free metal ion concentration will increase sharply as a simple ligand approaches saturation. However, since most naturally-occurring heterogeneous complexants possess similar complexation buffering properties, they can easily generate a "relay" action to buffer abrupt changes in the free metal ion concentration. Consequently, it has been proposed that humic substances play an essential role in the maintenance of life in aquatic systems [50] because they give the maximum possibility for all organisms to adapt to large perturbations of environmental conditions, leading to chemostasis. Existence of chemostasis is a necessary condition for the myriads of members (all life forms) of an aquatic ecosystem, interlocked by various feedback loops, in maintaining its homeostasis. Relatively unimportant humic substances thus play an important role in the homeostasis of an aquatic ecosystem!

3.5 References

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Chapter 4 The lability of chemically heterogeneous systems

4.1 Introduction

The distribution and behaviour (e.g. mobility and bioavailability) of metals in aquatic systems depends on the degree, and nature of their association with naturally-occurring complexants. The majority of complexing agents in natural waters are heterogeneous, polyfunctional macromolecules, such as the class of compounds known as humic substances. Since only certain metal species are bioavailable, an understanding of the metal distribution among different chemical forms (chemical speciation) is essential for predicting the potential beneficial or adverse environmental impacts [1].

The high analytical sensitivity of Anodic Stripping Voltammetry (ASV) with a Thin Mercury Film-Rotating Disk Electrode (TMF-RDE) allows for the determination of trace elements in natural waters with minimal sample treatment so that chemical equilibrium is not appreciably disturbed. "ASV labile" and "ASV non-labile" species [2] have sometimes been correlated with metal bioavailability and toxicity [3]. A critical study of the role of the size of macromolecular ligands and of dissociation kinetics of complexes in voltammetric techniques for complexation measurements in natural aquatic media has been presented by van Leeuwen et al. [4].

4.1.1 The nature of 'ASV-lability'

The application of Anodic Stripping Voltammetry to chemical speciation is based on the assumption that ASV can distinguish between *labile* and *non-labile* complexes. The use of the terms *labile* and *non-labile* in the context of ASV is unfortunate because 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 this sphere. *Lability* as a term applied to ASV has an **operational definition** related to the observed stripping current. Reduction of the metal ion during pre-electrolysis at the mercury electrode lowers its concentration near the electrode surface and disturbs the equilibrium between the metal complex and its components, as shown in reaction (2.1). A complex that dissociates rapidly enough to maintain equilibrium is called *labile*, and requires a rate of dissociation at least as rapid as the rate of mass transfer of the metal to the electrode surface. The measured current in ASV results from the reduction of metal ions supplied by dissociation of *labile* complexes. A non-labile complex is one that dissociates very slowly 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 [2], as shown in Figure 4.1. The timescale of the electrochemical measurement defines the metal species that can be measured and that can be called *labile* [2,4,5].

Figure 4.1 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.

4.1.2 The Rotating Disk Electrode

In Anodic Stripping Voltammetry, the analytical timescale of the electrochemical measurement is defined by the thickness of the diffusion layer [4,5]. Although an average diffusion layer thickness can be defined for a hanging mercury drop electrode [5], it is neither reproducible nor well-defined. A much better approach would be to use a

Rotating Disk Electrode (RDE). At high rotation rates, the electrode acts like a pump, transporting bulk solution towards the electrode surface by laminar convection. Close to the electrode surface the solution is thrown radially outwards from the disk, resulting in the formation of a thin, well-defined and reproducible mass transport regime immediately adjacent to the surface of the electrode, known as the Nernstian diffusion layer, in which diffusion is the form of mass transport. When the RDE is rotated at a high velocity, a **steady-state is rapidly** established, typically within 0.25 s [6]. The current is completely mass-transport controlled. It is a convective-diffusive system of mass transport. There is no contribution from charging the electrical double layer current. Therefore, under the **steady-state** condition, the recorded current may be unequivocally equated with the Faradaic current. The thickness of the diffusion layer can be precisely controlled by controlling the rotation rate of the electrode [7].

The RDE surface is uniformly accessible to all the ions in solution because the thickness of the Nernstian diffusion layer is the same over the entire disk surface. Regardless of the distance from the axis of rotation, the conditions for transport of material to any point on the disk surface are identical. This makes rotating disk electrode ideally suited for investigating kinetic reactions such as dissociation of metal 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 [2,8] using the method of Hale [9]. Shuman and Michael [2] 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. A TMF-RDE with Differential Pulse Anodic Stripping Voltammetry and Square Wave Anodic Stripping Voltammetry was used to determine dissociation rate coefficients and diffusion coefficients of metal complexes in samples of a river surface water [10], model solutions and snow [11,12]. However, the previous investigations were based on the assumption of equal diffusion coefficients for the free metal ion, M, and the complex, ML.

Figure 4.2 Schematic diagram of a Thin Mercury Film - Rotating Disk Electrode .

4.2 Theory

4.2.1 Anodic Stripping Voltammetry at a Rotating Disk Electrode

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 [13,14]. 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 association and dissociation rate coefficients, thus including the stability constant of the complex.

$$J(t) = D_M^{1/2} k_d^{1/2} c_{M,T} \frac{(\varepsilon^{-1} + K')^{3/2}}{K'(1 + K')} \exp(\Lambda^2 t) \operatorname{erfc}(\Lambda t^{1/2}) \quad (4.1)$$

where $c_{M,T} = c_M + c_{ML}$, $K' = Kc_L$, $\varepsilon = D_{ML}/D_M$ and Λ is the lability parameter as defined by equation (1.13) in Chapter 1.

In Anodic Stripping Voltammetry, the timescale of measurement is defined by the thickness of the Nernstian diffusion layer, δ . At a rotating disk electrode, the thickness of the diffusion layer, δ , is given by [7]:

$$\delta = 1.62 D^{1/3} \nu^{1/6} \omega^{-1/2} \quad (4.2)$$

where D is the mean diffusion coefficient, ω is the angular velocity (rad s^{-1}) and ν is the kinematic viscosity (viscosity/density). A typical value of ν for an aqueous solution is $10^{-6} \text{ m}^2 \text{ s}^{-1}$, which has been adopted for this work [11].

Combining equations (4.1) and (4.2) gives,

$$J(t) = D_M^{1/2} k_d^{1/2} c_{M,T} \frac{(\epsilon^{-1} + K')^{3/2}}{K'(1 + K')} \exp(\Lambda_{ss}^2 \delta^2) \operatorname{erfc}(\Lambda_{ss} \delta) \quad (4.3)$$

where Λ_{ss} is given by,

$$\Lambda_{ss} = \frac{k_d^{1/2} (1 + \epsilon K')}{D_M^{1/2} \epsilon K'} \quad (4.4)$$

Combining equation (4.4) and (1.10) for the relationship between time and the diffusion layer thickness, the lability condition given by equation (1.11) may be expressed equivalently as,

$$\Lambda_{ss} \delta \gg 1 \quad (4.5)$$

The limiting current is related to the flux by equation (4.6):

$$i_{lim} = nFAJ(t) \quad (4.6)$$

where n is the number of electrons involved in the electrode reaction, $c_{M,T}$ is the total concentration of metal in the bulk solution, F is the Faraday constant and A is the electrode surface area.

Combining equations (4.3) and (4.6) gives the limiting current at a rotating disk electrode.

$$i_{lim} = nFAD_M^{1/2} k_d^{1/2} c_{M,T} \frac{(\epsilon^{-1} + K')^{3/2}}{K'(1 + K')} \exp(\Lambda_{ss}^2 \delta^2) \operatorname{erfc}(\Lambda_{ss} \delta) \quad (4.7)$$

4.3 Experimental

4.3.1 Reagents and materials

Standard solutions (1000 $\mu\text{g/mL}$) of Zn(II), Cd(II) and Pb(II) were purchased from SCP Science. For formation of a Thin Mercury Film, a stock solution of 5000 $\mu\text{g/mL}$ $\text{Hg}(\text{NO}_3)_2$ was prepared by dissolving pure mercury in ultrapure nitric acid and diluting the solution with 1% (v/v) nitric acid. A stock solution of 2.0 M sodium acetate electrolyte solution was prepared by dissolving an appropriate quantity of sodium acetate trihydrate (ACS grade/BDH) in ultrapure water. This solution was then purified by electrolysis at -1.4 V vs. E_{SCE} for at least 110 h using a mercury-pool cathode to remove heavy metals. A stock solution of 2.0 M acetic acid was prepared by diluting ultrapure glacial acetic acid (Aristar grade) with ultrapure water. Ultrapure water of resistivity 18.2 $\text{M}\Omega\text{-cm}$ was obtained direct from a Milli-Q UF Plus water purification system (Millipore Corporation), fitted with a purifying column to remove organic matter. All test solutions (unless otherwise stated) were prepared by serial dilution of the above stock solutions with ultrapure water immediately prior to use.

4.3.2 Apparatus

Voltammetric measurements were carried out using the Bioanalytical Systems (BAS) 100B/W Electrochemical Analyzer, equipped with an RDE-1 rotating disk electrode (BAS), controlled by a 486 personal computer (Gateway 2000). The reference electrode was a Ag/AgCl electrode (BAS), filled with 3 M NaCl, and the counter electrode was a Pt wire (BAS). Analysis of voltammetric peaks was done using the BAS 100W windows

control software v2.3 (BAS). The data was transferred to a 486 computer and saved for processing.

4.3.3 Electrochemical parameters

Each sample was purged with nitrogen gas for 10 min. prior to use. The electrode rotation rate was set at 2000 rpm. LSASV: deposition time 180 s, equilibration time 15 s, step potential 2.5 mV. Deposition potential: Zn(II) -1.20V , Cd(II) -0.80V and Pb(II) -0.8V .

4.3.4 Formation of the thin mercury film

The glassy carbon electrode was prepared by polishing with 3 μm monocrystalline diamond paste (DP-Paste, M, Struers) prior to use. The mercury film was formed on the rotating disc electrode (RDE) by applying a potential of -1.0 V for 15 min., rotating the RDE at 2000 rpm [15] in a plating solution containing 50 mL ultrapure water, 0.12 M nitric acid and 5000 $\mu\text{g/mL}$ Hg^{2+} that had been deoxygenated for 15 min. using pure nitrogen gas immediately prior to this operation. A 10 min. deposition period was used, followed by a 5s quiescent period, 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 TMFE. The plating solution was then removed and the TMFE visually inspected. Non-uniform films and films containing bubbles were wiped off and the TMFE reformed.

4.4 Results and Discussion

Although pulsed techniques such as Differential Pulse and Square Wave Anodic Stripping Voltammetry are significantly more sensitive than Linear Scan Anodic Stripping Voltammetry, the peak height (and peak area) of pulsed techniques may be influenced by a number of factors such as adsorption and secondary effects during the stripping step as was shown in Chapters 2 and 3. Hence, all the experiments described in this Chapter were done using Linear Scan Anodic Stripping Voltammetry.

Figure 4.3 Modified Cottrell plot of Cd(II)-FA complexes in a model solution of Laurentian fulvic acid. HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.
● $c_{\text{Cd}} = 7.87 \times 10^{-7}$ mol/L with fulvic acid, □ $c_{\text{Cd,T}} = 7.87 \times 10^{-7}$ mol/L, $c_{\text{FA}} = 5.82 \times 10^{-4}$ mol/L. The solid lines represent data that was fitted to the Levich equation (equation 3.2).

Peak height is almost universally used as the measure of the amount of metal oxidized during the stripping step in Anodic Stripping Voltammetry. However, a broadening and flattening of the stripping peak in the presence of fulvic acids has been reported by Pinheiro et al. [16]. This effect has been attributed to changes in the effective metal-to-ligand ratio, and hence, the 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 [17].

Figure 4.4 Modified Cottrell plot of Cd(II)-FA complexes in a model solution of Laurentian fulvic acid. $C_{Cd,T} = 7.87 \times 10^{-7} \text{ mol/L}$, $C_{FA} = 5.82 \times 10^{-4} \text{ mol/L}$, HOAc/NaOAc buffer, pH 5.0, $I = 0.03 \text{ M}$, $T = 23^\circ\text{C}$. The solid line represents data that was fitted to equation (4.6).

Table 4.1 Diffusion coefficients and dissociation rate coefficients for Zn(II)-, Cd(II)- and Pb(II)-FA complexes in model solutions of Laurentian fulvic acid. HOAc/NaOAc buffer, pH 5.0, I = 0.03 M, T = 23°C.

$c_{M,T}$ (mol/L)	c_{FA} (mol/L)	$c_{M,T}/c_{FA}$	$\bar{D}$ (m ² /s)	k_d (s ⁻¹)
Zn(II)				
7.40×10^{-7}	5.82×10^{-4}	0.00127	3.3×10^{-10}	2.7×10^1
Cd(II)				
7.87×10^{-7}	5.82×10^{-4}	0.00135	3.6×10^{-10}	1.9×10^2
8.90×10^{-8}	1.16×10^{-4}	0.000765	3.3×10^{-10}	2.8×10^2
Pb(II)				
2.41×10^{-7}	1.16×10^{-4}	0.00207	3.9×10^{-10}	3.3×10^2
8.90×10^{-8}	1.16×10^{-4}	0.000765	2.4×10^{-10}	6.0×10^1

Typical results for the kinetic speciation in model solutions of Laurentian fulvic acid are presented in Figures 4.3 and 4.4 for Cd(II)-FA. Figures 4.3 and 4.4 are modified versions of the Cottrell plot, which are commonly used in pulse polarography to investigate electrochemical processes [18]. The graphs in Figures 4.3 and 4.4 have been adapted for Anodic Stripping Voltammetry, where $t^{-1/2}$ (s^{-1/2}) along the x-axis of a Cottrell plot has been replaced with its analogue for Anodic Stripping Voltammetry, the square root of the

angular velocity, $\omega^{1/2}$ ($s^{-1/2}$), which defines the thickness of the diffusion layer in, and hence, the analytical timescale of measurement in Anodic Stripping Voltammetry.

For labile complexes, the graphs should result in a straight line, passing through the origin. At extremely low rotation rates, when the diffusion layer becomes infinitely large and there is enough time for all complexes to dissociate, the curves approach the labile limit [13]. The deviation from linearity (i.e. curvature) at high rotation rates in Figures 4.3 and 4.4 probably results from a loss in lability as the thickness of the diffusion layer is decreased, suggesting that the Cd(II)-FA systems is *quasi-labile*. At very high rotation rates, the diffusion layer becomes extremely small, and there is no time for the complexes to dissociate and the system approaches the *static* (equation 1.5) limit.

An analysis of the lability of the Zn(II)-, Cd(II)- and Pb(II)-FA complexes is presented in Table 4.2. The data was fitted to equation (4.7) by non-linear regression analysis using the commercial software program NLREG v5.1 (Philip H. Sherrod). Following the criterion set by condition (1.11) the metal species are defined as labile if $\Lambda t^{1/2} \gg 1$, or equivalently $\Lambda_{ss}\delta \gg 1$ (equation 4.5). The results indicate that all the Cd(II)- and Pb(II)-FA systems are near the limit of lability ($\Lambda t^{1/2} \approx 1$, *quasi-labile*). The Zn(II)-FA system is non-labile ($\Lambda t^{1/2} < 1$). At lower metal loading conditions ($c_{M,T}/c_{FA} = 0.000765$), the Pb(II)-FA system also becomes *non-labile* ($\Lambda t^{1/2} = 0.28$). However, it is interesting to note that no change in lability was observed for the Cd(II)-FA complexes at lower metal loading conditions ($c_{M,T}/c_{FA} = 0.000765$), despite a significant reduction in the dissociation rate coefficient. The example for the Cd(II)-FA system highlights the

inherent distinction between *volume reactions* in the bulk solution and *flux reactions* at an interface. Dynamics is related to the rate of interconversion between M and ML in the bulk solution ($k_a t$ and $k_d t \gg 1$, dynamic), whereas lability is concerned with the rate of interconversion and the coupled diffusion of M and ML towards the interface ($\Lambda t^{1/2} \gg 1$, labile).

Table 4.2 Dynamic characteristics and labilities of metal complexes in model solutions of Laurentian fulvic acid. HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$, angular velocity 1000 rpm.

$c_{M,T}/c_{FA}$	K (mol^{-1}L)	$k_a^\dagger$ ($\text{M}^{-1}\text{s}^{-1}$)	k_d (s^{-1})	$\bar{\delta}^\ddagger$ (m)	t (s)	$\Lambda_{ss}\bar{\delta}$ ($\Lambda t^{1/2}$)
Zn(II)						
0.00127	2.0×10^5	8×10^6	2.7×10^1	1.1×10^{-5}	0.36	0.40
Cd(II)						
0.00135	8.1×10^4	3×10^7	2.8×10^2	1.1×10^{-5}	0.35	2.1
0.000765	1.9×10^5	3×10^7	1.9×10^2	1.1×10^{-5}	0.36	2.2
Pb(II)						
0.00207	1.6×10^6	8×10^8	3.3×10^2	1.2×10^{-5}	0.34	1.1
0.000765	8.8×10^6	8×10^8	6.0×10^1	9.9×10^{-6}	0.40	0.28

† calculated as 6/55 times the first-order rate coefficient for water exchange, k_w [19,20].

$^\ddagger \bar{\delta}$ calculated from equation (4.2) for an angular velocity of 1000 rpm.

Most natural complexants are significantly larger than the metal aqua complex, thus have smaller diffusion coefficients. As a rough approximation, the individual molecules of a given group of heterogeneous complexants (e.g. fulvic compounds) are often assumed to have similar sizes and a single diffusion coefficient [21]. However, heterogeneous complexants are usually polydisperse.

In Figure 4.3, a reduction in the slope is observed relative to that for the free metal ion. For *labile* complexes (i.e. complexes that obey condition 1.11, $\Lambda t^{1/2} \gg 1$), this may be attributed to a reduction in the diffusion coefficients of the metal-fulvic acid complexes. Diffusion coefficients for the Zn(II)-, Cd(II)- and Pb(II)-FA complexes were determined by fitting the experimental data to the Levich equation [7] for a rotating disk electrode (equation 3.2) by linear regression analysis. The most significant observation was an approximately 50% reduction in the diffusion coefficients relative to those of the Zn^{2+} , Cd^{2+} and Pb^{2+} aqua ions ($D_{\text{Zn}} = 6.38 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, $D_{\text{Cd}} = 6.90 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ and $D_{\text{Pb}} = 8.28 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ [6]). The large decrease in the diffusion coefficients suggests that Zn(II), Cd(II) and Pb(II) were bound as large, metal-fulvic acid complexes. For systems with a significant degree of *non-labile* behaviour, the diffusion coefficients were measured in the *labile* section of the curve (i.e. long t , short $\omega^{-1/2}$, when the thickness of the Nernstian diffusion layer is large and there is sufficient time for all the complexes to dissociate and diffuse towards the electrode surface), as shown in Figure 4.3. The measured diffusion coefficients shown in Table 4.1 are weighted averages of all metal species diffusing toward the electrode surface, as defined by equation (4.8) [22].

$$\bar{D} = \frac{D_M c_M + D_{ML} \sum_i c_{ML_i}}{c_M + \sum_i c_{ML_i}} \quad (4.8)$$

Hence, the mean diffusion layer thickness, $\bar{\delta}$, is defined jointly by M and ML as shown in Figure 4.5.

Figure 4.5 Concentration profile for a metal complex system under limiting conditions [4]. Dotted lines: M and ML are fully independent of each other (e.g. ML is an electroactive, fully inert complex) and diffuse separately, and 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, $\bar{\delta}$.

The influence of the diffusion layer thickness on the lability of the Pb(II)-FA system ($c_{M,T}/c_{FA} = 0.000765$) is highlighted in Figure 4.6, which shows how the Pb(II)-FA complexes move from the *labile* state to the *non-labile* state as the diffusion layer decreases and the thickness of the diffusion layer, and hence lability ($\Lambda_{ss}\delta$ or equivalently $\Lambda r^{1/2}$), decreases. Both the diffusion layer thickness and lability asymptotically approach 0 at infinitely short timescales, as predicted from equation (4.2).

Figure 4.6 ● Diffusion layer thickness, and □ lability ($\Lambda_{ss}\bar{\delta}$) as a function of the rotation rate of the electrode. $c_{Cd,T} = 7.87 \times 10^{-7}$ mol/L, $c_{FA} = 5.82 \times 10^{-4}$ mol/L, HOAc/NaOAc buffer, pH 5.0, $I = 0.03$ M, $T = 23^\circ\text{C}$.

4.5 Conclusions

The assumption of a very thin, well-defined and reproducible diffusion layer near the electrode surface was central to the development of the convective-diffusion hydrodynamic model of mass transfer to the rotating disk electrode, which were proposed independently by Levich [7] and Hale [9] for investigating the dissociation kinetics of metal complexes in well-stirred solutions. However, both approaches rely on the assumption of equal diffusion coefficients for M and ML. The kinetic approach presented in this chapter explicitly accounts for the *lability* of metal complexes and differences in the diffusion coefficients between M and ML. The work presented here highlights the importance of considering the influence of *lability* for interpreting voltammetric measurements. Furthermore, since the timescale of measurement determines the species in solution that will be measured by Anodic Stripping Voltammetry, the effective diffusion layer thickness should always be reported for the purpose of comparison with other work.

The main advantage of the rotating disk electrode is the unique ability to precisely control the thickness of the Nernstian diffusion layer, and hence, the analytical timescale of measurement. The rotating disk electrode achieves a steady state very rapidly (typically within 0.2 s [6]) because the electrode is continuously supplied with fresh electroactive species to within a fraction of a millimetre of the rotating disk by convection (and diffusion). Thus, the transport layer is almost as narrow as at a microelectrode. Despite the great disparity in size, the rotating disk electrode is voltammetrically similar to a

hemispherical microelectrode, and perhaps more importantly, the concentration gradient at the electrode surface is expected to be similar to that at the surface of biological membranes of microorganisms such as bacteria and algae.

By analogy with microelectrodes, microorganisms such as algae may be modelled as a microsphere, where [6]

$$\delta = l/r \quad (4.9)$$

where r is the radius of the microorganism (analogous to the radius of a spherical microelectrode).

For a fish, where the gill has been proposed as the active biological surface [23], the transport can be approximated as a laminar flow along a plane, where the plane is formed by the surface of the gill micro lamellae. According to Levich [24], the thickness of the diffusion layer, δ , is given by:

$$\delta = 3D^{1/3} \nu^{1/6} (y/\nu)^{1/2} \quad (4.10)$$

where D is the diffusion coefficient, ν is the kinematic viscosity of the medium, y is the distance from the entrance point of the fish gill, and v is the velocity of water flow over the gill surface.

When the cell surface is in equilibrium with the various metal species in the bulk solution (i.e. *labile* systems), then the identity of the metal species reacting with the cell surface is

of no biological significance because all of the metal species are equally bioavailable. Hence, for *labile* systems (i.e. equilibrium at the cell surface), free metal ion activity simply reflects the chemical reactivity of the metal, and hence, the extent of the metal's reactions with surface cellular sites (i.e. bioavailability). The results indicate that the Zn(II)-, Cd(II)- and Pb(II)-FA systems are near the limit of labile behaviour ($\Lambda_I^{1/2} \approx 1$)—at low metal-to-fulvic acid ratios, the Zn(II)- and Pb(II)-FA systems are *non-labile*. The main conclusion is that under conditions of excess ligand, such as those usually encountered in freshwaters, the Free Ion Activity Model may fail because the lability condition ($\Lambda_I^{1/2} < 1$, or equivalently $\Lambda_{ss}\delta < 1$) is not satisfied. Consequently, the free metal ion activity at the biological surface will be determined by the kinetics of the complexation reaction (condition 1.5) or the transport kinetics in the reaction layer outside the cell (condition 1.14) [25].

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Part 2:

***Volume Reactions – Kinetic studies in the
bulk solution***

Chapter 5 Kinetic studies and the Differential Kinetic Function

5.1 Introduction

The dominant tool for the analysis of metal ion speciation has been local the equilibrium (or pseudoequilibrium) approximation [1]. Complexation reactions in the bulk solution are assumed to be fast relative to other processes, and an equilibrium distribution among dissolved organic and inorganic species is assumed despite the fact that the total metal concentration may vary as a result of some controlling biogeochemical process. However, natural systems are not equilibrium systems. They are dynamic and often far-removed from equilibrium [1-5]; hence, equilibrium speciation methods may not apply [6]. Heterogeneous ligand systems, which are colloidal, polyelectrolyte ligands, can depart considerably in kinetic behaviour from simple ligands and can result in systems that are dynamic and far removed from equilibrium [1]. In some instances, the rates of biogeochemical reactions may be influenced, or even controlled by the rates of metal complexation reactions [5]. Hence, new kinetics-based chemical speciation methodologies are required to reflect more correctly the realities of freshwater systems, in which metal release is often kinetically controlled.

This chapter describes the application of a kinetic method for the speciation of metals. Adapting from the kinetic method proposed by Olson and Shuman [7], the following kinetic model has been developed [8,9] to investigate the dissociation kinetics of metal complexes, ML , where M is the metal aqua complex and L is a heterogeneous complexant, such as fulvic acid. The kinetic method has the advantage of providing an approximation to the kinetic behaviour of metal species in the natural environment

provided that the competing ligand does not produce extreme conditions that are entirely irrelevant to natural waters.

5.2 Theory

5.2.1 Competing Ligand Exchange Method [10,11]

Consider an aqueous mixture of n components, in which each component, ML_i , exists in equilibrium with its dissociation products, M and L_i (charges have been omitted for simplicity). The subscripted L_i represents different binding sites for the metal, M .

where k_a and k_d are the association and dissociation rate coefficients, respectively.

The sample is swamped by the addition of a large excess of a competing ligand, such as Chelex 100 chelating resin, resulting in the formation of the M-Chelex complex, and driving reaction (5.1) to the right.

Since the Chelex is added in large excess, reaction (5.2) is pseudo-first-order. If $K_{M-\text{Chelex}} \gg K_{ML}$ then M will be bound irreversibly to the Chelex and the net reaction represented by reaction (5.3) will lie far to the right.

If $k_{d, \text{Chelex}} \gg k_d$, then reaction (5.1) is the rate-determining step and the change in the concentration of ML_i can be described by a first-order rate law.

$$-\frac{dc_{\text{ML}_i}}{dt} = k_{d,i} c_{\text{ML}_i} \quad (5.4)$$

Integrating,

$$c_{\text{ML}_i}(t) = c_{\text{ML}_i}^{\circ} \cdot \exp(-k_{d,i} \cdot t) \quad (5.5)$$

where $k_{d,i}$ is the dissociation rate coefficient of the i^{th} ML complex, c_{ML}° is the initial concentration of ML_i complex and $c_{\text{ML}_i}(t)$ is the concentration of ML_i at any time, t .

If each complex, ML_i , dissociates simultaneously and independently, the total concentration of all complexes, c_{ML} , at any time, t , is given by a summation of exponentials (equation 5.6).

$$c_{\text{ML}}(t) = \sum_{i=1}^n c_{\text{ML}_i}^{\circ} \cdot \exp(-k_{d,i} \cdot t) \quad (5.6)$$

In the favourable case where the reactions can be described by equations (5.1) and (5.2), the rate coefficients measured for each component will be the dissociation rate coefficients of the corresponding complexing site.

5.2.2 *Disjunctive and adjunctive pathways*

Two reaction pathways for the overall ligand-exchange reactions may be considered [12]:

i) slow dissociation of ML to give M and L, followed by a fast reaction with the competing ligand, Chelex 100, which is called the disjunctive pathway (analogous to an S_N1 reaction), shown in reactions (5.1) and (5.2), and ii) direct attack by the competing ligand followed by loss of the original ligand, L, which is called the adjunctive pathway (analogous to an S_N2 reaction), shown in reactions (5.7) and (5.8):

The first-order rate coefficient of the disjunctive pathway is simply the rate coefficient for the slow step (reaction 5.1), dissociation of the metal complex, ML. Dissociation of ML is a fundamental process of the natural sample, and is the type of reaction for which the theory of ligand substitution processes can be reliably extrapolated over concentrations and pH [5]. If the complex-formation reaction is an elementary reaction, then because of detailed balancing, the measured dissociation rate coefficients are expected to be inversely related to the stability constants [12]. However, the observed rate coefficients for the adjunctive pathway are strongly influenced by the nature of the arbitrarily chosen probe ligand (e.g. steric and electrostatic factors, and protonation of the incoming ligand) since either formation or dissociation of the intermediate ternary

complex can be rate limiting. Consequently, very little relevant information can be inferred about the rates of processes in natural systems [13] from the adjunctive pathway. Both the disjunctive and adjunctive pathways may contribute to the observed, overall rate coefficient [14,15]. However, the experimental conditions may be chosen to favour one pathway over the other [14,15].

5.2.3 *The Differential Kinetic Function*

The influence of naturally-occurring heterogeneous complexants, such as fulvic acid, on the dissociation rate coefficients of the metal complexes will be studied by the Differential Kinetic Function (equation 1.21).

$$\log \theta = \Gamma \log K_o^* - \Gamma \log k_a + \Gamma \log k_d^* \quad (1.21)$$

5.3 *Experimental*

5.3.1 *Reagents and materials*

Standard solutions (1000 µg/mL) of Cd(II), Pb(II) and Cu(II) were purchased from SCP Science. A 2 M sodium acetate stock solution was prepared by dissolving an appropriate quantity of sodium acetate trihydrate (ACS grade/BDH) in ultrapure water. The sodium acetate (NaOAc) solution was purified of metals by electrolysis at -1.5 V vs. E_{SCE} for at least 48 h. A 2 M stock solution of acetic acid was prepared by diluting glacial acetic acid (HOAc) (Baseline, Seastar Chemicals, Vancouver, BC) with ultrapure water. The Laurentian FA was obtained from Fredriks Research Products (Amsterdam, The

Netherlands). The concentration of carboxylic and phenolic groups in the Laurentian fulvic acid is 11.6 mmol/g [16,17]. The stock solution of Laurentian FA was prepared by dissolving 1.0000 g of freeze-dried Laurentian FA into 1.00 L of ultrapure water. All test solutions were prepared by serial dilution of the above stock solutions with ultrapure water immediately prior to determination unless otherwise specified. Ultrapure water (18.2 M Ω -cm) was obtained direct from a Milli-Q Academic water purification system (Millipore Corporation, USA), fitted with an organic purification column to remove organic matter.

Chelex 100 resin (Bio-Rad, 100-200 mesh, sodium form) was equilibrated with a NaOAc-HOAc buffer at pH 5.0 ± 0.5 . The resin was kept in the NaOAc/HOAc buffer solution until the time of kinetic measurements because dryness of the resin affects the properties of the resin and causes changes in the pH of the sample during kinetic measurements. The Chelex 100 resin was taken out of the buffer solution and filtered just before use. The NaOAc-HOAc buffer was prepared as follows: 4.0 mL of 2.0 M sodium acetate trihydrate (BDH, Analar) (which had been purified of all metals by controlled-potential electrolysis for several days immediately prior to its use) was added to about 2 L of ultrapure water. The pH of the solution was adjusted to 5.0 ± 0.5 using ultrapure nitric acid (Baseline, Seastar Chemicals, Vancouver, BC).

5.3.2 Cleaning procedure

All containers used in this work were pre-cleaned by soaking in 10% HNO₃ (AR grade) for one week at room temperature, followed by a five-time rinse with ultrapure water. Finally, they were soaked in ultrapure water until they were used.

5.3.3 Fulvic acid model solutions

Model aqueous solutions were prepared by spiking ultrapure water with appropriate volumes of standard solutions of the metals and of the Laurentian fulvic acid solution (1.0219 g / L). The pH of all the model solutions was measured using a Fisher Scientific Accumet 925 pH / ion meter and adjusted to 5.0 ± 0.5 with dilute ultrapure nitric acid (Baseline, Seastar Chemicals, Vancouver, BC) or dilute sodium hydroxide (electrolytically pure) solutions. The changes in the pH between the beginning and the end of the experiment were found to be < 0.3 pH units.

5.3.4 Apparatus

Perkin-Elmer 4100ZL Zeeman atomic absorption spectrophotometer equipped with an AS-40 autosampler, an HGA-600 graphite furnace, and pyrolytically-coated graphite tubes (Perkin-Elmer) with integrated platforms were used. Table 5.1 shows the instrumental parameters used in the graphite furnace atomic absorption spectrometric determination of the metals: lead, copper and cadmium. For the determination of the metal concentrations, 20 μ L of the solution was injected into the graphite furnace, where it was dried, ashed and atomized. The signal was measured in the peak area mode. Each completed determination was followed by a 4 s clean-up cycle of the graphite tube at

2600°C. During the drying, ashing and clean-up cycles, the internal argon gas was passed through the furnace at 300 mL/min. The internal argon gas flow was interrupted during the atomization step, but was restored for the duration of the clean-up cycle.

Screw-cap Teflon cylindrical bottles (500 mL capacity) were machined to fabricate Reactors for the test solutions for the kinetic studies. Teflon bottles of 1 L and 2 L capacity were used as sample storage containers.

Table 5.1 Analysis lines and instrumental conditions for the Perkin-Elmer 4100ZL GFAAS.

Element	Wavelength	Ashing Temperature	Atomization	
	(nm)	(°C)	Temperature (°C)	Time (s)
Cd	326.1	300	1800	3
Pb	217.0	600	1700	3
Cu	324.7	1200	1900	5

5.3.5 Kinetic experiments

The dissociation kinetics was measured by GFAAS using Chelex 100 chelating resin as the competing ligand. Chelex 100 was added to a 300 mL sample solution placed in the Teflon Reactor, and the mixture was stirred with a Teflon-coated magnetic stirring bar. Test solutions for analysis were sampled from the Teflon Reactor through a nylon

membrane filter (placed at the end of the Reactor tube), which filtered the Chelex 100 resin and injected a clear filtrate into the graphite furnace. The initial time for the kinetic measurement was taken as the time at which the Chelex 100 resin first came in contact with the aqueous sample in the Teflon Reactor. The sum of the concentrations of all the kinetically distinguishable metal species remaining in the test solution, $c_{ML}(t)$, was measured as a function of time.

Figure 5.1 Experimental set-up for kinetic experiments.

5.4 Results and Discussion

The results for Pb(II), Cu(II) and Cd(II) in model solutions of Laurentian fulvic acid are presented in Figures 5.2, 5.3 and 5.4, respectively. The steeply falling section at the beginning of each curve can be attributed to the presence of the metal aqua complex and/or the rapid dissociation of weak inorganic and/or organic metal complexes that are *dynamic*. The slowly falling section of each curve is probably due to the slow dissociation of strong metal complexes. Hence, the fraction of metal complexes remaining at the end of each experiment represents the fraction of complexes that were not able to dissociate within the time of measurement. For these complexes, the assumption that $K_{M-Chelex} \gg K_{ML}$ is probably not valid.

The elongate nature of the curves in Figures 5.2, 5.3 and 5.4 suggest the presence of more than one kinetically distinguishable component, since none of the metal-FA systems appear to obey the pure, 1-component exponential decay model described by equation (5.5). The solid lines in Figures 5.2 – 5.4 represent data that was fitted to the kinetic model (equation 5.6) by non-linear regression analysis. The results for Pb(II), Cu(II) and Cd(II) in model solutions of Laurentian fulvic acid are presented in Tables 5.2 – 5.4, respectively. Equations (5.1) – (5.6) represent reactions in the bulk solution (volume reactions). Hence, equations (1.4) and (1.5) show that, under the experimental measurement time ($t = 2$ h), complexes with $k_d \gg 10^{-4} \text{ s}^{-1}$ have been defined as *dynamic* ($k_d t \gg 1$, condition 1.4) and complexes with $k_d \ll 10^{-4} \text{ s}^{-1}$ have been defined as *inert* ($k_d t \ll 1$, condition 1.5).

Figure 5.2 Dissociation kinetics of Pb(II)-FA complexes in model solutions of Laurentian fulvic acid using Chelex 100 as the competing ligand and graphite furnace atomic absorption spectrometry to measure the dissociation kinetics. $c_{Pb,T} = 7.24 \times 10^{-7}$ mol/L, pH 5.0, $T = 23^\circ\text{C}$. ● $c_{Pb,T}/c_{LFA} = 0.0427$; ■ $c_{Pb,T}/c_{LFA} = 0.00427$; ◆ $c_{Pb,T}/c_{LFA} = 0.000427$.

Two kinetically distinguishable components are shown in Tables 5.2, 5.3 and 5.4: *dynamic* complexes ($k_d \approx 10^{-2} \text{ s}^{-1}$) that dissociate rapidly and *inert* complexes ($k_d \approx 10^{-4} - 10^{-6} \text{ s}^{-1}$) that dissociate slowly relative to the analytical timescale of measurement (equations 1.4 and 1.5). Unfortunately, in the case of heterogeneous complexants, such as fulvic acid, there is no way to study the individual binding sites separately. The problem

lies in attempting to fit the kinetic data to a set of rate coefficient, k_i , and concentration, $c_{ML,i}^0$, parameters without any *a priori* knowledge about the number of sites. Since equation (5.6) is non-linear, this is a difficult task. Furthermore, the actual binding sites may be a nearly continuous distribution [18]. Hence, the measured rate coefficients do not necessarily imply the presence of only two distinct metal complexes. They only represent the **minimum** number of components require to describe the metal-FA systems. The metal complexes probably exist as distributions of individual metal complexes [19,20], but they group into recognizable individual sets which may be approximated by a small set of rate coefficients.

Tables 5.2 – 5.4 all show a decrease in the concentration of the *dynamic* component and an increase in the concentration of the *inert* component, suggesting that here is a relationship between the thermodynamic stability of metal complexes and their kinetic inertness (equation 1.2). The Pb(II)-, Cd(II)- and the Cu(II)-FA systems all show a reasonable trend towards increasing *static* behaviour with the decreasing metal-to-fulvic acid ratio, supporting the conclusion that the kinetic speciation model (equations 5.1 – 5.6) has chemical significance.

Figure 5.3 Dissociation kinetics of Cu(II)-FA complexes in model solutions of Laurentian fulvic acid using Chelex 100 as the competing ligand and graphite furnace atomic absorption spectrometry to measure the dissociation kinetics. $c_{Cu,T} = 7.24 \times 10^{-7}$ mol/L, pH 5.0, $T = 23^{\circ}\text{C}$. ● $c_{Cu,T}/c_{LFA} = 0.0467$; ■ $c_{Cu,T}/c_{LFA} = 0.00467$; ◆ $c_{Cu,T}/c_{LFA} = 0.000467$.

Figure 5.4 Dissociation kinetics of Cd(II)-FA complexes in model solutions of Laurentian fulvic acid using Chelex 100 as the competing ligand and graphite furnace atomic absorption spectrometry to measure the dissociation kinetics. $c_{Cd,T} = 7.24 \times 10^{-7}$ mol/L, pH 5.0, $T = 23^\circ\text{C}$. ● $c_{Cd,T}/c_{LFA} = 0.0467$; ■ $c_{Cd,T}/c_{LFA} = 0.00467$; ◆ $c_{Cd,T}/c_{LFA} = 0.000467$.

Table 5.2 Kinetically distinguishable components of Pb(II)-FA complexes in model solutions of Laurentian fulvic acid measured by graphite furnace atomic absorption spectrometry. $c_{\text{Pb,T}} = 7.24 \times 10^{-7}$ mol/L, pH 5.0, T = 23°C.

$c_{\text{Pb,T}}/c_{\text{LFA}}$	$C_1, \%$	$k_1 \times 10^2, \text{s}^{-1}$	$C_2, \%$	$k_2 \times 10^5, \text{s}^{-1}$
0.427	86 ± 5	3.4 ± 0.6	13 ± 3	66 ± 34
0.0427	39 ± 8	2.3 ± 1.5	59 ± 3	8.5 ± 2.0
0.00427	12 ± 2	0.43 ± 0.15	88 ± 2	1.2 ± 0.4

k_1 , and k_2 are the dissociation rate coefficients of the rapidly dissociating and the slowly dissociating components, respectively. % C_1 , and % C_2 , are the relative concentrations of the rapidly dissociating and the slowly dissociating components, respectively. The uncertainty represents the 95% confidence interval from the non-linear regression analysis.

Table 5.3 Kinetically distinguishable components of Cu(II)-FA complexes in model solutions of Laurentian fulvic acid measured by graphite furnace atomic absorption spectrometry. $c_{\text{Cu,T}} = 7.24 \times 10^{-7}$ mol/L, pH 5.0, T = 23°C.

$c_{\text{Cu,T}}/c_{\text{LFA}}$	$C_1, \%$	$k_1 \times 10^2, \text{s}^{-1}$	$C_2, \%$	$k_2 \times 10^5, \text{s}^{-1}$
0.467	81.6 ± 6	4.1 ± 0.8	18.4 ± 3	18 ± 6
0.0467	41.5 ± 6	5.3 ± 2.1	58.5 ± 2	8.5 ± 1.3
0.00467	15.3 ± 3	0.62 ± 0.25	84.7 ± 2	5.9 ± 4.9

k_1 , and k_2 are the dissociation rate coefficients of the rapidly dissociating and the slowly dissociating components, respectively. % C_1 , and % C_2 , are the relative concentrations of the rapidly dissociating and the slowly dissociating components, respectively. The uncertainty represents the 95% confidence interval from the non-linear regression analysis.

Although the inert component in Tables 5.2 – 5.4 has been attributed to the very slow dissociation of metal bound to the strong sites of the fulvic acid, another factor that should be considered is that the kinetics may also be limited by the gel structure of humic substances, such as fulvic acid, which are large porous gels. Diffusion through the gel is a slow process that can be kinetically limit both the association and dissociation kinetics of reactions (5.1) – (5.3)

Table 5.4 Kinetically distinguishable components of Cd(II)-FA complexes in model solutions of Laurentian fulvic acid measured by graphite furnace atomic absorption spectrometry. $c_{\text{Cd},T} = 7.24 \times 10^{-7}$ mol/L, pH 5.0, $T = 23^\circ\text{C}$.

$c_{\text{Cd},T}/c_{\text{LFA}}$	$C_1, \%$	$k_1 \times 10^2, \text{s}^{-1}$	$C_2, \%$	$k_2 \times 10^5, \text{s}^{-1}$
0.093	69 ± 12	5.1 ± 2.6	30 ± 5	31 ± 13
0.019	30 ± 10	0.54 ± 0.38	61 ± 8	15 ± 1
0.0047	14 ± 6	0.79 ± 1.43	89 ± 3	13 ± 1

k_1 , and k_2 are the dissociation rate coefficients of the rapidly dissociating and the slowly dissociating components, respectively. % C_1 , and % C_2 , are the relative concentrations of the rapidly dissociating and the slowly dissociating components, respectively. The uncertainty represents the 95% confidence interval from the non-linear regression analysis.

The analytical window for the Competing Ligand Exchange Method is simply the time from the start of the experiment to the time that has been arbitrarily chosen to terminate the experiment. The lower limit for the rate coefficients that can be measured by the Competing Ligand Exchange Method is simply the time that has been arbitrarily chosen to complete the experiment. The measurement of rapid kinetics is limited by experimental conditions such as the mixing rate of the added Chelex with the sample solution and the sampling rate (approximately 60 s between each data point). As a

consequence, the upper limit of experimentally measurable rate coefficients is approximately 10^{-2} s^{-1} . For a total reaction time of approximately 2 h, this represents an analytical window of measurable rate coefficients of $10^{-2} - 10^{-6} \text{ s}^{-1}$ for this technique. As a consequence, *dynamic* metal complexes with $k_d > 10^{-2} \text{ s}^{-1}$ cannot be experimentally distinguished from the metal aqua complex with this technique.

Figure 5.5 The Differential Kinetic Function for Pb(II)-FA complexes in model solutions of Laurentian fulvic acid using Chelex 100 as the competing ligand and graphite furnace atomic absorption spectrometry to measure the dissociation kinetics. $c_{\text{Pb},T} = 7.24 \times 10^{-7} \text{ mol/L}$, pH 5.0, $T = 23^\circ\text{C}$.

Figure 5.6 The Differential Kinetic Function for Cd(II)-FA complexes in model solutions of Laurentian fulvic acid using Chelex 100 as the competing ligand and graphite furnace atomic absorption spectrometry to measure the dissociation kinetics. $c_{\text{Cd},T} = 7.24 \times 10^{-7}$ mol/L, pH 5.0, $T = 23^\circ\text{C}$.

The analytical window may be extended by employing faster sampling techniques. A flow-through sampling system using Inductively-Coupled Plasma – Mass Spectrometry with solution nebulization has been used to investigate kinetic speciation of metals [21]. Rapid sequential scanning by the quadrupole mass spectrometer enables data points to be collected on the millisecond timescale. However, the experimentally measurable rate

coefficients are still limited to approximately 10^{-1} s^{-1} [21], suggesting that the rate of mixing of the Chelex (approximately 10 s for complete mixing) is still the limiting factor.

Figure 5.7 The Differential Kinetic Function for Cu(II)-FA complexes in model solutions of Laurentian fulvic acid using Chelex 100 as the competing ligand and graphite furnace atomic absorption spectrometry to measure the dissociation kinetics. $c_{\text{Cu,T}} = 7.24 \times 10^{-7} \text{ mol/L}$, pH 5.0, $T = 23^\circ\text{C}$.

The Differential Kinetic Functions for Pb(II), Cd(II) and Cu(II) in model systems of Laurentian fulvic acid are presented in Figures 5.5, 5.6 and 5.7, respectively. Because

equation (1.21) is strictly defined for the differential kinetic parameter, k_d^* , Figures 5.5, 5.6 and 5.7 were constructed by replacing k_d^* with the weighted average dissociation rate coefficient, $\bar{k}_d$, by analogy with equations (1.20) and (1.21).

$$\log \theta = \Gamma \log K_o^* - \Gamma \log k_a + \Gamma \log \bar{k}_d \quad (5.9)$$

where,

$$\bar{k}_d = \frac{\sum_i k_{d,i} c_{ML_i}}{\sum_i c_{ML_i}} = \frac{\sum_i k_{d,i} c_{ML_i}}{\bar{K}_{CM} \sum_i c_{L_i}} \quad (5.10)$$

The heterogeneity parameter, Γ , was the same ($\Gamma \approx 0.53$) for both Pb(II) and Cu(II). The value for Cd(II) was $\Gamma = 0.81$. The results are in relatively good agreement with the results obtained by pseudopolarography in Chapter 2 ($\Gamma_{Pb} = 0.44$ and $\Gamma_{Cu} = 0.39$), suggesting that the Differential Kinetic Function and the Differential Equilibrium Function are independent of the technique used. The slightly lower values of Γ obtained for the Differential Equilibrium Function by Anodic Stripping Voltammetry may be due to a loss in lability as discussed in Chapters 2 and 4.

5.5 Conclusions

The approach described in this chapter is a compromise between equilibrium and kinetic speciation. Although the system was perturbed during kinetic analysis, the species identified are those that existed at equilibrium. Each species had an associated rate

coefficient. The measured rate coefficients were strictly parameters of the experiment, not the sample. If the reaction mechanism is simple, a semi-quantitative connection between the experimental rate coefficients and related rate processes in natural samples may be established. However, even where the mechanism is more complicated the relative rates are probably still correctly diagnosed.

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**Chapter 6 A kinetic study of nickel speciation by Adsorptive
Cathodic Stripping Voltammetry**

6.1 Introduction

Living systems form examples *par excellence* of ordered dynamic systems far from equilibrium [1]. Additionally, the natural processes that determine the physical and chemical properties of aquatic systems are not in equilibrium. Disequilibria between metals and complexing agents in natural waters may arise from changes in natural as well as anthropogenic inputs, and from hydrological, geochemical and biological processes [2]. For such non-equilibrium systems, kinetic speciation would give a more realistic description of bioavailability of trace metals than metal speciation based on local equilibrium approximations. For nickel complexes in freshwaters, Cabaniss [3] has proposed that both kinetic and equilibrium models are required to predict metal partitioning in relatively inert systems such as Ni(II)-fulvic acid (FA) complexes.

In freshwaters, equilibrium assumptions for transition metal complexation are always hazardous, and for Ni(II) complexation, risky. Coordination equilibrium of transition metals in freshwaters, especially double exchange reactions, are typically slow in systems containing mixtures of strong and weak ligands and high concentrations of alkaline earth metals or mixtures of competing transition metals, such as are found in freshwaters. The complexation kinetics of $3d^8$ Ni(II) is known to be slow in water because of unfavourable changes in Ligand Field Stabilization Energy (LFSE) during complex formation [4]. This is in contrast to other divalent $3d$ -transition metals [5,6]. Although an abundant literature on the complex formation and dissociation reaction kinetics of Ni(II) with well-defined ligands is available, only a few studies concern conditions relevant to natural systems [3,24,7]. The slow Ni(II) complexation kinetics is likely to influence its speciation in

natural systems and to affect interaction of other metal ions with ligands [8]. The quotation shown below from Xue et al. of the Swiss Federal Institute for Environmental Science and Technology (EAWAG) captures our current knowledge on the influence of slow Ni(II) complexation kinetics in freshwaters.

“The speciation results after long equilibration only reflect the Ni speciation close to equilibrium, in particular the occurrence of strong ligands. The Ni distribution in natural waters may in many cases never really reach equilibrium but may rather be determined by the slow exchange rates between various ligands. New Ni inputs may persist for long periods in their original speciation and disturb the Ni distribution in a system. Ni inputs in the form of strong organic complexes (e.g., NiEDTA) may persist as such for long times before equilibrating with all available ligands. On the other hand, inputs of free Ni^{2+} ions (e.g., from acidic waters) would also react only slowly and may result in higher free $[\text{Ni}^{2+}]$ than predicted from equilibrium. The slow equilibration of Ni complexation may also affect the speciation of other metals that react faster with strong ligands.” [9]

Hence, coordination disequilibrium arising from double-exchange reactions in the multimetal (3d-transition metals) and the multiligand milieu of freshwaters are probably due to slow kinetics of metal exchange reactions rather than that of ligand exchange reactions.

In developing a model for Ni(II), considerable problems may arise because the equilibrium assumption may not be valid for Ni(II) complexation in freshwaters. The key

question to be asked is: “When is equilibrium between Ni(II) and the ligands in freshwaters established? Do the non-equilibrium concentrations of the nickel species provide a meaningful description of the true nickel speciation?” These questions are relevant because studies on Ni(II) complexation by Mandal et al. [10,11] have revealed that other 3d-transition metals such as Cu(II) and Co(II) (especially copper) strongly influence the *dynamics* of Ni(II)-FA complexes in model solutions and in freshwaters. In trace concentrations (ca $10^{-5} - 10^{-7}$ M) Cu(II), Co(II) were found to successfully compete with Ni(II) in unpolluted freshwaters ($C_{Ni,T} \approx 10^{-8}$ M) for the binding sites of NOM [10,11]. This is in contrast with the effects of major cations, Ca^{2+} and Mg^{2+} , on the Ni(II)-NOM binding, in which comparable effects are observed only when the Ca^{2+} and Mg^{2+} are present in massive excess (ca $10^{-3} - 10^{-5}$ M) over the nickel concentration (ca 10^{-8} M) [12]. The binding strength in the Ni(II)-NOM complexation arises from several factors: the intrinsic acidity of the Ni^{2+} ion and basicity of the NOM, and the Hard-Soft Acid-Base (HSAB) interactions between the soft acid (Ni^{2+}) and soft bases (e.g. S^{2-}), resulting in polarization of the anion (e.g. S^{2-}) and cation-anion mutual polarization (covalency). Another important contribution to the binding strength of 3d-metal-NOM complexes is Ligand Field Stabilization Energy (LFSE). For the 3d⁸ Ni(II) complex formation, the change in LFSE for Ni^{2+} in going from an initial octahedral $[Ni(H_2O)_6]^{2+}$ configuration to the intermediate, five-coordinate $[Ni(H_2O)_5]^{2+}$ configuration (activated complex), in order to accommodate an incoming ligand, L, is unfavourable, and entails a loss in the LFSE. The result is a loss in the lability of the NiL_6 complex, as observed.

Adsorptive Cathodic Stripping Voltammetry (AdCSV) with dimethylglyoxime (DMG) has been used for nickel determination [13-15] and equilibrium speciation [16,17] in natural waters. Recent voltammetric studies indicate that under marine conditions, more than 95% of the total nickel is organically complexed [16]. Langford and Gutzman [18] have used the Competing Ligand Exchange Method (CLEM) for kinetic speciation of nickel with the chromophore 4-(2-pyridylazo) resorcinol (PAR) as a competing ligand and spectrophotometric determination of the Ni(II)-PAR complex. The kinetic speciation of Ni(II)-FA complexes has been studied using CLEM with Chelex 100 as the competing ligand and Graphite Furnace Atomic Absorption Spectrometry (GFAAS) or Inductively-Coupled Plasma - Mass Spectrometry (ICP-MS) to measure the dissociation kinetics [19-22]. However, there are only two publications (both from this laboratory) in which the AdCSV method has been used to determine the kinetic speciation of nickel in natural waters [23,24]. In all these studies, the authors have assumed that the ligand exchange reactions of Ni(II)-FA complexes involve the dissociation of the initial complexes as the rate-determining step and have determined the rate coefficients for dissociation of these complexes.

The complexity and heterogeneity of naturally-occurring complexants, such as humic substances, in freshwaters presents problems in interpretation of kinetic results. The metal complexation properties were studied in model systems so that the results can be extrapolated from the laboratory to natural water conditions. In this study, model systems containing citric acid (CA) and a well-characterized fulvic acid solution as model ligands have been used. Citric acid has been used earlier as a model ligand to simulate the

discrete site models of binding by humic substances [25]. The fulvic acid concentration in model systems is intended to simulate Natural Organic Matter (NOM) in freshwaters. Adsorptive Cathodic Stripping Voltammetry was then applied to investigate the kinetic speciation of nickel in freshwater samples collected from the Sudbury (Ontario) area.

6.1.1 Adsorptive Cathodic Stripping Voltammetry

In this work, we have investigated rates of ligand exchange reactions for nickel complexes in model systems using CLEM with DMG as the competing ligand and AdCSV to measure the dissociation kinetics. Two reaction pathways for the overall ligand-exchange reactions may be considered: the disjunctive pathway (dissociation of the metal complex) and the adjunctive pathway (direct attack by the incoming ligand to form an intermediate complex) [1,2,4,26,27]. Herring and Morel [26] introduced this new terminology to denote this as a stoichiometric pathway (a sequence of elementary steps) as opposed to an intimate mechanism which deals with the activation of the rate-determining step. If the disjunctive pathway is followed, the measured rate coefficients can be related to a fundamental process in the natural environment—dissociation of the Ni(II) complex. However, the observed rate coefficients for the adjunctive pathway depend on the nature of an arbitrarily chosen probe ligand, and consequently, little information can be obtained about the processes in the natural environment. The theoretical and experimental conditions in which one of these pathways predominates have been investigated.

6.2 Theory

6.2.1 Ligand exchange reactions

The overall ligand exchange reaction of the initial nickel complex, NiL, with DMG as the competing ligand can be described by the equation:

The electrochemical measurements are based on adsorption of the Ni(DMG)_2 complex on the mercury electrode [16,17] and reduction of the adsorbed complex, which according to Ma et al. [14], proceeds by a stepwise reduction involving 10 electrons.

Pihlar et al. [13] have reported that the reaction of Ni(DMG)_2 reduction on a hanging mercury drop electrode is first-order with respect to the concentration of Ni^{2+} . The peak current as measured by AdCSV is proportional to the concentration of Ni(DMG)_2 adsorbed on the mercury electrode surface [14,28]. Provided that both adsorption of the Ni(DMG)_2 complex from the aqueous solution on the mercury electrode surface (equation 6.2) [29], and reduction of the adsorbed complex (equation 6.3) are fast, the increasing rate of the measured peak current corresponds to the rate of formation of $\text{Ni(DMG)}_2(\text{sol.})$, and hence, the rate coefficient calculated for this process (reaction 6.1) is k_{overall} .

The rate law for the overall reaction shown as reaction (6.1) is given by equation (6.4):

$$\frac{dc_{\text{Ni(DMG)}_2}}{dt} = k_{\text{overall}} c_{\text{NiL}} c_{\text{DMG}}^2 \quad (6.4)$$

No inference can be drawn from equation (6.4) about the molecularity of elementary reactions.

In the kinetic measurements, the condition that $c_{\text{DMG}} \gg c_{\text{Ni}}$ is maintained. Since $c_{\text{NiL}} < c_{\text{Ni}}$, $c_{\text{DMG}} \gg c_{\text{NiL}}$, and equation (6.1) is pseudo-first-order, the integrated form of the rate law is:

$$c_{\text{Ni(DMG)}_2}(t) = c_{\text{NiL}}^0 [1 - \exp(-k_{\text{observed}} t)] \quad (6.5)$$

where $k_{\text{observed}} = k_{\text{overall}} c_{\text{DMG}}^2$

It can be shown that:

$$i_p(t) = i_p^{\text{max}} \cdot [1 - \exp(-k_{\text{observed}} t)] \quad (6.6)$$

where $i_p(t)$ is the peak current at any time, t , and i_p^{max} is the maximum current in the absence of complexants. The observed rate coefficient, k_{observed} , can be calculated by fitting the kinetic data to the exponential rise model in equation (6.6).

If the overall reaction follows the disjunctive pathway, then:

Assuming a steady-state approximation for c_{Ni} (see Appendix A), one can obtain equation (6.7).

$$k_{disjunctive} = \frac{k_1^{disj.} k_2^{disj.}}{k_{-1}^{disj.} c_L + k_2^{disj.} c_{DMG}^2} \quad (6.7)$$

where c_L is the concentration of the initial ligand and $k_{disjunctive}$ is the rate coefficient for the disjunctive pathway. In this case, $k_{observed}$ will strongly depend on the relative concentrations of the initial ligand, L, and the competing ligand, DMG.

If the reaction follows the adjunctive pathway, then:

In this case, the rate coefficient for reaction (6.1) is given by equation (6.8), and $k_{overall}$ is independent of the concentrations of both the initial ligand, L, and the competing ligand, DMG (see Appendix B).

$$k_{adjunctive} = \frac{k_1^{adj.} k_2^{adj.}}{k_{-1}^{adj.} + k_2^{adj.}} \quad (6.8)$$

In general, the reaction schemes represented by reaction (6.1) may follow both the adjunctive and disjunctive pathways simultaneously, although one pathway may predominate under certain experimental conditions.

$$k_{\text{observed}} = k_{\text{adjunctive}} + k_{\text{disjunctive}} \quad (6.9)$$

If the concentrations of the competing ligand, DMG, and the initial ligand, L, are such that the condition $k_2^{\text{disj.}} c_{\text{DMG}}^2 \ll k_{-1}^{\text{disj.}} c_L$ is satisfied, then equations (6.7) and (6.9) can be rewritten as:

$$k_{\text{disjunctive}} = \frac{k_1^{\text{disj.}} k_2^{\text{disj.}}}{k_{-1}^{\text{disj.}} c_L} \quad (6.10)$$

$$k_{\text{observed}} = k_{\text{adj.}} + \frac{k_1^{\text{disj.}} k_2^{\text{disj.}}}{k_{-1}^{\text{disj.}} c_L} \quad (6.11)$$

In this case, a plot of k_{observed} versus $1/c_L$ will be a straight line with intercept equal to $k_{\text{adjunctive}}$ and the slope equal to $k_1^{\text{disj.}} k_2^{\text{disj.}} / k_{-1}^{\text{disj.}}$.

The hypothesis to be tested is that the observed rate coefficient of the overall reaction, k_{observed} , is equal to the dissociation rate coefficient of the initial complex NiL (called $k_1^{\text{disj.}}$) only if:

1) the disjunctive pathway predominates

$$2) k_2^{\text{disj.}} c_{\text{DMG}}^2 \gg k_{-1}^{\text{disj.}} c_L \quad (6.12)$$

If the above two conditions are satisfied, then:

$$\frac{dc_{\text{Ni(DMG)}_2}}{dt} = -\frac{dc_{\text{NiL}}}{dt} = k_1^{\text{disj.}} \cdot c_{\text{NiL}}$$

and hence,

$$k_{\text{observed}} = k_1^{\text{disj.}}$$

The disjunctive and adjunctive pathways can be distinguished experimentally by the dependence of the observed rate coefficient on c_L . If the reaction follows the adjunctive pathway the overall rate coefficient will not depend on c_L . If the disjunctive pathway predominates, the overall rate coefficient will strongly depend on c_L .

6.3 Experimental

6.3.1 Reagents and materials

A stock solution of 1000 $\mu\text{g/mL}$ nickel was prepared by dissolving pure nickel powder (SPEX, 99.999%) in ultrapure nitric acid (Ultrex II, JT Baker Inc.), diluting with ultrapure water containing 1% (v/v) nitric acid. A 0.1 M DMG stock solution was prepared by dissolving an appropriate amount of solid DMG (Fisher Scientific, certified) in absolute ethanol (Spectro grade). The HEPES (BDH, ACS grade) and sodium hydroxide (ACS grade, BDH) reagents were used to prepare the aqueous stock solutions of 2 M HEPES and 2 M sodium hydroxide, respectively. The BDH, Analytical Grade reagents was used to prepare the stock solution of citric acid. All solutions were prepared in ultrapure water of 18.2 $\text{M}\Omega$ resistivity obtained directly from a Milli-Q UF Plus water purification system (Millipore), fitted with a column to remove organic impurities in

ultrapure water. The ionic strength was kept constant throughout this work at 0.02 M using sodium hydroxide.

A well-characterized fulvic acid was collected from Armadale, Prince Edward Island, Canada and was extracted from a Bh horizon, by Dr. D.S. Gamble, Agriculture Canada, Ottawa [30,31]. The total number of phenolic and carboxylic groups determined by potentiometric titration were 3.9 mmol/g fulvic acid and 7.71 mmol/g fulvic acid, respectively [30-32]. The Armadale fulvic acid stock solution (1.0219 g/L) was prepared by dissolving an appropriate amount of the freeze-dried fulvic acid in ultrapure water. The stock solution was stored in the dark at 4 °C until use.

6.3.2 Apparatus

Voltammetric measurements were carried out using a Bioanalytical Systems (BAS) 100B/W Electrochemical Analyzer, with controlled-growth mercury electrode (CGME) stand (BAS), controlled by a 486 personal computer. A medium size (size 6) hanging mercury drop electrode was employed as the working electrode. The reference electrode was a Ag/AgCl electrode (BAS), filled with 3 M sodium chloride, and the counter electrode was a coiled platinum wire (BAS). Analysis of voltammetric peaks was done using the BAS 100W windows control software v2.1 (BAS). The deposition potential was -0.700 mV and the adsorption time 5 s. Square Wave Stripping Voltammetry was used with frequency of 100 Hz, scan rate 5 mV/s and pulse height 20 mV. All the measurements were performed under the nitrogen gas-flow condition.

6.3.3 Sampling sites and sampling protocol.

Water samples (6.6 L) were collected in June 1999 from several sampling sites in the Sudbury, Ontario area using pre-cleaned Teflon bottles. The sampling sites were chosen with a view to studying the applicability of the nickel speciation techniques developed in our laboratories using model solutions [12] to natural waters. The sites were chosen upstream and downstream of the nickel-tailing ponds, and outflows from the treatment tanks receiving effluents from nickel ore processing and smelting operations. A list of the sampling sites, pHs, conductivities, total nickel concentrations and concentrations of Dissolved Organic Carbon (DOC) is presented in Table 6.1. The DOC concentration of the filtered water samples was measured using a total organic carbon analyzer (O I Analytical Model 1010). The samples were filtered through 0.45 μm membrane filters (CN-6 Metrice, 47 mm diameter, Gelman, Ann Arbor, MI) in our laboratory within 24 h of the sample collection to filter out the particulate matter. These filters, made of mixed cellulose esters, had the advantage of providing filtrates having low blanks for the metals. Each 0.45 μm filter was able to filter approximately 500 mL of water sample before they were clogged. A subsample (250 mL) of the filtered samples was acidified with nitric acid (Ultrex II) to pH 1.5. The acidified sample was used to determine the total nickel concentrations using GFAAS. The remaining filtered samples were stored in the dark at 4°C until the kinetic experiments, and were used for the kinetic studies within 72 h of filtration.

Table 6.1 List of the Sudbury area sampling sites.

No.	Sampling Site	pH	Conductivity, S/m	$C_{Ni,T}$, mol/L	DOC, mg/L
1	Copper Cliff	6.2	0.292	2.5×10^{-6}	7.09
2	Kelly Lake	7.6	0.135	4.7×10^{-6}	6.84
3	Alice Lake	7.2	0.020	4.9×10^{-6}	6.03
4	Conservation Area	7.6	0.083	1.8×10^{-6}	6.47
5	Coniston Creek downstream	8.1	0.042	1.3×10^{-6}	6.98
6	Moose Lake treated discharge	7.9	0.167	0.9×10^{-6}	6.39
7	Moose Creek upstream	7.6	0.164	2.1×10^{-6}	3.51
8	Onaping River	7.2	0.221	0.6×10^{-6}	6.39

6.3.4 Kinetic experiments

The test solutions for kinetic measurements were prepared by mixing appropriate amounts of nickel and the initial ligand, L, (citric acid or fulvic acid) solutions. The mixtures were left overnight for equilibration at room temperature (23°C). The ionic strength and the pH were held constant for all experiments by adding 0.200 mL of 2 M NaOH and 0.175 mL HEPES 2M solutions to 20 mL of the sample to fix the pH at 8.3 and the ionic strength at 0.02 M for the kinetic experiments. The kinetic experiments were started immediately upon addition of DMG to the test solution. The peak current

was recorded every 25s and the recording was continued until no further change in the signal was observed and the current achieved a maximum value $i_p^{\max}$.

The citric acid model solutions contained 400 nM Ni^{2+} and 0.25 – 500 μM citric acid. The kinetic measurements were performed using the DMG concentrations ranging from 0.5 – 3 mM. Model solutions of the fulvic acid were prepared to contain 400 nM Ni^{2+} and up to 200 mg/L fulvic acid.

6.3.5 Data analysis

The overall reaction was pseudo-first-order, and the rate coefficients were calculated by fitting the experimental data to a one-component exponential rise model by non-linear regression analysis using the Marquardt-Levenberg algorithm. The calculations were performed using SigmaPlot 5 computer program (SPSS Science).

6.4 Results and Discussion

6.4.1 Citric acid model solutions

Figure 6.1 shows the kinetic curves i.e. $i_p / i_p^{\max} (\%)$ as a function of time for the test solutions containing varying concentrations of citric acid (up to 50 μM) and 1 mM DMG. For the complexes of nickel with citric acid, the experimental conditions were such that only one complex, $\text{Ni}(\text{Citrate})$, was formed (according to MINEQL calculations), and hence, the kinetic data were fitted to the one-component exponential rise model.

Figure 6.1 $i_p / i_p^{\max}$ ratio of Ni-citrate solutions containing various concentrations of citric acid as a function of time. $T = 23^\circ\text{C}$. $\blacklozenge$ no citric acid; $\bullet$ $c_{\text{CA}} = 16.7 \mu\text{M}$; $\square$ $c_{\text{CA}} = 27.3 \mu\text{M}$; $\blacksquare$ $c_{\text{CA}} = 36.4 \mu\text{M}$; $\blacktriangle$ $c_{\text{CA}} = 45.5 \mu\text{M}$. Error bars represent 95% confidence intervals.

Figure 6.2 Effect of DMG and citric acid concentrations on the observed rate coefficient, k_{observed} , for citric acid model solutions containing $c_{\text{Ni,T}} = 400 \text{ nM}$:
 ♦ no CA; ■ $c_{CA} = 0.25 \mu\text{M}$; ▲ $c_{CA} = 2.5 \mu\text{M}$; □ $c_{CA} = 25 \mu\text{M}$; ○ $c_{CA} = 250 \mu\text{M}$; ● $c_{CA} = 500 \mu\text{M}$. $T = 23^\circ\text{C}$. Error bars represent 95% confidence intervals.

Table 6.2 Observed rate coefficients for Ni-citric acid model systems. Results were calculated by fitting the kinetic data to the one-component exponential rise model using non-linear regression analysis. T = 23°C.

Citric Acid (μM)	$k_{\text{observed}} (\text{s}^{-1})$				
	0.5 mM DMG	1.0 mM DMG	1.5 mM DMG	2.0 mM DMG	3.0 mM DMG
0	0.12 ± 0.09	0.12 ± 0.020	0.12 ± 0.02	0.12 ± 0.08	0.12 ± 0.03
0.25	0.10 ± 0.01	0.097 ± 0.003	0.10 ± 0.01	0.11 ± 0.004	0.12 ± 0.003
2.5	0.053 ± 0.002	0.074 ± 0.008	0.11 ± 0.04	0.11 ± 0.03	0.11 ± 0.03
25	0.025 ± 0.003	0.055 ± 0.040	0.12 ± 0.04	0.12 ± 0.03	0.11 ± 0.02
250	0.0041 ± 0.0010	0.029 ± 0.008	0.079 ± 0.004	0.11 ± 0.03	0.11 ± 0.02
500	0.0010 ± 0.0006	0.030 ± 0.005	0.067 ± 0.02	0.11 ± 0.02	0.11 ± 0.08

The uncertainty represents the 95% confidence interval from the non-linear regression analysis.

The observed rate coefficients, k_{observed} , are presented in Table 6.2. Figure 6.2 clearly shows the general trend of the dependence of the rate coefficient for the overall reaction on the concentration of DMG and citric acid for $c_{\text{DMG}} < 2 \text{ mM}$. With increasing concentrations of DMG, the measured rate coefficient became constant, regardless of the concentration of DMG or citric acid. This observation can be interpreted in one of two ways: either the adjunctive pathway predominated (equation 6.8), or

$k_2^{\text{disj.}} c_{\text{DMG}}^2 \gg k_{-1}^{\text{disj.}} c_L$, i.e. the condition shown by equation (6.12) was satisfied, or the disjunctive pathway predominated.

Further kinetic measurements were performed using experimental conditions such that the rate coefficient depended on the concentrations of citric acid or DMG so that we could determine the experimental conditions under which one of these two pathways predominated and to calculate the contribution of $k_{\text{disjunctive}}$ and $k_{\text{adjunctive}}$ to the k_{observed} .

Figure 6.3 k_{observed} vs. $1/c_{\text{CA}}$ for citric acid model solutions containing $c_{\text{Ni,T}} = 400 \text{ nM}$ and $c_{\text{DMG}} = 1 \text{ mM}$. (—) linear regression curve (.....) 85% confidence limits. $T = 23^\circ\text{C}$.

The relationship expressed by equation (6.11) for the model systems of citric acid and DMG is presented in Figure 6.3. The non-zero intercept accounts for some contribution of the adjunctive pathway to the stoichiometric pathway of the overall reaction. The contribution of the adjunctive pathway became important for high CA-to-DMG ratios. For example, at $c_{CA} = 50 \mu\text{M}$ and $c_{DMG} = 1 \text{ mM}$ (the highest CA-to-DMG ratio employed in this study) the rate coefficient for the disjunctive pathway $k_{\text{disjunctive}} = 0.0037 \pm 0.0002 \text{ s}^{-1}$ was comparable with $k_{\text{adjunctive}} = 0.0059 \pm 0.0003 \text{ s}^{-1}$. The contribution of both pathways was equal for $c_{CA} \approx 30 \mu\text{M}$. With decreasing CA-to-DMG ratios, the contribution of the adjunctive pathway became less important. This means that for high concentrations of c_{DMG} (i.e. for the concentrations for which k_{observed} remained constant) the ligand exchange reaction (6.1) followed the disjunctive pathway and the observed rate coefficient equalled the dissociation rate coefficient of Ni-CA complex.

The ratio $k_{-1}^{\text{disj.}}/k_1^{\text{disj.}}$ is related to the conditional stability constant of the Ni(II)-CA complex by analogy with equation (1.2). For $k_2 = 0.12 \text{ s}^{-1}$ (the overall rate coefficient for the test solution in the absence of citric acid), the conditional stability constant of NiCA complex is $\log K'_{\text{NiCA}} = 5.8$, which is in reasonable agreement with the conditional stability constant at $\text{pH} = 8.3$ calculated using MINEQL, $\log K'_{\text{NiCA}} = 6.3$.

6.4.2 Fulvic acid model systems

Results for the dependence of the observed rate coefficient, k_{observed} , on the DMG and fulvic acid concentrations are presented in Figure 6.4. The approximation with one component fitted the experimental data for all the Ni(II)-FA test solutions except for the

one with the lowest fulvic acid and DMG concentrations. For this case, the exponential growth with two components fitted the experimental data better. Hence, these data were not included in Figure 6.4. The results are presented in Figure 6.4, which shows that the overall rate coefficient strongly depended on the fulvic acid and the DMG concentrations in the test solutions when $c_{\text{DMG}} \leq 3$ mM. For $c_{\text{FA}} > 50$ mg/L, the k_{observed} depended on c_{DMG} even if $c_{\text{DMG}} > 3$ mM.

In order to find out experimental conditions for which the contributions of the adjunctive and the disjunctive pathway were significant, the reaction was studied under the experimental conditions in which $c_{\text{DMG}} < 3$ mM. Figure 6.5 presents the k_{observed} versus $1/c_{\text{FA}}$ for $c_{\text{DMG}} = 1$ mM; the intercept represents the rate coefficient of the adjunctive pathway and the slope gives the rate coefficient of the disjunctive pathway. The rate coefficient for the ligand exchange reaction, k_{observed} , under the experimental conditions prescribed earlier can be obtained as follows:

$$k_{\text{observed}} = 0.19 \frac{1}{c_{\text{FA}}} + 0.0096 \quad (6.13)$$

where c_{FA} is in mg/L

From this equation the contributions of both pathways were calculated to be equal for fulvic acid concentration ≈ 20 mg/L or $c_{\text{DMG}}/c_{\text{FA}} \approx 0.05$ mmol/mg. With increasing DMG-to-FA ratios, the contribution of the adjunctive pathway became less important. For example, at $c_{\text{DMG}}/c_{\text{FA}} = 0.2$ nmol/mg and $c_{\text{DMG}} = 1$ mM, $k_{\text{disjunctive}}$ was four times larger than $k_{\text{adjunctive}}$. If we consider that the average fulvic acid concentration in natural waters is approximately 5 mg/L (assuming that approximately 50% of DOC in natural

waters is fulvic acid and the concentration of DOC is normally 5 – 15 mg/L), using 3 mM DMG as the competing ligand, the $k_{\text{disjunctive}}$ was found to be about 12 times higher than $k_{\text{adjunctive}}$. In this case, the observed rate coefficient did not depend on c_{DMG} and represented the average dissociation rate coefficients of Ni(II)-FA complexes. The conditional stability constant for the Ni(II)-FA complex ($\log K'_{\text{NiFA}} = 5.1$) was estimated from the slope of the equation (6.13), which is in reasonable agreement with the stability constants for Ni(II)-HA complexes reported by Burba [33] ($\log K' > 5.3$) and Sedlak et al. [34] ($\log K' = 3.1 - 4.2$). Since conditional stability constants should be compared only under identical experimental conditions (which is obviously not the present case) the agreement between these values seems reasonable.

As Langford et al. [1] have emphasized, ligand exchange kinetics are always recorded under experimental conditions determined by the reagent solutions whatever the pH, concentration, or ionic strength of the sample. The species identified are those of the conditions of the sample, but the rate coefficients obtained are not rate coefficients for processes that occur under the original conditions of the sample, but for processes occurring at the standardized condition imposed by the reagent solution. However, dissociation of ML complex in the ligand exchange reaction: $\text{ML} + \text{X} \rightarrow \text{MX} + \text{L}$ is a fundamental process of natural waters, and if the ligand exchange reaction follows the disjunctive pathway, the theory of ligand substitution processes allows for a significant degree of reliable extrapolation over concentrations and pH [1].

Figure 6.4 Dependence of the observed rate coefficient, k_{observed} , on DMG and fulvic acid concentrations for model solutions. $T = 23^{\circ}\text{C}$, $c_{\text{Ni,T}} = 400$ nmol/L. $\square$ no FA, $\bullet$ $c_{\text{FA}} = 0.5$ mg/L, $\blacktriangle$ $c_{\text{FA}} = 5$ mg/L, $\blacksquare$ $c_{\text{FA}} = 50$ mg/L, $\circ$ $c_{\text{FA}} = 200$ mg/L. Error bars represent 95% confidence intervals.

Figure 6.5 k_{observed} vs. $1/c_{\text{FA}}$ for fulvic acid model solutions containing $c_{\text{Ni,T}} = 400 \text{ nM}$ and $c_{\text{DMG}} = 1 \text{ mM}$. (—) linear regression curve (.....) 95% confidence intervals. $T = 23^\circ\text{C}$.

The results for the model systems demonstrate that the ligand exchange kinetics of nickel complexes with DMG as the competing ligand can follow both the adjunctive and the disjunctive pathways. The conditions under which one of these pathways may predominate depend on the composition of the sample—the relative concentrations of the initial ligand, L, and the competing ligand, DMG. If the concentration of the initial

ligand, L, is high compared to that of the competing ligand, DMG, then the contribution of the adjunctive pathway becomes important. In this case, the kinetics of the overall reaction is dominated by the formation of the intermediate binuclear complex. At low ligand concentrations of the ligand, L (or high nickel loadings), the ligand exchange reaction proceeds by the dissociation of the initial complex which becomes the rate-determining step and the observed rate coefficient is equal to the rate coefficient for dissociation of the initial complex, NiL. The correspondence of the observed rate coefficients and the conditional stability constant reported for the Ni-CA complex supports our interpretation of the ligand exchange reactions for NiL. This conclusion is consistent with that of Hering and Morel [26]. Langford et al. [1,18] and Cabaniss [3] have accepted (although not explicitly stated) that the ligand exchange reactions between the Ni(II)-FA complexes and PAR as the competing ligand follow the disjunctive pathway, which would be expected for higher Ni(II)-to-fulvic acid ratios ($C_{Ni}/C_{FA} > 100$ nmol/mg) that they have used.

Interpretation of the ligand exchange reactions for the Ni(II)-FA model systems should be done with caution. We have not considered the fact that, in these systems there may be a distribution of rate coefficients rather than a single value and that this distribution depends on the experimental conditions such as pH, ionic strength and temperature [18,32,35].

6.4.3 Kinetic speciation of nickel in freshwaters

Figure 6.6 presents the results for the kinetic speciation of Ni(II) in freshwaters from the Sudbury area. The quickly rising section at the beginning of each curve is due to free metal ions that were present in the original sample plus those generated by the dissociation of *dynamic* Ni(II) species. The slowly rising section of the curve is due to the slow dissociation of less dynamic nickel complexes which are strongly bound. The limiting value at the top of the each curve represents the total concentration of all free metal ions plus those that are generated by dissociation of all Ni(II) complexes present in the original sample. The lability of nickel species increases from the bottom curve to the top curve.

The solid lines in Figure 6.6 represent data that was fitted to equation (6.5) by non-linear regression analysis. The results are presented in Table 6.3. Under the experimental measurement timescale ($t = 2$ h), complexes with $k_d \gg 10^{-4} \text{ s}^{-1}$ have been defined as *dynamic* ($k_d t \gg 1$, condition 1.4) and complexes with $k_d \ll 10^{-4} \text{ s}^{-1}$ have been defined as *inert* ($k_d t \ll 1$, condition 1.5). Two kinetically distinguishable components were identified: *dynamic* complexes ($k_d \approx 10^{-2} \text{ s}^{-1}$) that dissociate rapidly and *inert* complexes ($k_d < 10^{-4} \text{ s}^{-1}$) that dissociate slowly relative to the analytical timescale of measurement. As discussed in Chapter 5, the measured rate coefficients do not correspond to two precisely defined and homogeneous site types [36,37]. Rather, they probably represent averages over distribution of similar sites which remain kinetically defined over the full range of conditions [18].

Figure 6.6 Dissociation kinetics of Ni(II) complexes in freshwater samples collected from Sudbury (Ontario). ● Copper Cliff, □ Coniston Creek, ◆ Falconbridge Conservation area, ○ Moose Creek, ■ Onaping River, ◇ Moose Lake.

Table 6.3 Kinetically distinguishable components in freshwaters collected from Sudbury (Ontario).

Sample	C ₁ , %	k ₁ × 10 ² , s ⁻¹	C ₂ , %	k ₂ × 10 ⁵ , s ⁻¹
1	87 ± 6	7.5 ± 1.9	13 ± 1	< 10 ⁻⁶ s ⁻¹
2	81 ± 3	17 ± 14	19 ± 1	< 10 ⁻⁶ s ⁻¹
3	82 ± 4	13 ± 6	18 ± 2	< 10 ⁻⁶ s ⁻¹
4	70 ± 4	8.1 ± 2.6	30 ± 1	3.0 ± 1.1
5	80 ± 4	8.8 ± 2.4	20 ± 1	2.0 ± 1.5
6	30 ± 2	7.7 ± 2.0	70 ± 1	1.5 ± 0.2
7	57 ± 3	6.0 ± 2.0	43 ± 1	8.3 ± 0.7
8	59 ± 3	7.3 ± 1.4	42 ± 1	< 10 ⁻⁶ s ⁻¹

k₁, and k₂ are the dissociation rate coefficients of the rapidly dissociating and the slowly dissociating species, respectively. % C₁, and % C₂, are the relative concentrations of the rapidly dissociating and the slowly dissociating species, respectively. The uncertainty represents the 95% confidence interval from the non-linear regression analysis.

Unlike the situation for model solutions, the concentration of complexing sites in real samples is generally not known. However, a rough estimate of the concentration of naturally-occurring complexants can be obtained from the concentration of Dissolved Organic Carbon, which was approximately 6 mg/L as shown in Table 6.1. Typical DOC

concentrations in freshwaters range from 0.1 – 10 mM [38], of which humic substances represent 40% to 90% of the DOC [38]. Table 6.3 does not indicate any statistical difference in the measured rate coefficients (k_1) for the *dynamic* components of the different samples. However, the relative concentration of the *dynamic* components (C_1) was found to increase with increasing metal-to-ligand ratio as shown in Figure 6.7, suggesting that at first the strong sites, and then the weak sites were occupied until a point was reached when a sufficient amount of the metal had been added to saturate all the strong sites. Beyond that point, the fraction of the dynamic component, C_1 , must remain unchanged (constant) as the Ni-to-DOC ratio varied, as would be expected. The results are consistent with bioassay toxicity tests using the alga *Selenastrum capricornutum* for the same samples [39], which also show the effect of Ni-to-DOC ratio on the toxicity of nickel in the freshwater samples, as presented in Figure 6.8. Both the results of the kinetic speciation and the bioassay toxicity tests indicate that the free Ni^{2+} ion concentration, and hence toxicity, depend on the Ni-to-DOC ratio, suggesting that the kinetic speciation model has chemical significance.

Although major cations are known to form weak complexes with humic substances, they are present in **large excess** relative to the concentrations of trace metals in freshwaters. The overwhelming amounts of major cations (e.g. Ca^{2+} and Mg^{2+}) that exist naturally in freshwaters results in the presence of a large amount of Ca^{2+} and Mg^{2+} in the electrical double layer of the NOM polyanions, screening the charge of the NOM polyanions and thereby significantly decreasing the total binding energy of the Ni(II)-NOM complexes. The influence of major cations such as Ca^{2+} and Mg^{2+} on metal speciation has been

demonstrated by Mandal et al. [12] who have shown that overwhelming amounts of major cations release large fractions of organically-bound nickel.

Figure 6.7 Concentration of free plus labile Ni(II) species, C_1 (%) , as a function of nickel-to-DOC ratio (mol/g) in freshwater samples collected from Sudbury (Ontario).

Figure 6.8 Algal yield as a function of nickel-to-DOC ratio (mol/g) [39].

Naturally-occurring complexants, such as humic and fulvic acids, are oligoelectrolytes with large, negative surface potentials that form polyanions as a result of deprotonated carboxylic and phenolic acid groups. Coulombic forces are effective over relatively large distances ($1/r^2$) and affect the free energies of ions even in dilute solutions such as unpolluted river water. Humic and fulvic acids are weak acids, and pH has a dramatic effect on their binding as with any weak acid whose apparent affinity for a metal increases with pH. This effect is exacerbated by the increase in coulombic attraction of the metal ion to the binding sites as the humates become deprotonated with increasing pH. When a cation reacts with an acidic functional group of a molecule with multiple

functional groups (e.g. humic and fulvic acid) the chemical free energy of interaction is augmented by the long-range coulombic attraction emanating from all the neighbouring, non-reacting, negative sites [4,40]. At relatively high pH, this polyelectrolytic effect can greatly strengthen the binding of metal ions by polyanions.

The change in free energy of reaction (ΔG°) between a metal, M, and the functional group, L, (charges have been omitted for simplicity) is the sum of the chemical free energy change ($\Delta G_{\text{chemical}}^\circ$) due to the reaction with the functional group itself and the coulombic free energy change ($\Delta G_{\text{coulombic}}^\circ$) due to electrostatic interactions between the metal and all the charges on the polyanion, as shown in equation (6.15) [4].

$$\Delta G^\circ = \Delta G_{\text{chemical}}^\circ + \Delta G_{\text{coulombic}}^\circ \quad (6.15)$$

At the experimental pH (pH $\approx$ 7.7) used, NOM develops anionic charges, which profoundly affect the binding energy of the Ni(II)-NOM complexes, by affecting both $\Delta G_{\text{chemical}}^\circ$ and $\Delta G_{\text{coulombic}}^\circ$ of equation (6.15). Both Ca^{2+} and Mg^{2+} (especially Ca^{2+}) also bind to specific binding sites of the NOM, forming covalent bonds. They greatly affect $\Delta G_{\text{coulombic}}^\circ$ by forming electrostatic bonds (ion-pair formation) because of their (overwhelming) mass of positive charge due to their massive presence in the electrical double layer of the polyanions of naturally-occurring complexants. By their presence in the diffuse, electrical double layer, they also have long-range coulombic interactions with

the NOM polyanions. Additionally, Ca^{2+} and Mg^{2+} interact with all the neighbouring non-reacting negative sites on the polyanions of NOM (the terms 'reacting' and 'non-reacting' have been used here in the context of forming chemical bonds). This results in massive screening of the charge on the fulvate and humate polyanions, resulting in substantial decrease in the electrostatic interactions, i.e. in the $\Delta G_{\text{coulombic}}^{\circ}$ term of equation (6.15). Since ion-ion interaction energy is inversely proportional to the interionic distance, such interactions are strong and long-range. All the above interactions result in a great reduction in ΔG° in equation (6.15), which is reflected in Figure 6.6.

6.5 Conclusions

The mechanistic model of ligand exchange reactions presented in this paper is reasonably consistent with kinetic and equilibrium studies. The results show the crucial role of the relative concentrations of the initial ligand and the competing ligand, and the nickel-to-fulvate loading, in the contribution of each pathway to the overall reaction. The DOC concentration in model systems for which the contributions of both pathways are equal is higher than the values reported for the typical DOC concentrations in freshwater systems (2-10 mg/L DOC) [4].

The kinetic experiments require control over crucial parameters such as temperature, pH and ionic strength. This means that the speciation is controlled by the history of the sample prior to the start of the experiment, but that the measured rate coefficients are specific to the experimental conditions. This limitation is not as serious for metal

complexes in freshwaters as it might be in other circumstances, since the reaction rates of octahedral complexes tend to be controlled by the rate of ligand dissociation (i.e. the disjunctive pathway), and extrapolation from one condition to another is relatively simple and reliable [18]. In particular, relative rates tend to remain the same. However, several other factors should also be considered when investigating freshwater systems.

Freshwaters are very complex systems with various chemical and physical characteristics. Several trace metals may be present and may compete for the binding sites of organic ligands [19], and the presence of alkaline earth metals, Ca^{2+} and Mg^{2+} (usually present in massive excess), may affect the kinetics of trace metal complexation in freshwaters [34,41]. Also, other organic complexants, i.e., other than humic substances, e.g., polysaccharides, proteins, may compete for binding the trace metals including the target ones. All the above competitions can affect the pathway of the overall reaction. The application of AdCSV to natural waters may also cause further complications because of possible adsorption of surface-active organic substances on the surface of the hanging mercury drop electrode [12,42]. Finally, the structures of chemically isolated humic and fulvic acids are normally different from those of humic substances present in freshwaters [34].

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Chapter 7 Conclusions

7.1 Conclusions

Now that I have reached the end of this journey of scientific discovery which started with the challenge of Morel and Herring [1], I look back at the tortuous meanderings of the voyage, and take stock of what I have accomplished. Here are some highlights.

The major progress in our understanding of trace metal interactions with aquatic microorganisms has come from an appreciation of the importance of the chemical speciation of metals. The most significant result to emerge is the universal importance of the free metal ion activities in determining metal uptake, nutrition and toxicity. In a system at equilibrium, free-metal ion activity simply reflects the chemical reactivity of the metal and a measure of the extent of the reaction with the cellular sites that is independent of the actual mechanisms of the reactions. Hence, physiological effects, such as enzyme inhibition, will directly depend on the free metal ion activity regardless of the particular species (e.g. free metal ions or other metal complexes) present—all *labile* complexes are equally bioavailable. However, if the equilibrium assumption at the surface of the cell membrane is not satisfied, then the Free Ion Activity Model cannot be applied, and the biological response will be determined by the kinetics of the complexation reactions in the bulk solution or the rate of metal transport in the reaction layer outside the cell.

The concepts presented in this thesis: 1) reaction kinetics in the bulk solution (volume reactions), and 2) the transport kinetics M and ML (flux reactions) towards an interface (e.g. a biological membrane or an electrode) represent fundamental properties of metal

systems in the aquatic environment. As Figure 1.4 clearly illustrates, the experimental approach and design mimics the reactions that aquatic organisms are likely to encounter in freshwaters. The main significance of this work is that the concepts outlined in this thesis are **directly** relevant to other metal speciation techniques and for predicting metal bioavailability.

For the metals that form amalgams with mercury (e.g. Zn, Cd, Pb and Cu), Anodic Stripping Voltammetry with a mercury electrode is a particularly useful technique for investigating metal bioavailability because the metal flux at an electrode are inherently similar to those at a biological membrane. The Competing Ligand Exchange Methods (volume reactions) presented in Chapters 5 and 6 are not able to measure sufficiently rapid kinetics to make their results directly relevant to bioavailability in the same way as voltammetry. However, the results obtained by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) using Chelex 100 as the competing ligand or Adsorptive Cathodic Stripping Voltammetry (AdCSV) using dimethylglyoxime as the competing ligand provide information that is chemically relevant. Furthermore, the kinetic speciation using AdCSV demonstrates a clear link between the fraction of *labile* nickel species and toxicity. Similar linkages between nickel speciation and nickel toxicity have been established for GFAAS [2] and Inductively-Coupled Plasma – Mass Spectrometry [3] using Chelex 100 as the competing ligand.

The distinction between volume reactions and flux reactions can also be used to explain the nature of the inherent difference in the analytical windows for dissociation rate

coefficients provided by Anodic Stripping Voltammetry ($10^{-3} - 10^2 \text{ s}^{-1}$) and Competing Ligand Exchange Methods ($10^{-6} - 10^{-2} \text{ s}^{-1}$). Whereas the timescale of measurement in Anodic Stripping Voltammetry is defined by the thickness of the Nernstian diffusion layer, in the Competing Ligand Exchange Methods it is simply the time that has been arbitrarily chosen to terminate the experiment. However, the measurement of rapid kinetics is limited by experimental constraints such as the mixing rate and the sampling rate.

In a complex-forming medium, the supply of metal species to an interface (e.g. a biological membrane or an electrode in voltammetry) is related to the association and dissociation kinetics, and mass transport. Metal complexes are *labile* if the kinetic flux of metal towards an interface is much larger than the diffusional flux, so that diffusion of the metal species is the rate-determining step. The complexes are *non-labile* if they do not have time to dissociate within the timescale of the measurement process. For both an electrode and aquatic organisms, the timescale of measurement is defined by the thickness of the Nernstian diffusion layer, δ .

The Differential Equilibrium and Differential Kinetic Functions presented in this thesis assume that there is no upper or lower limit of $\log K$ or $\log k_d$ for the binding sites of a complexant. However, real complexants (even extremely heterogeneous ones) are limited by a maximum $\log K$ and $\log k_d$ value. Expected shapes of the DEF and DKF curves for heterogeneous complexants are shown in Figure 7.1. The asymptotically horizontal section of the curves corresponds to the region in which complexation is controlled by the

weak sites. The asymptotically vertical section of the curves corresponds to the region where even the strongest sites are predominantly free. The intermediate zone is where the binding sites are partially occupied by metals.

Figure 7.1 Schematic diagram for various degrees of heterogeneity. a) Differential Equilibrium Function. b) Differential Kinetic Functions. Adapted from Buffle et al. [4].

The slope, Γ , of the Differential Equilibrium Function and the Differential Kinetic Function is related to the buffer intensity. The buffering action of natural complexants gradually increases with increasing metal concentrations (i.e. metal pollution) over several orders of magnitude. This behaviour is in stark contrast with model systems in which only one or two simple ligands are responsible for the total metal complexing capacity. The cooperative action of the binding sites of naturally-occurring complexants means that there is always a suite of binding sites that actively complexes the metal, some beginning to be occupied, some near their half titration point, and some sites close to saturation. As a consequence, naturally-occurring complexants have closely spaced binding energies which enables them to buffer metals over a large concentration range (several orders of magnitude) with only a gradual increase in free metal ion concentration. This chemostatic property of naturally-occurring complexants such as humic substances is ecologically important since it helps organisms to adapt to gradual changes in free metal ion concentrations in the natural environment. From the intrinsic evidence of their wide-ranging metal-buffering complexation reactions in the natural environment, the **GREAT** buffer of freshwaters, the ubiquitous humic substances, now begin to appear as a vital web in the complex fabric of homeostasis.

7.2 References

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GLOSSARY

Symbol	Definition	Symbol	Definition
A	Freundlich constant	K	thermodynamic equilibrium constant ($L \text{ mol}^{-1}$)
A	Electrode surface area (m^2)	$\bar{K}$	mean equilibrium constant ($L \text{ mol}^{-1}$)
c	concentration (mol/L)	K^*	differential equilibrium parameter ($L \text{ mol}^{-1}$)
c_L	concentration of ligand, L (mol/L)	K_o^*	constant (= value of K^* for $\theta = 1$) ($L \text{ mol}^{-1}$)
c_{FA}	concentration of fulvic acid (mol/L)	k_a	association rate coefficient ($L \text{ mol}^{-1} \text{ s}^{-1}$)
c_M	free metal ion concentration (mol/L)	k_a'	$= k_a c_L \text{ (s}^{-1}\text{)}$
$c_{M,T}$	total metal concentration (mol/L)	k_d	dissociation rate coefficient (s^{-1})
c_{ML}	concentration of complex ML (mol/L)	$\bar{k}_d$	mean dissociation rate coefficient (s^{-1})
$c_{ML}(t)$	concentration of complex ML at any time, t (mol/L)	k_w	rate coefficient for water exchange
c_{ML_i}	concentration of complex formed by site i (mol/L)	M	free metal ion (metal aqua complex)
$c_{ML_i}^o$	concentration of complex ML_i at time zero (mol/L)	ML	metal complex formed by M with ligand, L
D	diffusion coefficient ($\text{m}^2 \text{ s}^{-1}$)	ML_i	metal complex formed by M with site type i
$\bar{D}$	mean diffusion coefficient ($\text{m}^2 \text{ s}^{-1}$)	t	time (s)
D_M	diffusion coefficient of free metal ion, M ($\text{m}^2 \text{ s}^{-1}$)	T	temperature (K)
D_{ML}	diffusion coefficient of complex, ML ($\text{m}^2 \text{ s}^{-1}$)	Γ	slope of the Differential Equilibrium Function and the Differential Kinetic Function ($0 < \Gamma \leq 1$)
E	potential (V)	δ	thickness of the Nernstian diffusion layer
$E_{1/2}$	half-wave potential (V)	ε	$= D_{ML}/D_M$
i	current (A)	v	liquid flow velocity
i_{lim}	limiting current (A)	θ	Degree of site occupation of the ligands in a heterogeneous system ($= c_{ML}/c_L$)
i_p	peak current (A)	Λ	lability parameter
J	flux	Λ_{ss}	steady state lability parameter
J_{kin}	kinetically controlled flux	μ	Reaction layer thickness $= (D_M/k_a')^{1/2}$
J_{diff}	diffusion-controlled flux		

APPENDIX A – Disjunctive Pathway

The rate constants can be calculated for each of pathways assuming steady-state concentrations of intermediate species (i.e., $\text{LNi}(\text{DMG})_2$ or Ni).

If the reaction follows the **disjunctive pathway**, then the following reactions will be written:

$$\begin{aligned}\frac{dc_{\text{Ni}(\text{DMG})_2}}{dt} &= k_2^{\text{disj.}} c_{\text{Ni}} c_{\text{DMG}}^2 \\ k_1^{\text{disj.}} c_{\text{NiL}} - k_{-1}^{\text{disj.}} c_{\text{Ni}} c_{\text{L}} - k_2^{\text{disj.}} c_{\text{Ni}} c_{\text{DMG}}^2 &= 0 \\ c_{\text{Ni}} &= \frac{k_1^{\text{disj.}} c_{\text{NiL}}}{k_{-1}^{\text{disj.}} c_{\text{L}} + k_2^{\text{disj.}} c_{\text{DMG}}^2} \\ \frac{dc_{\text{Ni}(\text{DMG})_2}}{dt} &= \frac{k_1^{\text{disj.}} k_2^{\text{disj.}}}{k_{-1}^{\text{disj.}} c_{\text{L}} + k_2^{\text{disj.}} c_{\text{DMG}}^2} c_{\text{NiL}} c_{\text{DMG}}^2\end{aligned}$$

Finally:

$$k_{\text{overall}} = k_{\text{disjunctive}} = \frac{k_1^{\text{disj.}} k_2^{\text{disj.}}}{k_{-1}^{\text{disj.}} c_{\text{L}} + k_2^{\text{disj.}} c_{\text{DMG}}^2}$$

APPENDIX B – Adjunctive Pathway

For the reaction that follows the **adjunctive pathway**, the rate law is:

$$\frac{dc_{\text{Ni(DMG)}_2}}{dt} = k_2^{\text{adj.}} c_{\text{LNi(DMG)}_2}$$

$$k_1^{\text{adj.}} c_{\text{NiL}} - k_{-1}^{\text{adj.}} c_{\text{LNi(DMG)}_2} - k_2^{\text{adj.}} c_{\text{Ni(DMG)}_2} = 0$$

$$c_{\text{LNi(DMG)}_2} = \frac{k_1^{\text{adj.}} c_{\text{NiL}} c_{\text{DMG}}^2}{k_{-1}^{\text{adj.}} + k_2^{\text{adj.}}}$$

$$\frac{dc_{\text{Ni(DMG)}_2}}{dt} = \frac{k_1^{\text{adj.}} k_2^{\text{adj.}}}{k_{-1}^{\text{adj.}} + k_2^{\text{adj.}}} c_{\text{NiL}} c_{\text{DMG}}^2$$

and:

$$k_{\text{overall}} = k_{\text{adjunctive}} = \frac{k_1^{\text{adj.}} k_2^{\text{adj.}}}{k_{-1}^{\text{adj.}} + k_2^{\text{adj.}}}$$